



DESIGN AND DEVELOPMENT OF AN ELECTROSPUN POLYMERIC TRANSCATHETER HEART VALVE FOR TISSUE ENGINEERING

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*To my mother, Tania,
the embodiment of wisdom and strength.
Thank you for teaching me to see the gift in failure,
and for giving me the courage to put one foot in front of the next.

This is, and always was, for you.*

Declaration

I, *Rosslee Christie Guess*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

The growing global burden of valvular heart disease (VHD) remains a significant challenge to overcome. Despite the advancements in heart valve replacements and the ground-breaking introduction of transcatheter valve technologies, current solutions offer suboptimal therapy as they either require lifelong anticoagulation or have limited durability. The ideal replacement valve should mimic the functionality of its native counterpart, comprise of viable living tissue, and offer lifelong durability, also for young patients.

The central aims of this project were to design, manufacture, and evaluate two types of electrospun trileaflet transcatheter heart valve (THV) substitutes, one with non-degradable Pellethane® leaflets (PEL valves) and the other type using degradable DegraPol15® leaflets (DP valves). PEL and DP scaffolds were successfully electrospun after implementing a humidity control system and optimising input (ambient and processing) parameters; and were characterised for their morphological (fiber geometry and architecture), mechanical (static and dynamic), and hydraulic (wettability and permeability) properties. Six distinct valve manufacturing concepts (leaflet suturing, leaflet bonding, sandwich spinning, trileaflet preforming, inverted trileaflet preforming and submould assembly) were designed and prototyped for feasibility screening and selection. The selected concept was further employed to produce PEL and DP valves that were subsequently assessed for hydrodynamic function and durability by means of pulsatile flow analyses and accelerated fatigue testing.

The fibres of DP scaffolds were approximately three times thicker than those in PEL scaffolds (5.2 ± 1.3 vs $1.8 \pm 0.5 \mu\text{m}$) with correspondingly larger pores (16.3 ± 4 vs $8.0 \pm 1.7 \mu\text{m}$). All scaffolds were essentially randomly oriented, and porosities averaged at $75 \pm 5\%$. PEL scaffolds were approximately three times stronger than DP counterparts with slightly lower extensibility (550 vs 730%). From the six valve manufacturing concepts, the method involving the stitching of pre-cut leaflets onto the expandable stent (CoCr, 23 mm) after heat bonding an electrospun PEL skirt was selected. Four groups of valves, differing in average leaflet thickness ($n = 2\text{--}4$ per group), were produced for each material type. Under simulated aortic conditions, the PEL valves ($N = 10$) achieved remarkably high accelerated fatigue life of up to 260 million cycles (equivalent to 2.5 years), with a positive correlation between leaflet thickness and durability. The hydrodynamic parameters obtained for the PEL valves were well within ISO standards ($\text{EOA} > 1.25 \text{ cm}^2$, mean pressure gradient $< 10 \text{ mmHg}$, and $\text{RF} < 20\%$ at a cardiac output of 5 l/min). In the current embodiments, DP valves ($N=14$) were not able to withstand aortic pressures but demonstrated good hydrodynamic function at nominal pulmonary pressures.

Although much further study is required, the concepts described in this project contribute valuable insight into the manufacturing, performance, and durability of devices intended for transcatheter heart valve tissue engineering.

Graphical Abstract

1. SCAFFOLD DEVELOPMENT

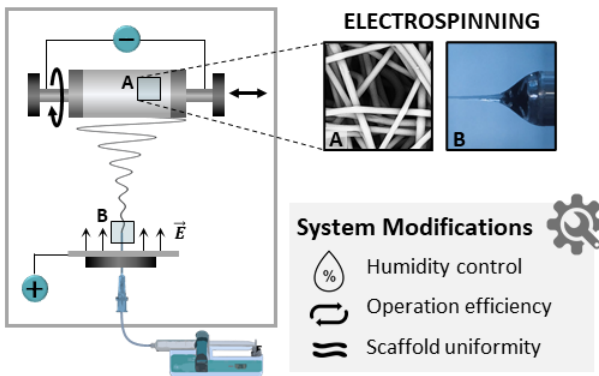
A. POLYMER SELECTION

Degradable DEGRAPOL® **Non-degradable** PELLETHANE®

B. PARAMETRIC INVESTIGATION

SOLUTION	PROCESS	AMBIENT
Concentration	Flow rate RPM	% RH

C. SCAFFOLD FABRICATION



D. SCAFFOLD CHARACTERISATION

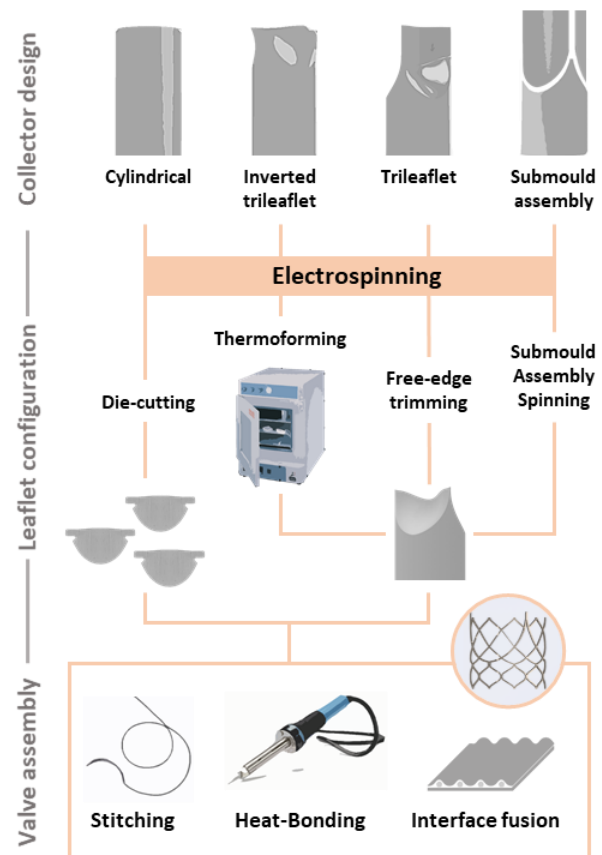
STRUCTURAL	MECHANICAL	HYDRAULIC
Fiber Diameter	Static tensile	Permeability
Pore Size	Cyclic tensile	Hydrophobicity
Orientation		
Porosity		

SCAFFOLD OUTCOMES

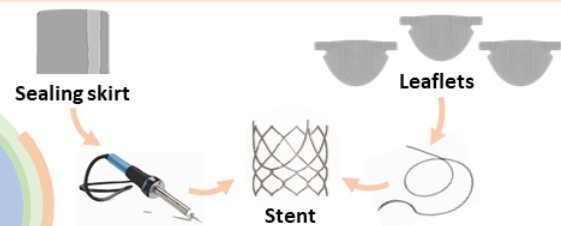
- ✓ ↑ Fiber uniformity
 - ✓ ↑ Pore size and porosity
 - ✓ ↑ Mechanical integrity
 - ✓ Viscoelasticity
 - ✓ ↑ Wettability
 - ✓ ↑ Permeability
- TARGET**
- ≈ Native valvular tissue
 - ≈ Current TEHV's

2. VALVE MANUFACTURING DESIGN

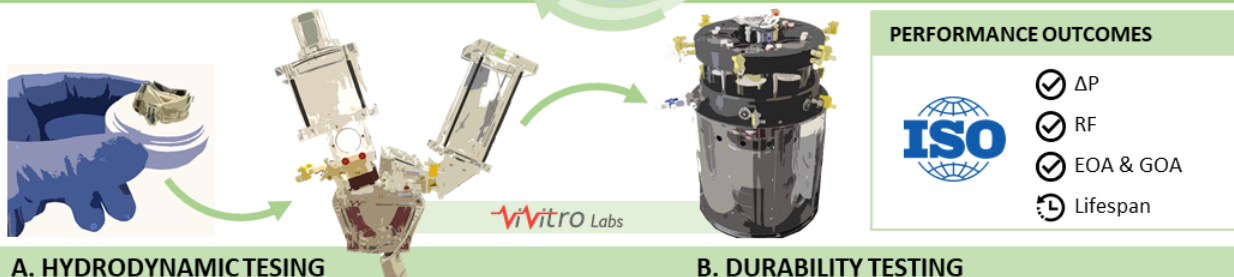
A. CONCEPT GENERATION & PROTOTYPING



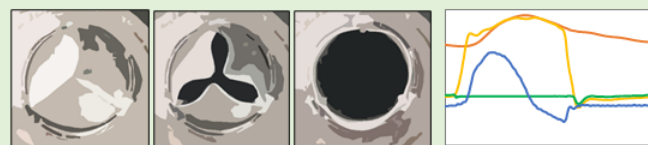
B. CONCEPT SELECTION



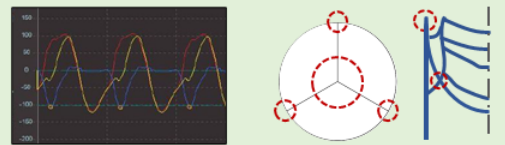
3. VALVE EVALUATION



A. HYDRODYNAMIC TESTING



B. DURABILITY TESTING



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List of Symbols

$\vec{E}$	Electric field strength
ρ	Density
m	Mass
E	Elastic modulus
ε_{max}	Maximum elongation
V	Voltage
A	Area
D_{fiber}	Fiber diameter
D_{pore}	Pore size
wt%	Solution weight percent (wt/wt)
OI	Orientation index
Q	Flow rate
Q_{rms}	Root mean square forward flow
μm	Microns
R^2	Coefficient of determination
P	Probability value
κ	Permeability
K	Modulus of toughness
ΔP	Transvalvular pressure gradient
t	Thickness
w	Width
l	Length
T	Time
d	Distance
γ	Surface tension
n	Number
N	Population number
r	Radius
n	Sample size
N	Sample population size
Da	Dalton
SD	Standard deviation

List of Abbreviations

ANSI	American National Standards Institute
ASTM	American Society for Testing and Materials
AV	Aortic valve
BDI	Bending deformation index
BPV	Bioprosthetic valves
CFP	Closing flow period
CHCl ₃	Chloroform
CO	Cardiac output
CRU	Cardiovascular Research Unit
DMAc	N,N-Dimethylacetamide
DMF	N,N-Dimethylformamide
DP	DegraPol®
DP15	DegraPol15®
EC	End of closure
ECM	Extracellular matrix
EOA	Effective orifice area
ES	End of systole
FR	Flow rate
GOA	Geometric orifice area
HV	Heart valve
ISO	International Organisation for Standardisation
LPM	Litres per minute
NA	Not applicable
NR	Not reported
PD	Pulse duplicator
PEL	Pellethane®
PEU	Polyester urethane
PI	Pinwheeling index
PV	Pulmonary valve
RF	Regurgitant fraction
RH	Relative humidity
RHD	Rheumatic heart disease
RMS	Root mean square
RVCT	Rapid valve-closing time
RVCVI	Rapid valve-closing velocity index
RVOT	Rapid valve-opening time
RVOVI	Rapid valve-opening velocity index
SAT	Strait Access Technologies
SD	Standard deviation
SEM	Scanning electron microscope
SS	Start of systole
TAVI	Transcatheter aortic valves
TCD	Tip-to-collector distance
TE	Tissue engineered
TETHV	Tissue engineered transcatheter heart valve
THF	Tetrahydrofuran
THV	Transcatheter heart valve
TPU	Thermoplastic Polyurethane
UPy-PC	Ureidopyrimidinone-polycarbonate
UPy-PCL	Ureidopyrimidinone-polycaprolactone
UTS	Ultimate tensile strength
VHD	Valvular heart disease

1 Introduction

1.1 Heart valve anatomy and biomechanics

The primary function of heart valves (HVs) is to direct the unidirectional flow of blood throughout the cardiac cycle (illustrated in Figure 1.1) while facilitating optimal flow efficiency with negligible regurgitation¹. Thus, heart valves play a vital role in ensuring the sustained functioning and survival of all biological structures and processes within the body. Heart valves are essentially comprised of passive tissues that act in response to inertial forces exerted by the surrounding blood. The inertial forces, generated by alternating contractions of cardiac muscle, direct HVs to open and close with each cardiac cycle². While pumping 3-5 litres of blood per minute during a cardiac cycle, heart valves are subject to cyclic, tensile, shear and flexural loading forces. Resultant blood flow velocities reach up to 1.35 ± 0.35 m/s within the aortic valve, and transvalvular pressures generally reach 80 mmHg. The highly dynamic motions, large anisotropic deformation and complex surface geometries of the thin valvular leaflets contribute largely to the challenges faced in developing a prosthetic heart valve that accurately mimics the intricate behaviour of its native counterpart^{1,3}.

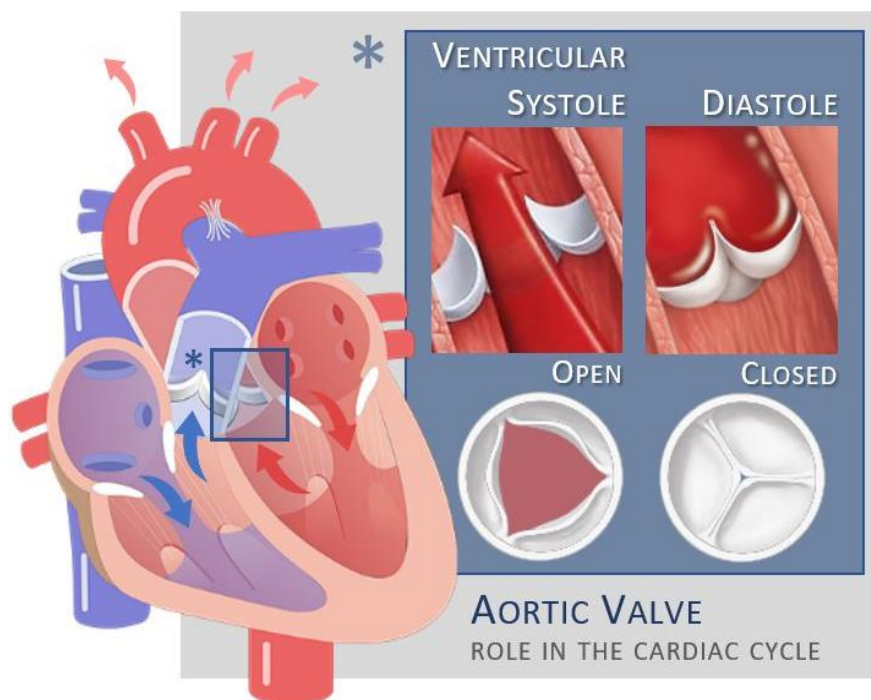


Figure 1.1: Illustrative diagram of heart valve anatomy showing the location and basic biomechanics of the aortic heart valve.

1.2 Disease state fundamentals

1.2.1 Cardiovascular Disease

Over the past few decades, cardiovascular disease (CVD) has prevailed as the leading cause of annual deaths globally⁴. Constituting an estimated 31% of global fatalities, the current status of the CVD epidemic looms as an immensely threatening global health phenomenon, which is particularly severe on low- and middle-income countries (LMICs) where the rise in prevalence persists⁵. While approximately 75% of CVD related deaths occur in LMICs⁶, it is the poorest of these populations where the magnitude and severity of this health burden is greatest⁷. This can be attributed to the lack of access to well-equipped screening facilities and basic disease management resources within these communities⁸. Furthermore, owing to the general lack of education at primary, secondary and tertiary levels, basic health awareness among these vulnerable populations remains largely undeveloped, which has been directly associated with CVD fatality rates⁷. Moreover, the growing prevalence of CVD worldwide has led to a general shortage of cardiovascular specialists required to meet the clinical demands in both private and public sectors⁹. To this end, the prevailing lack of access to essential healthcare resources is being exacerbated by the growing need for specialist clinicians in cardiology¹⁰. Currently, the highest prevalence of CVD occurs in young adult populations in LMICs, who essentially account for the majority of the global workforce. Thus, the economic implications of this disease are not confined to LMICs but significantly impact high-income countries as well⁴.

1.2.2 Heart valve disease

Of the most prevalent CVDs, the emerging epidemic of valvular heart disease (VHD) has become one of critical concern worldwide¹¹. Until the 1950s, valvular disease was predominantly presented with rheumatic aetiology. Thereafter the predominance of valvular disease in developed countries shifted to a degenerative aetiology¹². From a study conducted by Coffey *et al.*¹³. Degenerative valvular disease is primarily acquired by populations over the age of 65 years and presents a rapid increase in prevalence with advancing age. In the context of the global population aging phenomenon, where it has been predicted that the population of over 60 years of age is going to triple by 2050¹⁴, the corresponding trajectory of degenerative VHD incidence is distressing¹¹.

In developing countries, such as South Africa, the burden of VHD manifests primarily as rheumatic heart disease (RHD) – a condition that poses a significant threat to children, adolescents, and young adults¹⁵. It is estimated that the young adult patient population, specifically in developing countries, will triple by 2030¹⁶, which would impose great strain on the already overstretched and under-resourced healthcare facilities within these regions¹⁷. The strong correlation between VHD prevalence and population growth points to the concerning projection of morbidity and mortality rates associated with the disease¹⁸.

While the first prize would be to achieve disease prevention and basic management *before* surgical intervention is necessitated; the complex interdependence of sociology, economics, politics, and socioeconomics involved

in achieving a shift of this nature on a global scale is an implausible objective. Ultimately, achieving this would require dedicated cooperation from various inherently unpredictable global industries. For this reason – it is important that surgical interventions are kept at the frontline of advancement; and that alternative treatment strategies continue to be innovated, investigated, and developed. To achieve the development of an effective treatment strategy – that not only markedly improves outcomes for individuals, but also alleviates the colossal burden of this disease for future generations on a global scale – international research collaboration, coordinated resource alliances, and multidisciplinary involvement is critical¹⁷

1.2.3 Heart valve disease treatment and challenges

Primary treatment strategies for VHD include surgical valve repair and total valve replacement; implemented according to the type and severity of a patient's condition¹⁹. To date, the number of heart valve replacement (HVR) procedures performed annually has risen to over 300,000 worldwide. Consequent to the projected rise in VHD prevalence over the coming years, by 2050 the annual demand for HVR procedures in developed countries alone is projected to rise to approximately 850,000²⁰, while for developing countries the need is predicted to be far greater with estimates reaching into the millions²¹.

Currently treatment of VHD relies largely on surgical valve replacement, by either mechanical or bioprosthetic valves (BPVs). While these replacement therapies have proven to greatly improve patient survival and revive quality of life²², each therapy presents inherent drawbacks and limitations²³. Thromboembolism is a common and severe potential side effect of mechanical valve replacement, which requires administration of lifelong anticoagulation medication. Calcification and structural deterioration are inevitable progressions for BPV implants, which necessitates repeated replacement surgeries approximately every 10 to 15 years²⁴. Ultimately, these ramifications jeopardize patient morale and general wellbeing²⁵.

Furthermore, replacement procedures for both mechanical and surgical bioprosthetic valves involve open-heart surgery; a highly invasive procedure that demands a long-recovery period. For this reason, elderly patients in frail conditions or with compromising comorbidities are mostly deemed unfit to undergo surgical valve replacement. Thus, with the rapid growth in aging populations, the incidence of patients that are not eligible for open-heart surgery is increasing²³. Additionally, postoperative depression has been found to manifest following open-heart surgery²⁶, indicating the burdensome impact not only a patient's physical condition, but also on their emotional wellbeing. To address the many issues associated with open-heart surgery, transcatheter valve implantation procedures were developed. However, as transcatheter valves typically comprise of biological tissue they too are susceptible to tissue degeneration and are subsequently limited in durability²⁷. As the inherent complications associated with HVR are exacerbated in developing countries (due to infrastructural, educational and healthcare deficits), replacement failure and consequent death is a harsh, but inevitable reality for the majority these populations²⁸.

Additional complications manifest within paediatric and young adult populations, for whom current heart valve replacement strategies do not provide as favourable results as in adult populations²⁹. For example, the process of calcification and subsequent degeneration of BPVs has been found to be markedly accelerated in younger patients³⁰. Moreover, the inability for growth, repair and remodelling of both mechanical and biological HV prostheses – essential for accommodating the growth and maturation of a child over time – creates a perpetual anatomical complication. As a result, reoperation is required over shorter periods of time, not only due to children outgrowing their prostheses, but also due to premature HVR failure as a result of rapid degradation. Having to endure numerous surgeries over the course of one's life is without question deeply traumatic for any child endure³¹. Evidently, the importance of developing an alternative strategy for the replacement of heart valves that overcomes associated limitations is critical.

1.3 Existing solutions

Amidst the eruption of medical advancement during the post-second world war period, significant developments in cardiac surgery emerged, such as the break-through success of the first heart valve replacement procedure in 1952^{32,33}. However, 70 years later, diseased HVs continue to be replaced by prostheses that closely resemble concepts born in the 1960s and that manifest similar inherent complications³⁴. Nevertheless, there has been a slow undercurrent of evolution in heart valve replacement technologies over the years, which ultimately led to the breakthrough technique of transcatheter aortic valve implantation (TAVI) that was clinically realised in 2002³⁵. With the advancement of TAVI technologies and increasing efforts toward the development heart valve interventions, HVR therapies are seeing a positive turn in trajectory. The central objectives of HVR that remain to be met include achieving analogous physiological flow patterns; reducing thrombogenicity; optimizing haemodynamic performance; and improving durability²⁷. Currently, cardiac valve prostheses can be categorised as (1) surgical valves, which include mechanical and bioprosthetic valves (BPVs); and (2) transcatheter valves, which include bioprosthetic and polymeric valves.

1.3.1 Surgical valves

Surgical valve replacement involves an invasive open-heart surgery; wherein the diseased valve and surrounding calcifications are excised, and the valve prosthesis is subsequently sutured into the decalcified valve annulus.

1.3.1.1 Mechanical Valves

To date, the development of mechanical valves have progressed through three main designs: caged-ball, single tilting disc, and bileaflet valves²³ (Figure 1.2AC). The first total valve replacement, which involved the use of a caged-ball valve, was performed in 1960 and was a significant breakthrough for cardiovascular surgery worldwide³⁶. However, while the caged-ball valve presented good durability and minimal risk of mechanical failure, the model was discontinued in 2007, due to its suboptimal haemodynamics and elevated risk of thromboembolism. Currently, the bileaflet design is the most commonly used mechanical valve compared to

the single tilting-disc prosthesis³⁷. More recently, a novel design featuring a trileaflet configuration (Figure 1.2D) has demonstrated promising *in vivo* outcomes, such as low thrombotic potential due to its superior haemodynamics over bileaflet mechanical valves³⁸. However, further investigation is required for this technology to be clinically endorsed.



Figure 1.2: Mechanical heart valve prostheses. A) Starr-Edwards ball-cage valve, B) Björk-Shiley tilting-disc valve, and C) St Jude Medical Regent bileaflet valve. D) Novel trileaflet mechanical heart valve prosthesis. E) Thrombosed bileaflet mechanical valve prosthesis (adapted from Zenses *et al.*²⁷, Blot *et al.*³⁹, Sun *et al.*⁴⁰, Schaller *et al.*³⁸, and Callisen *et al.*⁴¹, respectively).

Regardless of the mechanical valve type, life-long anticoagulation therapy is currently a mandatory prescription for all recipients, which while preventing thrombus formation, raises risk of bleeding complications⁴². Thromboembolic complications associated with these devices is largely attributed to the complex and unsteady flow profiles induced at the leaflet hinge points⁴³. Achieving more physiologic flow profiles remains the greatest challenge for mechanical valve design optimization. Ultimately, long-term durability of mechanical valves is the primary advantage over bioprosthetic valves, where valve degeneration is a greater issue³⁷.

1.3.1.2 Bioprosthetic valves

A characteristic feature of BPVs, which comprise primarily of biological tissue³⁷, is their trileaflet structure that closely mimics the geometry of their native counterpart. Surgical bioprostheses can be divided into two categories, namely, stented or stentless valves (Figure 1.3). Stented bioprostheses are composed of three leaflets typically derived from porcine valve leaflets or bovine pericardium that are mounted on a flexible frame²³. While the frame provides essential structural support to the leaflets, hot spot stresses at the stent attachment sites significantly reduces tissue longevity, leading to premature tissue deterioration⁴⁴.



Figure 1.3: Surgical bioprosthetic heart valves. Stentless: (A) Medtronic Freestyle⁴⁵, (B) St. Jude Toronto SPV (adapted from Yong *et al.*⁴⁶); Stented: (C) Medtronic Hancock II (adapted from Kheradvar *et al.*³⁷, (D) St. Jude Epic™ (adapted from Mylotte *et al.*⁴⁵). E) Severe calcific stenosis of a bioprosthetic porcine AV (adapted from Rodriguez-Gabella *et al.*⁴⁷).

Stentless bioprostheses, on the other hand, are constructed from porcine aortic wall and valve tissues⁴⁸. The greater degree of flexibility achieved with this valve construct significantly enhances distensibility and reduces leaflet stress in comparison to stented valves. However, despite the improvement in haemodynamics and biomechanical properties, stentless valves have not demonstrated superior clinical outcomes at short- and mid-

term compared to stented bioprostheses⁵⁰. Due to the excessive complexity associated with implanting stentless valves, stented bioprostheses remain the preferred replacement substitute⁵¹.

Prior to implantation, biological tissues used in both stented and stentless valves undergo chemical fixation treatments to reduce immunogenicity and mitigate calcification. However, no established fixation techniques have proven to fully circumvent these limitations⁵². Consequently, calcification, structural deterioration, and subsequent valve failure (portrayed in Figure 1.3E) is an inevitable outcome for BPVs. The replacement of BPVs is thus required within a 10–15-year period⁵³, while for children and pregnant women these processes are accelerated, and reoperation is required more frequently³⁷. Limited durability is considered the greatest obstacle to advancing BPV technology and thus remains a focal point of associate research⁵³.

1.3.2 Transcatheter valves

1.3.2.1 Transcatheter heart valve replacement

Transcatheter valves consist of three leaflets, primarily made of bovine or porcine pericardium, that are mounted on a metal stent in a trileaflet-valve configuration²⁷. The implantation technique involves the insertion of a specialized flexible delivery catheter, which transports the crimped valve to the aortic annulus via a vascular route, under fluoroscopic guidance. Currently, vascular access through the femoral artery (transfemoral route) accounts for more than 90% of all TAVI cases⁵⁴. Alternative access routes include the apex of the heart (transapical), ascending aorta (transaortic), and the axillary artery (transaxillary)⁵⁵. Upon correct placement, the valve is deployed using one of two techniques depending on the valve type. These comprise balloon-expandable valves (Figure 1.4A) and self-expandable valves (Figure 1.4B and C)⁵⁶.

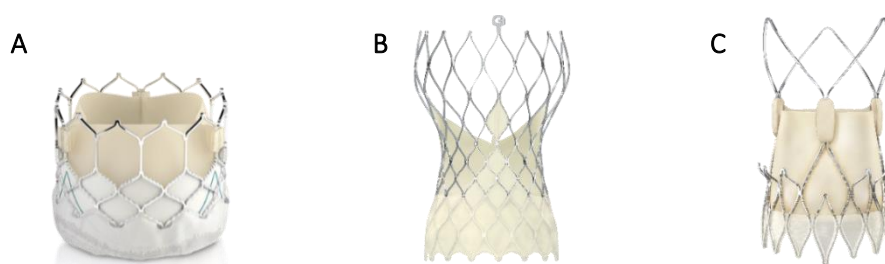


Figure 1.4: Transcatheter bioprosthetic heart valve. A) Edwards SAPIEN 3 balloon-expandable valve. B) Medtronic CoreValve Evolut R. C) Boston ACURATE neo™ TF (taken from Gonska *et al.*⁵⁷, Neylon *et al.*⁵⁴, and Costa *et al.*⁵⁸, respectively)

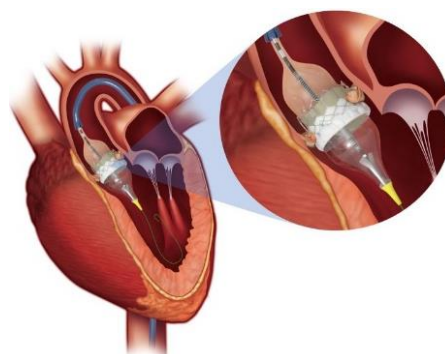


Figure 1.5: Illustration of balloon-expandable valve. Complete balloon inflation and expansion of the valve across the stenotic aortic valve (adapted from Kerrigan *et al.*⁵⁹)

Balloon-expandable valves are crimped on a balloon catheter and deployed by balloon inflation (Figure 1.5). Cobalt-chromium supersedes stainless steel as the preferred stent material for balloon-expandables, as it permits a reduced strut thickness, which increases flexibility and conformability, without compromising the mechanical properties; subsequently allowing for a smaller introducer sheath, and improved deliverability⁶⁰. Self-expandable valves, on the other hand, are housed in the catheter, and deployed using a retraction mechanism. The key to achieving this technique is the use of a shape-memory alloy stent (e.g., Nitinol) that due to its superelastic properties and shape memory property that gets triggered with heat exposure, rapidly expands as the catheter is gradually retracted (Figure 1.6)²⁷.

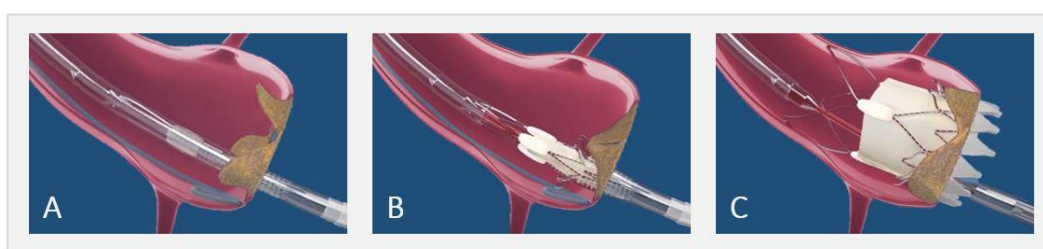


Figure 1.6: Self-expandable transcatheter valve. New generation top-down deployment approach featuring self-expandable ACURATE neo™ valve⁶¹. A) Positioning of valve delivery system in the aortic annulus. B) Initial antegrade retraction of the catheter. C) Final retrograde retraction and complete valve expansion across the native stenotic valve.

1.3.2.2 Current challenges for transcatheter heart valve replacement

While the transcatheter technique provides the solution to minimising procedural invasiveness and has demonstrated areas of superior haemodynamic performance compared to surgical valves, the technology presents a new line of complications and weaknesses that need urgent mitigation before it can be adopted as the gold standard for all patient groups requiring aortic valve replacement⁶². Common complications associated with TAVI development include: paravalvular regurgitation, valve malpositioning and migration, vascular complications, valve degeneration⁵⁶, and above all, cardiac rhythm disturbance – a conduction disorder which requires treatment with a permanent pacemaker, and occurs in up to 30%-50% of patients⁶³.

The occurrence of paravalvular regurgitation (PVR) is a contributing factor to the adverse effects outcomes of TAVI procedures⁶⁴. Unlike surgical valves, transcatheter valves must accommodate noncalcified aortic annuli and consequent anatomical irregularities. This can cause complications with valve positioning and anchorage, resulting in nonsymmetric deployment, valve migration, and subsequent PVR⁶⁵. To address these issues, the integration of sealing skirts and/or cuffs were introduced to TAVI valve design to reduce paravalvular leakage. In addition, recent generations typically incorporate control arms for improved valve anchorage, as well as retrieval/repositioning mechanisms to improve the fidelity of deployment procedures⁶².

In the earlier stages of TAVI development, a high incidence of vascular complications, owed to vascular damage inflicted during catheter delivery, contributed significantly to the adverse clinical outcomes of these procedures⁶⁶. However, after the focus of TAVI development turned to minimising delivery profiles, the routine use of 22-24F introducer sheaths was soon substituted for smaller profiles of up to 14-16F which facilitated the shift toward less problematic TAVI operations²⁷, leading to the plummet in incidence of vascular complications⁵⁴.

Despite the rapid development of TAVI technologies and the considerable progress that has been made toward improving the safety and efficacy of these procedures²⁷, the use of biological tissue in TAVI valves introduces durability constraints akin to those of surgical bioprostheses⁶⁷. Thus far, the persistence of structural valve degeneration due to progressive calcification, immunologic damage, and leaflet fatigue, limits the development of TAVI technologies to expanding the reach in vulnerable populations, rather than addressing the vexing issue of long-term valve durability⁵².

1.3.3 Polymeric valves

The investigation of flexible polymeric materials for fabricating heart valve substitutes emerged in the 1950s⁶⁸, and saw its first clinical application in 1960⁶⁹. Silicone rubber, PTFE (Teflon®), PET (Dacron®), polyvinylchloride and polyurethane (PU) were of the first polymers used to fabricate polymeric valves and undergo *in vitro* and *in vivo* testing⁷⁰. While none of these valves proved to be eligible for human implantation, due to rapid material degradation and thrombogenicity, PUs displayed the most promise as an alternative to biological leaflet tissues⁷¹. Their promising mechanical properties, good biocompatibility, versatile processability, and affordable manufacturability resulted in research on PUs to gain widespread popularity⁷². However, over the years that followed, the promise of superior durability and improved biostability remained an unmet expectation of PUs at the time⁷³. Clinical evidence of premature failure exposed fundamental shortfalls of PU valves that would require deeper investigation and more advanced technologies⁷⁴. PU degradation mechanisms, such as mineralization, oxidation, hydrolysis and environmental stress-cracking, were identified as being integral to the material's limited long-term efficacy⁷⁵.

Subsequent to the progress made toward overcoming these limitations, a variety of novel polyurethanes have emerged as candidate materials for flexible valve leaflets, including polyester urethanes⁷³ (e.g., Lubrizol Estane®), polyether urethanes (PEUs) (e.g., Angioflex®), (silicone)polyurethanes (known as Avcothane™-51) polycarbonate urethanes (PCUs) (e.g., Bionate®)⁷⁶, and more recently, siloxane poly(urethane-urea)s (SiPUUs) (known as LifePolymer™)⁷⁷.

Early *in vivo* outcomes of valves made of polyester urethane and PEU reported high susceptibility to rapid hydrolysis and oxidative degradation respectively, thus deducing inadequate durability⁷⁸. In contrast, PCU-based valves (Figure 1.7B-C) tested under *in vivo* conditions have demonstrated superior hydrolytic and oxidative stability indicating improved resistance to degradation⁷⁰. The favourable outcomes demonstrated by PCU valves set a new precedent for research to follow⁷³.

Concurrently, significant advances in synthesis methods and structural modification of polymers launched a new generation of biomaterials with enhanced properties⁷³. Herein, the investigation of novel surface modification techniques gained increasing relevance on the premise that improving the surface characteristics of biomaterials significantly influenced its biocompatibility⁷⁹. Concurrently, the effect of a biomaterial's surface topography on certain cell behaviours (e.g., cell adhesion, proliferation, differentiation, and apoptosis) was

realised. From this, it was conceived that by introducing topographic features at nanoscale, and thereby creating a ‘cell-friendly’ surface on polymers, the biostability, thromboresistance and haemodynamic performance of polymeric valves could be significantly improved⁸⁰. Another advantage of nanocomposite polymers is the ability to isolate the favourable properties and mitigate the inherent weaknesses of two or more materials to achieve superior mechanical and biological stability⁷².

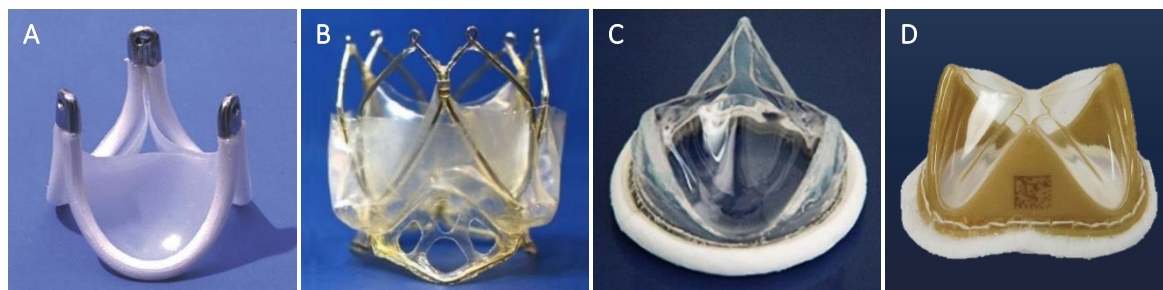


Figure 1.7: Polymeric heart valves. A) The Reul-Haussinger valve (Avcothane-51 (PU/PDMS)). B) The TRIKSKELE valve (PCUU/POSS). C) The ADIAM® PCU valve. D) Foldax Tria Aortic Valve (PiSUU) (adapted from, Jenney *et al.*⁸¹, Daebritz *et al.*⁷⁰, Kutting *et al.*⁸², and Rahmani *et al.*⁸³, respectively).

In the wake of the more recent advances in polymer synthesis and biomaterial modification, widespread development of novel polymeric valves was evoked²⁷. One such example is the Strait Access Technologies (SAT) TAVI polyurethane valve, which was reported to demonstrate superior durability, reduced thrombogenicity and other favourable performance outcomes such as minimized PVL and effective valve anchorage⁸⁴ (Figure 1.8). With this, the development of polymer scaffolds for tissue engineering applications was conceived as an alternative approach to prosthetic heart valve design⁸⁵.



Figure 1.8: Polymeric transcatheter aortic valve prosthesis. Left: before crimping and deployment. Right: after deployment by balloon-expansion. developed by Strait Access Technologies (adapted from Scherman *et al.*⁸⁶).

More recently, a novel surgical polymeric valve comprising flexible SiPUU leaflets (known as the Foldax® Tria Aortic Valve) (Figure 1.7D) was developed, and was reported to fulfil essential properties for heart valves leaflets, such as “low dynamic modulus, high tensile strength, minimal creep, and excellent biostability” under thorough *in vitro* and *in vivo* evaluation⁸¹. The valve is currently undergoing its first clinical investigation, and with preliminary evaluations indicating favourable early outcomes⁸⁷, the goal of establishing polymeric valves in a clinical setting has been brought closer to a reality.

1.4 Tissue Engineering

1.4.1 Concept fundamentals

The field of tissue engineering emerged in the late 1980s, with the term first officially being coined at the 1988 National Science Foundation in Granlibakken⁸⁸. However, it was only years later that the concept of tissue engineering, as we understand it today, was fully defined in the landmark article, *Tissue Engineering*, by Langer and Vacanti⁸⁹. Tissue engineering was therein defined as an interdisciplinary field where the principles of both engineering and life sciences are applied to develop biological substitutes that replace, restore, maintain, or improve the functioning of native tissues or organs⁸⁹. Since the inception of the field, tissue engineering strategies have been contingent on three interrelated factors, which known as the “triad of tissue engineering”, includes (1) scaffolds, (2) cells, and (3) signals⁹⁰ (Figure 1.9).

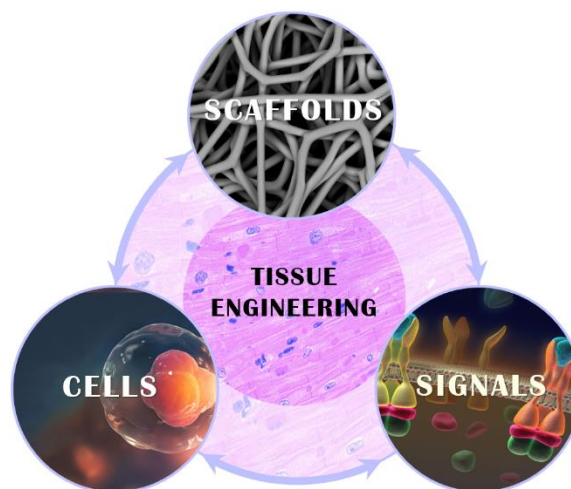


Figure 1.9: The triad of tissue engineering. The combination of cells, scaffolds, and signal for engineering functional tissue

1.4.1.1 Cells

Cells, being the founding constituents of all living tissue, play a vital role in creating tissue substitutes. The recent escalation of study on cell manipulation and stem cell differentiation has revealed an infinite vista of potential in the field, and expanded the cell pools for all tissue engineering applications⁹⁰. Autologous cells are currently considered the favourite cell type as they are non-immunogenic, but due to them being limited in supply, investigation into the use of allogeneic or xenogeneic cell sources has gained more attention. To date, human embryonic and adult mesenchymal stem cells are the most commonly used cell type in tissue engineering⁹¹. However, stem cell-based tissue engineering is a growing paradigm, which is anticipated to secure its position in clinical practice over the coming years. Moreover, the discovery of induced pluripotent stem cells, which enables the provision of a large source of autologous cells⁹², has given rise to new possibilities in the stem cell industry, and alternative prospects for engineering functional tissues⁹³.

1.4.1.2 Signals

Following cell seeding, cell survival and extracellular matrix (ECM) synthesis within engineered scaffolds requires specific biological signals. Cell responses such as proliferation, migration, and differentiation, to name a few, are induced by an intricate interaction of signals generated in the surrounding cell microenvironment. In tissue engineering, primary cell signals include growth factors, mechanical stimulation, oxygen levels, and ECM molecules, of which unique combinations are required to engineering different tissues⁹⁰. Essentially, the goal of integrating various cell signals is to simulate the biochemical processes and physiological conditions that influence the cell's behaviour and manipulate a scaffold's propensity to generate functional tissue.

To generate the physiological loading conditions required for the various stages of tissue growth and maturation, the use of bioreactors was introduced to tissue engineering practice⁹⁴. Bioreactors are designed to provide a controlled, dynamic culturing environment wherein biological substitutes are generated from engineered constructs. Apart from providing the "classic" conditions, such as pH and temperature, bioreactors are designed to facilitate mechanical conditioning, such as tensile, compressive and shear loading; control hydrostatic pressure; and regulate perfusion conditions⁹⁵. Moreover, bioreactors have been proposed to standardise, control, and automate culture conditions, which is essential for achieving reproducible outcomes, and a prerequisite for bringing tissue engineered products to the clinic.

1.4.1.3 Scaffolds

Achieving cohesion between the three elemental factors of tissue engineering depends largely on the properties of the engineered scaffold⁹⁶. Scaffolds are primarily required to support cells and/or growth factors and facilitate appropriate cell activities; provide structural and functional support immediately upon implantation; and encourage neotissue ingrowth and remodelling⁹⁷. With recent advancements in scaffold engineering technologies, the variety in biomaterials and manufacturing strategies has evolved dramatically since the field was conceived. Conversely, the objective of tissue engineering has remained precisely the same, along with the fundamental criteria for designing TE scaffolds, which include:

- (i) **Material selection** – the choice of scaffold material is crucial as it will largely determine the selection of manufacturing and material processing techniques implemented, as well as govern the range of material properties achievable for the engineered construct ⁹⁸
- (ii) **Manufacturing technology** – to achieve clinical and commercial viability, this should be cost-effective and capable of upscaling from small-scale prototyping to large-scale batch production in order for the tissue engineered construct ⁹⁹
- (iii) **Scaffold architecture** –comprising ideally of a highly porous, three-dimensional structure, with interconnected pore networks to encourage cellular penetration, facilitate diffusion of nutrients and oxygen throughout the construct, and allow waste removal ¹⁰⁰.

- (iv) **Mechanical properties** – which should essentially be comparable to the anatomical site, strong enough to endure surgical handling during implantation, and be sufficient for the construct to function effectively from the time of implantation until the remodelling process is complete ¹⁰¹.
- (v) **Biocompatibility** – the degree to which cells adhere to the scaffold surface, migrate onto and through the scaffold, proliferate, and ultimately lay down a new matrix, all while functioning normally ⁸⁸.
- (vi) **Biodegradability** – since the intention for TE scaffold implants is not to persist permanently, but rather be replaced over time by the body's own cells; for which the rate of degradation must be synchronous with that of tissue formation ¹⁰²

1.4.2 Heart valve tissue engineering

1.4.2.1 Criteria overview

The optimal characteristics for an ideal heart valve prosthesis was defined in as early as the 1960s by a renowned pioneer in heart valve surgery, Dr D. E. Harken¹⁰³. Described as the “*ten commandments*” for heart valve prosthesis design, these criteria remain the global industry benchmark, which to date, no existing device has sufficiently fulfilled¹⁰⁴. Among these criteria, the most pertinent include sufficient functional and structural durability; lack of antigenicity and absence of thrombogenicity; resistance to infection; and potential for growth regarding congenital heart valve disease¹⁰⁵.

It was really only when the concept of tissue engineering was applied to the development of heart valve replacement strategies that the possibility of fulfilling these criteria was realised¹⁰⁶. As originally proposed, the main objective of tissue engineering is to generate tissue/organ substitutes that mimic the structure, function and mechanical behaviour of its native biological counterpart¹⁰⁶. To achieve this objective in heart valve tissue engineering, numerous key biological and functional criteria, including those listed in Table 1.1, need to be met.

Table 1.1: Key criteria for heart valve tissue engineering

BIOLOGICAL	FUNCTIONAL
▪ Biocompatible ▪ Biodegradable ▪ Non-immunogenic ▪ Non-thrombogenic ▪ Facilitate tissue growth and remodelling ▪ Accommodate growth and maturation	▪ Appropriate pulsatile flow dynamics ▪ Functional durability ▪ Synchronous leaflet action ▪ Efficient opening behaviour ▪ Smooth and complete coaptation ▪ Suitable degradation characteristics

1.4.2.2 Current strategies

Since the emergence of heart valve tissue engineering (HVTE), several different approaches to producing tissue engineered heart valves (TEHVs) have emerged, which are broadly categorised as *in vitro*, *in vivo*, and *in situ* HVTE (illustrated in Figure 1.10). Figure 1.11 presents the outcomes obtained from different studies that investigate the respective approaches^{107,108}.

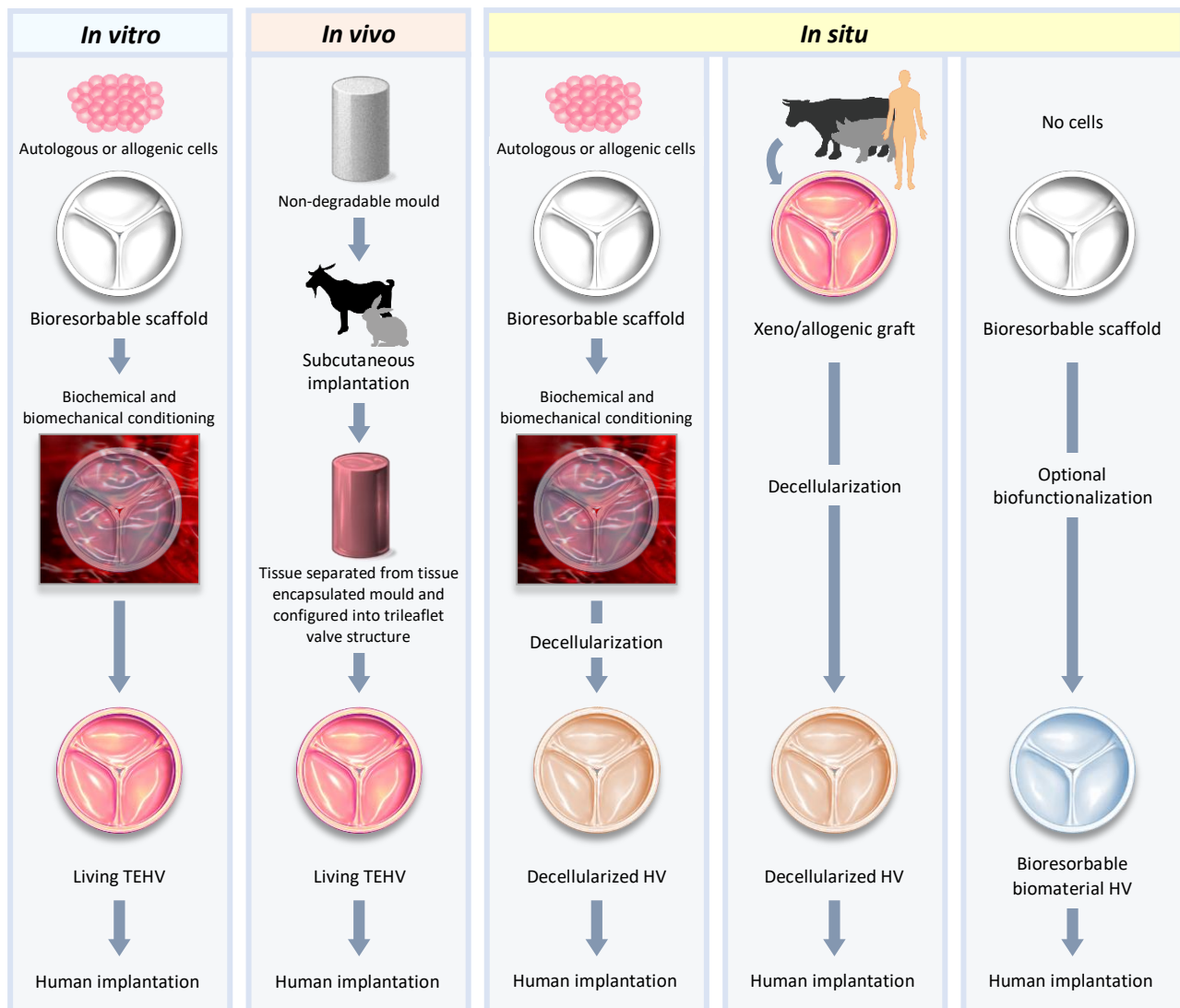


Figure 1.10: Illustrative diagram of different heart valve tissue engineering strategies known as *in vitro*, *in vivo*, and *in situ*.

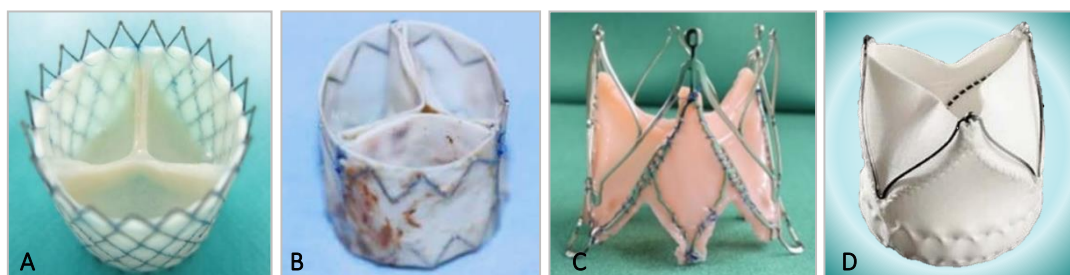


Figure 1.11: Different approaches to engineering tissue heart valves. A) *In vitro* TEHV¹⁰⁹. B) *In vivo* TEHV¹¹⁰. C) *In situ* TEHV based on decellularized engineered tissues¹¹¹ and D) bioresorbable polymer scaffolds¹¹⁰.

In vitro tissue engineering

In vitro tissue engineering involves the use of a cell support device (scaffold) seeded with cells and/or growth factors which gets introduced to a controlled environment wherein cells are prompted to deposit ECM components and subsequently generate viable living tissue for implantation⁸⁸. The conditions of the cell culture environment play a critical role in the maturation of the tissue construct, and ultimately determines its architectural and functional expression. The key to obtaining an effective *in vitro* grown tissue, is to generate

culturing conditions that simulate the physiological environment corresponding to the *in vivo* implant site, such that the functionality of the engineered implant corresponds seamlessly to its native counterpart. To induce this level of functionality extensive culturing is required, for which a complex integration of biological, chemical and/or mechanical stimuli is necessary. Upon implantation, the presence of cells in the engineered construct provide physical and biochemical cues that along with the body's natural ability to regenerate, contribute to tissue development and remodelling¹¹². While the ineffable complexity of human physiology continues to impede the translation of clinical trials to clinical application, the *in vitro* technique has provided an invaluable platform from which strides are being made toward achieving regenerative therapies for commercialization¹¹³.

***In vivo* tissue engineering**

The *in vivo* approach was first investigated in the 1960s, and involved using the body as a 'bioreactor', whereby the foreign-body response was exploited to grow autogenous tissue on a tissue-die for autologous transplantation¹¹⁴. The approach has since been refined, but the basic principle still applies, which involves the subcutaneous implantation of a non-degradable mould, which becomes fully encapsulated by a fibrotic collagen-rich matrix, at which point it is harvested and subsequently transplanted as a non-immunogenic, non-toxic, autologous tissue replacement¹¹⁵. However, this method is highly invasive, requires long-term *in vivo* tissue formation time (a minimum of 4 months in humans), and presents limited controllability over the tissue thickness encapsulating the mould. For these reasons, the technique is considered unsuitable for emergency cases and unlikely to be clinically endorsed¹¹⁶.

***In situ* tissue engineering**

A more recent progression in tissue engineering is the *in situ* approach, which utilises the body's own biological resources and innate reparative capability by introducing cell-free scaffolds that facilitate host cell recruitment and guide the regeneration of functional and structural tissues¹¹⁷. This main advantage of this approach is that the intermediate cell culturing phase associated with the *in vitro* approach, is obviated¹¹⁷. In this way, the *in situ* approach aims to produce an off-the-shelf implant that is more accessible and economical¹⁰⁷. To obtain functional living tissue *in situ*, the scaffold must not only function adequately upon implantation, but it must also provide specific instructions to control cell recruitment and differentiation, and prompt tissue formation.

1.4.2.3 TETHV manufacturing strategy review

The primary manufacturing methods that have been investigated for the production of TEHV include: (1) moulding, (2) 3D bioprinting, (3) melt electrowriting (MEW), and (4) electrospinning⁷⁶.

Moulding

The moulding technique achieves the construction of complex polymeric structures by introducing a polymeric solution or melt into a geometrically relevant mould, after which the polymer is solidified over time and subsequently removed from the mould (Figure 1.12A). To fabricate constructs comprising of multiple materials, a multistep approach can be used whereby different polymers are introduced to the mould in a sequence of time points. For example, Weber *et al.*¹¹⁸ employed multistage injection moulding to generate a

multicomponent heart valve comprised of fibrin and elastin-like recombinant gels. The proof-of-concept study aimed to replicate the heterogeneity of native heart valves (Figure 1.12B). An advantage of moulding is the high viability for embedded cells due to the mild processing conditions involved. However, the practical implications associated with constructing highly heterogeneous structures (such as repeated opening and closing of the mould) are limiting. Moreover, multicomponent constructs are limited to exhibiting isotropic behaviour within each material phase, unlike native heart valves which behave anisotropically across each tissue layer⁷⁶.

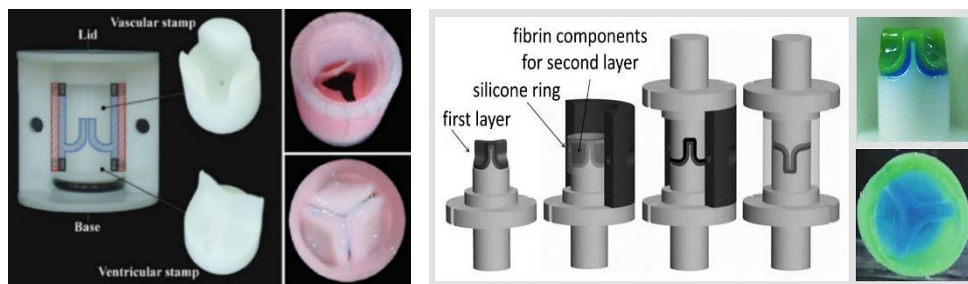


Figure 1.12: Moulding approach to heart valve tissue engineering. A) Typical moulding process (images adapted from Flanagan *et al.*¹¹⁹). B) Multistep moulding approach (images adapted from Weber *et al.*¹¹⁸)

3D Bioprinting

Derived from the principles of rapid prototyping/additive manufacturing techniques, 3D bioprinting involves layer-by-layer printing of functionalized biomolecules embedded in cytocompatible biomaterials to generate functional 3D mimetic tissue constructs¹²⁰. Biomaterials consisting of living cells, ECM proteins and other biofunctional components are referred to as 'bioinks' and are synthesised to accommodate the fabrication of geometrically complex structures with intricate tissue architecture and surface topographies¹²¹. By varying the biological composition and controlling the distribution of cell-based bioinks; as well as optimizing printing parameters and tuning ambient conditions; 3D bioprinted constructs can be generated with highly specific biochemical and biomechanical properties for the facilitation of tissue growth and remodelling¹²².

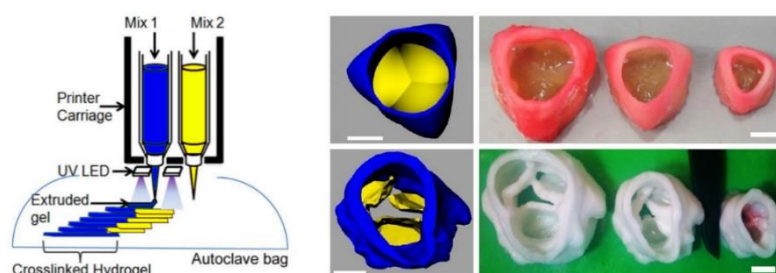


Figure 1.13: 3D printed aortic valve. Adapted from Hockaday *et al.*¹²³

Hockaday *et al.*¹²³ fabricated aortic valve constructs constituting polyethylene glycol diacrylate (PEGDA). The study investigated the feasibility of 3D printing multiple cross-linkable inks to produce a heterogeneous heart valve construct and demonstrate customizability by achieving scalable anatomical accuracy (Figure 1.14). Subsequent research involved 3D printing a trileaflet valve construct using a cell-encapsulated biological hydrogel¹²⁴. The study demonstrated that by optimizing hydrogel composition and properties, and tailoring printing techniques, constructs with high cell viability, geometric accuracy and remodelling potential is achievable¹²⁴. In a study conducted by Coulter *et al.*¹²⁵, a 3D-bioprinted silicone-based heart valve with leaflet

reinforcement features was produced using a multi-material additive technology (Figure 1.13). While the study does not involve the fabrication of biomaterials with regenerative capacity, the techniques used demonstrated a promising model for further development and future translation of 3D bioprinted TEHVs ¹²⁵.



Figure 1.14: 3D bioprinted silicone-based heart valve. Adapted from Coulter *et al.*¹²⁵

Despite the immense potential of 3D bioprinting and the recent advancements in the field, the application of this technology is relatively young, and presents numerous challenges that need to be overcome before 3D printed valves can be brought to the clinic. Current challenges include synthesizing printable biodegradable material that facilitate cellular activity; achieving the intricate patterning of native ECMs, maintaining long-term functionality throughout the growth and remodelling phase; and uncertainties regarding *in vivo* biostability and potential cell toxicity of bioink implants ¹²¹.

Melt Electrowriting (MEW)

A recent technology that innovatively merges the principles of electrospinning and additive manufacturing, referred to as melt electrowriting (MEW), has been investigated for the design and fabrication of scaffolds for TEHVs ¹²⁶. This technique enables the production of highly-organised fibrous scaffolds and thereby extensive control over microstructural and mechanical properties. Saidy *et al.*¹²⁶ demonstrated the tunability of tensile, anisotropic, and viscoelastic properties of MEW scaffolds. Furthermore, scaffolds exhibited regenerative potential, and when shaped into a trileaflet valve construct for a proof-of-concept evaluation, adequate hydrodynamic performance was achieved. Due to its recent emergence, the technology must still undergo extensive investigation to verify its true potential in the landscape of TEHV development.

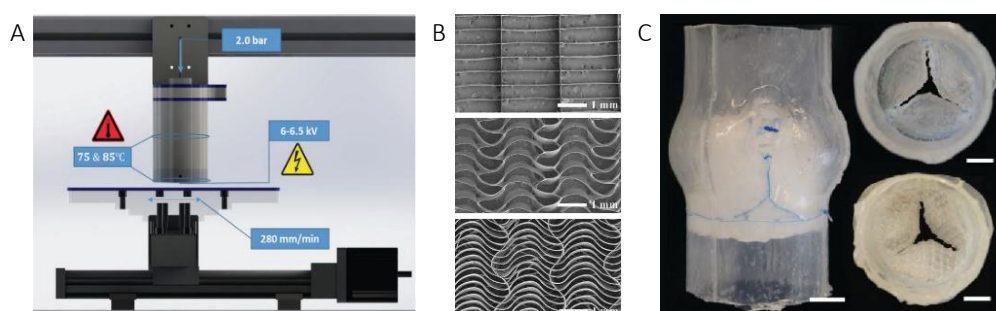


Figure 1.15: Melt electrowriting for HVTE. A) Illustration of melt electrowriting (MEW) setup. B) SEM images of PCL MEW scaffolds. C) MEW scaffold sutured as single leaflets into a silicone root (image adapted from Saidy *et al.*¹²⁶)

Electrospinning

Electrospinning is a remarkably versatile material manufacturing technique that has been widely used in HVTE. As electrospinning formed a fundamental component of the current study, a comprehensive investigation into its development and application in HVTE was conducted, which is thoroughly discussed in Chapter 2 to follow.

1.5 Research Overview

1.5.1 Background and motivation

The development of transcatheter heart valve substitutes derived from biomaterial scaffolds with regenerative capacity offers the promise of an exciting alternative to current heart valve replacement therapies. Recent progress toward fulfilling this pursuit has led to the innovation of numerous novel technologies and intriguing strategies that provide a broad foundation on which to base future developments in this field. However, to date, no proposed strategy has been clinically successful, and findings reported across different research groups are largely contradictory, ambiguous, or relatively inconclusive. As many questions remain unanswered and unprecedented challenges persist, the quest to define the optimal strategy for engineering tissue heart valves for percutaneous delivery remains in uncharted territory¹²⁷.

For HVTE to be feasible for clinical application, respective clinical outcomes must be advantageous over commercially available heart valve replacement technologies. Thus, the challenges that must be surmounted before HVTE can be clinically realised not only includes those specific to the HVTE approach, but also those inherent to conventional heart valve substitutes¹²⁷. Accordingly, TEHVs must demonstrate superior hydrodynamics and biocompatibility; minimally invasive deliverability; absence of pathologic processes such as thrombosis, inflammation, and calcification; accommodate somatic growth of the recipient; and provide indefinite durability. Accomplishing this goal would provide extraordinary relief to the growing burden of heart valve disease globally and contribute to the advancement of healthcare as a whole^{127,128}.

Considering the immense number of challenges and multifactorial complexities that HVTE research entails, the scope of this project was confined to three primary aspects of TEHV development. These included (1) biomaterial scaffold design, (2) valve manufacturing design, and (3) valve performance characterisation.

1.5.2 Research aims and objectives

The overall objective of this research was to develop biostable and biodegradable electrospun trileaflet transcatheter heart valve substitutes composed of biomaterial scaffolds characteristically suitable for tissue engineering, and with appropriate hydrodynamic functionality and sufficient durability to verify its feasibility for *in vivo* investigation. To achieve this objective, the three aspects constituting the project scope were addressed as three distinct phases of research and development, each with its own aim and objectives.

Biomaterial scaffold development

Aim 1: To develop biostable and biodegradable electrospun scaffolds that are microstructurally and mechanically suitable for heart valve tissue engineering applications.

- 1a. Design and implement a humidity control system into the electrospinning setup.
- 1b. Establish suitable conditions to electrospin Pellethane® (PEL) and DegraPol® (DP) scaffolds, respectively.

- 1c. Establish techniques to effectively tune the architecture of electrospun PEL and DP scaffolds.
- 1d. Establish techniques to enhance the tensile mechanical properties of electrospun PEL and DP scaffolds.
- 1e. Evaluate the viscoelastic behaviour of PEL and DP scaffolds.
- 1f. Investigate the effect of scaffold alignment on the viscoelastic behaviour of electrospun scaffolds.
- 1g. Investigate the influence of fiber architecture on the surface wettability of electrospun scaffolds.
- 1h. Investigate the influence of fiber architecture on scaffold permeability.

Valve manufacturing design

Aim 2: To establish an effective process for manufacturing a functional trileaflet transcatheter heart valve composed of electrospun polymer scaffolds and an expandable metal stent.

- 2a. Conduct a systems analysis on the typical production process of an electrospun tissue engineered transcatheter heart valve (TETHV).
- 2b. Establish key requirements and criteria for producing an ideal TETHV.
- 2c. Identify project-specific requirements for the development of electrospun trileaflet THV constructs.
- 2d. Identify a set of manufacturing operations and electrospinning strategies to potentially incorporate into the valve manufacturing process.
- 2e. Generate conceptual valve manufacturing procedures.
- 2f. Evaluate the conceptualised procedures through concept prototyping and screening.
- 2g. Select the most feasible concept to be carried through to the next phase of development.

Valve performance characterisation

Aim 3: To assess the effect of leaflet material properties and average leaflet thickness on the performance characteristics of the biostable and biodegradable electrospun trileaflet THV constructs, respectively.

- 3a. Manufacture PEL and DP valves using the selected manufacturing process established in Phase 2.
- 3b. Characterise the structural and mechanical characteristics of the PEL and DP valve leaflet scaffolds.
- 3c. Establish feasible hydrodynamic operating conditions for testing the PEL and DP valves, respectively.
- 3d. Perform hydrodynamic testing on the PEL and DP valve samples in compliance with ISO 5840 standards.
- 3e. Perform accelerated fatigue tests on the valve samples that survive hydrodynamic testing.
- 3f. Analyse the fatigue mechanisms and failure modes demonstrated by the PEL and DP valve samples.
- 3g. Draw conclusions on the suitability of the PEL and DP valves for their respective translation into HVTE.

In general, most aspects of biomaterial development, valve manufacturing design, and valve performance testing are interrelated and chronologically intermingled. Valve manufacturing is largely reliant on scaffold development, and scaffold development is largely reliant on feedback from valve testing, and so on. Thus, the various aspects explored in this project were not addressed sequentially, but rather in an ad hoc manner, which was steered by insights gained from experimental feedback, design iterations, and research discoveries made along the way.

2 Scaffold Development

2.1 Introduction

Based on insights from the early years of research to the outcomes of more recent investigations, it has been widely established that the structural and mechanical aspects of engineered scaffolds are central to engineering functional tissue heart valves^{98,129,130}. The scaffold architecture plays a fundamental role in facilitating tissue growth by providing a microenvironment that supports cellular activity and features specific cues to promote appropriate cell behaviour, ECM deposition, and tissue remodelling. Furthermore, the architectural properties of scaffolds can greatly influence the mechanical behaviour and degradation characteristics of engineered constructs. The mechanical properties of scaffolds are essential to providing structural support to cells to facilitate tissue ingrowth, while conducting the haemodynamics loading regime of the native biological environment¹²⁹. Despite the large body of research that has been dedicated to understanding the parameters that encompass the structural and mechanical attributes of scaffolds, no consensus has been reached on defining the optimal scaffold properties for TEHVs^{131,132}. However, remarkable innovations continue to surface within the field, and while clinical success remains an elusive target, the global surge in research on scaffold fabrication and optimization techniques for HVTE is renewing the promise of a future with TEHVs in clinical practice^{108,115}.

2.1.1 Source of scaffold materials

The primary sources from which TEHV scaffolds are derived include (1) biological and (2) polymeric scaffolds³.

2.1.1.1 Biological scaffolds

The closest representative to native valvular tissue, and hence an ideal material for heart valve substitution, is of allogenic origin¹³³. In HVTE, the use of allogenic material as a starter matrix involves the decellularization of valvular tissue acquired from human donors, followed by reseeding with autologous cells. However, despite the advantage of its inherent resemblance to native tissue, the availability of donor valves is unpredictable and greatly limited worldwide. For this reason, current research on biological scaffolds focuses on xenogeneic tissue¹³⁴. Xenogeneic tissue is mostly obtained from porcine, ovine or bovine subjects, and decellularized enzymatically or with detergents¹³⁴. Complete decellularization is critical to avoiding any adverse immunogenic response upon implantation; conversely, it is crucial that the fibre morphology of the ECM is preserved such that its inherent mechanical properties and physicochemical characteristics are retained. Despite the multitude of decellularization and fixation techniques investigated, adverse outcomes, such as ECM damage, reduced porosity, increased stiffness and density, mechanical instability, and inadequate biocompatibility prevail¹³⁵. Additionally, a potential for disease

transmission persists, and *in vivo* repopulation complications have shown to cause reverse remodelling, leading to scaffold degradation and premature valve failure¹³⁶. Moreover, the excessive time and cost implications associated with this method, specifically the *in vitro* recellularization phase, are considered unfeasible for translation into routine clinical practice¹³⁷. While investigations into biological scaffolds for TEHV continue, the unavoidable limitations associated with their application have motivated researchers to turn their efforts toward investigating the alternative approach of engineering scaffolds derived from polymers³.

2.1.1.2 Polymer scaffolds

The de novo construction of scaffolds using natural and/or synthetic polymers is an approach that has garnered great attention over the past two decades⁹⁸. Research on polymeric biomaterials has been the source of remarkable innovation in tissue engineering, advancing researchers dramatically toward producing the optimal scaffold for TEHV fabrication. The allure of polymer-based scaffolds lies in its remarkable tailoring capacity, which grants unlimited control over scaffold characterization and optimization. Given the ability to manipulate its architecture, enhance its mechanical properties, and incorporate biological queues to guide cellularization and tissue regeneration¹³⁸, polymeric scaffolds are considered promising candidates for achieving long-term TEHV durability, while circumventing immunogenicity and thrombogenicity¹³⁹. Moreover, both natural and synthetic polymers are unlimited in availability which is a unique advantage distinct to this approach, and one worth capitalizing on⁹⁸. Polymeric scaffolds are typically categorized based on the polymer source used, which include synthetic and natural polymers. Conveniently, respective biomaterial groups exhibit advantageous and disadvantageous properties that counteract one another, for which reason composite scaffolds that yield more refined characteristics have gained research interest⁸⁸.

Natural Polymers

Studies on TEHV scaffold fabrication from natural materials have investigated biopolymers such as collagen, hyaluronic acid, elastin, fibrin, alginate, chitosan, silk and poly-4-hydroxybutyrate (P4HB) to name a few⁹⁶. Biopolymeric scaffolds contain natural cellular adhesion sites, which promote cellularization and tissue growth, and are less likely to induce a toxic immune response¹⁴⁰. However, studies show that when derived purely from biological materials, scaffolds present characteristically weak matrices and poor mechanical properties¹³⁵, indicating that as autonomous materials they are functionally insufficient for TEHV scaffold construction¹⁴¹.

Synthetic Polymers

Synthetic scaffolds, on the other hand, present innate advantages pertaining particularly to mechanical characteristics and durability⁹⁶. Numerous synthetic polymers for TEHV fabrication have been explored, including polyglactin¹⁴², polyglycolic acid (PGA)¹⁴³, poly-ε-caprolactone (PCL)¹⁴⁴, polylactic acid (PLA)¹⁴⁵, copolymer of PGA and PLA (PLGA)¹⁴⁶, polyhydroxyalkanoate (PHA)¹³⁰, polyether urethane (PEU)¹⁴⁷, polyester urethane urea (PEUU)¹⁴⁸,

polycarbonate urethane (PCU) and bis-urea modified carbonate (PC-BU)¹⁴⁹. The superior controllability associated with synthetic scaffold production allows for customization and fine-tuning of the resultant mechanical properties. In that way, producing repeatable and reproducible outcomes is more viable¹⁴⁴. Furthermore, synthetic polymers coalesce with a wide range of fabrication techniques; can possess biodegradable properties with tuneable degradation rates; and can typically be processed rapidly and cost-effectively¹³⁵. However, replicating the structural and biological composition of native valves with synthetic materials remains an unmet challenge. Further drawbacks associated with synthetic biomaterials include the potential to induce an immune response when long term mechanical properties change as the polymer degrades over time¹⁵⁰; as well as limited potential for cellular response regulation¹⁵¹. For this reason, the notion was conceived to integrate both materials to produce a composite scaffold; thereby obtaining desirable mechanical properties and improved durability with the synthetic component, while impeding an immune response and promoting tissue formation with the biological component¹³⁵. An example of this, that has demonstrated promising clinical outcomes for TEHVs, is the fabrication of PGA meshes coated in P4HB (PGA/P4HB) described in Hoerstrup *et al.*¹⁵².

2.1.2 Types of scaffold materials

The main types of scaffold materials used in heart valve tissue engineering include: decellularized xeno/allogenic ECM, decellularized tissue engineered matrices, and biodegradable polymer scaffolds.

Decellularized xeno/allogenic ECM scaffolds

The decellularization process involves removing the cell component of living tissue to produce acellular starter matrices for orthotopic implantation¹⁵³. The process was developed to produce an alternative to glutaraldehyde-treated bioprostheses, primarily focusing on reducing the immunological response without affecting cellular infiltration, and ensuring the collagen structure and biomechanical properties of the ECM are preserved¹⁵⁴. Several decellularization methods have been formulated¹⁵⁵ and further validated in animal models, where promising recellularization and tissue remodelling outcomes were observed¹⁵⁶. Contrary to preclinical results, clinical investigations of xenogenic decellularized tissues resulted in dramatic adverse responses such as stenosis and thickening¹⁵⁷; severe valve degeneration; and most controversial of all, rapid inflammation and valve calcification that led to three paediatric fatalities¹³⁶. Consequently, the use of allogenic material for clinical application was declared preferential. Clinical studies on decellularized allogenic replacement valves have, however, reported conflicting results regarding their capacity for complete recellularization¹⁵⁸ and long-term durability¹⁵⁹. Considering the restriction of donor shortage for suitable allogenic materials, the pertinency of this strategy is limited⁹⁸.

Decellularized tissue engineered scaffolds

To evade the dependence on donor-derived valves and integrate transcatheter application, Dijkman *et al.*¹⁶⁰ investigated the feasibility of decellularizing in-vitro grown TEHVs, based on biodegradable synthetic scaffolds and

homologous cells. Central to the aim of this method is to provide largely available, standardised off-the-shelf valve replacements. Results obtained from early studies showed reduced leaflet thickening and retraction compared to previous reports on living TEHVs¹⁶¹. The method also verified that off-the-shelf availability would be feasible, and that transcatheter implantation was perfectly viable¹⁶². More recent preclinical trials demonstrated good functionality and recellularization¹⁶³. Although leaflet shortening was reported in long-term outcomes¹⁶⁴, data from an *in vitro* study conducted by Sanders *et al.*¹⁰⁹, investigating an optimised valve geometry, showed a considerable decrease in leaflet retraction and improved functionality. Nevertheless, clinical validation is needed to evaluate the translation of these outcomes from *in vitro* to *in vivo* models.

Biodegradable polymeric scaffolds

Biodegradable polymeric scaffolds intended for this approach are considered as target-specific biomaterials, designed specifically as growth factor devices that upon implantation induce the recruitment of endogenous cells; guide and regulate cell migration; and thereby promote tissue formation and remodelling⁹⁷. After providing structural and mechanical functionality immediately upon implantation, scaffold degradation and endogenous tissue formation should transpire synchronously, resulting in a fully biological replacement construct over time. A distinct advantage of this approach is that the laborious decellularization process is bypassed. Achieving this more streamlined approach would undoubtedly shift the paradigm of valvular disease management globally, but with related research still in its infancy, this prospect remains uncertain⁹⁸.

2.1.3 Scaffold design parameters

The greatest hurdle in HVTE scaffold design lies in mimicking the architectural, functional, and biological properties of native heart valve tissues¹⁶⁵. To assess whether these requirements have been met, engineered scaffolds must be appropriately characterised. For TEHV scaffold design, the most important characteristics to define include the (1) microstructural characteristics, (2) mechanical properties, (3) surface properties, (4) degradation characteristics, and (5) biocompatibility.

2.1.3.1 Scaffold microstructure

For the application of tissue reconstruction, a scaffold's physical characteristics play a critical role. The ultimate objective of scaffold design is to achieve a structure, at nano- to macroscale, that mimics the architecture and morphology of the native ECM. The ECM primarily provides structural support and tensile strength to the tissue – two fundamental contributions to the functionality of living tissue. To initiate and encourage key processes of ECM reconstitution, such as cell adhesion, migration, proliferation, and specialisation, providing sufficient attachment sites for cell surface receptors, by maximising the surface-to-volume ratio, is critical to scaffold design¹⁶⁶.

To facilitate cell ingrowth, uniform cell distribution, and nutrient diffusion, scaffolds should be highly porous, comprise of an open, interconnected geometry, and provide a large surface area to promote cell-cell and cell-substrate interactions. Key characteristics to consider in scaffold design include fiber diameter, pore size, and porosity. Scaffold pore size is especially important due to its pivotal influence on cellular activity¹⁶⁷. When pores are too small, cells are unable to penetrate the scaffold which leads to surface pore occlusions, impeded infiltration, and irregular ECM deposition (Figure 2.1). The impact of pore size on tissue growth has been demonstrated in numerous studies that investigate pore size optimization for engineering various tissues¹⁶⁶. Pore size requirements vary according to the intended application of the scaffold and the selected cell source¹⁶⁸. The cell types typically used for HVTE are listed in Table 2.1 with reference to their estimate range in size.

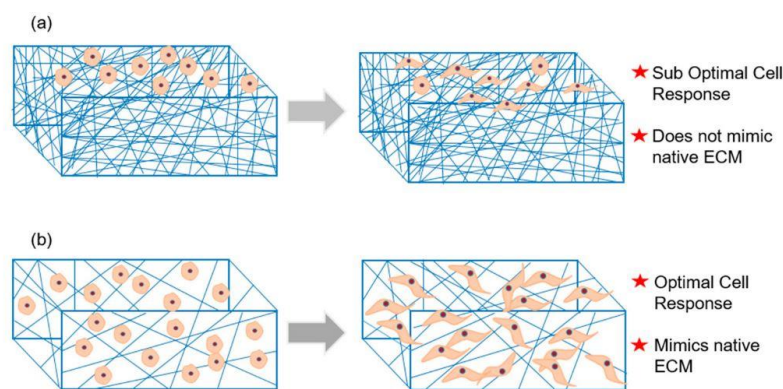


Figure 2.1: Schematic illustration showing effect of scaffold porosity on cell infiltration. (a) The densely packed network of fibers reduces cell infiltration, resulting in suboptimal cell response. Whereas (b) increased porosity and a high surface-to-volume ratio promotes cell infiltration, leading to a more favourable cell response (adapted from Ameer *et al.*¹⁶⁸).

While maximising porosity and pore size greatly enhances cellular response, it conversely compromises the scaffold's mechanical properties. It is therefore important in scaffold design to determine the optimal interplay between pore size and mechanical integrity. Studies on TEHV scaffolds have reported average pore sizes ranging between 10 – 240 μm that demonstrate adequate cell infiltration and migration¹⁶⁹. Hence, there remains no “golden standard” or optimal value for the average pore size of scaffolds for TEHVs.

Table 2.1: Cell size estimates reported or referenced in literature

Cell type	Cell size	Reference
Valvular interstitial cells (VICs)	10 – 80 μm	Tandon <i>et al.</i> ¹⁷⁰
Valvular endothelial cells (VECs)	5 – 15 μm	Monaghan <i>et al.</i> ¹⁷¹
Endothelial cells (ECs)	10 – 25 μm	Ameer <i>et al.</i> ¹⁶⁸
Fibroblasts	5 – 15 μm	Dhandayuthapani <i>et al.</i> ¹⁶⁶
Myofibroblasts	6 – 30 μm	Tomasek <i>et al.</i> ¹⁷²

2.1.3.2 Mechanical Properties

The mechanical properties of engineered scaffolds are integral to the success of regenerative tissue constructs as they must withstand and conduct the loads experienced in the biological environment. It is essential for a scaffold to retain mechanical integrity after implantation for appropriate cellular activity and tissue ingrowth to occur effectively. To validate the mechanical integrity of a scaffold, a few key properties must be characterised, including:

- (i) *tensile strength* – maximum tensile stress a material can withstand before failure
- (ii) *maximum strain* – maximum deformation a material can withstand before fracture
- (iii) *elastic modulus* – ratio of tensile stress (σ) to the corresponding measured strain (ϵ)
- (iv) *yield strength* – stress at which a material begins to plastically deform
- (v) *fatigue life* – number of fluctuating stress-strain cycles endured before failure¹⁶⁶

Engineering biomimetic tissues requires the mechanical properties to replicate those of the native tissue. The greatest challenge to overcome in HVTE has been to mimic the properties of the heart valve leaflets. To accommodate the mechanical loading that varies spatially and temporally within leaflet tissues, scaffolds must exhibit mechanical anisotropy and heterogeneity, high recoverability, and low compressibility¹⁷³. As shown in Figure 2.2B, native HV leaflets demonstrate higher tensile strengths and elastic moduli in the circumferential direction compared to the radial direction. Furthermore, scaffold fibers must withstand large rotations and strains, and demonstrate non-linear stress-strain behaviour. The challenge in replicating the mechanical behaviour and integrity of native leaflets is further intensified by the disproportionate relationship between leaflet thickness and the applied environmental loads¹⁶⁹. For instance, aortic valve leaflets, which experience maximum pressures of approximately 120 mmHg in the aorta, range between 260–480 μm in thickness along the central and peripheral coaptation regions, and between 590–860 μm along the basal regions¹⁷⁴.

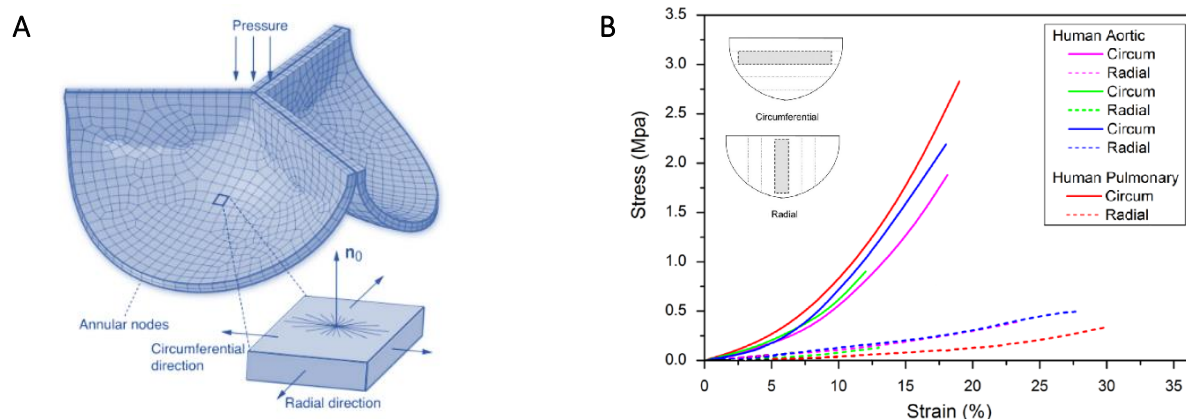


Figure 2.2: Mechanical characterisation of trileaflet valves. A) Finite element model of a trileaflet heart valve indicating its complex mesh structure and multi-directional loading (adapted from Oomen *et al.*¹⁷⁵). B) Stress-strain curves for human valves in the circumferential and radial directions (adapted from Hasan *et al.*¹⁷⁶ and Balgud *et al.*¹⁷⁷)

2.1.3.3 Degradation Characteristics

Biodegradation of polymers have greatly influenced the landscape of biomaterial synthesis for tissue engineering applications¹⁶⁶. Biodegradation involves cleavage of hydrolytically or enzymatically sensitive bonds, resulting in polymer erosion, scaffold deterioration, and material resorption. Scaffold degradation occurs through mechanisms involving physical, chemical and/or biological processes, which can be manipulated to control the rate and nature of degradation. Thus, degradation characteristics can be tuned so that scaffold degradation is synchronous with neotissue formation⁸⁹. In this way, the transfer of functional loads from that degrading scaffold to reconstituted tissue can be synchronised to sustain mechanical and structural integrity of the construct throughout the tissue growth phase. Alternatively, nondegradable polymeric scaffolds, which are biologically stable, can be used to provide permanent structural support to the ingrown tissue¹⁷⁸.

2.1.3.4 Biocompatibility

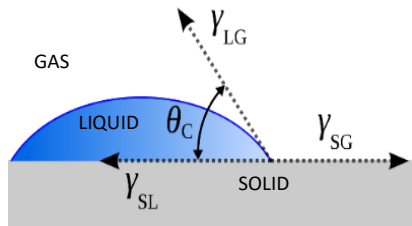
Biocompatibility refers to a material's ability to perform a designated function in a biological environment without eliciting any undesirable local or systemic immune responses. Biocompatible materials should induce the most favourable cellular or tissue response upon implantation and demonstrate optimal performance relative to its clinical role¹⁷⁹. In the context of scaffolds for tissue engineering, biocompatibility refers to a scaffold's ability to effectively support cellular activities necessary for tissue growth and remodelling. Key factors that influence a scaffolds biocompatibility include polymer chemistry and structural morphology which are governed by polymer synthesis and processing techniques, and can be enhanced by optimising processing conditions, applying surface treatments, and performing physical and chemical surface modifications¹⁶⁶. Although it does not pertain to the scope of the current study, it was important to consider for prospective *in vivo* investigations.

2.1.3.5 Surface wettability

The surface of a biomaterial is the first aspect of an implant to come in contact with the biological components of the *in vivo* environment and is thus a definitive factor in determining its biological response upon implantation¹⁸⁰. Surface wettability has been found to be indicative of a biomaterials biocompatibility¹⁸¹ and is therefore an important parameter to characterise for the evaluation of biomaterials prior to *in vivo* study¹⁸². Wettability corresponds to the ease with which a liquid adheres to a surface which is typically evaluated by measuring the contact angle of a liquid droplet deposited on a solid surface¹⁸³. According to Young's theory, the contact angle is governed by the interfacial tension of a three-phase system defined as solid/gas, solid/liquid, and liquid/gas, as shown in Figure 3.12, and is given the relationship¹⁸⁴:

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cos \theta_C \quad (1)$$

where θ_C is the contact angle, and γ_{SG} , γ_{SL} , and γ_{LG} denotes the interfacial tensions corresponding with the solid/gas, solid/liquid, and liquid/gas phases, respectively.



Contact angle	Degree of wetting	Hydrolytic evaluation
$\theta = 0^\circ$	Perfect wetting	Hydrophilic
$0 < \theta < 90^\circ$	High wettability	
$90 \leq \theta < 150^\circ$	Low wettability	Hydrophobic
$150^\circ \leq \theta \leq 180^\circ$		Superhydrophobic
$\theta = 180^\circ$	Non-wetting	

Figure 2.3: Contact angle of an ideal three phase system (adapted from Nogalska *et al.*¹⁸⁵)

As Young's equation was derived purely for ideal solid surfaces (*i.e.*, smooth and homogeneous) two mathematical models derived from Young's equation, known as the Wenzel and Cassie-Baxter model, were developed to incorporate the influence of surface roughness on surface wettability¹⁸⁵. Each model describes one of two distinct interaction states that exist. As illustrated in Figure 3.13, the Wenzel model describes a state in which the liquid makes complete contact with the surface by conforming to the surface interface¹⁸⁶. The Cassie-Baxter model refers to a state where the liquid suspends on the surface roughness peaks and entraps underlying air pockets¹⁸⁷.

While these models provide valuable insight for evaluations on surface wettability, their application is limited to solid surfaces, and do not account for the dynamic wetting behaviour associated with porous substrates where the droplet permeates into the porous structure causing a decrease in contact angle over time¹⁸⁸. Dynamic wetting of porous substrates is driven by the simultaneous spreading and penetration of deposited liquids¹⁸⁸. Penetration can be defined as the infiltration of liquid into the porous network, which results in the gradual depletion of the droplet volume¹⁸⁹. This, in conjunction with the spreading action, results in the droplet size and corresponding contact angle to recede over time. The rate at which the angle recedes can be used to determine the wetting kinetics of a porous substrate¹⁹⁰, and thus provide a measure of dynamic wetting efficiency. Biomaterials with enhanced dynamic wettability have shown to promote cellular adhesion, infiltration, and growth^{182,191} which is key to the engineering of functional tissue.

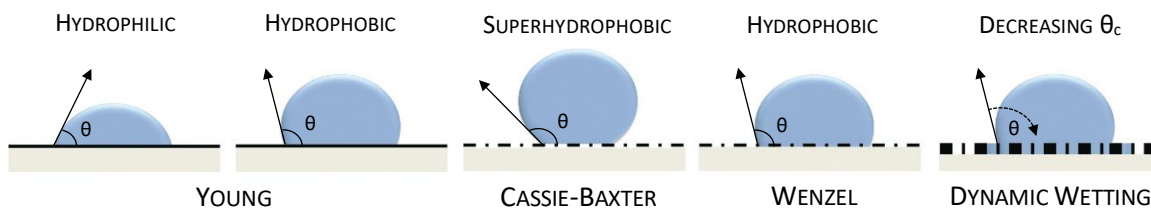


Figure 2.4: Schematic representation of principle contact angle measurement models

2.1.3.6 Scaffold permeability

Permeability can be defined as the ability of a liquid to flow through a structure and is regarded as the capacity of a material to transport nutrients and metabolites to and from cells. It is therefore an essential parameter for the *in vivo* performance of engineered tissues, as inadequate nutrient diffusion and cellular waste transport leads to limited cell migration and tissue ingrowth within scaffolds¹⁹². However, an increase in permeability generally results

from a higher scaffold porosity, which suggests a compromise in mechanical strength¹⁹³. Thus, finding the right balance between permeability and mechanical properties is an important consideration when designing porous scaffolds intended for dynamic load bearing functions.

2.1.3.7 Scaffold thickness

The deformation behaviour of leaflets under dynamic loading relies largely on its flexural properties, of which include bending stiffness and strength¹⁹⁴. The bending stiffness of a material can be evaluated using the Euler-Bernoulli beam theory, which defines it as the product of the elastic modulus (E) and area moment of inertia (I)¹⁹⁵. For a rectangular cross-section of the leaflet, this can be numerically expressed as:

$$EI = E \cdot \frac{bh^3}{12} \quad (2)$$

where b is the cross-sectional width, and h denotes the leaflet thickness. This shows that a leaflet's bending stiffness is linearly proportional to its elastic modulus and scales cubically with the leaflet thickness¹⁹⁶. Therefore, in principle, the thickness of leaflets has a significantly greater influence on the bending behaviour of leaflets compared to the elastic modulus^{197,198}. Studies on the relative effect of elastic modulus and leaflet thickness on valve function have reported congruence with beam theory by demonstrating that leaflet thickness is a key determinant of valve performance¹⁹⁷. In support, it has been widely reported that the flexural behaviour of leaflets during dynamic loading is largely dependent on leaflet thickness^{148,198-200}.

In general, thin, flexible leaflets demonstrate better fluid dynamics as their low resistance to deformation facilitates efficient opening behaviours which results in lower pressure gradients and reduced fluid shear stresses¹⁹⁹. However, thin leaflets with too much flexibility experience larger strains than stiffer materials. Consequently, thinner leaflets typically suffer limited durability due to the effects of strain accumulation and plastic deformation^{201,202}. Therefore, stiffer materials are considered more favourable in terms of reduced strains and increased valve durability. However, thicker leaflets inherently possess a high bending stiffness and are therefore more flexurally restricted. As a result, valves with thicker leaflets are predisposed to presenting suboptimal open and closing behaviours¹⁹⁴. Furthermore, the large forces required to overcome the bending resistance of stiffer leaflets result in elevated pressure gradients, closing volumes, and transvalvular energy losses, which can manifest as valve dysfunction¹⁹⁷. Moreover, valve materials subjected to high closing volumes can suffer limited durability as they endure excessive structural loads which can induce premature leaflet wear and mechanical fatigue²⁰².

This conflicting phenomenon is a major design challenge in HVTE and has led to extensive research on the topic. Nevertheless, leaflet thicknesses reported among prominent research groups over the years have been relatively inconsistent (see Table 2.2). To this end, the optimal leaflet thickness for HVTE remains undefined and illusive⁷⁶.

Table 2.2: Summary of average leaflet thicknesses reported in studies on heart valve tissue engineering

Year	Author	Scaffold material	Thickness (μm)
2020	Uiterwijk <i>et al.</i> ¹³²	Electrospun biodegradable UPy-PC	500 ± 20
2020	Fioretti <i>et al.</i> ²⁰³	Electrospun PC-BU	406 ± 41
2017	Capulli <i>et al.</i> ²⁰⁴	Electrospun P4HB/Gelatin	250 – 600
2017	Kluin <i>et al.</i> ¹⁴⁹	Electrospun PC-BU	400
2016	Sanders <i>et al.</i> ¹⁰⁹	Non-woven PGA	1000
2016	Del Gaudio <i>et al.</i> ²⁰⁵	Electrospun PCL	640 ± 50
2008	Del Gaudio <i>et al.</i> ¹⁴⁴	Electrospun PCL	840 ± 140

2.1.4 Scaffold fabrication strategies

Derived from either natural, synthetic, or composite sources, polymeric scaffolds for tissue engineering applications typically take one of three forms: porous foam, hydrogels, and fibrous scaffolds¹⁴¹, as depicted in Figure 2.5.

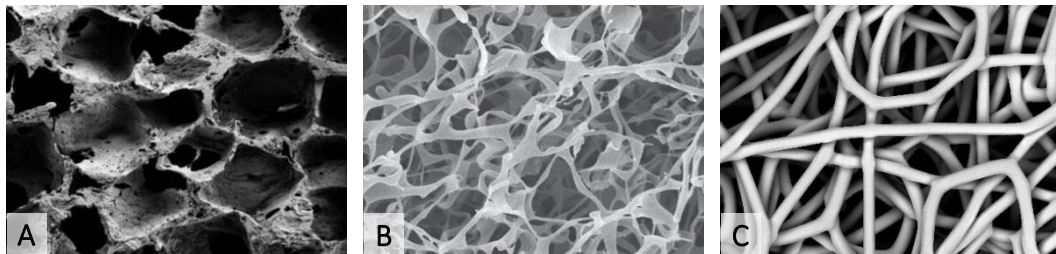


Figure 2.5: SEM images representing three primary structures of engineered polymer scaffolds. (A) Porous scaffold (adapted from Park *et al.*²⁰⁶), (B) hydrogel (adapted from Xia *et al.*²⁰⁷) and (C) fibrous scaffold.

2.1.4.1 Porous scaffolds

Sponge or foam porous scaffolds comprise of an interconnected homogenous pore network with which allows continuous circulation of nutrients and metabolic waste throughout the matrix to enable cell colonisation and tissue growth¹⁶⁹. Multiple approaches have been investigated to fabricate sponge/foam porous scaffolds for TEHV which have achieved positive results²⁰⁸. However two limitations inherent to these scaffolds include a limited capacity to replicate complex geometries and insufficient elasticity¹⁶⁹.

2.1.4.2 Hydrogels

Hydrogels are swollen hydrophilic polymer chain networks, capable of holding large amounts of water which enables efficient diffusion of nutrients, oxygen, and water-soluble metabolites through the structure²⁰⁹. The structural similarity of hydrogels to ECM contributes to its high cellular viability and growth factor delivery efficacy¹⁶⁹. The main limitation of conventional hydrogels is their insufficient mechanical properties, which undergo further weakening with cell seeding¹⁴¹. Although their stiffness can be improved by crosslinking⁷⁶, densely cross-linked gels may limit cell movement, reducing its capacity for cellular infiltration²¹⁰. For this reason, hydrogel systems are mainly implemented as cell carriers for improved seeding efficacy and biocompatibility²¹¹.

2.1.4.3 Fibrous Scaffolds

Fibrous scaffolds are currently the golden standard for TEHV materials²¹². Comprised of a network of tailored micro- and/or nanoscale fibers, the architecture of fibrous scaffolds closely mimics the microstructure of the natural ECM, and can provide high porosity with controlled pore sizes to facilitate cell activity³. Three techniques are used for the fabrication of fibrous scaffolds: phase separation, self-assembly and electrospinning¹⁴¹. Above all, electrospinning is the most commonly used approach for producing TEHV scaffolds due to its simplicity, versatility, controllability, and cost-effectiveness³. The versatility of electrospinning has led to the innovation of customised systems and novel techniques to fine-tune the architectural properties of electrospun and achieve complex valve geometries²¹³. Currently, electrospinning leads as the most promising method for producing TEHVs, having established a strong position in clinical studies and with demonstrations of promising *in vivo* outcomes^{76,132}.

2.1.5 Electrospinning principle

Electrospinning is a material manufacturing technology that generates nonwoven scaffolds composed of fine polymer fibers with diameters ranging in micro- to nanoscale²¹⁴. Electrospinning systems typically consist of three primary components, including a high voltage power supply, a solution feed system, and a fiber collector, typically in the form of a metal plate or rotating cylinder²¹⁵, as illustrated in Figure 2.6,

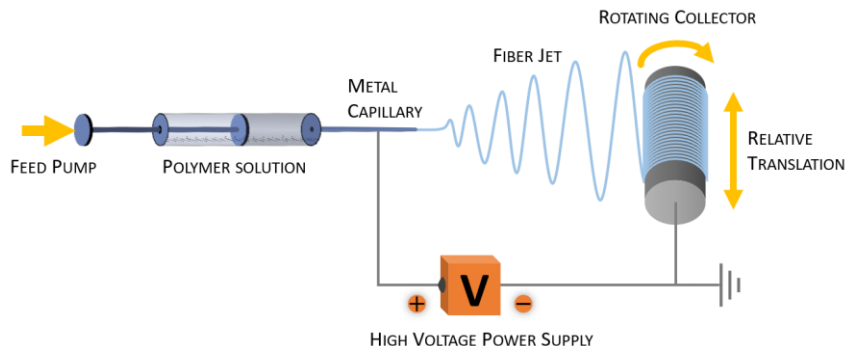


Figure 2.6: Generic electrospinning system featuring a rotating cylindrical collector.

The process involves applying voltage to a metal needle being fed a conductive polymer solution which becomes electrically charged and is subsequently accelerated across an electric field onto a collecting surface of opposite polarity positioned at an optimal distance from the needle²¹⁶. The formation of the fiber jet relies on the interaction between the opposing forces generated by the surface charges induced on polymer droplet, and the reciprocal repulsive charges generated by the applied electric field. When the applied voltage reaches a critical value, the electric field generates electrostatic forces that overcome the surface charge of the polymer solution, at which point the ejection of the charged fiber jet is initiated. By increasing the applied voltage, the droplet on the spinneret tip undergoes further stretching until it eventually deforms into a stable Taylor cone, from which the jet elongates and thins dramatically²¹⁶ (illustrated in Figure 2.7). After a small distance of stable travelling, the polymer jet

experiences a whipping instability which induces rapid bending and stretching of the fiber, observed as an expanding helix, and leads to evaporation of the solvent before depositing on the collector in a 'random' scheme²¹⁷. These instabilities play a crucial role in electrospinning as they ultimately govern the resultant matrix architecture. The onset and behaviour of these instabilities is governed by the solution properties and process parameters²¹⁷.

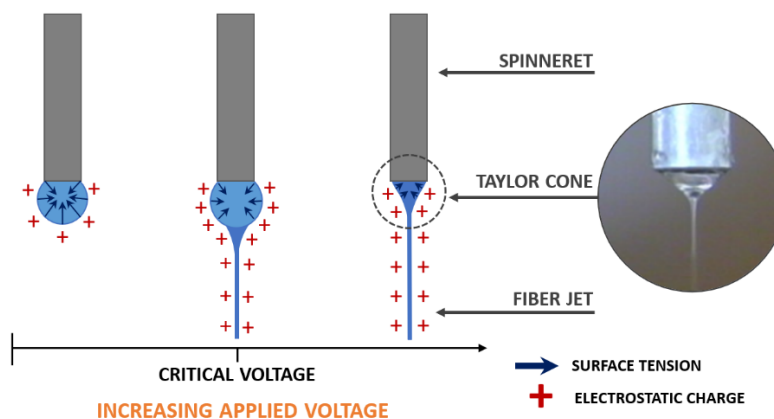


Figure 2.7: Schematic illustration of the Taylor cone formation process

2.1.6 Electrospinning parameters

The fiber thickness and morphology is governed by three groups of parameters including (I) polymer solution properties, which include solvent selection, solution concentration, viscosity, and conductivity; (II) processing parameters, which include applied voltage, solution feed rate, tip-to-collector distance and collecting speed; and (III) ambient conditions, including temperature and relative humidity³. By adjusting these parameters, scaffold architectures can be tailored to obtain specific mechanical properties¹.

2.1.6.1 Solution parameters

Solvent Selection

Solvent choice contributes significantly to fiber formation and resultant morphology. Generally, two principal factors are considered when selecting a solvent, which include polymer solubility and the solvent's boiling point²¹⁸. The solvent's boiling point gives signifies its volatility, which influences the evaporation rate of the solution during jet flight. Highly volatile solvents are unfavourable due to their low boiling points and high evaporation rates, which leads to rapid drying at the needle tip and consequent solution flow blockage. Solvents with very low volatility are also avoided due to their high boiling points which prevents solvent evaporation, resulting in wet landing (the deposition of solvent-containing fibers on the collector) and subsequent fiber fusion²¹⁹. Selected solvents should therefore be volatile and have a moderate boiling points, such that the evaporation rates are high enough to ensure complete evaporation from the fiber jet upon reaching the collector to result in smooth, continuous dry fibers²²⁰.

Solution concentration

Fiber formation in electrospinning is contingent to the phenomenon of uniaxial stretching of a charged jet²¹⁸. Varying the solution concentration has proven to significantly affect the stretching behaviour of the charged jet. For instance, when the solution concentration is low, the effect of the applied electric field and low surface tension causes polymer chain entanglements to break into fragments before reaching the collector. This fragmentation causes the formation of droplets or beaded nanofibers. Increasing the solution concentration increases the solution viscosity, which improves chain entanglement between polymer chains²²¹. With an applied electric field, the chain entanglements overcome surface tension to form stable bead-free uniform fibers, at which point the critical concentration value is reached. Beyond a certain limit the solution viscosity becomes too high, which impedes flow through the capillary tip, resulting in the formation of defective or beaded fibers²²². Critical values for generating smooth fibers differ dramatically among polymer solutions, and vary relative to applied processing parameters²¹⁸.

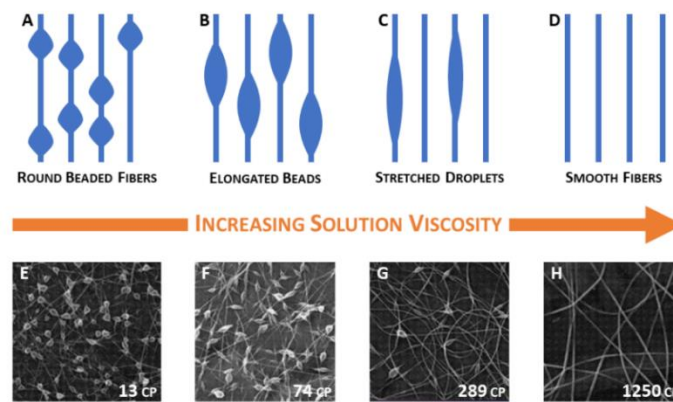


Figure 2.8: Influence of increasing solution viscosity on electrospun fiber morphology. (A-D) schematic representation, and (E-H) SEM images (adapted from Fong *et al.*²²³)

Solution conductivity

Solution conductivity is critical not only to Taylor cone formation, but also fiber diameter control. Taylor cone formation depends largely on the electrostatic force of the fluid surface charge induced by the applied external electric field. Solutions with low conductivity are unable to conjure sufficient surface charge for the induction of a Taylor cone, and therefore lack electrospinnability. Increasing the conductivity increases the surface charge induced on the droplet, allowing Taylor cone formation to ensue. However, when the conductivity exceeds a critical value, Taylor cone formation is hindered and electrospinning becomes defective²¹⁸. The influence of electrostatic and Coulomb forces governs the elongating and thinning of the jet, as well as the fiber whipping behaviour. The stretching and bending in the whipping region become amplified when induced surface charges increase, resulting in fluid jets drawn into the nanoscale²²⁴. Thus, by applying an external electric field and increasing the solution conductivity, the diameter of deposited fibers can be significantly reduced²²⁵.

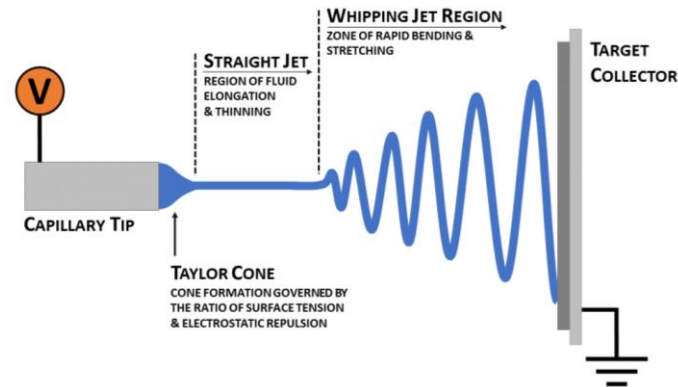


Figure 2.9: Schematic illustration of fiber jet behaviour across an electric field.

2.1.6.2 Process parameters

Applied voltage

The electrospinning process begins when the solution becomes charged by an applying a voltage²²¹. As previously described, when a polymer solution is fed through a metal capillary subjected an applied voltage, the fluid will initially deform from a spherical droplet into a Taylor cone, and upon reaching a critical value the fiber jet will reach nanoscale. The threshold of applied voltage in electrospinning is solution dependent, but the behaviour of fiber formation relative to varying the applied voltage can be generalised²²⁶. The stretching of the polymer solution correlates to the charge repulsion within the polymer jet, thus attributing reduced fiber diameters to an increased applied volatage²²⁷. As shown in Figure 2.12, increasing the voltage beyond the threshold value will lead to the formation of beaded nanofibers, followed by a spray of discrete particles, known as electrospaying.

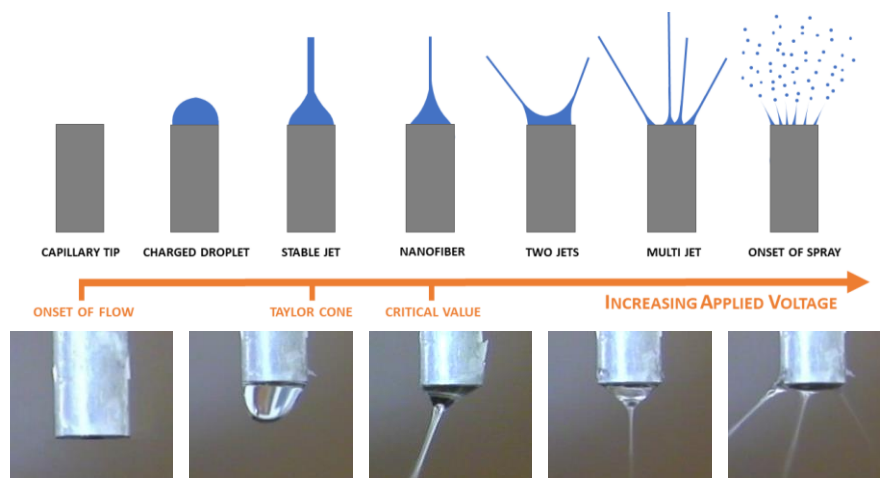


Figure 2.10: Influence of increasing applied voltage on fiber formation.

Solution flow rate

The flow rate of the solution is essential to tailor cone formation and thus significantly impacts fiber jet morphology. In essence, increasing and decreasing the flow rate causes fiber jet diameters to increase and decrease respectively. When the flow rate is too high, solvent evaporation becomes ineffective due to the excessive solution

volume of large-diameter fluid jets. This leads to incomplete drying of deposited fibers, unspun gravity-induced droplets, fiber beading and ribbon-like defects²²⁰. Flow rates below a certain limit will result in retracted jets which are jets that emerge directly from inside the capillary with no sign of droplet or cone formation. Jets of this nature are highly unstable and result in the formation of fibers with a wide range of diameters²²⁸.

Tip-to-collector distance

The effect of varying the distance between the capillary tip and collector has proven to substantially influence fiber formation and morphology. The tip-to-collector distance (also referred to as deposition distance) has a fundamental impact on the deposition time, which largely determines the whipping interval and evaporation period²²⁹. Thus, short working distances can lead to wet fiber landing which results in the fusion of adjacent fibers (Figure 2.11B). Large areas of fused fibers diminishes scaffold porosity and subsequently hinders cellular infiltration during *in vitro/in vivo* culturing. An optimal working distance should therefore be established to ensure complete solvent evaporation occurs before the fiber deposits on the collector²³⁰. Additionally, variations in the deposition distance influences the fiber diameter, whereas the distance is increased, diameters are generally observed to decrease²²¹. As with the parameters described previously, the optimal and threshold values associated with the tip-to-collector distance vary broadly among different polymers and electrospinning conditions²¹⁴.

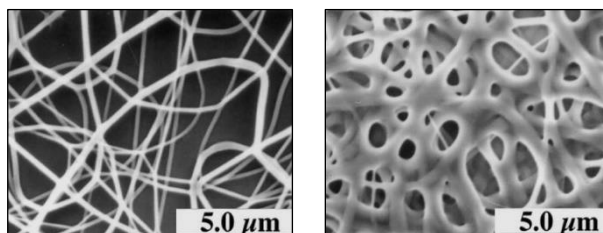


Figure 2.11: Influence of deposition distance on scaffold morphology. SEM images of A) dry fibers, and B) fused fibers from wet landing (adapted from Buchko *et al.*²³⁰).

Relative humidity

The relative humidity (RH) affects the electrical conductivity of the ambient environment, which influences the solidification process of the charged fiber jet²²⁶. The nature of this process and its effect on fiber diameter is dependent on the chemical properties of the polymer²¹⁸. Generally, lowering the relative humidity results in decreased diameters, while raising the humidity produces larger fiber diameters. Varying the humidity also plays a role in controlling the surface porosity of single fibers when binary solvent systems are used^{231,232}. At a very low humidity, volatile solvents tend to dry at a much higher rate, which can lead to premature evaporation of the solvent from the capillary tip causing an unstable Taylor cone and irregular fiber deposition²¹⁴. Conversely, due to its influence on ambient electrical conductivity, increasing the relative humidity can assist the discharge of the fiber jet. However, beyond a certain limit, increasing the humidity will hinder the solidification process of the polymer jet resulting in beaded fibers, wet fiber landing and scaffold fusion, and may prevent electrospinning altogether²³³.

2.1.7 Polymer fundamentals

For this project, two commercially available synthetic polymers were investigated for their application in electrospinning, including non-degradable Pellethane® 2363-80AE (PEL) and biodegradable DegraPol® (DP15), of which both classify as thermoplastic polyurethane elastomers (TPUs)¹²⁹.

2.1.7.1 Thermoplastic elastomers

Thermoplastic elastomers (TPEs) are generally categorised as physically crosslinked elastomers whose individual polymer chains are linked by weak inter/intramolecular interactions, such as hydrogen bonds, dipolar forces, crystalline regions or glassy domains²³⁴. The nature of these interactions imparts the elastic response characteristic of these elastomers. Physical crosslinking also facilitates biomedical safety by obviating the risk of cytotoxicity from unreacted chemical crosslinkers, inherent to chemical crosslinking processes²³⁵. Owing to the intrinsic reversibility of physical crosslinks, TPEs can be melted and reshaped which reinforces its extensive utility in bioengineering^{129,234}.

Structurally, TPEs are linear block copolymers, typically composed of two microphase-separated domains with the general AB structure. “A” typically constitutes a hard crystalline segment which provides structural rigidity; and “B” constitutes a soft amorphous segment which imparts flexibility²³⁶. Structures of this nature can sustain varying degrees of deformation under stress and recover to their original state when unloaded. Considering the dynamic loading endured by heart valve tissues, the elastic attribute inherent to this class of polymers make them highly favourable for engineering HV biomaterials¹²⁹.

2.1.7.2 Polyurethane elastomers

Family to TPEs, polyurethane elastomers (PUs) encompass a large class of polymers with remarkably diverse chemical compositions, physical properties, and degradation profiles¹²⁹. Mechanical flexibility and good blood compatibility are prominent features of PUs, which in conjunction with their diverse compositions and tuneable mechanics has reinforced their role as one the most widely exploited polymers for biomedical applications after half a century of use in clinical settings²³⁴.

PU structures share the common backbone composition of TPEs, mostly with a hard segment formed by diisocyanates and chain extenders, and a soft segment formed from polyols based on ester, ether, carbonate, or siloxane linkages^{234,237}. As depicted in Figure 2.12, the hard and soft segments alternate n times to form a structure of repeated units which quantifies its molecular weight²³⁸. A common characteristic of PU structures is the presence of urethane linkages along the large molecular chains and hydrogen bonding between adjacent urethane groups²³⁹. Traditionally, chemical reactions are introduced purely between molecules, while physical reactions are formed to link molecular chains²⁴⁰. The amalgamation of physical and chemical-crosslinking facilitates the elastic behaviour of PU materials, while simultaneously enhancing its mechanical strength and resistance to degradation²³⁶.

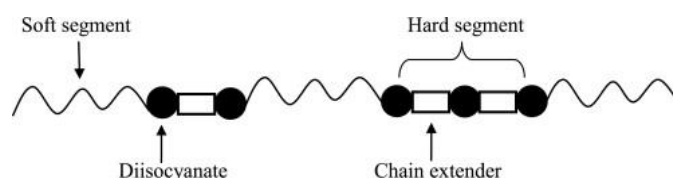


Figure 2.12: Schematic representation of a typical PU structure (adapted from Yilgör *et al.*²⁴¹)

Having been originally developed for long-term implant devices²³⁹, efforts to synthesise biostable PUs with long-term *in vivo* durability were hampered by innate vulnerabilities to biodegrade^{75,234}. As a result, their susceptibility to biodegrade provoked extensive inquiry into the culprit mechanisms which led to the emergence of a new line of research on synthesising PUs with controlled biodegradability¹²⁹. To this end, research toward understanding the mechanisms of biodegradation not only yielded novel PUs with enhanced biostability, but also generated an entirely new class of bioresorbable PUs, catered specifically for engineering regenerative tissue constructs²³⁴.

2.1.8 Polymer specification

2.1.8.1 Pellethane®

Pellethane® is a family of medical grade thermoplastic polyurethane elastomers (TPUs), commercially available in several compositions that differ primarily in soft block length²⁴². PEL is a linear segmented polyetherurethane (EU) with a polyether-based soft segment and hard segment constructed from an aromatic diisocyanate and chain extender²⁴². Each composition is graded by its nominal Shore hardness, which ranges from 70A to 80D²⁴³. For this study, Pellethane 2362-80AE® was used, which is composed of a hard segment made from 4,4'-methylenediphenyl diisocyanate (MDI) and chain extender 1,4-butanediol (BDO), and a soft segment made from poly(tetrahydrofuran) (PTMEG), as illustrated in Table 2.3^{244,245}.

Pellethanes are mostly renowned for their high resistance to abrasion wear; high mechanical strength, good hardness to flexibility ratio, resistance to non-polar solvents, and hydrolytic stability^{243,246}. The advantageous physical and chemical properties of PEL has generated extensive research centred on its use in cardiovascular devices and biomaterial implants^{244,247}. However, from as early as 1981, when Parins *et al.*²⁴⁸ first reported on the degradation of PEL, the inherent susceptibility of these polymers to degrade *in vivo*²⁴⁹ remains unresolved and has greatly limited its application for long-term implant devices^{244,250}.

The *in vivo* degradation of PEL has been attributed to the inherent biphasic nature of its soft and hard segment composition. When introduced to a biological environment long-term, both phases undergo relative chemical and physical changes which alters the polymer structure and results in material degradation²⁵¹. Specifically in cardiovascular applications, adverse outcomes such as hydrolysis, oxidation, environmental stress cracking, and calcification have been associated with the degradation of PEL implant materials²⁵². However, the biostability and long-term degradation mechanisms of PEL have primarily been studied in the form of films, sheets and other

nonporous structures²⁵³. For this reason, long-term *in vivo* studies on its degradation behaviour in porous form would be required to define its susceptibility to any adverse biological response, and in this way, could more reliably qualify its suitability for TE applications²⁵⁰.

Table 2.3: Component structures and chemical composition of Pellethane®

Component	Name	Abbreviation	Chemical Structure
Polyether	poly(tetrahydrofuran)	PTMEG	
Junction unit	4,4'-diphenylmethane diisocyanate	MDI	
Chain extender	1,4-butanediol	BDO	
Composition			

2.1.8.2 DegraPol15®

DegraPol® is a biodegradable polyesterurethane (AU) block copolymer²⁵⁴, commercially available in three compositions, namely DP0, DP15, and DP30, each composed of varying hard and soft segment ratios. By controlling the weight fraction of the crystallizable segment, the mechanical properties and degradation rate of DP can be independently controlled to produce functional materials for specific applications^{255,256}.

For this study, a modified DP synthesis developed by Buschmann *et al.*²⁵⁷, called DegraPol15® (DP15), was selected for investigation due its characteristically softer, more elastic properties and degradation rate of ~2 months which is considered favourable for HVTE applications. DegraPol15® is composed of poly(3-(R-hydroxybutyrate)-co-(ε-caprolactone))-diol as the hard crystalline segment and poly(ε-caprolactone-co-glycolide)-diol as the soft amorphous segment²⁵⁷. Research has revealed DP15 biomaterials to be good carrier systems for growth factors, cells, and other stimuli, and to possess promising healing properties *in vivo*^{257,258}. These findings highlight the potential of DP15 as a prospective candidate for developing functional biomaterials for TE application. Several studies have investigated the use of DP15 as electrospun scaffolds for tissue engineering applications^{258,259}, however, currently no research has been reported that explores its application in engineering heart valve replacement materials.

Table 2.4: Component structures and chemical composition of DegraPol15®

Component	Name	Chemical Structure
Hard segment	poly(3-(R-hydroxybutyrate)-co-(ε-caprolactone))-diol	
Junction unit	2,2,4-trimethylhexane-diisocyanate	
Soft segment	poly(ε-caprolactone-co-glycolide)-diol	
Composition		

2.2 Materials and Methods

This section describes the materials and methods employed to manufacture, characterise, and tailor electrospun scaffolds for their application as materials for HVTE. The primary objective of scaffold development was to generate electrospun scaffolds morphologically suitable for HVTE applications, and mechanically sufficient to withstand hydrodynamic loading conditions associated with native cardiac valve environments.

2.2.1 Materials

Pellethane was purchased from Dow Chemicals (Michigan, USA), and DegraPol was generously supplied by ab medica S.p.A (Lainate, Italy). All the solvents, including Tetrahydrofuran (THF), N,N-Dimethylformamide (DMF) and Chloroform (CHCl₃), and N,N-Dimethylacetamide (DMAc), were purchased from Sigma-Aldrich (Pty) Ltd, RSA. Applicable polymer and solvent properties are referenced in Appendix A, Table A1 and Table A2, respectively.

2.2.2 Electrospinning procedure

2.2.2.1 Polymer Solution Preparation

All electrospinning solutions were prepared by dissolving the polymer-solvent mixtures with a magnetic stirrer running at 350 rpm at room temperature, over a 12–24-hour period. After mixing, solutions were rested for an hour to allow air bubbles to dissolve and slight heat generated from stirring to dissipate. Once rested, solutions were transferred to the syringe for electrospinning. The operating procedure employed to conduct the electrospinning experiments is detailed in Appendix A. Solution concentrations were expressed as weight-by-weight percentage (wt%) of polymer to solvent. Polymer masses were measured with a high-precision analytical balance (METTLER TOLEDO®), and solutions were prepared by pipetting solvents into glass bottles fitted with cover sleeves. Due to the degradable nature of DP15 and its characteristic time-dependency with exposure to solvents, DP solutions were prepared a maximum of 24 hours prior to electrospinning and discarded 48 hours thereafter.

2.2.2.2 Equipment Setup

The electrospinning rig employed for scaffold production was custom designed and assembled in-house. Key components of the experimental setup, depicted in Figure 2.13, included: (A) a computer equipped with a screen, (B) two high-voltage power supply units of opposite polarity (ES30N–20W, Gamma High Voltage Research, USA); (C) a high precision syringe pump (Fusion 100, Chemyx Inc., USA) secured on a lab jack; and (D) a central electrospinning module, featuring a rotating-collector unit fixed on an XY linear translating stage, and a mobile bracket with a slotted needle housing to mount the spinneret and auxiliary plate electrode. The electrospinning module was housed in a wooden cabinet equipped with sealed glass doors to achieve better environmental control.

The cabinet interior was painted matte black to facilitate observations of the Taylor cone and the whipping action of the fiber. In addition, a (D) humidity control system was developed to regulate the ambient relative humidity in the electrospinning module. Additional modifications were made to the system apparatus over the course of the project to improve operating efficiency and process repeatability.

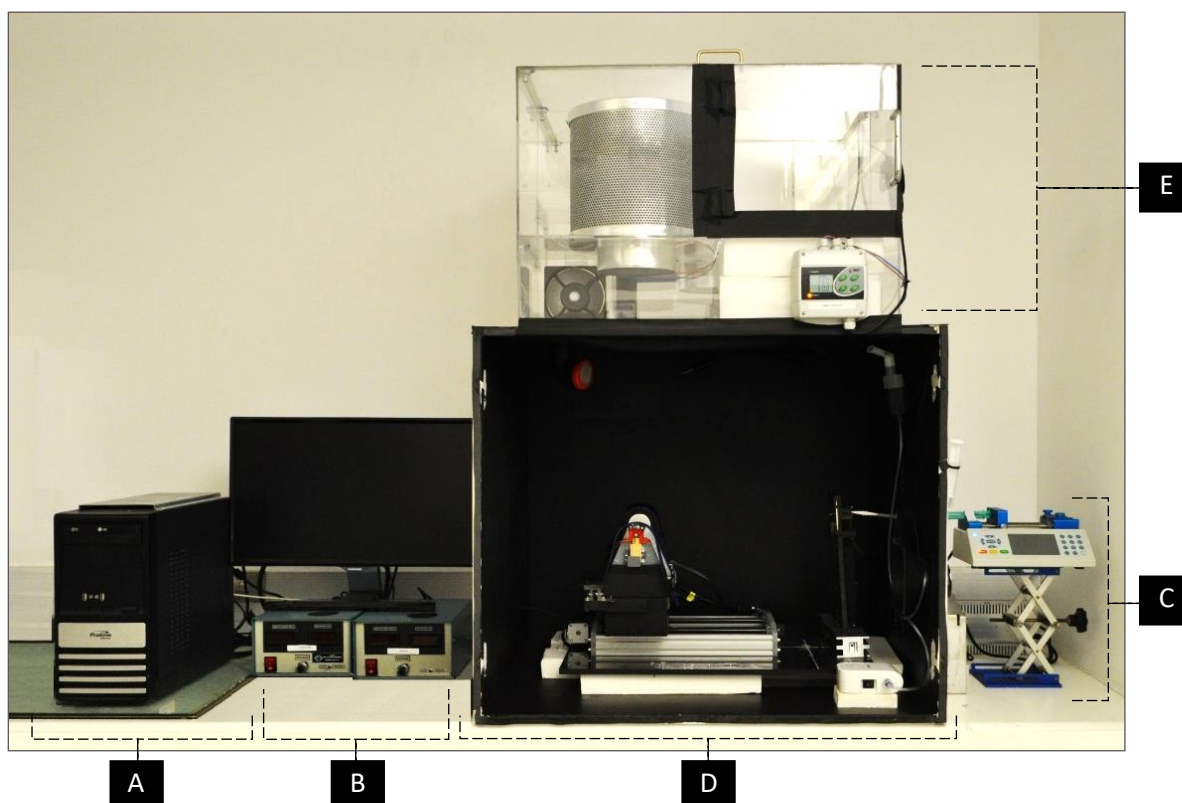


Figure 2.13: Electrospinning rig setup. Key components includes a (A) computer and user interface, (B) positive and negative power supply, (C) syringe pump, (D) central electrospinning module and, (E) humidity control unit.

2.2.2.3 Equipment Configuration

Fiber collector system

The frame bordering the fiber collector (fiber collector unit) was built from PMMA sheets to focus fiber deposition onto the collector by preventing the surrounding structures from interfering with the electric field generated between the collector and the needle. The fiber collector was mounted to the frame via a stainless-steel axle, which was connected to a belt drive mechanism partitioned on one end of the unit. Plastic ball bearings were installed in the axle housing to facilitate smooth rotation of the collector. The collector unit was mounted on a XY-platform for two-way perpendicular translation; and the translating stage was placed on a vibration damping pad to dissipate vibrational energy and reduce mechanical noise generated by the various moving components. Using Arduino software, an Arduino Uno microcontroller board equipped with standard motor controllers, was programmed to control the stepper motors to drive the rotation of the collector and translation of the collector unit.

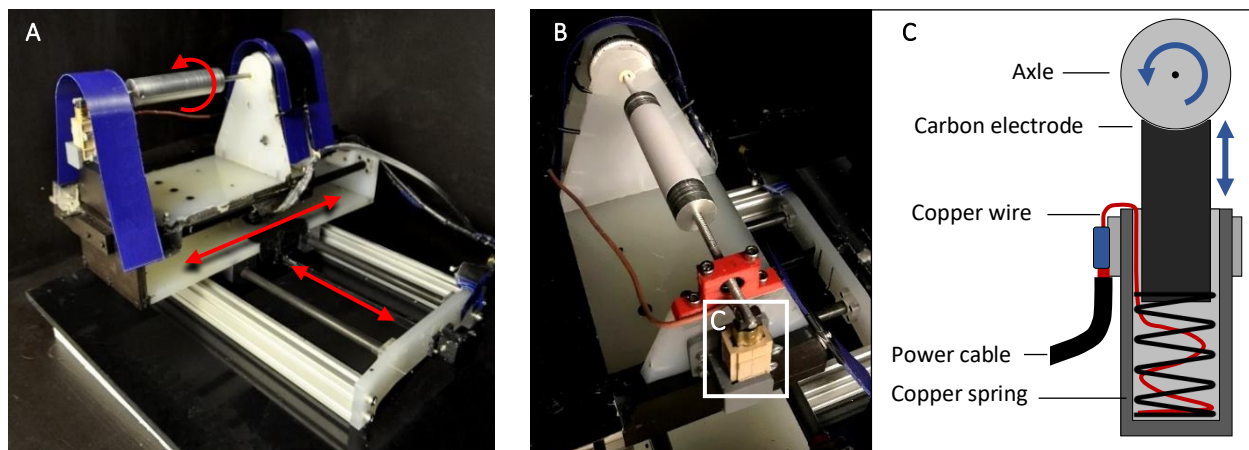


Figure 2.14: Fiber collector system setup. A) Fiber collector unit on XY-platform, showing directions of relative movement. B) Fiber collector chassis with (C) spring-loaded electrical conducting mechanism.

Finally, the collector was supplied with electrical current via a spring-loaded electrode module, depicted schematically in Figure 2.14C. The spring action of the electrode was designed to accommodate the small fluctuations in vertical displacement of the axel due to flexions caused by the rotation of the collector and load imbalances within the unit. Another primary function of the module was to maintain electrical continuity between the conducting element and the rotating surface of the axel.

Solution Feed System

The spinneret assembly was composed of a stainless-steel blunt tip needle connected to a 10ml two-part syringe via a flexible Teflon® tube. A 3D-printed PLA bracket was employed to mount the needle and auxiliary plate electrode (Figure 2.15). The syringe pump was located outside the rig cabinet.

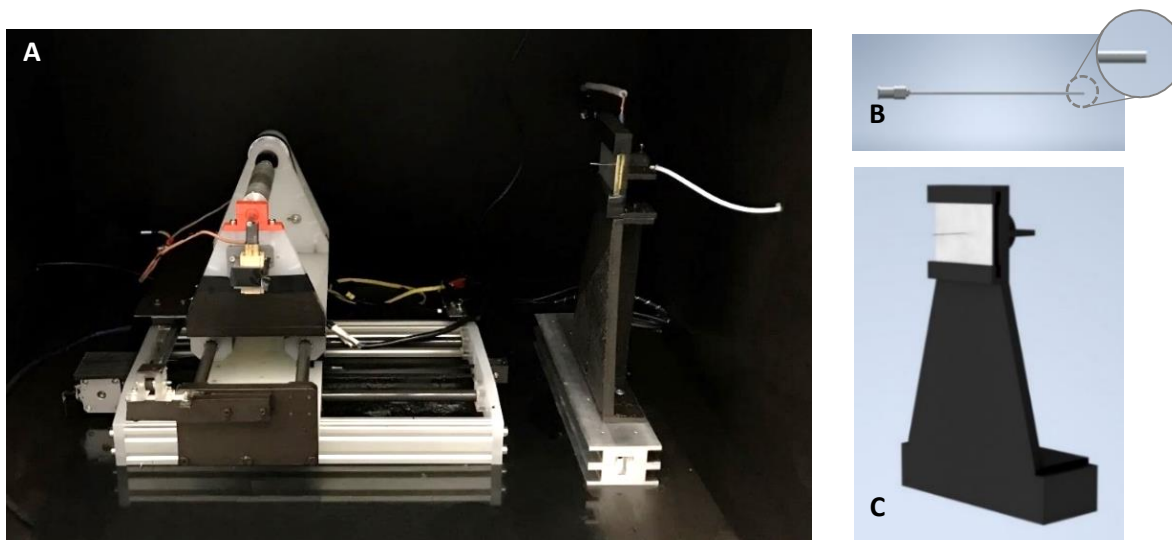


Figure 2.15: Solution feed system components. A) Solution feed system and collector unit configuration. B) Blunt-tip needle. C) Mobile bracket featuring spinneret needle and auxiliary plate electrode.

2.2.2.4 Humidity Control System

The humidity control system comprised of two subsystems programmed to function interactively to regulate the relative humidity of the working environment. The subsystems involved a dehumidification system and humidifying module. The systems were collectively controlled by implementing a thermo hygrometer featuring a digital interface, two relay outputs, and an external sensor probe (COMET H3531, COMET System, s.r.o.).

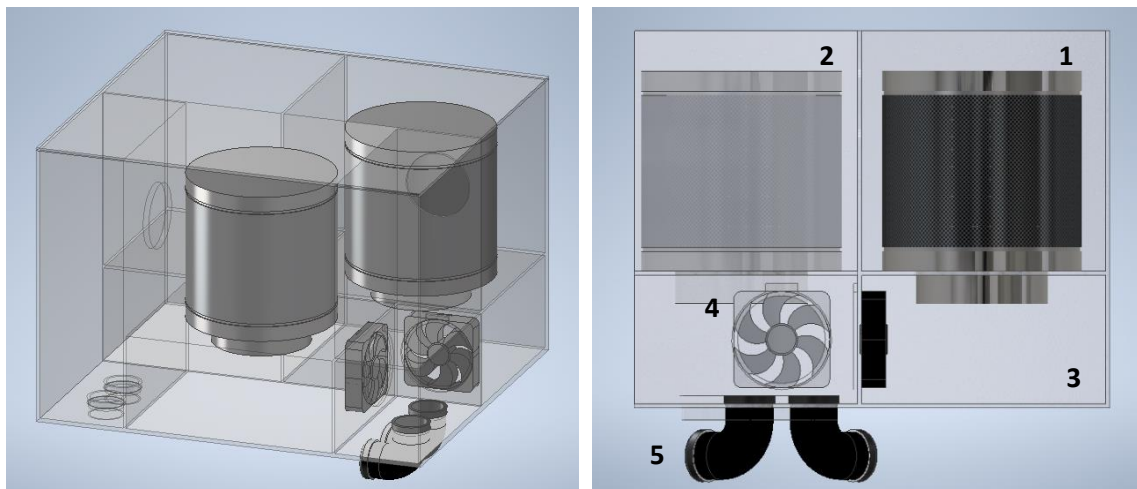


Figure 2.16: Humidity control system dehumidification unit. The unit comprised of a (1) desiccant filter, (2) carbon filter, (3) PMMA box, (4) two fans, and (5) two swivel air diffusing ducts.

Dehumidification subsystem

The dehumidifying system components included (1) a desiccant filter, comprised of a cylindrical filter cartridge containing a drying agent (moisture indicating silica gel crystals); (2) an activated carbon air filter cartridge; and (3) a PMMA box featuring a configuration of compartments designed to direct air flow through two distinct cycles that work to dehumidify or normalise the environmental conditions, respectively (illustrated in Figure 2.17). To control the direction of airflow, (4) brushless DC fans were installed in the vent holes leading from each cycle to the outlet compartment. Hinged lids were incorporated to assist in isolating the air cycles by automatically closing and opening relative to the respective fan activity.

Airflow from the humidity system outlet was channelled into the electrospinning rig via two vents fitted with elbow ducts capped with a passive air diffusion lid. The diffusing ducts were employed to direct airflow away from the whipping region of the electrospun fibers and reduce the airflow rate generated by the fan. In this way, laminar airflow was preserved within the electrospinning working environment throughout the experimental procedure. Airflow in electrospinning systems has been established to disrupt fiber deposition patterning and consequently lower the repeatability of scaffold structures²⁶⁰. Thus, applying techniques to impeding airflow was necessary.

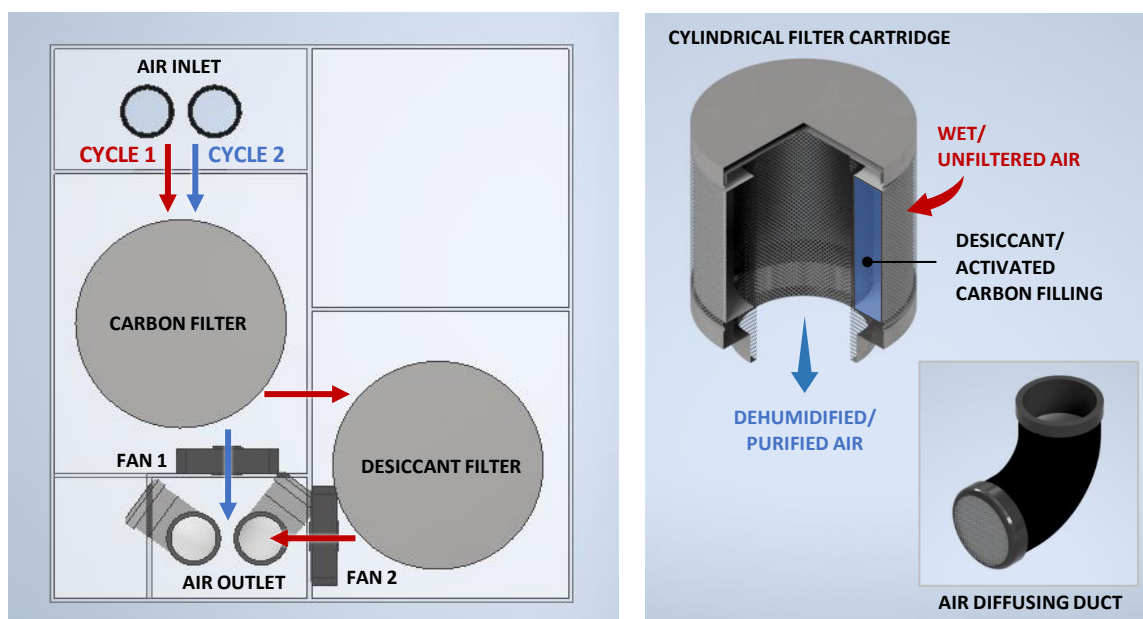


Figure 2.17: Humidity control airflow cycle. Cycle 1 represents the dehumidification system and Cycle 2 the normalising system.

Humidification subsystem

An ultrasonic humidifying unit (C803 Compressor Nebuliser, OMRON, SA) was employed to achieve environmental humidification. The basic mechanism of ultrasonic humidifiers involves the use of high-frequency vibrating diaphragms to generate aerosol particles from a solution reservoir. The humidifying unit was connected to the output relay of the humidity monitor and programmed to activate when the humidity dropped below a specified value. The control unit was positioned inside the electrospinning cabinet, with the water reservoir mounted on the wall relative in height to the air ducts for the effective dispersal of moisture to the working environment. Deionised water was utilised to minimise the number of ions introduced to the system. Increased ion concentrations in the air cause electrospun jets to discharge more rapidly, which results in a lower surface charge density that manifests as an impeded whipping action^{261,262}.

2.2.3 Electrospinning parametric investigation

Parametric investigations were carried out for both PEL and DP scaffold production, respectively. The objective for both studies was to quantify the influence of varying selected parameters on the resultant physical scaffold properties, and thereby optimise the electrospinning conditions to produce structurally and mechanically suitable scaffolds for their application as leaflet materials for valve construction and hydrodynamic testing.

2.2.3.1 Parametric studies for Pellethane® scaffolds

The processing parameters investigated for PEL scaffold development include the rotational speed of the collector (collecting speed) and solution feed flow rate. Rotational speeds of 500, 1000, and 1500rpm were investigated at

flow rates of 3–6 ml/hr. To study the influence of these variables on the structural and mechanical properties of resultant scaffolds, supplementary processing parameters were fixed. These included a solution concentration of 16 wt% in a 50/50 THF/DMF (wt/wt) solvent mixture, an applied potential of 20 kV across a collection distance of 25 cm, and a translating speed of 3mm/min with a translational distance of 100 cm. A needle size of 19G was employed for all PEL experiments, and the ambient relative humidity was regulated at 45%. All experiments were performed at room temperature. Table 2.5 below presents a summarised description of the experimental tests and corresponding sample sizes produced for this investigation.

Table 2.5: Electrospinning parametric test summary and sample size specification for Pellethane scaffold development

Needle size	RH	Distance	+ kV	- kV	RPM	Flowrate (ml/hr)	<i>n</i>	<i>k</i>	<i>N</i>
19G	45%	28 cm	17	3	500	3, 4, 5, 6	4	4	16
					1000	3, 4, 5, 6	5	4	20
					1500	3, 4, 5, 6	4	4	16

n = sample size per group, *k* = number of groups, *N* = sample population

The solution concentration and solvent composition were established based on previous studies that report effective parameters for electrospinning PEL scaffolds²⁶³⁻²⁶⁵. The remaining constants were established by conducting a qualitative prescreening study wherein each variable was investigated over a range of values referenced from literature. From this, unsuitable parameters that resulted in improper jet formation, inadequate whipping motions, and/or excessive fiber fusing were exposed; and moreover, a set of effective parameters found to consistently produce stable Taylor cones, effective whipping actions, continuous fibers, and minimal wet landing, were discovered and assigned as constants for proceeding quantitative parametric investigations.

2.2.3.2 Parametric studies for DegraPol® scaffolds

The parameters explored to structurally tune DP scaffolds included the solution concentration, flow rate, collecting speed, working distance and ambient RH. For all DP experiments, a potential difference of 25 kV was applied across a working distance of 28 cm. Solution concentrations of 15 and 18 wt% under ambient relative humidities between 25–45% were investigated for their influence on scaffold microstructure. Flow rates between 1–4 ml/hr at collecting speeds of 500–1500 rpm were investigated for their influence on scaffold architecture, porosity, and mechanics.

Table 2.6: Parametric investigation summary for the development of DP scaffolds

Parameter Study	Parameter 1	Parameter 2	<i>n</i>	<i>k</i>	<i>N</i>
Solution wt% and relative humidity	15 wt%	30, 35, 40, 45%	3	4	12
	18 wt%	25, 30, 35%	3	3	9
Collecting speed and flow rate	500 rpm	1, 2, 3 ml/hr	3	3	9
	1000 rpm	1, 2, 3, 4 ml/hr	3	4	12
	1500 rpm	2, 3, 4 ml/hr	3	3	9

n = sample size per group, *k* = number of groups, *N* = sample population

2.2.4 Microstructural characterisation

Investigations on the influence of matrix morphology features, such as fiber diameter, orientation, and pore size, have demonstrated significant correlations with the mechanical behaviour and cellular response of engineered tissue scaffolds²⁶⁶. Characterising the structural aspects of the electrospun scaffolds fabricated in this project was thus critical to determining their suitability as substrates for engineering tissue heart valve constructs.

2.2.4.1 Image Acquisition

Scanning Electron Microscopy

Scanning electron microscopy was performed to obtain micrographs suitable for the architectural quantification and analysis of scaffolds. Three different scanning electron microscope (SEM) instruments were employed due to periodic equipment evaluation and replacement over the course of the project (see Figure 3.4). These included the JCM-6000 NeoScope™ Desktop SEM and JSM-T200 InTouchSope™ SEM from JEOL Ltd. (Tokyo, Japan), and the Phenom™ Pro Desktop SEM (Thermo-Fischer Scientific Inc., USA). The majority of SEM images were captured under low-vacuum (LV) mode, in which charging on the specimen is neutralised by the introduction of air into the chamber. This enables non-conductive specimens to be observed in their native state, which obviated the need for electrospun scaffold samples to be sputter coated prior to SEM analysis. This allowed for more rapid evaluation which improved the efficiency of scaffold optimisation. Samples that were analysed under high-vacuum (HV) mode were sputter coated in gold (Polaron SC7640, Quorum Technologies, England).

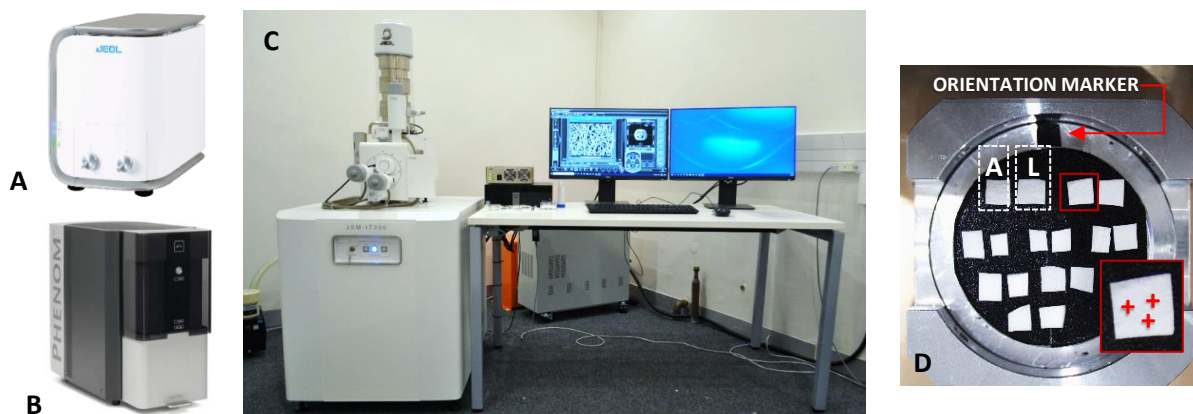


Figure 2.18: Scanning electron microscopes operated in this study. A) JCM-6000 NeoScope™ Desktop SEM (JEOL Ltd., Japan); B) Phenom™ Pro Desktop SEM (Thermo-Fischer Scientific Inc., USA); and C) JSM-IT200 InTouchSope™ SEM (JEOL Ltd., Japan). D) Scaffold specimens mounted to the SEM sample holder, with imaging sites (+), and abluminal and luminal orientation (right to left).

SEM sample preparation

Sample preparation involved cutting two square specimens (approximately 4 x 4 mm) from three locations along the length of the scaffold, followed by mounting to the SEM stub with conductive adhesive tape. Samples were oriented on the stub such that the luminal and abluminal surfaces could be observed, and the circumferential

direction was vertically aligned for orientation consistency. SEM micrographs were captured at three distinct locations on each sample (Figure 2.18D) at three different magnifications suitable for computational analysis.

Magnification selection

To determine the appropriate magnification settings for SEM imaging, the protocol described by Hotaling *et al.*²⁶⁷ on how to effectively use DiameterJ (a plugin used to measure fiber diameter and further described in section 2.2.4.5) to obtain reliable results was followed. Briefly, fiber structures were required to be at least 10 pix in diameter and no greater than 125 pix; and diameters were not to exceed sizes greater than 10% of the smallest dimension on the image. The protocol was carried out by measuring fiber diameters under live SEM observation at various magnifications and comparing the recorded values against the reported specifications. A unique magnification range was thereby determined for each sample group. For simplicity, three common magnifications were established to be suitable for the analysis of respective sample groups. However, due to the overall disparity between PEL and DP fiber diameters, the range of suitable magnifications corresponding to each material group conducted with the same instrument was determined to be distinct (summarised in Table 2.7).

Table 2.7: Summary of image magnifications utilised for image processing and analysis

	Phenom™ Pro	JCM-6000plus NeoScope™	JSM-IT200 InTouchSope™	N
Pellethane®	400x, 700x, 1000x	–	–	280*
DegraPol®	320x, 370x, 520x	100x, 200x, 500x	200x, 300x, 500x	500*

N = sample population

* 2 images were captured at each site per sample for each magnification. Samples were obtained from three locations of each scaffold, and three scaffolds constituted one sample group

2.2.4.2 Image Processing Software

ImageJ software was employed to process, measure, and analyse the scaffold micrographs acquired from the respective SEM instruments. ImageJ is an opensource Java image processing program (NIH Image, USA) with a wide range of applications, used extensively in biomedical and biomaterials research communities^{268,269}. In particular, ImageJ features highly pertinent functions for characterising electrospun polymeric scaffolds²⁶⁶, and is thus widely utilised for research in tissue engineering²⁶⁷.

2.2.4.3 Image Segmentation

The first step to SEM image analysis involves segmenting an image into a binary foreground and background graphic. The precision of segmentation is fundamental to the accuracy of morphological parameter estimates, since fiber measurements are derived directly from the segmented framework geometries²⁶⁶. Numerous segmentation tools have been developed for ImageJ to assist in obtaining adequately segmented images for fiber analysis²⁷⁰. However, due to the wide variability of image properties between SEM instruments, system settings, user experience, sample preparation protocols, and fiber compositions²⁶⁷, the application of fully automated

segmentation algorithms has been found to produce decidedly invalid network compositions^{270,271}. The best condition for image segmentation has thus been proposed to involve an adaptive algorithm comprised of instructions that can be tailored to the specific image set being processed²⁷⁰. To this end, semi-automated segmentation algorithms with specific adaptive instructions were compiled to expedite the segmentation process and reduce bias but preserve some aspects of manual intervention. Considering the variation in image size, resolution, and tonal range properties inherent to each SEM system employed, adopting an adaptive segmentation strategy was particularly necessary.

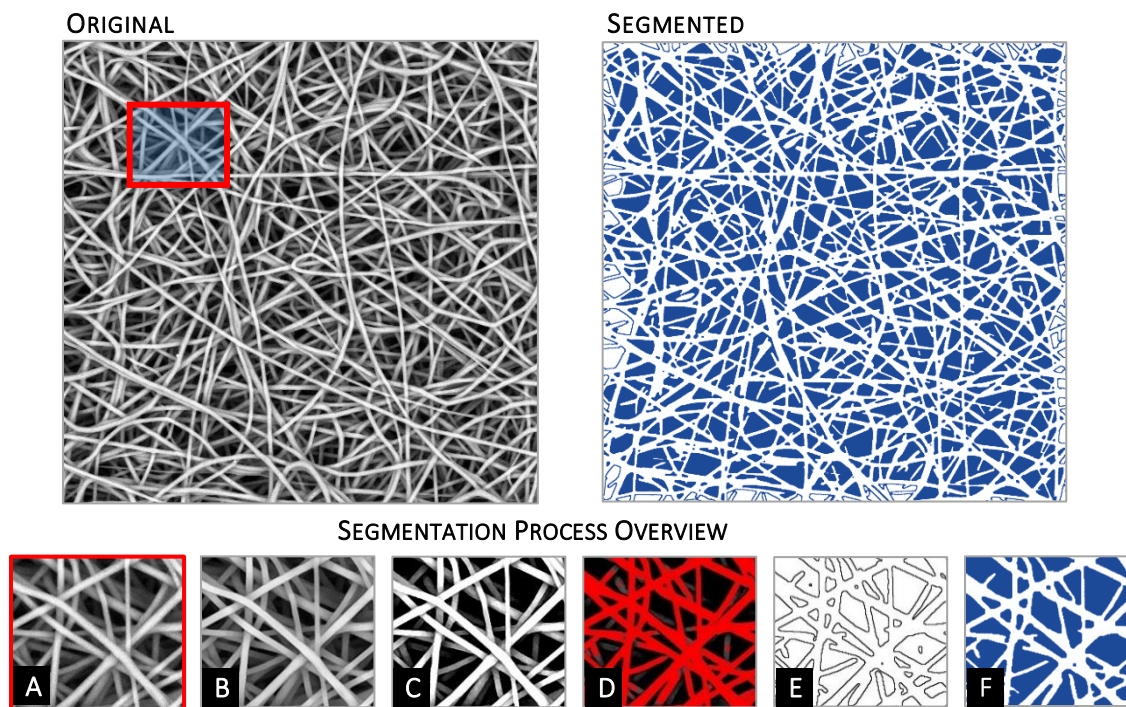


Figure 2.19: Simplified segmentation procedure. : A) Original image. B) Cropping and conversion to 8-bit. C) Histogram equalisation, median filtering, and outlier removal. D) Auto thresholding with Otsu method. E) Find edges. F) Fill holes.

The segmentation algorithms compiled for this study included a sequence of morphological operators derived from the methodology developed by D'Amore *et al.*²⁶⁶ with minor code adaptations tailored to segment images from different image sets. The algorithm that formed the basis of each segmentation macro involved the following series of instructions (illustrated in Figure 3.6): firstly, the original image was cropped to a specified size, followed by being converted to an 8-bit graphic. Thereafter, histogram equalization was performed to enhance the contrast of the surface fibers relative to the underlying pores. Median filtering (radius = 3) followed by removal of bright and dark outliers (radius = 2 and threshold = 50) was employed to reduce noise and define structure edges. Subsequently, auto thresholding through global min error method was performed to separate the fiber network from the background and thus produce a binary graphic. After thresholding, images underwent smoothing, sharpening, and despeckeling to further define the structure edges and remove isolated pixels from the binary fiber network.

Thereafter, the structure edges were isolated using the “find edges” prompt, after which the bordered holes were filled to produce the final segmented image.

2.2.4.4 Image processing for variability correction

Due to the different system configurations and operating features provided by each SEM machine, images acquired from each instrument exhibited varying degrees of resolution and tonal depths. Low-resolution images were found to be problematic when running through automated analysis tools. Due to low-resolution pixilation, severe noise concentrations were encountered along the boundaries of the demarcated fiber network when running through segmentation algorithms and applying the particle analysis tool led to significant error in pore partitioning, and subsequent failure to produce meaningful results (depicted in Figure 2.20A-B). To overcome this issue, a pre-processing macro was compiled to reduce the pixel noise, enhance structure definition, and increase tonal depth to pronounce surface fibers. The principle morphological operators used to compile this macro included noise reduction (despeckeling), smoothing, sharpening, median filtering, histogram equalisation, window/level tuning and removal of isolated pixels (outlier removal). With this, substantial refinement of low-resolution images was achieved, such that their segmented counterparts comprised of defined binary boundaries and representative network structures (as shown in Figure 2.20C-D). By applying this macro to every image sample, segmentation outcomes were essentially standardised.

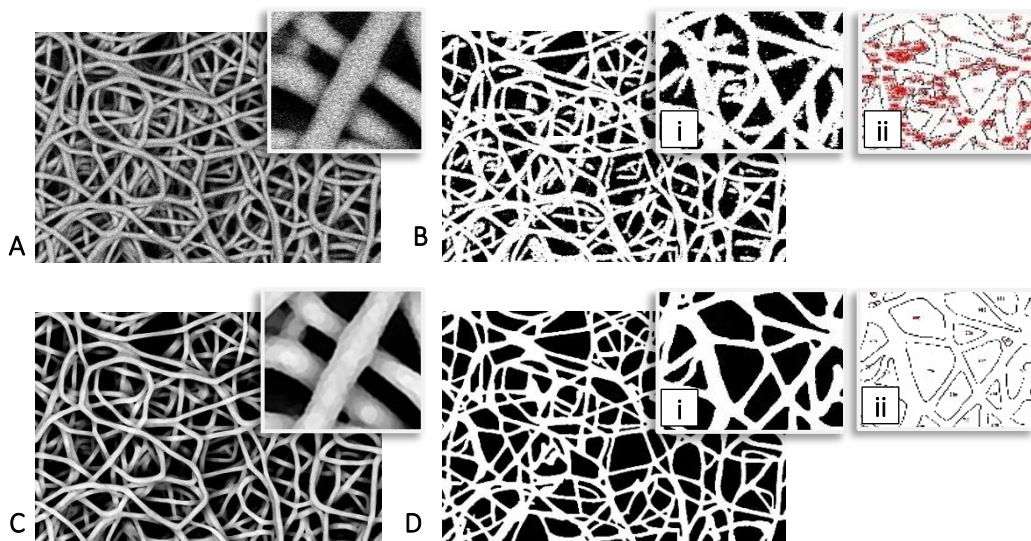


Figure 2.20: SEM image processing technique for resolution variability correction. A) Original low-resolution image. B) Corresponding segmented image exhibiting (i) noise at structure edges, and (ii) invalid pore partitioning with the application of the particle analysis tool. C) Pre-processed image using compiled macro. D) Corresponding segmented graphic showing (i) smooth linear structural boundaries and (ii) representative pore demarcations.

2.2.4.5 DiameterJ Analysis

Scaffold fiber geometries were characterised with DiameterJ, an open source fiber measurement plugin for ImageJ, which is widely used as a tool for biomaterial characterisation in the field of tissue engineering²⁶⁷. The algorithm was developed to achieve the efficient automation of measuring micro- to nanofiber diameters in scanning electron micrographs. The DiameterJ plugin includes a number of algorithms with different computational outputs that characterise key scaffold properties. Computed properties include fiber diameter geometries, pore composition, fiber orientation, intersection density, and estimated fiber length^{267,272,273}. The utilised outputs from DiameterJ analyses performed in this study included fiber diameter and pore size estimates.

2.2.4.6 OrientationJ Analysis

Scaffold fiber orientation was characterised using OrientationJ, a plugin for ImageJ that measures the orientation of an image both qualitatively and quantitatively²⁷⁴. The OrientationJ Distribution tool was used to calculate the distribution of orientations for each SEM image and produce multichromatic graphics of the fiber directionality. The protocol for interpreting the orientation graphics is illustrated schematically in Figure 2.21. To produce orientation visualisations for the electrospun scaffolds, such as those presented Figure 2.22, the Gaussian gradient structure tensor was applied with the Gaussian window equal in pixel length to the mean fiber diameter, the hue equal to orientation, saturation equal to coherency, and brightness corresponding to the original-image.

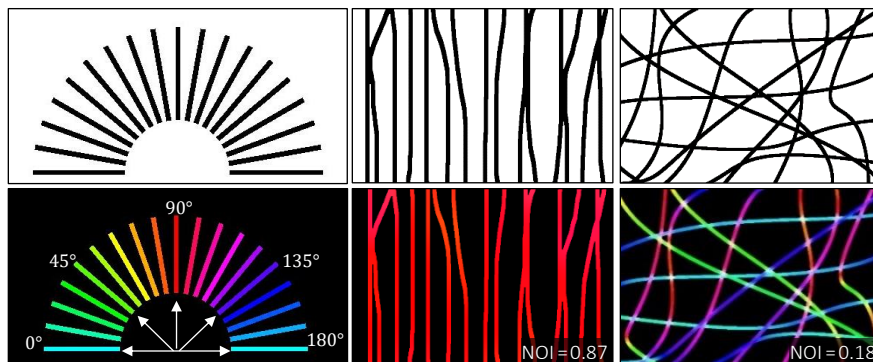


Figure 2.21: Schematic illustration for interpreting fiber orientation visualisation.

The degree of alignment was determined using the Dominant Direction tool, which equates the overall coherency of the image to a normalised orientation index (NOI). The NOI is a normalised derivation of the orientation index (OI), which can be described as the angle that contains one-half of the total area under the orientation distribution curve, representing 50% of the total number of fibers²⁷⁵. The formula for this relationship is given by:

$$NOI = \frac{90 - OI}{90} \times 100\% \quad (3)$$

such that values close to 1 indicate a high alignment, and values close to 0 suggest more random orientation²⁶⁶.

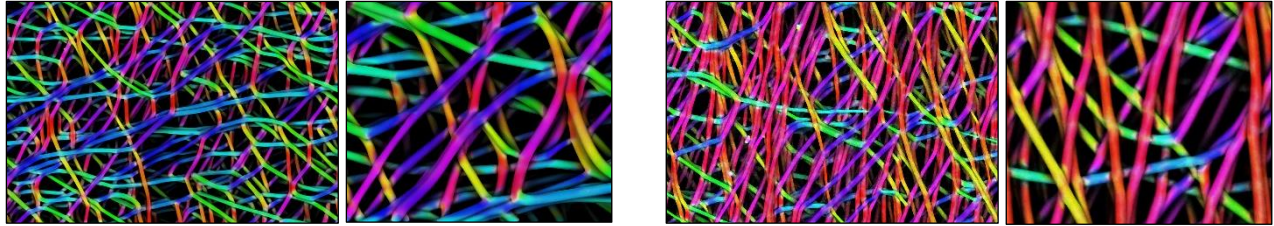


Figure 2.22: Orientation visualisation analysis examples. Orientation visualisation of electrospun scaffolds of randomly oriented (left) and highly aligned fibers (right).

2.2.5 Mechanical characterisation

Uniaxial tensile tests were conducted to mechanically characterize the electrospun scaffolds. Although biaxial testing would better mimic native *in vivo* loading conditions and thus provide more physiologically relevant material mechanics²⁷⁶, the majority of available published research on valve leaflet biomechanics presents uniaxial tensile data⁵³. This can be attributed to the simplicity of tensile test protocols, accessibility of standard equipment, and straightforward data processing procedures. Two types of tensile tests were conducted to mechanically characterise the electrospun scaffolds. Quasi-static tensile tests were performed to characterise the tensile mechanical properties of the scaffolds, and cyclic tensile tests were conducted to characterise the viscoelastic properties of the scaffolds.

2.2.5.1 Sample preparation

Tensile dog-bone specimens were die-cut from electrospun scaffold sheets with a steel rule die (Packtech Tooling, RSA) and manual hand press machine. Dog-bone geometry, illustrated in Figure 2.23A, was compliant with ASTM standards (gauge length of ~22mm and width of 4.75mm). Samples were cut in either longitudinal and circumferential directions (Figure 2.23B), vacuum dried and stored under vacuum until mechanically tested. Prior to mounting the specimen in the test machine, the mean thickness (t_0) of each sample was measured with a digital micrometer (Mitutoyo Corporation, Japan).

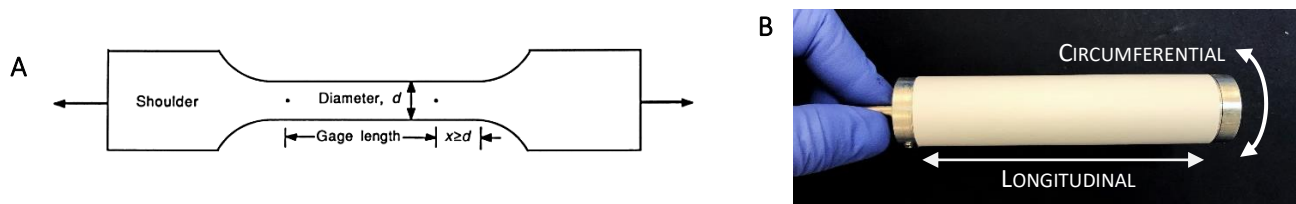


Figure 2.23: Mechanical testing specifications. A) Typical dog-bone specimen geometry²⁷⁷. B) Sample scaffold orientation.

2.2.5.2 Experimental Setup

Tensile testing was performed on a universal tensile test machine (Instron® 5544, Norwood, USA) equipped with a 10N load cell. Self-aligning grips were used to mount the specimen between the fixed and movable member, to ensure that the load applied through the centreline of the grip assembly translated colinearly with the long axis of

the specimen in tension. In this way, any torsional deformation induced by slight grip misalignment was minimised. Rubber lined jaws were used to fix the specimens between the grips. These were effective in preventing slippage without damaging the sample; and maintained even stress distribution throughout the specimen during testing. Once clamped, the gauge length was adjusted to remove any slack prior to starting the test procedure. For all tests, a pre-strain of 3% was applied to induce a state of pretension proposed to emulate that of aortic valve tissue in the native environment²⁷⁸.

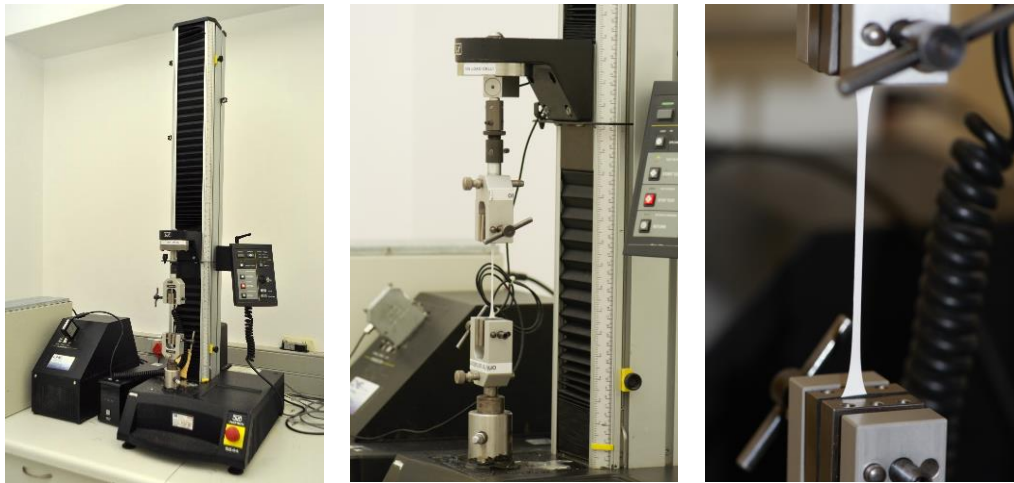


Figure 2.24: Mechanical testing apparatus. Instron® 5544 Tensile Tester.

2.2.5.3 Tensile Strength Testing

Uniaxial tensile strength testing was performed in accordance with ASTM D882 designations, developed for tensile testing thin plastic films with thicknesses below 1mm²⁷⁹. The ASTM D882 protocol is widely accepted as common practice for conducting tensile tests on scaffold specimens²⁸⁰.

Test procedure

A summary of the test sample groups that were mechanically characterised for electrospinning parametric investigations is provided in Table 2.8. Scaffold samples were preconditioned at a strain rate of 1.67 %/sec up to 7% strain for 10 cycles. Preconditioning parameters were obtained from those reported by Argento *et al.*²⁸¹ in a study on the mechanical characterisation of scaffolds for heart valve tissue engineering. After preconditioning, the clamp height was adjusted to remove any slack, after which the gauge length and cell load were reset and a pre-strain of 3% was applied. The system was zeroed immediately before initiating the test. Data acquisition was calibrated to capture readings at time intervals of 0.17 sec, and for the test to stop when a sudden drop in stress was recorded (load drop of 50%).

The test specimen was extended at a constant crosshead speed of 50 mm/min (3.75%/sec strain rate) until rupture. Generally, strain rate selection would be governed by the physiological conditions subjected at the native site, however native aortic heart valves are subjected to strain rates excessive of 440%/sec and 1600%/sec in the

circumferential and radial direction respectively^{194,282}. Replicating these values with typical tensile test equipment is unfeasible. Thus, no standard strain rate has been established for evaluating TEHV scaffolds. Consequently, those reported in literature range between 4–12%/sec²⁸³. For this project, the speed of testing employed for tensile strength tests was selected with reference to the ASTM D882 designations for the predefined specimen geometry²⁷⁹, and further cross-referenced with correlating literature to substantiate the chosen value.

Table 2.8: Summary of sample test groups for scaffold tensile strength characterisation

Study Description	Material	Number of groups	Sample size/group	direction
<i>Processing Parameter Influence</i>				
Solution concentration	DP	2	n = 6	C, L
Rotational velocity	DP, PEL	3, 3	n = 4	C, L
Flow rate	DP, PEL	4, 4	n = 4	C, L
<i>Structural property influence</i>				
Fiber alignment	DP, PEL	3, 2	n = 6	C, L
Fiber diameter	DP, PEL	3, 3	n = 6	C, L
Porosity	DP, PEL	3, 3	n = 6	C

Analytical method

The raw data captured by the Instron software was imported to Excel and plotted as stress-strain curves. All tensile mechanical properties were determined in accordance with standard engineering principles and are depicted for reference in Figure 2.25²⁷⁷. The maximum tensile stress and corresponding elongation

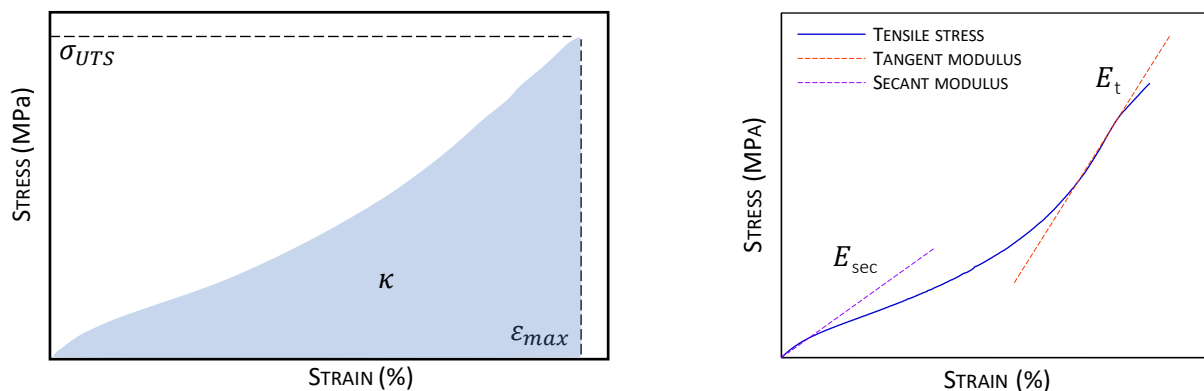


Figure 2.25: Tensile stress-strain curve interpretation. Stress-strain curves obtained from tensile testing indicating the primary properties obtained for mechanical analysis.

obtained from the stress-strain curve were defined as the ultimate tensile strength (UTS) and maximum strain (ϵ_{max}), respectively. Young's modulus was evaluated as the 20% secant modulus (E_{sec}), which is typically used to characterise the "useful elastic" region for TPEs of a more flexible grade^{284,285}, as well as the tangent modulus (E_t). The modulus of toughness (κ) was calculated from the area under the stress-strain curve²⁸⁶. Areas were approximated by numerical integration using the trapezoidal rule. Mechanical anisotropy was calculated as the ratio between properties obtained from the circumferential and longitudinal direction.

2.2.5.4 Cyclic Tensile Testing

Experimental procedure

The cyclic tests conducted on both DP and PEL scaffolds were performed at a constant strain rate of 12 %/sec up to a peak displacement of 50% for up to 50 cycles. To evaluate the influence of rotational speed on the viscoelastic behaviour of PEL and DP scaffolds, cyclic tests were performed on scaffolds spun at 500, 1000, and 1500 rpm ($n = 6$ per group) in both longitudinal and circumferential directions ($n = 3$ per direction). Raw data was first plotted as stress-strain curves, as depicted in Figure 2.26A. To evaluate the stress softening behaviour of each sample, the variation in the peak reaction stress (R_c) captured over successive cycles throughout the cyclic loading period was plotted as a function of cycle number. To compare the results obtained between each group from, the variation in peak stress for each cycle with respect to the first cycle was equated and then divided by that of first cycle to normalise the value. The mathematical expression of this relationship is given by:

$$R_c = \frac{\sigma_{max}(c_1) - \sigma_{max}(c_n)}{\sigma_{max}(c_1)} \quad (4)$$

where R_c is the variation in peak reaction stress, and $\sigma_{max}(c_1)$ and $\sigma_{max}(c_n)$ are the maximum stress values corresponding to the first and n^{th} cycle. respectively.

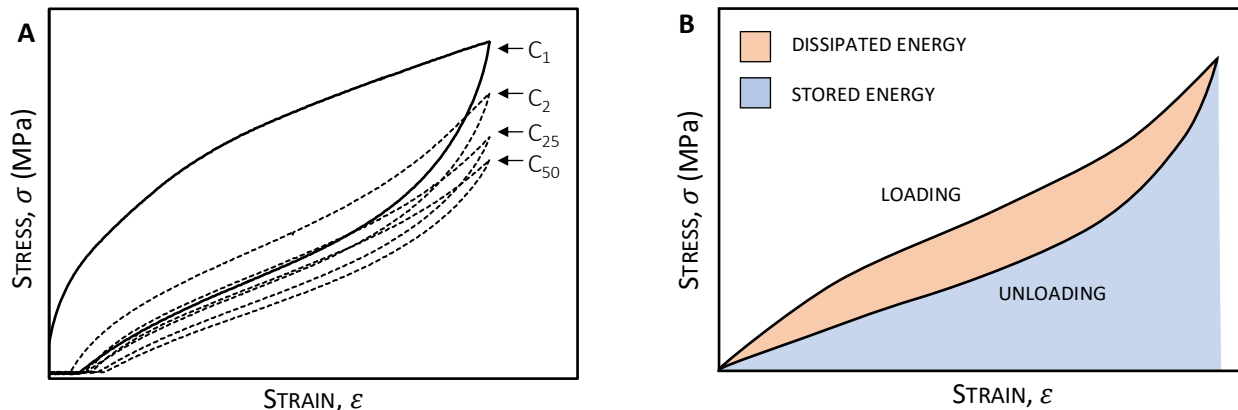


Figure 2.26: Cyclic stress-strain curve interpretation. A) Stress-strain curve demonstrating the variation in hysteresis and cyclic stress over successive cycles. Only four hysteresis loops designated by their cycle numbers (C_n) are presented. B) Stress-strain curve showing the loading cycle path and the corresponding strain energy relationships

Hysteresis was quantified for each scaffold as a percentage of the energy dissipated with respect to the strain energy stored during the loading phase, referred to in literature as the dissipated energy index^{287,288}. The dissipated energy was determined from the area bounded between the loading and unloading curve (illustrated in Figure 2.26B) by calculating the difference between the work done during loading and unloading deformation. The numerical formula for the dissipated energy index (hysteresis ratio, h) is thus expressed as:

$$h = \frac{U_{load} - U_{unload}}{U_{load}} \quad (5)$$

where U_L and U_U are the work done (strain energy/unit volume) during loading and unloading, respectively.

2.2.6 Porosity determination

2.2.6.1 Experimental procedure

A liquid displacement method based on the principle of densitometry was employed for the determination of scaffold porosity. For this method, test samples were prepared by cutting round specimens (8 mm diameter) from the electrospun scaffolds and measuring the thickness of each sample using a digital micrometer. The densitometric weighing technique was performed with an analytical balance and density determination kit (METTLER TOLEDO® Balance XS105 DualRange, Microsep (Pty) Ltd, SA) in accordance with standard operating protocols²⁸⁹. The two-step procedure included (1) measuring the mass of the scaffold in air, followed by (2) measuring the densitometric mass in an auxiliary liquid. DP scaffolds were weighed in Ethanol, which is highly effective in instantly penetrating the mesh interstices and eliminating entrapped air, and causes an ineffectual degree of swelling²⁹⁰. PEL scaffolds were weighed in hexane as they experience less swelling in hexane than in ethanol (3 vs 12 wt% increase)²⁹¹.

2.2.6.2 Analytical method

Scaffold porosity was quantified using the following equation:

$$P = 1 - \frac{V_f}{V_T} \quad (6)$$

where P denotes the porosity, and V_f and V_T represents the bulk fiber and total volume, respectively. The total volume refers to the combined volume of the fibers and interstitial pore space of the samples, which was measured from the scaffold thickness and circular area of the sample ($n=6$ per study group). The bulk fiber volume is equivalent to the volume of the liquid displaced by the submerged sample after the pore space is entirely infiltrated. The fiber volume was calculated in accordance with the verified mathematical equation defined for volume determination of solids using the specified densitometry apparatus and respective protocols, defined as:

$$V_f = \alpha \cdot \frac{m_{s,air} - m_{s,liquid}}{\rho_{liquid} - \rho_{air}} \quad (7)$$

where $m_{s,air}$ and $m_{s,liquid}$ is the mass of the sample in air and liquid respectively, ρ_{liquid} and ρ_{air} is the density of the auxiliary liquid and air, respectively, and α is the weight correction factor (0.99985) that accounts for the atmospheric buoyancy of the adjustment weight. Thus, the final expression used to calculate porosity was:

$$P = 1 - 4\alpha \cdot \frac{m_{air} - m_{liquid}}{\pi d^2 t (\rho_{liquid} - \rho_{air})} \quad (8)$$

where d and t are the diameter and thickness of the test specimen.

2.2.7 Permeability study

2.2.7.1 Experimental setup and equipment design

A large tank filled with distilled water was set up in an elevated position to exert a fixed hydraulic pressure onto a scaffold sample mounted in a watertight holder (Figure 2.27). The height of the water level to the holder outlet was held constant at 130 cm, subjecting a pressure of 100 mmHg on the sample, equivalent to peak differential pressures in the aortic environment. The volume of the water perfusing through the specimen was measured at various timepoints to quantify the permeability of the scaffold sample.

The sample holder was designed to mount the specimen by compressing the outer edge of the scaffold between two rubber O-rings and a sealing washer. A compressive force was generated by applying torque to the outlet screwcap. To avoid scaffold torsion, a metal sealing washer was positioned between the scaffold and contact-loading component. Owing to minimal friction between the washer and rotating surface, the applied torque translated as linear compressive force from the washer to the sample. For additional sealing precaution, waterproof adhesive tape was applied to the thread of the connector fitting head. To verify that the sample holder was effective in sealing, a solid polymer film was mounted in the holder and connected to the permeability rig for testing. The solid specimen was subject to a hydraulic pressure of 120 mmHg while the holder was observed for signs of leakage.

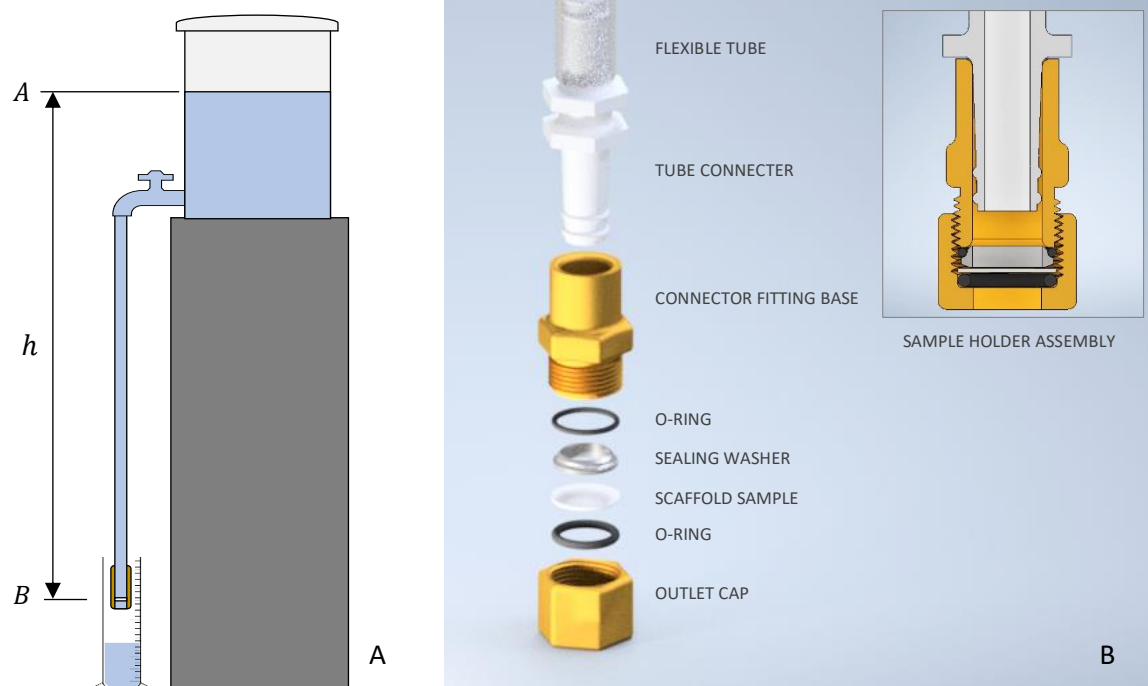


Figure 2.27: Permeability testing rig setup. A) Schematic illustration of testing rig. B) Rendered images of the sample holder subcomponents and assembly

2.2.7.2 Experimental Procedure

Electrospun specimens were cut from using a round die cutting tool and manual hand press machine. Prior to testing, the samples were pre-saturated by shaking in DI water for 15 mins. Once saturated, the scaffold thicknesses were measured using a digital microscope (TECHGEAR® EagleScope) featuring measurement software ($n=4$ locations per image). Thereafter, samples were mounted in the holder and secured to the pipe leading from the water tank. A 200 ml measuring cylinder was employed to collect the perfused water, from which volume readings were recorded at successive time intervals over a 60 min period. PEL and DP samples from three different porosity groups were tested to evaluate the influence of porosity on scaffold permeability ($n=3$ per group, respectively).

2.2.7.3 Analytical method

Scaffold permeability was calculated in accordance with Darcy's law²⁹², which describes permeability as a quantitative measure of the resistance of a porous material to fluid flow, mathematically expressed as:

$$k = \frac{Q}{A} \cdot \frac{\Delta x}{\Delta P} \quad (9)$$

where k is the permeability in Darcy units (D), Q is the volumetric flow rate, A is the flushing area of the sample perpendicular to fluid flow, Δx is the thickness of the scaffold, and ΔP is the applied pressure head directing the fluid flow through the scaffold. With reference to the method developed by Li and Mak²⁹³, the additional pressure drop across the scaffold was calculated using Bernoulli's equation²⁹⁴:

$$\frac{P_A}{\rho g} + \frac{V_A^2}{2g} + h_A = \frac{P_B}{\rho g} + \frac{V_B^2}{2g} + h_B + h_{LB} \quad (10)$$

where V , P , and h refers to the fluid velocity, pressure, and height at points A and B respectively, ρ is the density of the fluid, and h_{LB} is the frictional head loss in the system. By rearranging the equation to express the frictional head loss as a function of volumetric flow, and equating it to the pressure drop across the scaffold as follows:

$$\Delta h_L = \frac{Q_{B1}^2 - Q_{B2}^2}{2g\pi^2 r^4} = \frac{\Delta P}{\rho g} \quad (11)$$

the permeability according to Darcy's law was expressed as:

$$k = \frac{\Delta x}{A \cdot M_{B2}} \cdot \frac{2\pi^2 r^4}{(M_{B1}/M_{B2})^2 - 1} \quad (12)$$

where M denotes the mass flow rate and k has a unit of measurement of m^4/Ns . To determine the permeability behaviour of scaffolds as a function of time, the variation in the permeability measured at successive time intervals ($k(t)$) with respect to the first permeability measurement ($k(t_0)$) was calculated. To compare the values obtained for DP and PEL scaffolds ($n=3$ per sample group), this variation was normalised by its division of $k(t_0)$. The normalised relationship can thus be given by:

$$k(t) = \frac{k(t_0) - k(t_n)}{k(t_0)} \quad (13)$$

2.2.8 Wettability study

The wettability of each sample was determined by measuring the water contact angle (θ_c) using the sessile drop method. A digital microscope (TECHGEAR® EagleScope, China) mounted on a height-adjustable imaging booth was used to capture the images, and ImageJ software was used to measure the contact angles. The method was performed by dispensing 5 μ l water droplets ($n=4$) at various locations on the scaffold surface. Each droplet was video recorded for up to 2 minutes to assess the wetting behaviour over time. For static contact angle measurements, images were captured after 60s, by which time the droplet would be in static equilibrium ($n=6$ per group). For dynamic wetting analyses, still images were captured from the video footage every 5 seconds until the droplet was fully absorbed into the scaffold. The contact angles were measured for each image such that the change in contact angle over time could be determined.

2.2.9 Statistical analysis

Statistical analyses and data reporting of both scaffold and valve evaluations were performed using JMP Student Edition (JMP®, Version 12.1.0, SAS® Institute Inc., Cary NC, USA) and Microsoft Excel® (Microsoft 365®, 2016), respectively. All data, including experimental results and in-text citations, are expressed as mean $\pm$ standard deviation (SD). The statistical models employed for data analyses were chosen based on the sample distribution of the respective data sets. Parametric statistics were employed for normally distributed data and nonparametric tests were applied for non-normal data. Sample distributions were tested for normality using a Shapiro-Wilk goodness-of-fit test and which was corroborated by assessing the corresponding QQ plot. For normally distributed data, statistical analyses of single comparisons were performed using one-way analysis of variance (ANOVA) and Student's t-test methods, and for multivariate comparisons, two-way or full factorial ANOVAs was modelled. For nonparametric data, single comparisons were conducted using Kruskal Wallis H tests and multiple pairwise comparisons were performed using Wilcoxon Signed-rank tests. For linear-curve fitting, parametric data was modelled with least squares regression and nonparametric data was modelled with Theil-Sen regression. For nonlinear curve-fitting, a variety of regression models were employed, from which the curve of best fit was established. These included power, logarithmic, and reciprocal regression models. The strength and direction of association between parameters was quantified using Pearson's r and Kendall's Tau for parametric and nonparametric data sets, respectively. The p-value estimates obtained across all models were assumed statistically significant at 0.05 or less ($P \leq 0.05$).

2.3 Results and Discussion

This section presents the experimental results obtained for the development of non-degradable Pellethane and degradable DegraPol electrospun scaffolds for their application as materials in engineered heart valve constructs. The following sections include studies on the effect of electrospinning parameters on the morphology and mechanical properties of the scaffolds, and the evaluation of the permeability and surface wettability of scaffolds with distinct microstructures.

2.3.1 Microstructural properties

For the optimisation of PEL scaffolds, the influence of the flow rate and collecting speed on scaffold microstructure was investigated, and for the optimisation of DP scaffolds, the influence of solution concentration, relative humidity, flow rate, and collecting speed was investigated. The characterisation of microstructural properties includes average fiber diameter, pore size, orientation, and porosity.

2.3.1.1 Effect of relative humidity and solution concentration

The initial focus of process optimisation for DP scaffold production was on generating scaffolds with smooth, uniform fiber diameters by evaluating the effect of solution concentration (15 and 18 wt%) and relative humidity on fiber morphology and scaffold architecture. The effect of relative humidity was studied by fixing the flow rate and rotational speed at 2 ml/hr and 1000 rpm, respectively, and then modulating the moisture content of the electrospinning working environment by means of a humidity control system, described in section 3.3. From initial investigations, it was found that the range of operable RH conditions differed depending on the solution concentration of the polymer. For 15 wt% DP solutions, scaffold production was achievable in RH conditions between 30–45%, while 18 wt% DP solutions were only spinnable in RH conditions between 25–35% RH.

As shown in Figure 2.28, the microstructural characteristics of the scaffolds were strongly affected by changes in RH. From Figure 2.28(C and F), it can be seen that scaffolds spun from 15 wt% solutions at 45% RH, and 18 wt% solutions at 35% exhibited unfavourable features such as wet landing, interfiber fusion, nonuniformity, and high packing densities. Whereas for 15 and 18 wt% solutions spun at 30 and 25%, respectively, scaffolds comprised of highly nonuniform fibers, but with a significantly lower packing density compared to scaffolds spun at higher RH conditions. The optimal RH environment for producing scaffolds composed of smooth, uniform fibers and large pores was 35% for 15 wt% solutions (Figure 2.28B), and 30% RH for 18 wt% solutions (Figure 2.28E).

When the RH increases, the solvent evaporation rate decreases, while the conductivity of the working environment increases due to the polarization of water molecules in the air under the applied electric field²⁹⁵. This reduces the stretch on the fibers which can cause an increase in fiber diameter. At the same time, the charge separation of the

water molecules in the medium can induce high-conductivity paths across the working distance, resulting in the splitting of solution to form secondary jets that can manifest as thin web-like structures, and thereby result in a reduction in fiber diameter. The combined effect of these phenomena ultimately results in scaffolds composed of highly nonuniform fibers and a reduction in pore size and porosity²⁹⁶. On the other hand, when the RH is low, the solvent can evaporate too quickly, leading to a rapid increase in polymer viscosity which inhibits the voltage-induced stretching of the polymer chains, causing an increase in fiber diameter. This effect can be often accompanied by excessive clogging at the needle tip, which leads to the formation of thin web-like structures, as demonstrated by scaffolds in Figure 2.28(A and D).

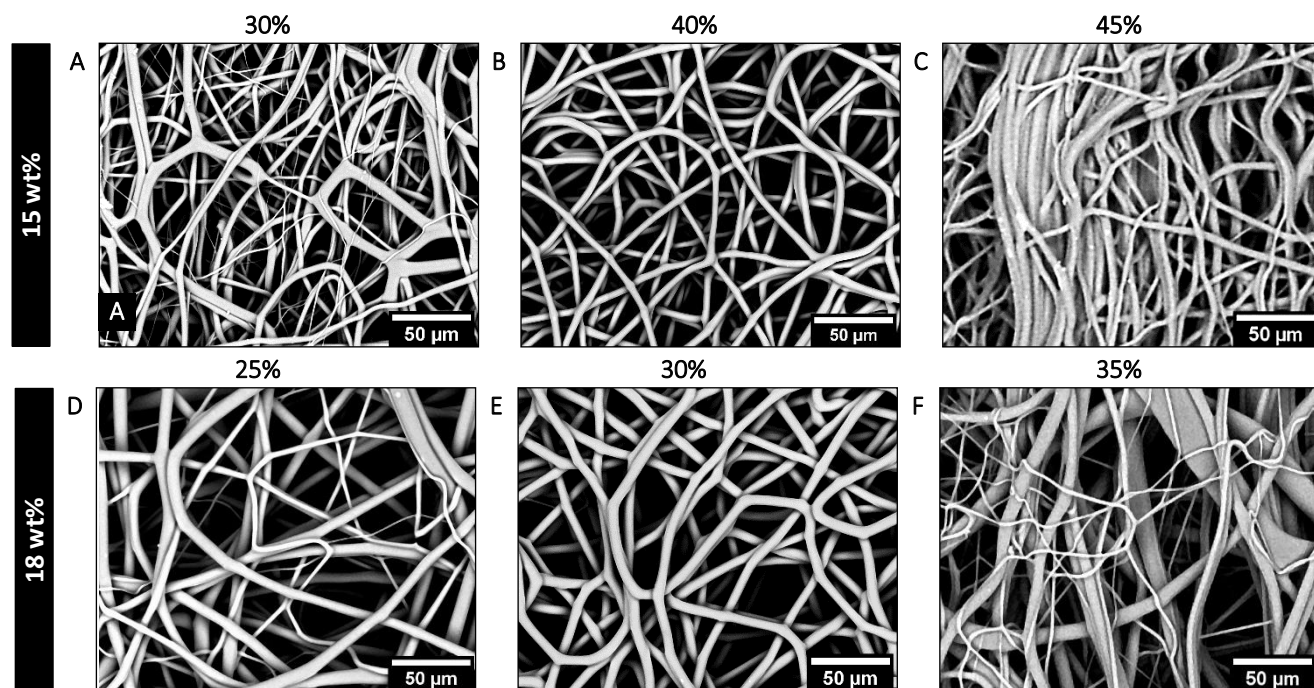


Figure 2.28: Influence of relative humidity on electrospun DP scaffolds. (A) 30, (B) 35–40, and (C) 45% RH for 15 wt% solutions (top), and (D) 25, (E) 30, and (F) 35% RH for 18 wt% solutions.

The average microstructural properties quantified for the respective scaffold groups are provided Table B, and depicted graphically in Figure 2.29. For 15 wt% DP, decreasing the RH from 45 to 40% resulted in a near twofold decrease in fiber diameter, from 5.4 ± 1.1 to $3.5 \pm 0.5 \mu\text{m}$ ($P < .0001$), while decreasing the RH beyond 40% led to an increase in fiber diameter, where at 25% fibers averaged at $5.5 \pm 0.7 \mu\text{m}$ ($P < .0001$). Similarly, for 18wt% solutions, decreasing the RH from 35 to 30% yielded fibers 1.5 times smaller (9.5 ± 0.5 vs. $6.2 \pm 0.8 \mu\text{m}$, $P < .0001$), while a further decrease in RH to 25% led to 20% increase in average diameter ($P = 0.004$).

As expected, the effect of RH on scaffold porosity closely corresponded to its effect on pore size, as shown in Figure 2.29, where the average pore size and porosity follow similar trends as a function of RH. 15 wt% solutions spun at 35 and 40% RH comprised of significantly larger pores of up to two times greater than scaffolds spun at 30 and 45%, and demonstrated porosities over 10% higher, respectively ($P < .0001$). For 18 wt% solutions, no significant

difference in the pore size and porosity was found between scaffolds spun at 25 and 30%, whereas scaffolds spun at 35% RH demonstrated significantly lower pore sizes and porosities of up to 1.5 and 1.2 times smaller than those spun at 30% RH, respectively. From these results, it could be concluded that the optimal environment for enhancing fiber uniformity, minimising fiber diameter and maximising pore size and porosity was 40% RH for 15 wt% solutions, and 30% RH for 18 wt% solutions.

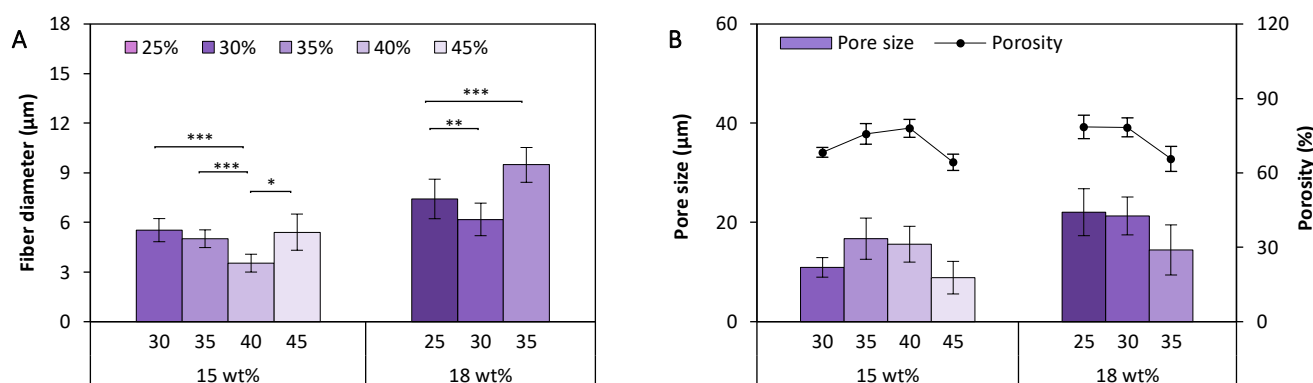


Figure 2.29: Effect of relative humidity on DP scaffold microstructure. (A) fiber diameter, (B) pores size and porosity for 15 and 18 wt% DP solutions. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Regarding the effect of solution concentration on scaffold microstructure, it was found that for the same flow rate and rotational speed, the average fiber diameter of scaffolds spun from 15 wt% solutions was half the size of those spun from 18 wt% at their respective optimal RH conditions (3.5 ± 0.5 vs. 6.2 ± 1.0 μm, $P < .0001$). Likewise, the average pore size of scaffolds from the lower concentration group was approximately 1.4 times smaller than the higher concentration group (15.6 ± 3.6 vs. 21.3 ± 3.8 μm, $P = 0.001$). Interestingly, despite being composed of significantly smaller pores, no discernible difference in scaffold porosity was found between the two solution groups (75.7 ± 1.5 vs. $78.3 \pm 1.6\%$, $P = 0.08$), which could be attributed to the influence of fiber packing density on the resultant porosity, or their respective ratios of pore size to fiber diameter, which was slightly higher for the lower concentration group (4.4 vs 3.4, respectively).

2.3.1.2 Effect of flow rate and rotational speed on fiber diameter

As shown in Figure 2.30, increasing the flow rate led to a clear increase in fiber diameter at each rotational speed, for both PEL and DP scaffolds. At 500 rpm, increasing the flow rate from 3 to 6 ml/hr resulted in a twofold increase in the mean diameter of PEL scaffolds (1.6 ± 0.3 to 3.0 ± 0.7 μm, $P < .0001$), and between flow rates of 1 and 3 ml/hr, the mean diameter of DP fibers increased by over 50% (4.9 ± 0.3 to 7.5 ± 0.4 μm, $P < .0001$). For both polymer groups, the influence of flow rate on fiber diameter diminished with increasing collecting speed. This was evaluated by performing linear regression analyses on the relationship between flow rate and fiber diameter for each collecting speed, where the slopes and strength of association decreased with increasing speed on both accounts, as shown in Figure 2.30(A and B). Averaged across all speed groups, an increase in flow rate from 3 to 6 ml/hr

provoked a linear increase in fiber diameter from 1.5 ± 0.3 to $2.5 \pm 0.6 \mu\text{m}$ ($R^2 = 0.61$, $P < .0001$), and for the DP scaffolds, increasing the flow rate between 1 and 4 ml/hr led to a linear increase from 4.4 ± 0.7 to $6.9 \pm 0.4 \mu\text{m}$ ($R^2 = 0.72$, $P < .0001$). The effect of flow rate on DP fiber diameter was slightly stronger than for the PEL scaffolds. Averaged across all flow rate groups, it can be seen that an increase in rotational speed led to a slight decrease in fiber diameter for both groups, however in both case the effect was fairly weak. On average, DP fibers were approximately three times larger than PEL fibers at each flow rate and collecting speed, respectively.

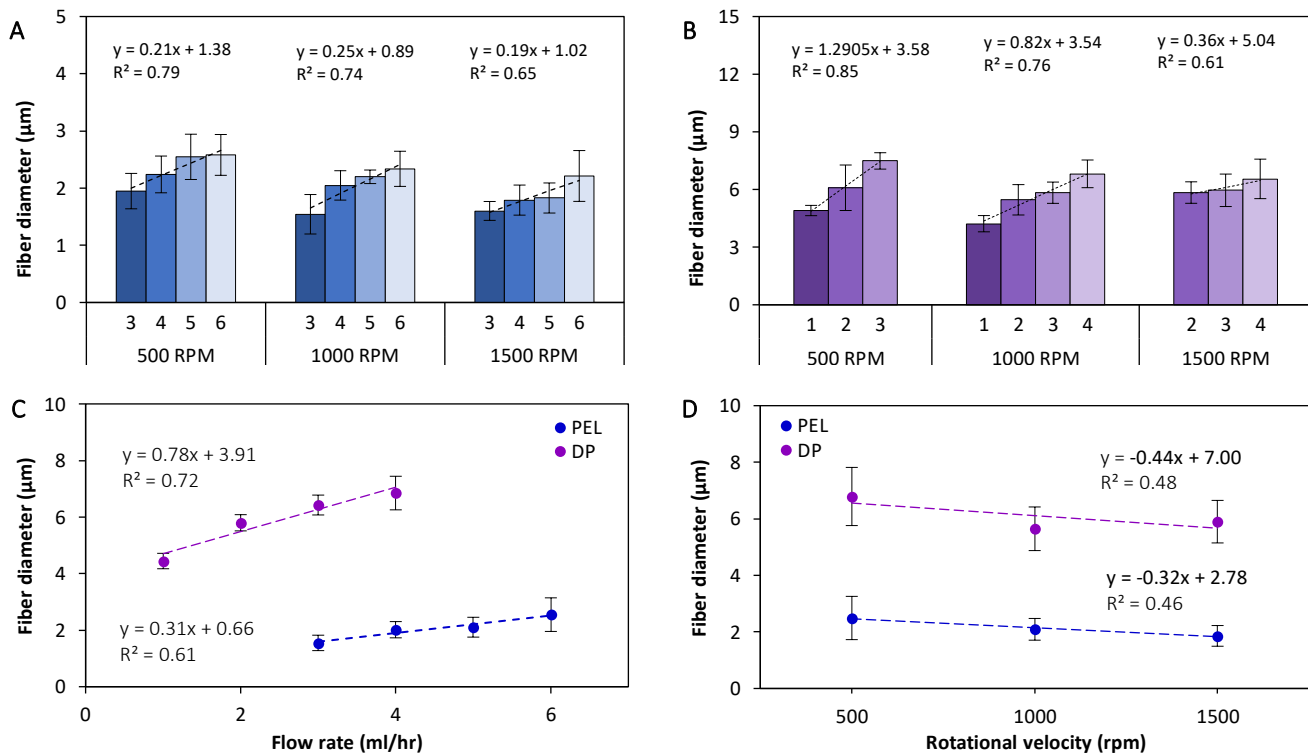


Figure 2.30: Influence of rotational speed and flow rate on PEL and DP fiber diameter.

2.3.1.3 Effect of flow rate and rotational speed on pore size and porosity

The effect of flow rate on pore size at respective rotational speeds is shown in Figure 2.31, and SEM images showing the change in pore size with increasing flow rate at a fixed rotational speed of 1000 rpm for PEL and DP scaffolds are presented in Figure 2.32. Interestingly, it can be seen that for the PEL scaffolds, an increase in flow rate provoked a decrease in pore size at each rotational speed, whereas for the DP scaffolds, an increase in flow rate led to an increase in pore size, respectively. The relationship between flow rate and porosity corresponded strongly with the relationship between flow rate and pore size for both PEL and DP scaffolds, with the exception of DP scaffolds spun at 1500 rpm, which showed no notable trend between flow rate and porosity. Overall, for PEL scaffold production, the smallest average pore size and porosity resulted from applying a flow rate of 6 ml/hr and collecting speed of 500 rpm, while the largest pore size and porosity was achieved by applying a flow rate of 3 ml/hr at 1500 rpm (pore size: 6.6 ± 0.5 vs. $9.8 \pm 1.2 \mu\text{m}$, $P < .0001$; and porosity: 64.2 ± 2.5 vs. $82.2 \pm 2.9\%$, $P = 0.001$). For DP scaffold

production, the smallest average pore size and porosity was associated with flow rates between 1 and 2 ml/hr with a collecting speed of 500 rpm, whereas the largest average pore size and porosity was achieved with a flow rate of 3 ml/hr at 1000 rpm (pore size: 14.7 ± 2.5 vs. 23.2 ± 3.3 μm , $P < .0001$; and porosity: 64.3 ± 4.4 vs. $83.1 \pm 3.6\%$).

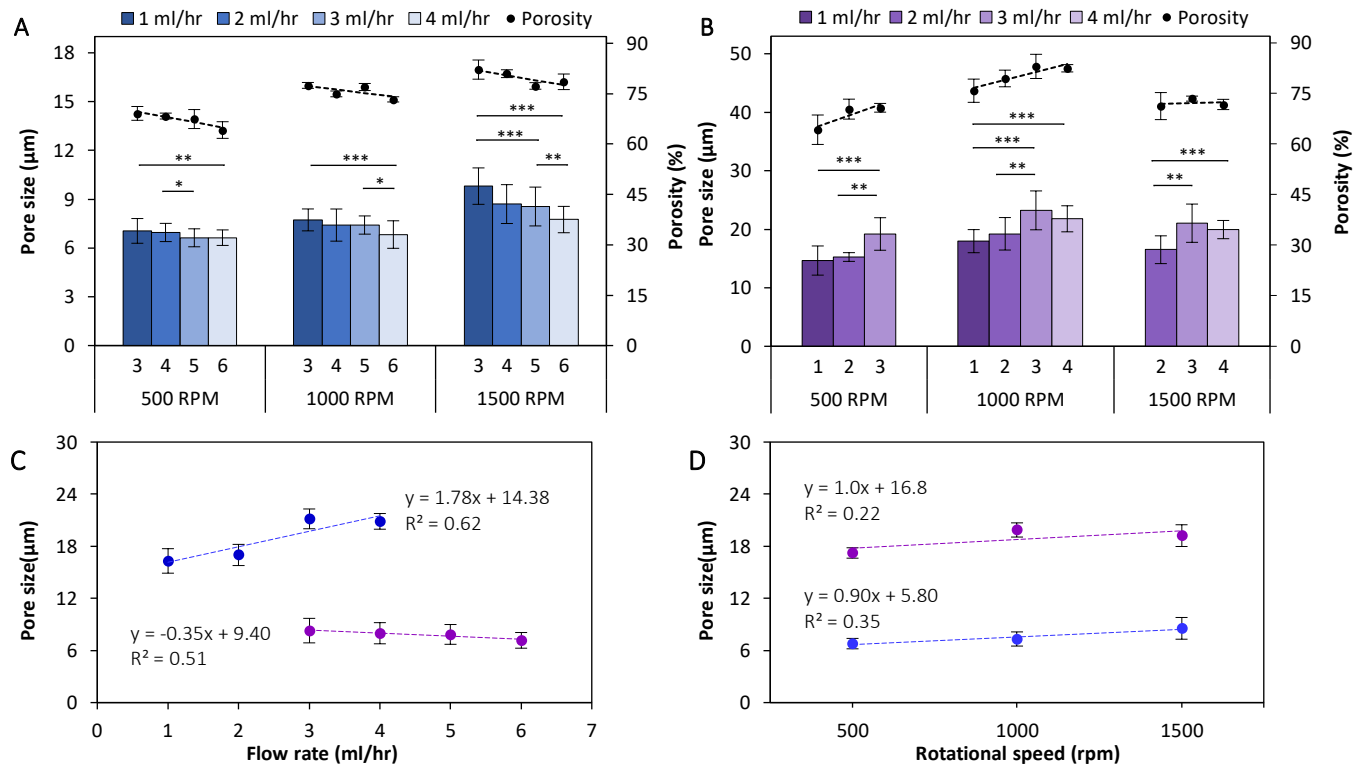


Figure 2.31: Effect of flow rate and rotational speed on scaffold pore size and porosity. Mean effect on A) PEL and B) DP scaffolds. Average effect on C) PEL and D) DP scaffolds. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

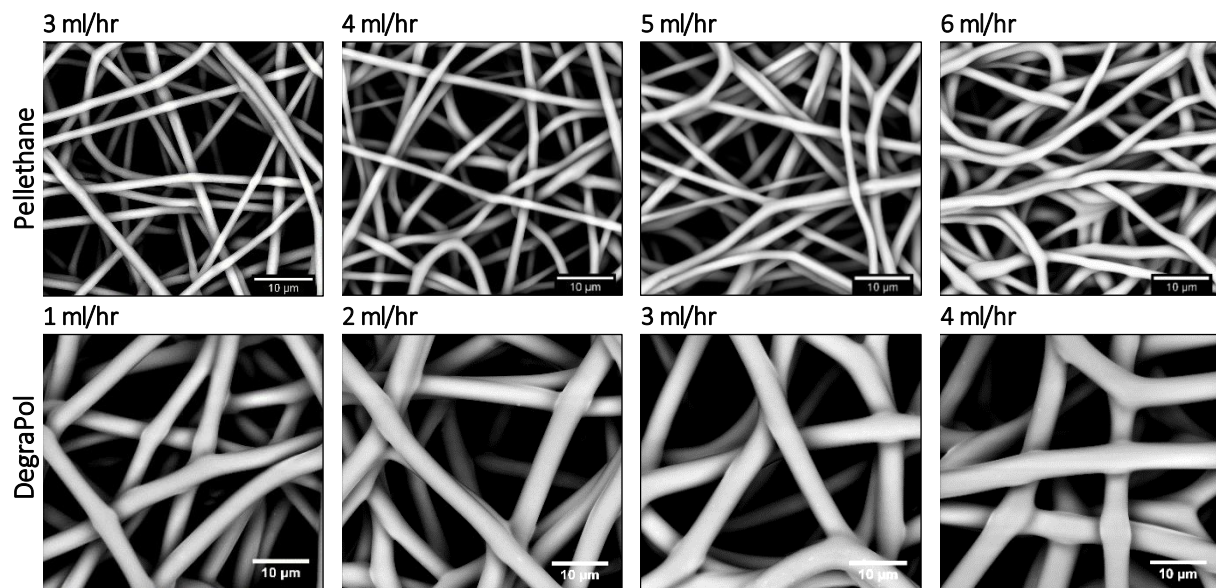


Figure 2.32: Influence of rotational speed on porosity. SEM images of scaffold samples electrospun at different flow rates.

The relationship between flow rate and pore size for samples averaged across all collecting speeds is shown in Figure 2.31. The figure highlights the distinct difference in correlation between pore size and flow rate demonstrated between the PEL and DP scaffolds, where a negative linear relationship with moderate association was determined for the PEL scaffolds ($m = -0.35 \text{ ml/hr}/\mu\text{m}$, $R^2 = 0.51$, $P < .0001$), while a strong positive linear relationship was determined for the DP scaffolds ($m = 1.78 \text{ ml/hr}/\mu\text{m}$, $R^2 = 0.72$, $P < .0001$). Averaged across all flow rates, average pore sizes demonstrated a slight increase with increasing collector speed for both polymer groups, but in both cases the association was fairly weak ($R^2 = 0.35$, $P = 0.0004$ and $R^2 = 0.22$, $P = 0.003$).

2.3.1.4 Effect of rotational speed on fiber orientation

Increasing the rotational speed led to an overall increase in fiber alignment in the circumferential direction for both scaffold groups, where between 500 and 1500 rpm the OI demonstrated a fourfold increase for the PEL scaffolds (0.09 ± 0.02 to 0.36 ± 0.07 , $P < .0001$) and 3.6 factor increase for the DP scaffolds (10.4 ± 6.0 to $38.2 \pm 5.3\%$, $P < .0001$) (Figure 2.33(A and C)). The relationship between collector speed and orientation was also depicted by plotting the distribution of orientation histograms, as presented in Figure 2.33(B and D). The narrow peak observed for 1500 rpm indicates a high degree of alignment, while the broader peaks for 1000 and 500 rpm indicate random alignment.

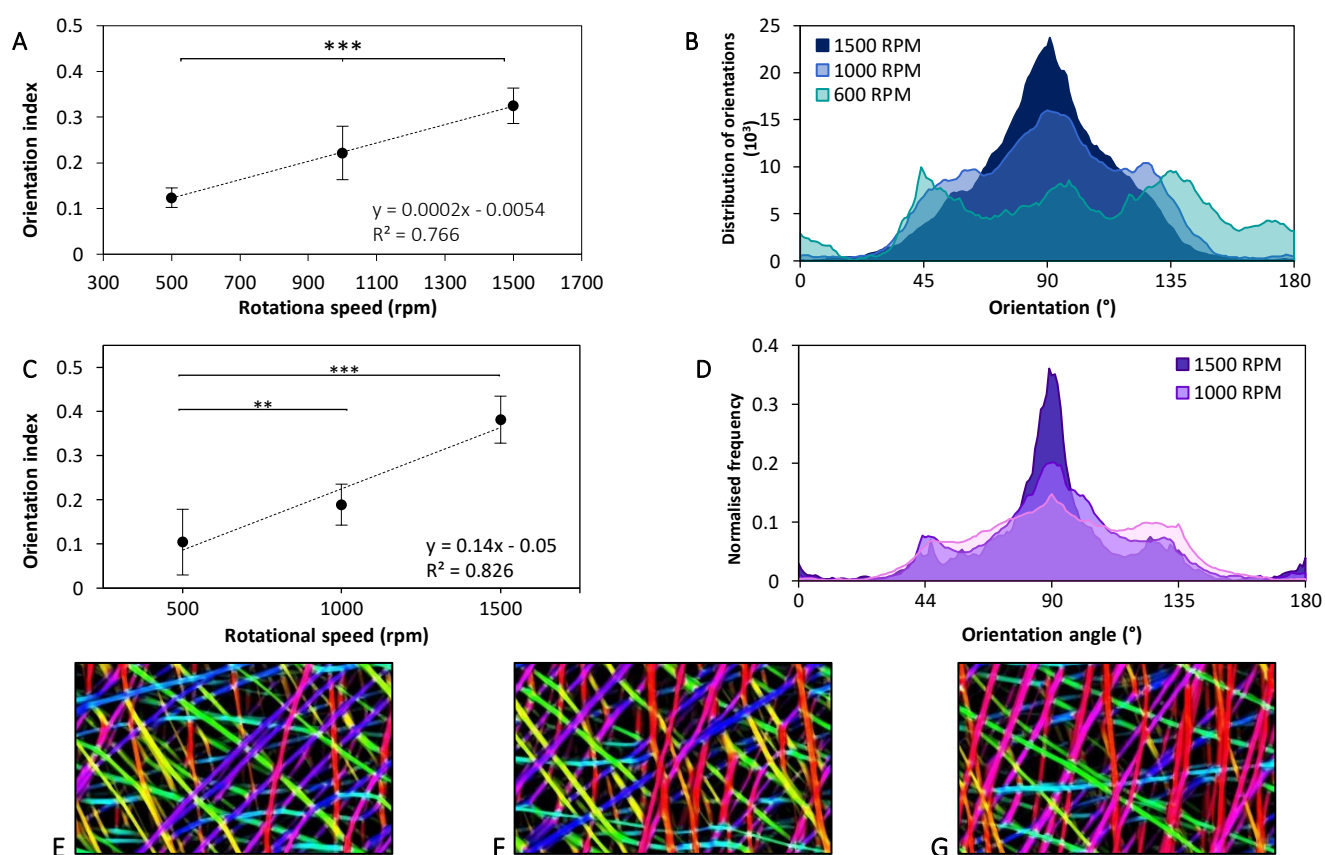


Figure 2.33: Influence of rotational speed on scaffold fiber alignment. Mean orientation index plotted as a function of rotational speed for PEL (A) and DP (C) scaffolds. Distribution of orientations plotted for PEL (B) and DP (D) scaffolds spun at (E) 500, 1000 and 1500 rpm. Examples of orientation visualisations performed for PEL scaffolds spun at (E) 500, (F) 1000, and (G) 1500 rpm.

Orientation visualisations, shown in Figure 2.33C, were performed for visual analysis, which demonstrated accordance with the described relationship. In general, scaffolds spun at 1500 rpm were dominated by red, pink, and purple fibers, indicating a high degree of alignment, whereas scaffolds spun at 500 rpm featured more blue, green, and yellow fibers, indicating random orientation.

2.3.2 Mechanical properties

The mechanical properties of PEL and DP scaffolds were evaluated for samples spun at flow rates of 3 to 6 ml/hr at collecting speeds of 500, 1000, and 1500 rpm. Representative stress strain curves obtained at each speed are presented in Figure 2.34 and average stress-strain curves for each experimental group are provided in Figure B1.

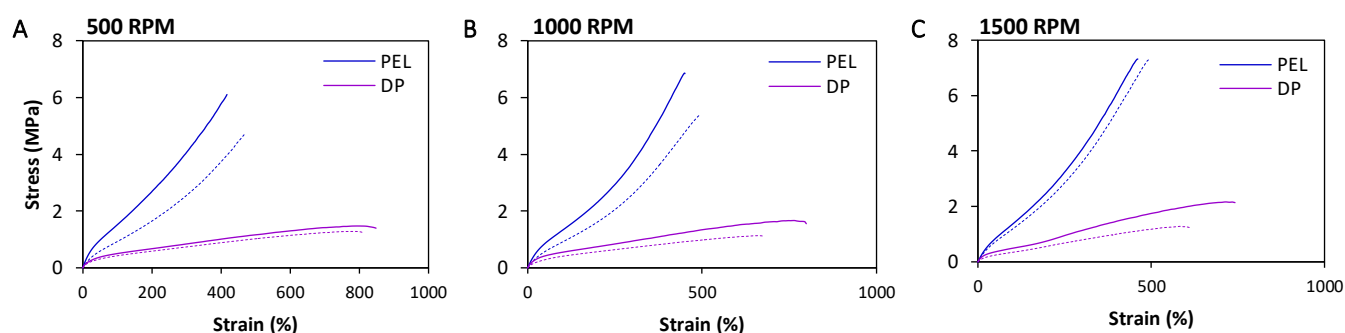


Figure 2.34: Average stress strain curves for PEL and DP scaffolds spun at A) 500, B) 1000, and C) 1500 rpm, tested in the circumferential (solid lines) and longitudinal (dashed lines).

For both polymers, increasing the rotational speed led to a clear increase in ultimate strength, stiffness, and toughness in the circumferential direction. This could be attributed to the fact that high rotational speeds lead to the enhanced stretching of fibers²⁹⁷, giving rise to higher fiber alignment in the circumferential direction, as was demonstrated in section 2.3.1.4. Thus, with increased alignment along the loading direction, the tensile properties of the scaffold are likely to improve²⁹⁸. In particular, increasing the collecting speed from 500 to 1500 rpm led to a 40% increase in UTS (4.9 ± 0.3 to 6.9 ± 0.6 MPa, $P < .0001$), 25% increase in Young's modulus (1.4 ± 0.1 to 1.8 ± 0.1 , $P = 0.001$), and 30% increase in toughness ($P < .0001$) for the PEL scaffolds. For the DP scaffolds, the overall effect of the collecting speed on UTS was slightly weaker, where between 500 and 1500 rpm, the DP scaffold UTS increased by only 25% (1.4 ± 0.2 to 1.8 ± 0.3 MPa, $P = 0.003$). On the other hand, DP scaffold toughness was strongly affected by collecting speed, where scaffolds spun at 1500 rpm obtained an average toughness approximately 40% greater than those spun at 500 rpm ($P < .0001$). While the collecting speed had no clear effect on maximum elongation for the PEL scaffolds ($P > 0.05$), for the DP scaffolds, increasing the speed between 500 and 1500 rpm led to a significant increase in maximum elongation from 665 to 763% ($P < .0001$). In general, the difference in mechanical response between scaffolds spun at 1000 and 1500 rpm was not significant for both polymers.

Regarding the relationship between collecting speed and mechanical anisotropy, an interesting phenomenon was observed from the PEL scaffolds. While both PEL and DP scaffolds demonstrated an increase in strain anisotropy

with an increase in collector speed (Figure 2.35E), the strength, stiffness, and toughness anisotropy of the PEL scaffolds decreased significantly with an increase in speed, illustrated by the stress-strain curves in Figure 2.34, and indicated in Figure 2.35(D, F, and H) by the increase in respective anisotropy ratios (AR) quantified for each group. This was highly unexpected since the scaffolds spun at higher speeds demonstrated greater degrees of fiber alignment, which is commonly associated with greater anisotropy^{299,300}. On other hand, DP scaffolds behaved predictably by increasing in anisotropy with increasing speed across all mechanical aspects, as shown Figure 2.35.

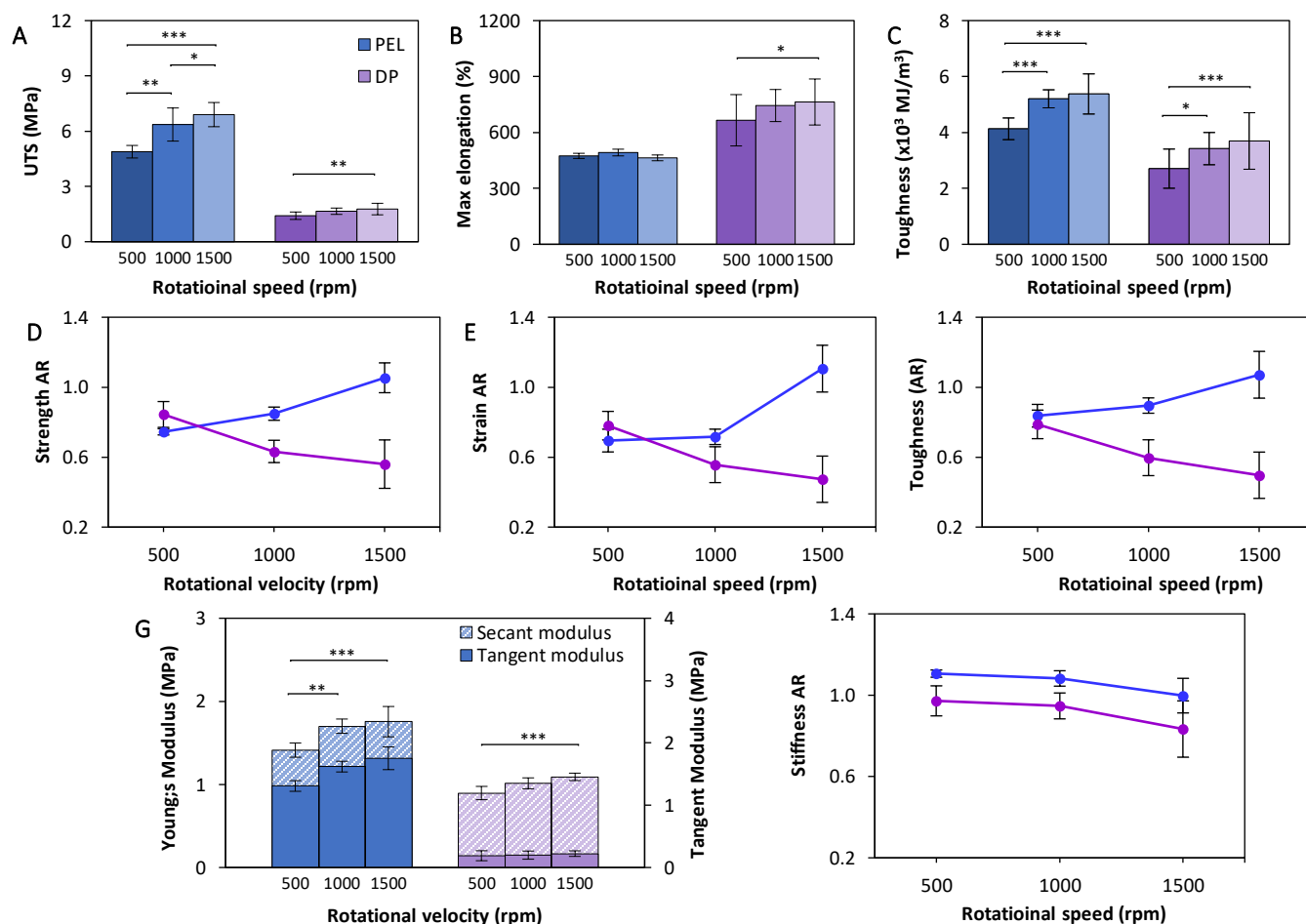


Figure 2.35: Influence of collecting speed on mechanical properties of PEL and DP scaffolds. A) Ultimate tensile strength, B) percent elongation, C) toughness, D) strength AR, E) strain AR, F) toughness AR, G) Young's modulus and tangent modulus, and H) stiffness AR. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Averaged across all speed levels, the PEL scaffolds demonstrated a mean UTS nearly four times greater than the DP scaffolds (6.1 ± 1.6 vs. 1.6 ± 0.4 , $P < .0001$). While the mean Young's modulus for the PEL scaffolds was only 1.6 times higher than the DP scaffolds (1.6 ± 0.2 vs. 1.0 ± 0.2 MPa, $P < .0001$), the corresponding tangent modulus was over seven times higher (1.6 ± 0.2 vs. 0.2 ± 0.1 MPa, $P < .0001$). Finally, DP scaffolds achieved maximum elongations almost 250% more than the PEL scaffolds (724.5 ± 204.0 vs. $476.9 \pm 27.4\%$, $P < .0001$). To conclude, these results highlight the distinct difference in mechanical behaviour and loading capacity between the two polymer groups, but that the mechanical properties of the scaffolds can be enhanced by increasing the rotational speed.

2.3.3 Viscoelastic properties

Valvular leaflet tissues are known to be highly nonlinear, strongly anisotropic, and present viscoelastic behaviour, such as strain rate dependence and hysteresis^{76,129,176,282}. The viscoelastic aspect of valve leaflets can be attributed to its free arrangement of collagen and elastin fibers which are saturated with fluids and can deform in all directions³⁰¹. It is generally considered that the solid component contributes the elastic response to loading and the fluid component contributes the viscous response, such that the overall response is viscoelastic. However, it has been shown that after a certain number of preconditioning cycles, the response of native leaflets reach a steady state, at which point the loading and unloading phases adopt distinct nonlinear responses that are insensitive to loading rate³⁰². In this way, it can be said that the viscous effect disappears after a certain amount of preloading, whereafter they can be characterised as hyperelastic materials in loading, and separate nonlinear materials in unloading³⁰³. This behaviour is typically known as pseudoelasticity, and is considered an integral aspect of the functionality of heart valves leaflets, making it an important characteristic to replicate in scaffolds for HVTE^{301,302}.

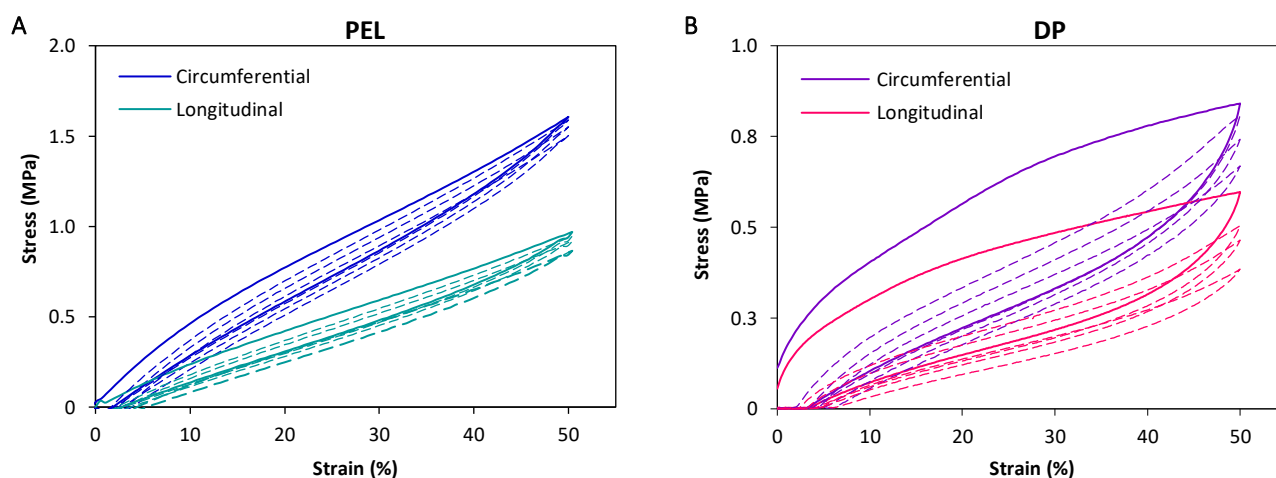


Figure 2.36: Average stress-strain curves obtained from cyclic tensile tests performed on (A) PEL and (B) DP scaffolds in the circumferential and longitudinal direction. Only the 1st, 2nd, 15th, and 50th cycles are presented to illustrate the evolution of hysteresis and variation in stress response to cyclic loading over successive cycles.

Average stress-strain curves obtained from the cyclic tensile tests performed on PEL and DP scaffolds with similar microstructures are shown in Figure 2.36. From the figure, it can be seen that the viscoelastic behaviour of the scaffolds manifested as hysteresis loops which appeared to shift down and become increasingly narrow as the number of cycles increased. Furthermore, both groups exhibited viscoelastic anisotropy where the scaffolds demonstrated higher peak stresses and stiffness in the circumferential direction, while exhibiting lower degrees of hysteresis in the longitudinal direction.

Compared to the PEL scaffolds, the DP scaffolds clearly experienced a larger degree of stress softening, illustrated in Figure 2.36 by the significant stress drop between the first and second loading cycles, after which the variation

in cyclic stress response gradually diminished. Stress softening is a typical mechanical response of rubber-like elastomers, which is referred to as the Mullins effect³⁰⁴, or in the case of soft tissues, is known as the preconditioning effect³⁰⁵. On both accounts, the phenomenon is largely attributed to a change in internal structure, such as changes in the hard and soft domain microstructure of the polymer due to internal sliding of macromolecular chains; or the disruption in matrix configuration due to the relative motion of connected fibers and subsequent damage of bonded junctions³⁰⁶⁻³⁰⁹. Thus, the high degree of stress softening exhibited by the DP scaffolds was likely the culmination of a dramatic, permanent change in the molecular structure and morphology of the scaffold fibers. On the other hand, the PEL scaffolds demonstrated a remarkably low degree of stress softening, shown by the slight drop in stress between the first and second cycles, early stabilisation of the variation in cyclic stress, and consistently narrow hysteresis loops. This indicates that the internal friction of the PEL scaffolds was significantly lower than the DP scaffolds, resulting in a lower loss in mechanical energy during cyclic deformation. Furthermore, the PEL scaffolds reached peak stresses and elastic moduli nearly two times higher than the DP scaffolds in both directions. To this end, the PEL scaffolds clearly possessed inherently superior molecular and structural recoverability over the DP scaffolds, as well as greater strength and stiffness.

Further investigation into the viscoelastic properties was conducted on PEL and DP scaffolds electrospun at 500, 1000, and 1500 rpm, respectively. The purpose of this study was to establish the optimal working parameter for minimising the degree of stress softening and hysteresis, while maximising stiffness and strength. Figure 2.37A shows the average stress-strain curves obtained from the 50th loading cycle performed by each scaffold group, and Table 2.9 summarises the viscoelastic properties obtained from the stress-strain curves.

In general, scaffolds spun at higher speeds were mechanically stiffer, yielded greater peak stresses, and were more anisotropic compared to those spun at lower speeds. For the PEL scaffolds, an increase in rotational speed from 500 to 1500 rpm led to a 1.3 factor increase in peak stress response, from 1.2 ± 0.3 to 1.6 ± 0.2 MPa ($P=0.02$), and a 1.4 factor increase in elastic modulus from 1.6 ± 0.5 to 2.2 ± 0.3 MPa ($P=0.04$). Regarding the DP scaffolds, the effect of rotational speed on the viscoelastic response was more notable. On average, scaffolds spun at 1500 rpm demonstrated a mean peak stress over 75% higher than those spun at 500 rpm (0.4 ± 0.1 to 0.7 ± 0.2 MPa, $P=.0001$), and the mean elastic modulus increased by over 65% (0.8 ± 0.2 to 1.3 ± 0.2 MPa, $P<.0001$).

For both PEL and DP scaffolds, the collecting speed had no clear effect on their resultant hysteresis ratios. On average, DP scaffolds demonstrated ratios approximately two times greater than PEL scaffolds (19.1 ± 2.2 vs $10.2 \pm 2.1\%$, $P<.0001$), and were significantly different across each speed level, as illustrated in Figure 2.37C.

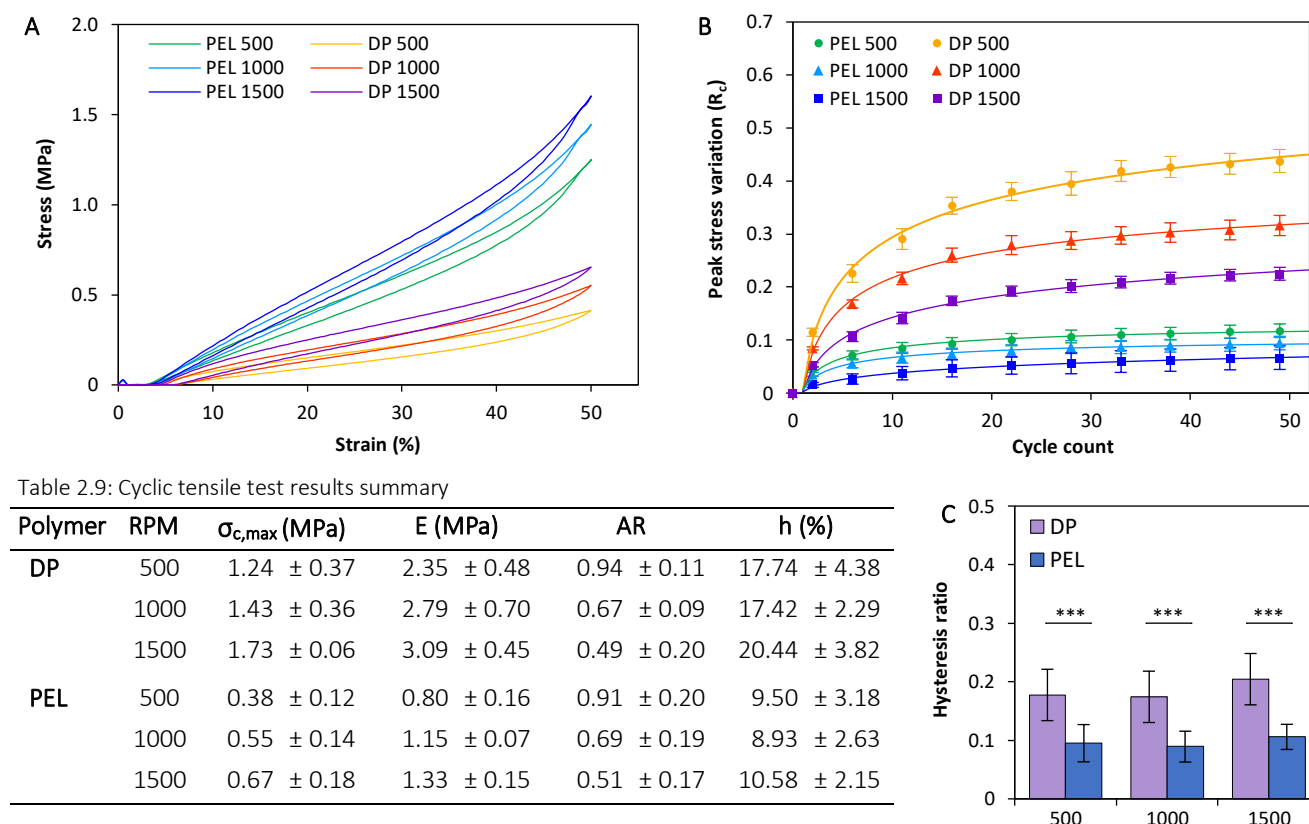


Figure 2.37: Cyclic tensile test results. A) Hysteresis loops obtained for PEL and DP scaffolds spun at 500, 1000, and 1500 rpm. Here only the 50th cycle is presented. B) Normalised variation in peak stress (R_c) of the six scaffold groups as a function of cycle number up to 120 cycles (R_c is presented for every 13th cycle). For each group, the relationship between R_c and cycle number was fit with a logarithmic curve. *** $p < 0.0001$.

The stress softening behaviour demonstrated by the scaffold groups was evaluated by plotting the variation in peak stress (R_c) as a function of cycle number, for which good fits were found for each plot with natural logarithmic regression, as shown in Figure 2.37B. The figure clearly demonstrates the different stress softening behaviours between the respective polymers. The PEL scaffolds curves are closely clustered and reach a relatively stable plateau after roughly 30 loading cycles. On average, the R_c increased by 7–8% for the PEL scaffolds, implying that over 90% of the scaffolds resistance to stretch was preserved. In contrast, the curves for the DP scaffolds were widely dispersed, suggesting the degree of stress softening had a stronger association with fiber alignment. Scaffolds spun at 500 rpm demonstrated the fastest rate of stress softening, where after 50 cycles the R_c increased by over 40%, which can be interpreted as a 40% decrease in the scaffolds resistance to deformation. Scaffolds spun at 1000 and 1500 rpm demonstrated relatively lower degrees on stress softening, where the R_c decreased by 25 and 30%, respectively. Furthermore, DP scaffold curves maintained a positive gradient after 50 cycles, indicating that the specimens were still undergoing cyclic softening. Cyclic softening is a mechanistic aspect described as the decrease of resistance to deformation during cyclic loading, which accumulates as permanent material damage³¹⁰.

2.3.4 Results summary

- Relative humidity played an influential role in DP scaffold production where electrospinning DP fibers was achievable within a relatively narrow range of RH parameters. For 15 w% DP solutions, a relative humidity of 40% was optimal and for 18 wt% DP solutions, the optimal RH was 30%.
- On average, fiber diameters generated from 18 wt% DP solutions were approximately 60% larger than those of 15 wt%, and corresponding pore sizes were 1.5 times larger.
- Fiber diameter demonstrated a positive correlation with flow rate and a negative correlation with collecting speed for both PEL and DP solutions.
- On average, PEL fiber diameters were approximately three times smaller than DP fibers.
- The average pore size of DP scaffolds correlated positively with flow rate, whereas PEL scaffolds demonstrated no clear trend between pore size and flow rate.
- On average, DP scaffold pores were two times larger than those in PEL scaffolds.
- Scaffold porosities among all scaffold groups were essentially comparable and averaged at $75 \pm 5\%$.
- A positive correlation between fiber orientation and collecting speed was found for both types of scaffolds.
- PEL scaffolds were approximately three times stronger than scaffolds spun with 15 wt% DP solutions, and six times stronger than scaffolds spun with 18 wt% DP solutions.
- PEL scaffolds were approximately two times stiffer than DP scaffolds, while DP scaffolds were over two times more extensible.

3 Valve Manufacturing Design

This chapter describes the process undertaken to design and evaluate various methods for manufacturing a trileaflet heart valve substitute. Figure 3.1 illustrates the engineering design cycle pursued for this design study, which involved defining the design scope and identifying pertinent design requirements; generating concepts; concept prototyping; prototype evaluation; and final concept selection. The concept refinement and testing phases are described in Chapter 4 to follow.

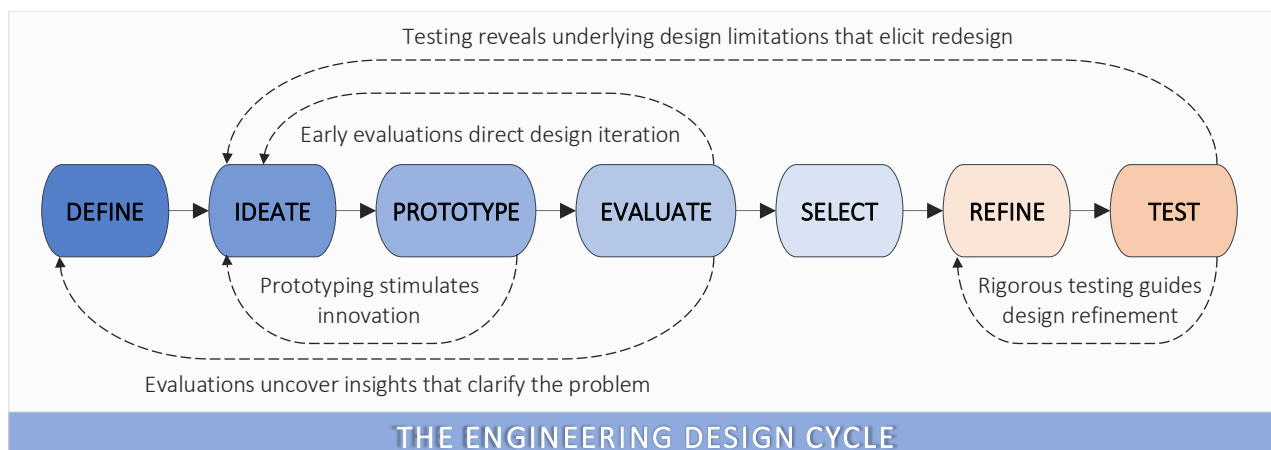


Figure 3.1: Schematic diagram of the engineering design cycle.

3.1 Problem analysis

A fundamental aspect of transcatheter heart valve (THV) development, on par with material selection, is the design of the manufacturing procedure. In a broad sense, this involves devising a system of operations whereby raw materials (operational inputs) are efficiently and effectively converted into functional deliverables (operational outputs). Ultimately, the individual processes that form part of the manufacturing procedure as whole, should interact cohesively to yield outputs that meet the physical, functional, and safety requirements specified for the device. Thus, the focus of this study was to devise an effective procedure for manufacturing a trileaflet heart valve substitute from electrospun polymer scaffolds and an expandable stent.

3.1.1 Tissue engineered heart valve system analysis

In general, THVs are composed of 3 primary components, including a stent, a paravalvular sealing skirt, and three flexible leaflets, as depicted in Figure 3.2. In order to produce a tissue engineered transcatheter heart valve (TETHV), additional aspects need to be considered, such as the incorporation of biological components and tissue processing strategies. Over the years, it has been widely established that each aspect pertinent to HVTE is rooted in an elaborate network of interacting microprocesses, that since the inception of the field remain largely misunderstood and difficult to define^{108,131,311}. Moreover, developing TEHVs that facilitate percutaneous implantation presents even greater challenges, as additional operational capabilities and more

demanding functional requirements need to be met³¹¹. Thus, the major challenge in manufacturing an electrospun THV lies in establishing a technique to join electrospun materials to a metal stent in a way that preserves the structural and mechanical integrity of the scaffolds, facilitates the configuration of the trileaflet structure, and generates functionally robust constructs by providing strong connections between the individual valve components. These three design challenges form the foundation of this investigation.

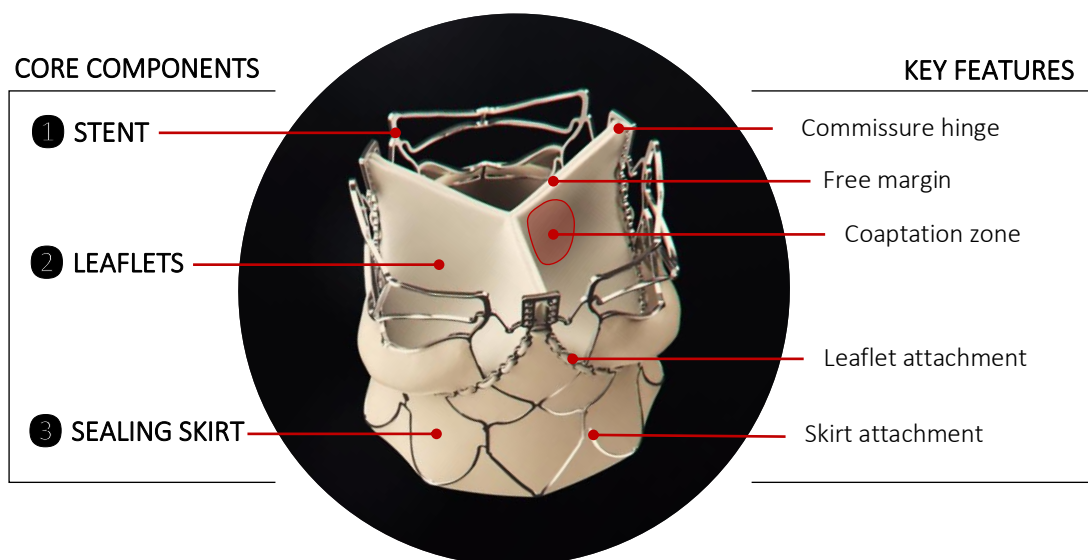


Figure 3.2: Core components of a transcatheter TEHV. The stent, leaflets, and sealing skirt form the core components of a transcatheter heart valve, each of which comprise of various key design features.

To gain a more holistic perspective of the TEHV development landscape and thereby broaden contextual insight to facilitate the ideation phase, an entity relationship diagram (ERD) was modelled (presented in Figure 3.3). The diagram illustrates the relations and interactions between all the system components, processes, parameters, and high-level system actors throughout the TEHV development cycle. To summarise, the physical components were identified as the stent, leaflets, sealing skirt, biological factors, and transcatheter delivery system. The primary operating procedures were identified as stent production, scaffold fabrication, valve manufacturing, delivery device production, tissue processing, and device implantation. The chief system actors were established as the device development team, manufacturers, patients, surgeons, stake holders and regulatory bodies.

As has been emphasised in recent reviews by leading researchers in the field, such as Fioretta *et al.*³¹² and Mirani *et al.*¹⁰⁸, and was made clear from the ERD, in considering the vast multitude of interrelated factors that need to be characterised, standardised, and verified long-term to meet all the requirements proposed for TEHV application, adopting a collaborative, interdisciplinary approach is essential to succeeding in moving HVTE into the clinic.

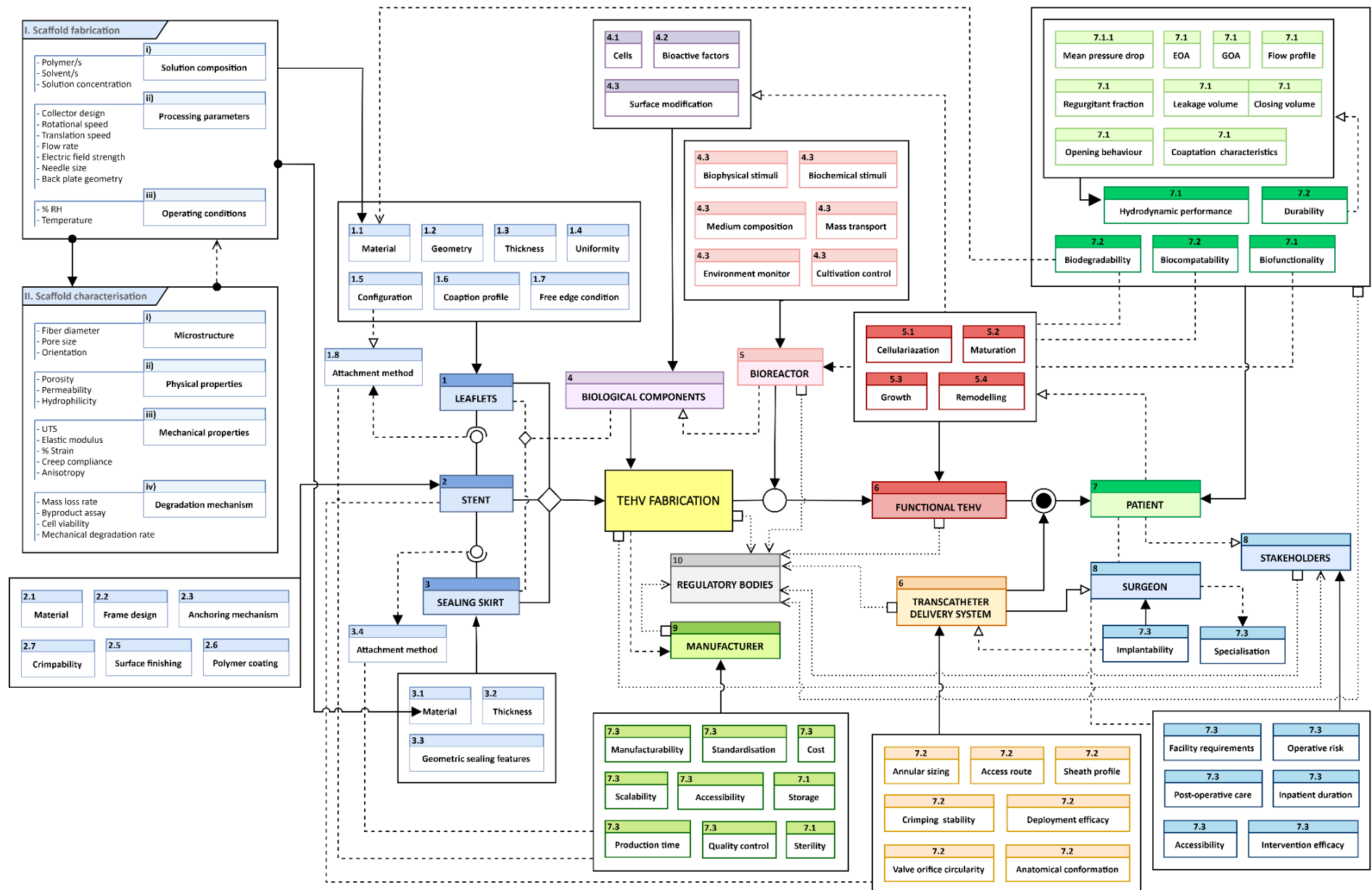


Figure 3.3: TEHV systems analysis diagram. Entity relationship diagram illustrating network of interactions between systems, operations, components, parameters, users, and actors involved in the design, manufacturing, testing, and verification of an electrospun transcatheter tissue engineered heart valve.

3.1.2 Electrospinning systems for heart valve tissue engineering

Researchers to date have investigated a wide range of electrospinning systems to yield optimised scaffolds for the substitution of native valvular tissues. In line with scaffold optimisation, methodologies focused on optimising valve-shaping processes and stent anchoring techniques have been widely explored³¹³. Multiple research groups across the globe have succeeded in fabricating functional heart valve substitutes from electrospun scaffolds, many of which reached preclinical testing, while only a recent few have undergone clinical investigation³¹⁴. None so far have proven long-term mechanical stability or adequate biocompatibility to be introduced to the clinical setting⁷⁶. However, with rapid technological developments and recent innovations in electrospinning, numerous groups are producing constructs with increasing physical resemblance to native heart valves and edging gradually toward achieving the mechanical and biological outcomes needed to survive the biological demands of the cardiac cycle⁷⁶.

Central to the scope of this project is the design and development of a feasible technique to fabricate trileaflet valve constructs from electrospun material with transcatheter application and regenerative capacity. A critical phase of the design process involved researching all the relevant techniques that have been conceptualized and studied to date. For the purpose of this research, applied electrospinning methodologies for TEHV construction were organised into three principal categories including: (I) conventional electrospinning, (II) double-component deposition technique (DCD) and (III) automated rotary jet spinning. The practical application of each method differs widely among independent research groups and encompass technical schemes unique to each study. It was therefore necessary to supplement knowledge on the principal approaches, with comprehensive examination of studies featuring unique techniques.

In addition, a table was compiled that provides an overview of the materials, manufacturing methods, evaluation assessments, and main findings reported in the respective studies that investigated the application of electrospinning methods for TEHV production (Table 3.1). By this, a more representative range of theoretical insight was established prior to commencing with the concept design and development phases.

3.1.2.1 Conventional electrospinning

Conventional electrospinning refers to the generic setup of apparatus featuring either a stationary flat plate collector or rotating mandrel that translates relative to the spinneret. The design of the collector plays a significant role in establishing appropriate electrospinning parameters and assembly techniques for valve fabrication.

The use of cylindrical collectors to produce tubular scaffold structures for tissue engineering application is a well-established technique. Kluin *et al.*¹⁴⁹ achieved the fabrication of a trileaflet valve structure from a tubular scaffold by fixing the scaffold to a crown-like reinforcement ring featuring suture holes along the ring base and

commissure posts. The technique involved suturing the electrospun tube onto the inner circumference of the support ring, prior to folding the tube from the ring base such that the free edge lines up with the commissures. By wedging the free edge into the top of the slotted commissure posts, the desired leaflet shape and final structure was attained. The preclinical *in vivo* application of this valve reported sustained functionality and structural integrity over 12-month follow up period with no signs of thrombus formation. However, explant examinations revealed severe leaflet thickening and development of microvasculature at the leaflet tip, which is considered a potential risk (Figure 3.4). Nevertheless, from a manufacturing design perspective, considering the valves mostly remained functional and intact over the test period, this fabrication technique warranted employment for further investigative procedures¹⁴⁹.



Figure 3.4: Approach to fabricating a tissue engineered heart valve from an electrospun polymer tube. A) Valve manufacturing process. B) H&E analysis of leaflet explants showing variable interleaflet thickness (adapted from Kluin *et al.*¹⁴⁹)

Another technique applied by Fioretta *et al.*²⁰³ for a preclinical study, involved mounting an electrospun tube onto a nitinol stent with sutures distributed along the leaflet hinge line and at the commissures. The leaflets were subsequently moulded into a predefined shape by applying custom-made polyetheretherketone (PEEK) inserts with an optimised geometry for enhanced physiological performance (Figure 3.5). The scaffold-insert assembly was incubated at 37°C for 5 days to ensure the leaflets retain the imposed shape. *In vitro* validations showed adequate leaflet mobility, valve functionality and valve durability. However, *in vivo* tissue growth and remodelling of pre-seeded valves lead to fusion of adjacent leaflets at the commissures. Both pre-seeded and non-seeded groups showed substantial thickening at the leaflet base and hinge region, as well as intervalve and interleaflet variability in leaflet length, thickness, and gene expression. Severe regurgitation and early signs of calcification was also reported. Although unfavourable *in vivo* outcomes were demonstrated, the findings were attributed primarily to the adverse cellular response of pre-seeding with bone marrow mononuclear cells (BMMCs). One outcome to highlight for design consideration is the fusion of adjacent leaflets at the commissures which was suggested to have promoted leaflet curling and shortening (refer to Table 3.1 for additional details on these studies).

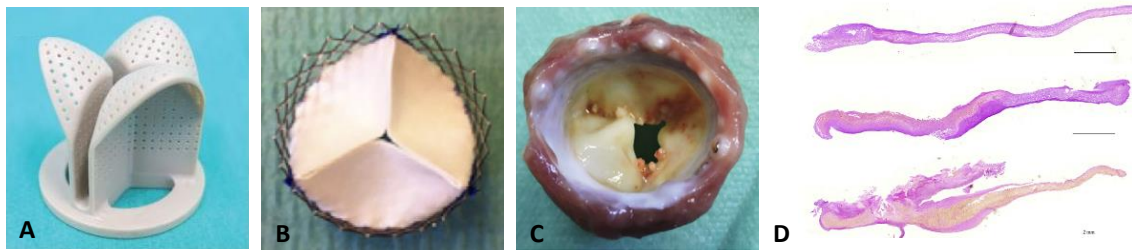


Figure 3.5: Tissue engineered electrospun THV. A) PEEK inserts used to shape leaflets. B) Test valve. C) TEHV explanted after 18 weeks. D) Histologic analysis of 3 leaflets explanted after 24 weeks (adapted from Sanders *et al.*¹⁰⁹ and Fioretta *et al.*²⁰³).

A simpler technique recently implemented whereby an electrospun scaffold was assembled into a trileaflet heart valve structure by: (i) cutting scaffold tube longitudinally to create a flat sheet, thereafter (ii) using a metal cutter to cut out a predefined trileaflet profile. (iii) The scaffold was then wrapped around a 3D printed stent and finally (iv) fixed to the stent using melted PCL (process depicted in Figure 3.6)³¹⁵. Valve performance was tested *in vitro* using a pulse duplicator system, with valves subjected to physiological pulmonary pressures for 10 hours. Valve functionality was reported near-comparable to commercial valves, and constructs showed structural rigidity and sturdiness. While this study proved preliminary effectiveness, long-term studies would need to be conducted to obtain more reliable outcomes and verify feasibility for clinical application³¹⁵.

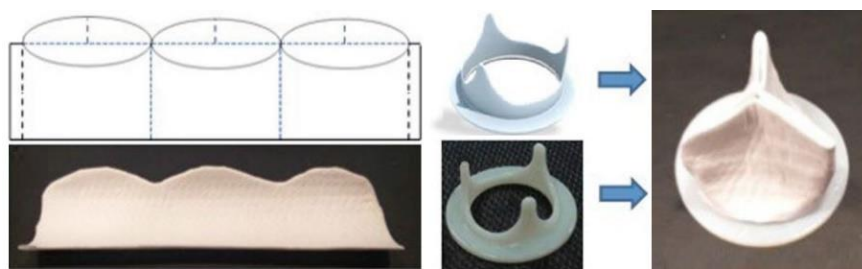


Figure 3.6: Cut-and-wrap method for TEHV fabrication. Adapted from Hasan *et al.*³¹⁵

3.1.2.2 Preform electrospinning

Preform electrospinning refers to the use of customized target collectors designed to produce electrospun scaffolds that upon removal from the mandrel retain a predefined form. A key advantage of this approach is its potential for automation, to optimize manufacturing repeatability, permit standardization, reduce production time, and thereby warrant commercial application. Furthermore, it could potentially circumvent any intermediate steps, such as suturing and/or heat-bonding, that are otherwise required to assemble a functional construct comprised of multiple components. Despite its proposed potential, this approach presents a multitude of challenges that are largely attributed to the inhomogeneous electric field distribution induced on moulds with complex geometry, which significantly influences fiber deposition resulting in nonuniform mass accumulation of the polymer scaffold. Numerous research groups have aimed to combat these challenges, and subsequent explorations have generated a range of innovative approaches. These approaches were subcategorised as a) direct preform spinning and b) preform submould assembly spinning.

Direct preform electrospinning

A pivotal report on fabricating a bioresorbable trileaflet stentless valve using the method of direct electrospinning onto a custom-made heart valve-shaped mandrel was realised by Del Gaudio *et al.*¹⁴⁴. The aluminium collector used was modelled according to design parameters obtained from commercial BPVs. The scaffold was electrospun at very low rotational speeds of 0.3-0.4 rpm which was proposed to compensate for the effect the complex geometry of the mandrel induced on polymer deposition. Leaflet thickness measurements revealed relative uniformity, and SEM micrographs showed random fiber deposition with an absence of bead defects. The study thus demonstrated the technical feasibility of electrospinning a functional bioresorbable heart valve from a preform mould. Hydrodynamic testing was conducted with a pulse duplicator system under conditions representative of the native infant pulmonary site. The valve showed synchronous but suboptimal opening, and valve closure revealed slight pinwheeling. As the valves were operated under hypotensive conditions, it was reported that further work on optimising the electrospinning parameters and valve design was necessary to verify the viability of this method.

Preform submould assembly spinning

Another study conducted by Van Lieshout *et al.*³¹⁶ reported on a novel approach to electrospinning a stentless trileaflet valve featuring sinuses. The study focused on determining whether producing a geometrically complex valvular scaffold with homogenous thickness and sufficient mechanical integrity was achievable by electrospinning. Due to the complex geometry of the aortic valve with reference to the leaflet curvature, hemispherical sinuses and triangular coaption area, the mandrel was designed to comprise of four submoulds, including three leaflet-sinus structures featuring mostly convex contours, and a corresponding trileaflet insert mould. The approach involved electrospinning the three leaflet structures individually, after which they were assembled with the insert mould and jointly electrospun to form the aortic wall (Figure 3.7). After the moulds were removed, excess material from the coaption region was excised to define the leaflet free edge.

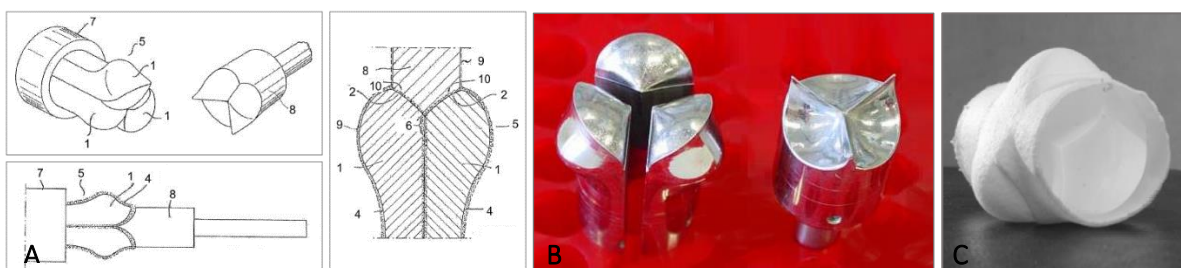


Figure 3.7: Preform assembly electrospinning. A) Schematic depiction of the submould spinning assembly process. B) Aluminium submoulds. C) Representative outcome using this approach (adapted from Baaijens³¹⁷ Van Lieshout *et al.*³¹⁶).

Upon physical examination nonuniform polymer deposition was observed throughout the scaffold structure, showing exaggerated thinning in the leaflet belly region and line of connection between the leaflets and aortic root. The scaffold ruptured at all sites where thinning was observed within 6 hours of pulse duplicator testing under aortic conditions. Hydrodynamic analysis also exposed improper functioning due to incomplete opening of one of the valve leaflets, which was later attributed to high material stiffness in regions of greater thickness. Moreover, histologic analysis revealed low permeability and resultant poor cellular penetration. The study thus

highlights the critical influence of scaffold thickness uniformity on the functional performance, mechanical integrity, and consequent durability of a TEHV construct.

Despite early reporting of adverse outcomes, the approach was since adapted and markedly refined by the Xeltis research group⁷⁶. Its recent implementation in a preclinical study carried out by Soliman *et al.*³¹⁸ yielded impressive outcomes. Favourable haemodynamic performance was achieved, reporting acceptable peak pressure gradients and mild regurgitation after 24-month follow up. Conduit diameters, measured at three critical levels of each implant indicated freedom from luminal narrowing or obstruction over the two-year study period. Overall performance outcomes indicated good midterm durability and great promise for future development. However, in a follow-up *in vivo* study conducted by Bennink *et al.*³¹⁹, valves explanted over a 12-month period presented large leaflet tears and significant leaflet retraction, as shown in Figure 3.8. Histologic analyses of the leaflets revealed substantial neointimal thickening at the leaflet base with distal tapering, as well as a presence of small fibrin thrombus and marginal calcification at the leaflet tips. Individual leaflets expressed variable degree of inflammation, with the greatest expression at the leaflet hinge point. Overall, leaflet inflammation was reported as mild, but the degree of leaflet retraction and shortening was considerable.

On the other hand, the conduit wall exhibited complete neointimal coverage, improved compliance, and an absence of calcification and luminal narrowing. The scaffold absorption rate revealed to be slower in the leaflets than in conduit wall which in considering respective inflammatory activity observed, supports the notion that slower degradation rates induce more favourable cellular behaviour³²⁰. Although the outcomes of this study present numerous contraindications, prior reports on the adequacy of the valve's functionality and durability indicate that the method used to fabricate the valve remains a viable solution, while rather the morphological architecture and biological properties of the leaflet scaffold should be recused.

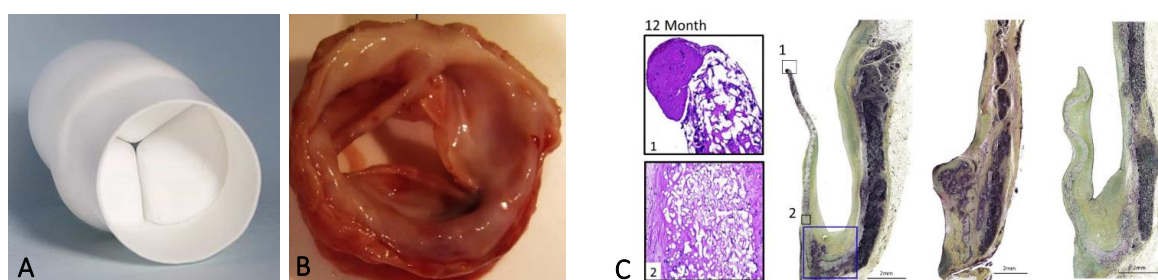


Figure 3.8: Xeltis Pulmonary Valved Conduit. A) Image of XPV conduit prior to implantation. B) Gross image of Xeltis Pulmonary Valved Conduit after 12 months of *in vivo* function. C) Histologic images of leaflet explants at 12 months showing significant variability in length, thickness, and degree of inflammation between leaflets (right). C-1) Small fibrin thrombus at leaflet tip. C-2) Leaflet base region exhibiting greater neointimal formation (adapted from Bennink *et al.*³¹⁹ and ³²¹).

3.1.2.3 Double-component deposition

Despite the diversity in manufacturing methods reported and respective preclinical achievements, the degree of control over structure-function outcomes for engineered tissue constructs remains largely limited across the board²¹³. In an attempt to overcome this limitation, D'Amore *et al.*²¹³ introduced an adaption to electrospinning, that utilizes two electrodes of varying conductivity to manipulate the induced electrostatic field such that fiber

deposition is advantageously oriented along the surface of the rotating collector. This would thereby enable leaflet thickness and microstructural control as well as potentially permit stentless valve construction. The first electrode employed in this technique acts as the primary target collector with geometric resemblance to the native heart valve. The second electrode, made from materials of lower conductivity, acts as a shielding component to reduce mass accumulation in regions where fiber deposition would otherwise be concentrated.

The double component deposition (DCD) technique was employed to produce a variety of heart valve structures for structural analysis and functional testing. The heart valve shaped component was machined from an aluminium alloy (Figure 3.9A), while the shield component was injection moulded using ABS (Figure 3.9D). The leaflet thickness measurements obtained from the valves revealed that spatial polymer deposition uniformity was achieved and produced results comparable to native valve leaflets (Figure 3.9B). This finding demonstrated the capacity to modulate and control leaflet macrostructure.

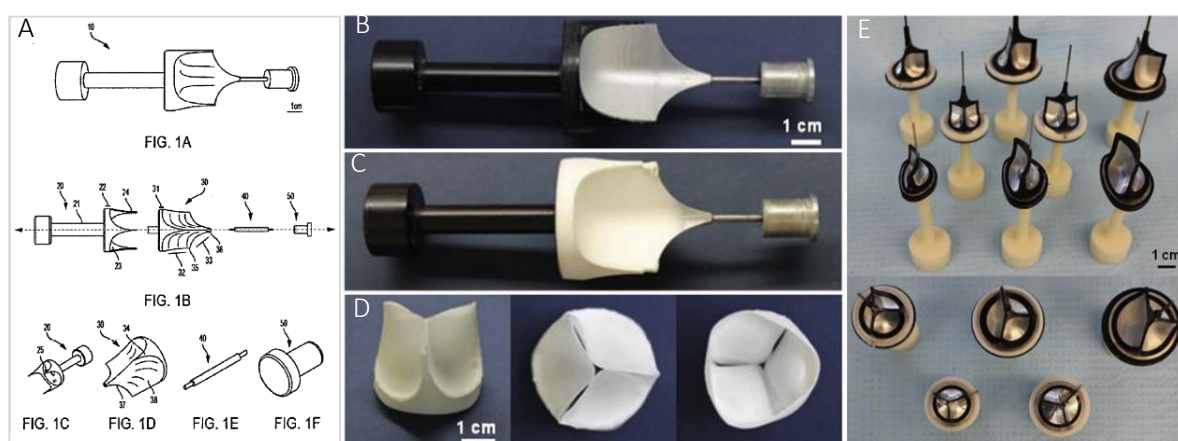


Figure 3.9: Double component deposition (DCD) technique. A) Figure illustrations for patent application. B) Heart valve-shaped aluminium alloy collector. C) Electrospun outcome prior to removal from the mandrel. D) DCD electrospun trileaflet valve construct. E) Non-conductive shielding components (adapted from D'Amore *et al.*²¹³ and Bae *et al.*²³¹, Badhwar *et al.*³²²).

The mechanical analysis and microstructural characterisation of the leaflet scaffolds revealed that controlling the translational velocity of the mandrel determined the number of fiber intersections per area, which correlated strongly with the bending rigidity of the scaffold, while varying the tangential velocity dictated fiber alignment and consequent mechanical anisotropy. These results demonstrated that the DCD processing method enhanced the capacity to control in- and out-of-plane mechanics and thereby produce structurally and functionally tuneable scaffold constructs. Subjected to pulse duplicator testing, the valves demonstrated adequate pulsatile flow under physiologic pressure conditions, and valve functionality was reported consistent with *in vitro* performance requirements for standard valve prostheses. Cumulatively, these findings present DCD as a promising technique for constructing TEHVs, but extensive investigation on this approach is further required to be validated for application in a clinical setting²¹³ (see Table 3.1 for summary details on this study)

3.1.2.4 Automated rotary jet spinning

The automated rotary jet system, introduced by Capulli *et al.*²⁰⁴, is a rapid production technique for fabricating biomimetic trileaflet heart valve scaffolds (referred to as JetValves) with a capacity for cell-free *in situ*

application. The technique involves a two-step mandrel collection process, using mandrels of customized shapes and sizes, whereby solution jets are extruded from a rotating nozzle to form a horizontal “fiber extrusion plane” (illustrated in Figure 3.10). The heart valve-shaped mandrels are translated vertically through the fibre extrusion plane while rotating at an angle relative to the horizontal plane. A key objective of this study was to achieve a more stream-lined fabrication method for generating functional TEHVs and thereby overcome the production rate limitations of electrospinning which is a major barrier to clinical translation²⁰⁴.

In vitro functional assessment of the JetValves revealed good structural integrity but a high regurgitation fraction (30%), which was later attributed to mounting discrepancies from the pulse duplicator setup. In an acute preclinical study, successful transcatheter delivery was achieved and demonstrated good haemodynamic performance with minor regurgitation. Explants revealed pliable intact leaflets with no evidence of early onset thrombosis or initial immunogenicity, indicating acute safety and compatibility. Moreover, JetValve constructs were reported to be easily incorporated into the stent and implanted without post processing or *in vitro* preconditioning. The overall outcomes indicate that this process offers a promising alternative to producing clinically relevant TEHVs. However, due to the limited timescale of the preclinical evaluation (15 hours) under mild pulmonary flow conditions, long-term preclinical evaluation involving the harsher conditions of the aortic site would need to be conducted before future clinical application can be realised.

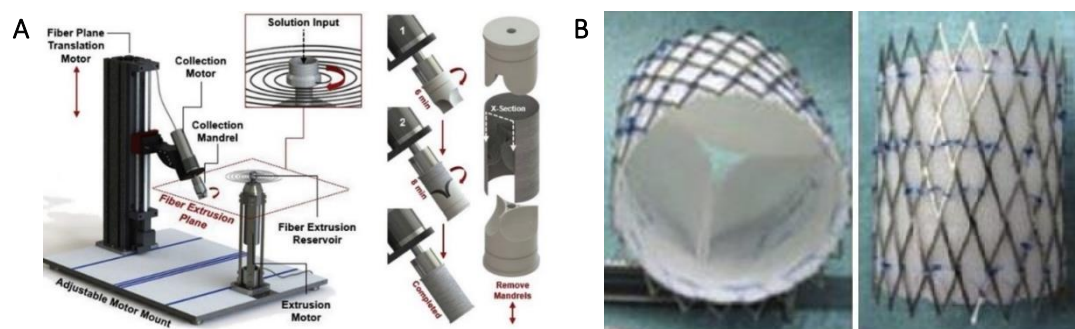


Figure 3.10: Automated rotary jet spinning. A) Illustration of automated rotary jet spinning process. B) Anchored JetValve (adapted from Capulli *et al.*²⁰⁴)

Table 3.1: Application of electrospinning methods for TEHV production

DATE	MATERIAL	STENT	METHOD	METHOD DETAILS	STENT INTEGRATION AND/OR LEAFLET SHAPING	EVALUATION	MAIN FINDINGS	REF.
2020	PCL	Biodegradable zinc-aluminium	Classic	Cylindrical rotating mandrel wrapped with aluminium foil	Tube-in-tube method	Preclinical (foetal lamb model)	Mild mechanical anisotropy of PCL scaffold. Hemodynamic testing showed complete opening and closure, trivial regurgitation, and a proper flow profile. Successful percutaneous in utero implantation, and delivery of foetal lamb alive at term. Severe post-operative implications observed include bleeding, bradycardia, pericardial effusion, and stent migration.	Zakko, 2020
2020	PC-BU	Nitinol	Classic	Cylindrical rotating mandrel	Cylindrical mesh mounted to stent using suturing technique, prior to moulding leaflets into more physiological shape by applying geometrically optimized custom-made PEEK inserts and incubating at 37°C for 5 days.	Preclinical (ovine model)	In vitro validation of adequate leaflet mobility, valve functionality and valve durability. Cell cultured valve scaffolds showed homogenous cell distribution. Heterogenous in vivo valve and leaflet remodelling was observed in both study groups. Pre-seeded valves exhibited fusion of adjacent leaflets at the commissures, severe regurgitation, and early signs of calcification. Both groups showed substantial leaflet base and hinge region thickening, as well as intervalve and interleaflet variability in leaflet length, thickness, and gene expression.	Fioretti, 2020
2019	PCUU	Biodegradable magnesium	DCD	Direct spinning onto trileaflet valve-shaped mandrel	Mounting of scaffold to stent achieved using sutures distributed at 6 locations	Preclinical - acute (swine model)	Acute in vivo function upon transcatheter implantation was achieved. No evidence of cellular infiltration or early degradation in leaflets. The stent showed early surface oxidation and fibrin sheath formation, but no bulk degradation. No thrombogenic or inflammatory reaction was detected up to the 12-hour end point. Difficulties with handling the stent during surgery was reported. Research limitations include small study population and acute time constraints.	Coyan, 2019
2018	PEUU	Stentless	DCD	Direct spinning onto trileaflet valve-shaped mandrel	DCD technique features conduction shielding components that assist in manipulating mass accumulation by orienting fiber deposition to enable leaflet thickness control and stent-less valve construction.	Quantification of manufacturing outcome controllability and hydrodynamic performance testing in pulse duplicator.	Spatial polymer deposition uniformity was achieved and comparable to native valve leaflets, thus demonstrating the ability to modulate and control leaflet macrostructure. Control of mandrel translational velocity dictated fiber intersections per area which would be correlated to bending rigidity, while tangential velocity variations dictated fiber alignment and consequent mechanical anisotropy. Results indicated enhanced control over in- and out-of-plane scaffold mechanics. Pulsatile physiologic flow was achieved, and valve functionality was reported consistent with in vitro performance requirements for standard valve prostheses.	D'Amore, 2018
2018	PCL-based UPy (conduit wall) PC-based UPy (leaflets)	Stentless	Phased preform assembly spinning	Four-part mandrel comprising submoulds that are electrospun individually, prior to being fit together and jointly electrospun.	NR	Preclinical (ovine model)	Presence of leaflet tears and fragmentation was observed macroscopically. Detection of substantial neointimal thickening at leaflet base. Presence of small fibrin thrombi and marginal calcification was observed at the leaflet tips. Individual leaflets expressed variable degree of inflammation, with greatest amount at hinge point. Leaflet infolding with free edge retraction and shortening was also observed. Conduit wall exhibited complete neointimal coverage, improved compliance, and absence of calcification. Scaffold absorption rate revealed to be slower in leaflet than in conduit wall.	Bennink, 2018
2017	PCL-based UPy (conduit wall) PC-based UPy (leaflets)	Stentless	Phased preform assembly spinning	Same as above	Electrospun leaflets mounted to frame via suture method	Preclinical - acute and midterm (ovine model)	Favourable haemodynamic performance was achieved, reporting overall acceptable peak pressure gradients and mild regurgitation after 24-month follow up. Conduit diameters, measured at three critical levels, indicated freedom from luminal narrowing or obstruction over two-year period. Overall performance outcomes indicated good midterm durability.	Soliman, 2017
2017	UPy-polyester urethane	Self-expandable nitinol frame	Classic	NR	Electrospun leaflets mounted to frame via suture method	Preclinical - acute (ovine model)	Good acute haemodynamic performance was observed and demonstrated comparable data to commercially available valve prostheses. Mostly mild regurgitation was detected, with three cases reported as severe.	Miyakazi, 2017
2017	PC-BU	PEEK reinforcement ring	Classic	Climate-control apparatus were implemented	Electrospun tubes were sutured onto the inner circumference of the support ring, which features suture holes along the ring base and up the commissure posts. By folding the tube externally to encase the ring and wedging the free edge into the top of the slotted commissure posts, the trileaflet configuration was achieved.	Preclinical (ovine model)	Sustained valve functionality over 12-month follow up period. Explanted valves revealed extensive host cell colonisation throughout entire leaflet. Endogenous neotissue formation was observed, exhibiting increasingly mature organization, synthesis of collagen and elastin, and de novo formation of mature elastic fibers. Near-complete endothelialisation was achieved by the study end point. No thrombus formation, calcification or chronic inflammation was detected. Slow resorption was observed to be cell driven such that structural integrity of the scaffold was warranted for the duration of tissue formation.	Kluin, 2017
2017	P4HB/gelatin	Nitinol	Automated rotary jet spinning	See in-text description for details	JetValve construct was hydrated prior to stent anchoring via suture method.	Preclinical - acute functional assessment (ovine model)	Functional assessment in vitro revealed good structural integrity but a high regurgitation fraction (30%), which was later attributed to mounting discrepancies. Successful transcatheter delivery was achieved and demonstrated good haemodynamic performance with minor regurgitation. Explants revealed pliable intact leaflets with no evidence of thrombus formation onset or initial immunogenicity, indicating acute safety and compatibility.	Capulli, 2017
2008	PCL	Stentless	Direct preform	Custom-made trileaflet heart valve-shaped mandrel, electrospun at very low rotational speeds (0.3-0.4 rpm) to compensate for the complex geometry and favour uniform polymer deposition.	NR	Hydrodynamic performance testing in pulse duplicator	Technical feasibility of electrospinning a functional bioresorbable heart valve was demonstrated. Under physiological conditions resembling the infant pulmonary site, the valve showed synchronous suboptimal opening, and valve closure revealed slight rotation of leaflets. Regardless, proper functioning was reported, with good repeatability and no evidence of structural damage.	Del Gaudio, 2008
2006	PCL	Stentless	Phased preform assembly	Phased preform mould assembly (Xeltis BV)	Excess material is excised from inner leaflet region to the commissures, to obtain a uniform triangular coaption profile.	Hydrodynamic performance testing in pulse duplicator and in vitro conditioning in bioreactor	Improper functioning was reported due to detection of an immobile leaflet, attributed to high material stiffness. Non-uniform polymer deposition was observed, showing exaggerated thinning in belly region and line of connection between the leaflets and aortic root. The scaffold eventually ruptured at all sites where thinning was observed. Histologic analysis revealed low permeability and resultant poor cellular penetration.	Van Lieshout, 2006

3.1.3 Requirements analysis

Based on insights gained from the system analysis, and findings obtained from the literature review, a comprehensive list of requirements pertinent to TETHV development was compiled (Table 3.2). The list includes requirements that were specific to this project, as well as standard requirements that were either relevant or irrelevant to the current study. Annotation symbols were used to differentiate between the requirements which are defined in the table legend. As this research did not involve *in vivo* studies, all biological criteria were not applicable. Nevertheless, they were important to consider for guiding design thinking toward the prospective translation of this work to *in vivo* investigation.

Table 3.2: List of project-specific and standard requirement for TETHV development

Legend	
★	Requirements specific to this project
●	Standard criteria identified as project requirements
○	Standard criteria not applicable to this project
Physical requirements	
Leaflets	
★	The leaflets must be compatible with different attachment techniques, such as suturing and heat bonding.
●	The scaffolds must be uniform in thickness to minimise nonuniform stress distribution and achieve synchronous and symmetric leaflet action.
●	The mechanical properties of the scaffolds must be equal to or exceed those of native leaflet tissues.
●	The scaffold must have high strength, pliability, and elasticity.
●	The scaffold must be porous to facilitate ECM deposition and tissue ingrowth.
●	The scaffold must be permeable to facilitate nutrient diffusion and metabolic waste removal.
○	The leaflet free-edge must be smooth and free of notable irregularities.
○	The scaffold must demonstrate mechanical anisotropy comparable to native valve leaflets.
○	The scaffold must exhibit viscoelastic behaviour.
○	The scaffold must demonstrate a balance between hydrophilic and hydrophobic properties to enhance biocompatibility (fluid affinity) while promoting cell-cell and cell-substrate interactions (cell affinity).
Sealing skirt	
●	The skirt material must be porous to facilitate tissue ingrowth.
●	The skirt must be thin to facilitate maximum radial displacement for optimised delivery sheath profiles.
●	The skirt must be sufficiently elastic to fully recover (both structural and mechanically) from the compressive and tensile stresses imposed during stent crimping and expansion.
Stent	
★	The stent must feature suture holes along a continuous line around the frame to facilitate leaflet fixation.
●	The stent must be radially symmetric to provide an appropriate geometric area for receiving a trileaflet structure.
○	The stent must comprise of a metal with high strength, stiffness, and elongation properties.
Functional requirements	
Leaflets	
●	The valves must perform rapid opening and complete closure during systole and diastole, respectively.
●	The leaflets must remain intact and fixed to the stent during operation.
●	The peripheral zones of apposition between the leaflets must be symmetric.
○	The central point of the coaptation zone must coincide with the central point of the stent/aortic root.
○	The leaflets shall perform synchronous opening and closing motions.

Sealing skirt

- * The skirt must be fixed to the abluminal surface of the frame to facilitate tissue ingrowth and subsequent integration with the tissue of the surrounding anatomical structure upon implantation.
- * The skirt material must be compliant with joining techniques such as heat bonding and suturing.
- The skirt must effectively seal the area between the abluminal surface of the frame and the wall of the surrounding structure to prevent paravalvular leakage.
- The skirt must remain intact and fixed to the stent during operation.

Stent

- The stent material must maintain physical and chemical integrity in the biological environment.
- The stent must provide high radial strength and stiffness and prevent elastic stent recoil.
- The stent must be capable of collapsing to the desired delivery profile, and expanding to the required operating condition, under the radial forces applied during crimping and balloon inflation, respectively.
- The stent must not suffer acute fracture during crimping, delivery, or deployment
- The stent must operate effectively in an over-deployed state.
- The stent must demonstrate a flexural response to leaflet action to increase load transfer from the leaflets to stent and thereby reduce stress concentrations at the leaflet commissures.

Performance requirements

- The *in vitro* hydrodynamic performance of the valves must be tested in compliance with ISO 5840
- The valve must perform mean transvalvular pressures below 10 mmHg.
- The EOA must be greater than or equal to 1.2 cm², tested at nominal aortic conditions.
- The valves must perform <20% regurgitant fraction.
- The leaflets must remain intact and operational.
- The opening and closing behaviour of the valves must not deteriorate over time.
- * The valve must be functional for a minimum of 140 thousand cycles.
- The valve must withstand crimping and expansion for transcatheter delivery
- The valve must perform appropriately at hypo-, normo-, and hyper hypertensive operating conditions.

Manufacturing requirements

- * The physical components of the valve must be manufactured from available raw materials.
- Manufacturing operations must be reproducible.
- The manufacturing process should be capable of mass production.
- The raw materials and manufacturing equipment should be sterile.
- Manufacturing operations should have a low reliance on skilled labour.
- The manufacturing process must be standardisable.
- The manufacturing process should be semi-automated.
- The manufacturing process should be cost-effective and time efficient.

Biological criteria

- The valve component materials must be biocompatible and nonimmunogenic
- The valve leaflet and skirt materials must facilitate cell activities, ECM deposition, and tissue ingrowth
- Accommodate somatic growth and maturation
- Biodegradable polymer with suitable degradation characteristics (e.g., appropriate degradation rate, non-toxic degradation products, etc)
- Appropriate degradation rate to provide structural and mechanical stability throughout the neotissue growth and remodelling phase
- Prevent fusion between leaflets at the commissure hinge

3.2 Manufacturing methods and materials

The valve manufacturing concepts generated in this study were all based on a common sequence of operations, which included the (1) production of the skirt and leaflet scaffold materials, (2) production of the stents, and (3) the assembly of the respective valve components, as depicted in Figure 3.11.

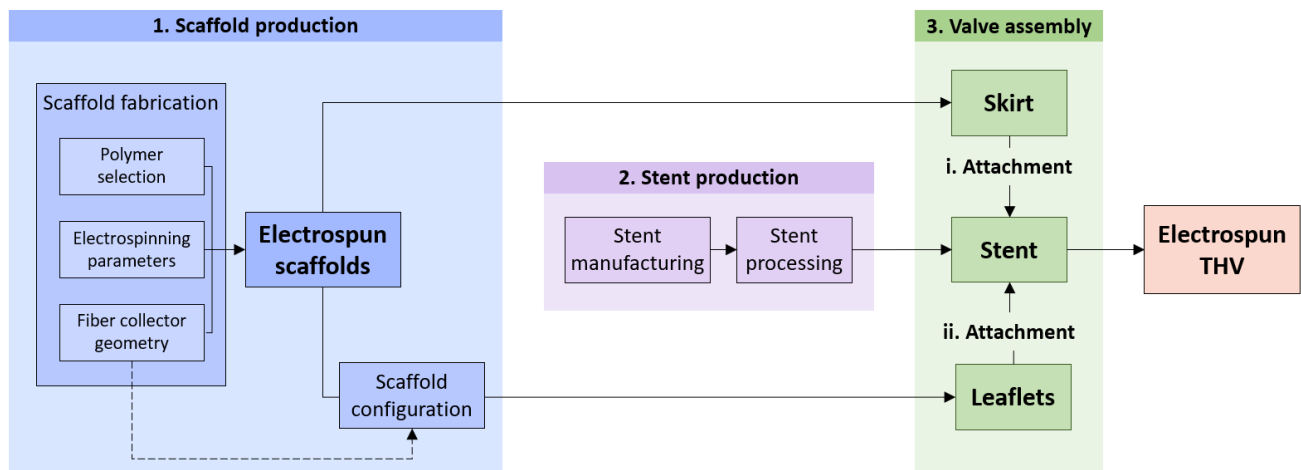


Figure 3.11: Schematic diagram of valve manufacturing operations sequence.

3.2.1 Scaffold production

The scaffold production process encompassed two subprocesses, including (1) scaffold fabrication and (2) leaflet configuration. The objective of the former was to electrospin scaffolds for leaflet and skirt materials, while the objective of the latter was to configure the leaflet scaffolds into a three-dimensional trileaflet valve structure. In general, these processes were interrelated, where the output of subprocess 1 governed the approach to subprocess 2, or the input of the subprocess 2 determined the output of subprocess 1.

3.2.1.1 Scaffold fabrication

The process of scaffold fabrication involved three primary considerations, including the selection of the polymer, electrospinning process parameters, and fiber collector geometry. For the purpose of rapid prototyping, Pellethane was selected for its high electrospinnability, and the electrospinning parameters were selected based on the parametric study described in the previous chapter. Briefly, scaffolds were electrospun using a 16 wt% Pellethane solution in DMF/THF (1:1) which was fed at a flow rate of 3 ml/hr through a 19G needle, supplied with 17 kV, and set at a tip-to-collector distance of 28 cm. The collector was supplied with a voltage of -3 kV and run at a rotational velocity of 1000 rpm and translational speed of 2 mm/min. The operating environment was maintained at 45% relative humidity for the duration of the experiment. Only slight adjustments were made to these parameters between experiments to accommodate the effect of the different collector geometries on the performance of the fibers. In this way, the geometry of the fiber collector, which was identified as a key design factor for developing different valve manufacturing processes, could be investigated independently.

3.2.1.2 Fiber collector design and production

A fundamental aspect of electrospinning is the geometry of the collector onto which the fibers deposit. Not only does the shape of the collector determine the final shape of the electrospun scaffold, but more importantly, it plays an influential role in creating the electric field, which largely governs the flight path, deposition pattern, and overall spread of electrospun fibers across the collector surface ³²³. Furthermore, the repeatability of scaffold production is highly dependent on the stability of the electrospinning process; and the stability of the electrospinning process is highly dependent on the uniformity of the electric field distribution, which is in turn directly impacted by the geometry of the collector ³²⁴.

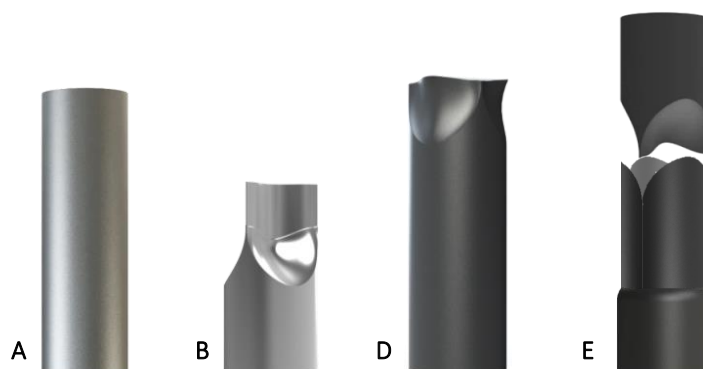


Figure 3.12: Rendered models of the fiber collector concept designs. A) cylindrical collector, B) trileaflet collector, C) inverted trileaflet collector, and D) trileaflet-submould assembly.

For this study, four different collector geometries were investigated, including a cylindrical collector, a trileaflet-shaped collector, an inverted trileaflet-shaped collector, and a trileaflet-submould assembly, as illustrated in Figure 3.12. The cylindrical and trileaflet collectors were provided by SAT for this study. The design and manufacturing of the inverted trileaflet collector and trileaflet-submould assembly form part of this study. Both of these concepts were modelled in Solidworks (2019 Student Edition) and manufactured using an inhouse assembled 3D printer (Prusa i3, DIY Electronics) with carbon fiber PLA (CFPLA) filament. The CFPLA filament was used for its good electrical conductivity and low thermal expansion properties.

Inverted-trileaflet collector design

The inverted-trileaflet collector was designed to overcome the challenges associated with employing a target collector with a complex shape involving convex, concave, and flat surfaces. Discontinuities in the geometry of conducting collectors causes different current densities to accumulate throughout the element, which creates nonuniform electric field distributions across the collector surface ³²⁵. In general, sharp convex edges create distinctly stronger electric fields compared to planar surfaces, while almost no charge accumulates at sharp concavities, causing local “voids” in the electric field. In electrospinning, the presence of local maxima and minima in the electric field causes a fluctuation in attractive and repulsive forces which influences the flight path of the fibers ³²⁶. This leads to the uneven deposition fibers, where fibers are prone to accumulating on the sharp corners and edges of the collector, while being hampered from depositing on low profile regions ^{213,316,327}. On the other hand, collectors comprised primarily of contiguous convex surfaces with no sharp

protruding features have been found to facilitate more uniform layering of electrospun fibers ³¹⁷. For this reason, the redesign of the collector was focused on reducing the geometric discontinuities between the deep concave profile of the leaflet belly and coaptation zone with the pronounced edges of the commissures. The idea was herefrom conceived to invert the leaflet geometry across the plane coinciding with the leaflet attachment line, such that when the leaflets are reverted they adopt the original closed-position of the valve.

To create this model, the SAT P-TAVR CAD model was imported to Solidworks from which the leaflet surface feature was exported as a component entity and converted into a new part (Figure 3.13A–C). The leaflet profile was inverted by reflecting the circumferential curvatures across a working plane coinciding with the edges of the commissures and the inflection point at the base of the scallop (Figure 3.13D). The modified surface was subsequently triplicated, assembled into an adjoining trileaflet configuration (Figure 3.13E), and extruded from the base to form the body of the collector (Figure 3.13F).

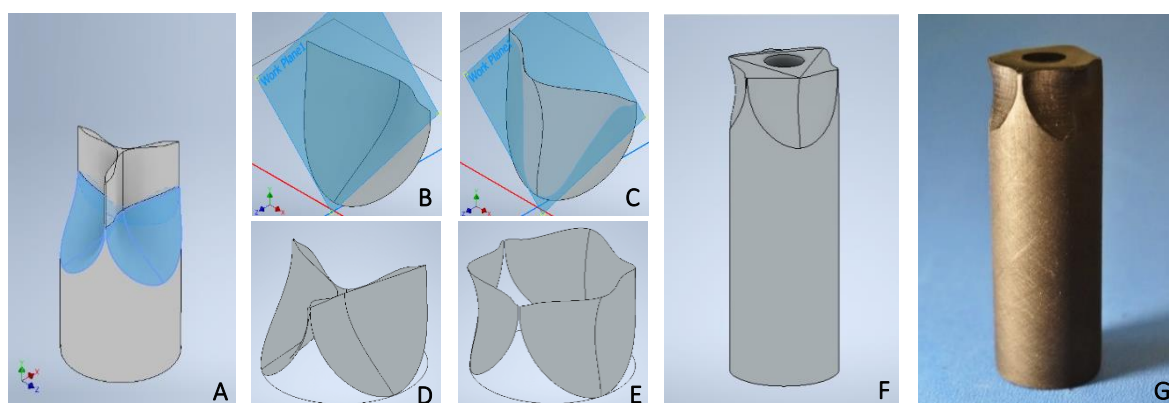


Figure 3.13: CAD modelling process to produce the inverted trileaflet HV-shaped collector. involving extracting and manipulating leaflet surface geometry to configure a solid body component for 3D printing the inverted trileaflet-HV mould

Trileaflet-submould assembly design

The trileaflet-submould assembly concept was based on a design patented by Baaijens³¹⁷, which was developed to manufacture an artificial trileaflet heart valve composed of electrospun scaffolds, commercially known as the Xeltis restorative pulmonary heart valve (XPV). The method was invented to facilitate the production of a three-dimensional trileaflet-shaped preform with the process of electrospinning, by subdividing the desired shape into submoulds that comprise purely of convex surfaces and that fit together to jointly form the mould. In this way, the submoulds can be electrospun separately and in assembly.

Based on this approach, three subcomponents were designed to jointly form the desired shape of the trileaflet-shaped heart valve. These included a leaflet-shaped submould, a trileaflet subassembly holder, and an arterial wall component, as shown in Figure 3.14. The leaflet-shaped submould was modelled by importing the surface geometry from the SAT P-TAVR model and configuring it into solid component featuring a slot in the base to facilitate its attachment to the electrospinning mandrel, as well as the leaflet subassembly holder. The subassembly holder was designed to secure the configuration of leaflet submould assembly by incorporating a housing wall which provides radial stability, and protruding features on the base of the holder, onto which the leaflets submoulds can be fixed by applying press fit. Lastly, the arterial wall component was developed to mate

with the leaflet subassembly along the leaflet attachment hinge to form the interleaflet triangle and anterior wall. As shown in Figure 3.14C, a blind hole was incorporated in the centre of the component to prevent it from contacting the opposing structures comprising the coaptation zone.

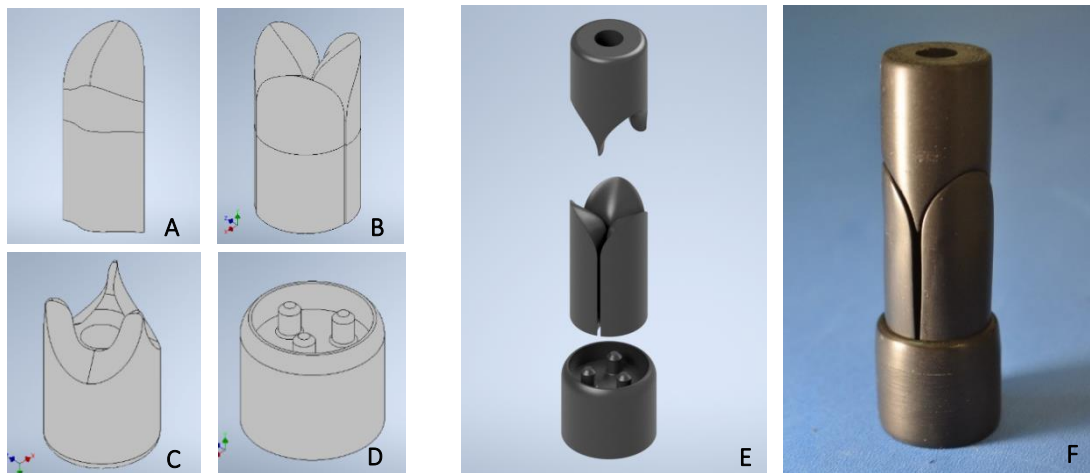


Figure 3.14: CAD models comprising the trileaflet-submould assembly. A) Leaflet-shaped submould. B) Leaflet submould assembly. C) Arterial wall component. D) Leaflet subassembly holder.

3.2.1.3 Leaflet scaffold configuration

In order to obtain a trileaflet valve structure, the leaflet materials needed to be fashioned into the appropriate form and configuration, depicted in Figure 3.15. Based on the approaches that have been investigated in previous studies, five methods were identified as being suitable for exploration and prototyping. These included: leaflet die-cutting, tube shaping, thermoforming, preform electrospinning, and submould assembly spinning.

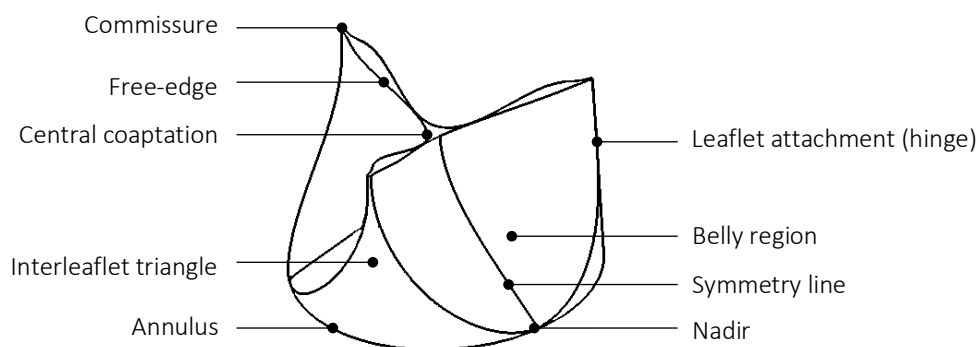


Figure 3.15: Characteristic physical features of trileaflet heart valve leaflets

Leaflet die-cutting

A common approach to configuring trileaflet structures is by cutting individual leaflets from a sheet of material and attaching them separately to a stent using a suitable joining technique. To investigate this method, electrospun scaffold sheets were produced from electrospun scaffold tubes which they were cut longitudinally and flattened. Thereafter, a steel rule die-cutter and manual hand press were employed to cut the leaflets from the scaffold sheets (Packtech Tooling, RSA). The leaflet-cutter geometry was designed by SAT as part of an early

investigation toward optimising leaflet designs for BPVs. As shown in Figure 3.17, the main leaflets features included (A) the commissure ears, designed to facilitate the attachment of the leaflets to the commissure posts; (B) the offset leaflet attachment line, and (C) the free-edge profile, designed for optimised coaptation³²⁸.

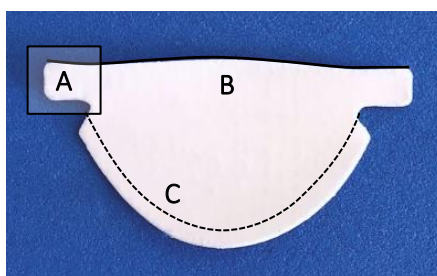


Figure 3.16: Pre-cut electrospun leaflet. A) Commissure ears. B) Leaflet free-edge. C) Leaflet attachment.

Tube shaping

A more simple approach to forming a trileaflet structure is to mount a tubular scaffold to the inside of a rigid stent/wall specifically at the commissures and along the leaflet attachment line using a suitable joining technique. This method has been used by multiple research groups to construct TEHVs from different materials, including *in vivo* engineered tissue¹¹⁰, electrospun scaffolds^{149,329}, and decellularized engineered tissue³³⁰.

Thermoforming

Thermoforming takes advantage of the ability of thermoelastic polyurethanes to be shaped and moulded with controlled exposure to heat. In general, this approach involves heating a material to a pliable forming temperature and applying a mould to obtain a specific shape. Although this technique is not commonly employed in TEHV research, there have been a few investigations that report the effective application of this strategy to configure a trileaflet structure from porous PU scaffolds^{331,332}.

Preform electrospinning

As discussed in section 3.1.2.2, preform electrospinning refers to the use of a target collector that mimics the shape of the desired trileaflet structure, so that the scaffold is electrospun directly into a trileaflet configuration. To investigate this strategy, a trileaflet-shaped mould machined from Aluminium alloy was provided by SAT.

Preform submould assembly spinning

As discussed in more depth in section 3.1.2.2, this method involves the application of leaflet-shaped submoulds that are electrospun separately and then joined together to be electrospun as a whole. To investigate this approach, a trileaflet-shaped submould assembly needed to be designed, modelled, and manufactured.

3.2.2 Stent production

Regarding transcatheter heart valves, the role of the stent is essential to fulfilling the operational requirements for percutaneous implantation. Stents serve as frames onto which the skirt and leaflets are attached, and on which they rely for structural support during implantation and operation. The stents used in this study were provided by Strait Access Technologies (SAT) by whom they were designed, manufactured, and processed.

3.2.2.1 Manufacturing and processing

The stents were manufactured from 23mm cobalt-chromium (CoCr) tubes (MP35N, MINITUBES, France) using the StarCut Tube Laser Cutting System (ROFIN-BAASEL Lasertech GmbH & Co. KG, Germany). Once cut, the stents were pickled and electropolished to remove oxide layers and yield smooth strut surfaces to minimise abrasion damage to the leaflets. With reference to Figure 3.17, the main design aspects of the stent were (G) the central scallops featuring suture holes to facilitate leaflet attachment; (E) the commissure posts featuring a slot and suture holes to facilitate leaflet attachment at the commissure hinge; and (F) six suspended arms designed to elevate during balloon-expansion and attain supra-annular anchorage³²⁸. Additional design parameters include the (A) outer diameter, (B) inner diameter, (C) stent height, and (D) skirt boundary height.

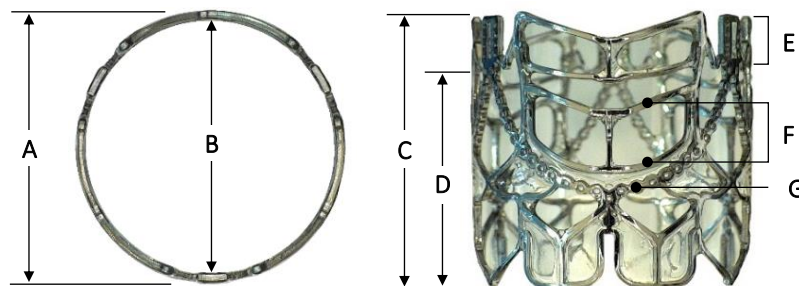


Figure 3.17: Key parameters and physical features of the stent. (A) outer diameter (OD); (B) inner diameter (ID); (C) stent height; (D) skirt boundary height; (E) commissure posts; (F) expandable anchoring arms; (G) central scallop ring featuring suture holes; and (H) rigid support struts.

3.2.2.2 Precoating

To achieve interfacial bonding between the scaffolds and stent by heat bonding, the stents were provided with a polymer precoating to which the scaffolds could be thermally welded. To coat the stents provided for this project, a simple dipping-suspension procedure was developed. Briefly, the stents were submerged into a solution of 5 wt% Pellethane® in DMAc (Sigma-Aldrich (Pty) Ltd. (RSA)) and continuously rolled for 5 seconds. The dipped stents were then suspended on a drying rack (Figure 3.18A) and placed in an oven at 60°C for 20 minutes or until dry. This process was repeated 6–8 times to obtain the desired thickness of 180–200 µm. The precoating layer was examined using a digital microscope (Ash Technologies Inspex 3, UNITRON® SA), and measured for thickness using the integrated image processing software (Figure 3.18C–D).

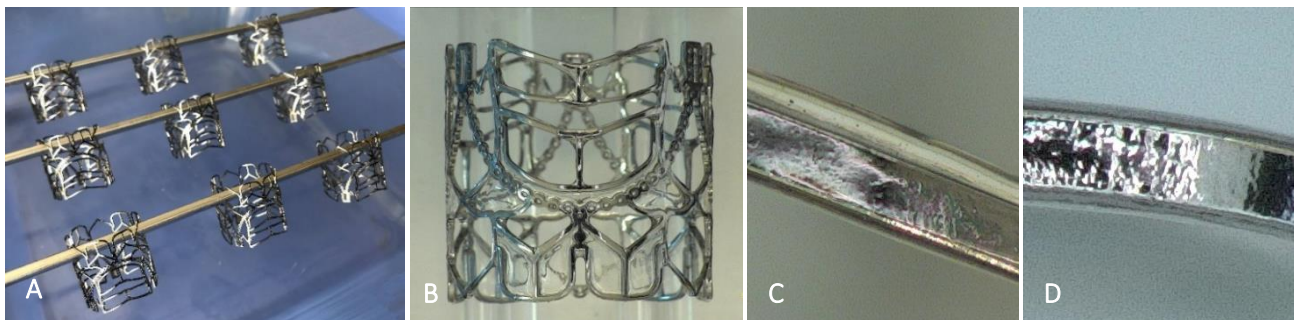


Figure 3.18: Pre-coating process and thickness evaluation. A) Suspension rack holding dipped stents. Images of (B) precoated stent, (C–D) oblique struts, E) suture attachment hole, and F) frame circumference.

3.2.3 Valve component assembly

The valve component assembly process encompassed two main subprocesses, including (1) the attachment of the skirt to the stent, and (2) the attachment of the leaflets to the stent. The primary objective of here was to establish suitable joining techniques that yield strong and durable joints between the scaffold and stent materials, capable of withstanding the operational loads. This required a multi-material structural design approach, which brought forth the challenge of joining materials with inherently different physical properties so that load transfer throughout the construct is efficient and the presence of stress concentrations is minimised¹³⁵. To date, the most widely adopted joining techniques to configure electrospun scaffolds into stented trileaflet valve structures include surgical sutures³³³ and heat-application welding (heat bonding)³³⁴. Other techniques include interface fusion³¹⁶ and integrating mechanical fastening mechanisms into the structure of the stent³²⁹. For this study, the joining techniques that were explored include suturing, heat bonding, and interface fusion. As stent design was not within the scope of this project, the mechanical fastening technique was not applicable. The techniques investigated for skirt attachment include heat bonding and interface fusion, and those investigated for leaflet attachment include suturing and heat bonding.

3.2.3.1 Skirt attachment techniques

Heat bonding

Heat bonding (also referred to as thermal welding) is a widely used to join thermoplastics for a wide range of applications in numerous different sectors^{335,336}. In particular, heat bonding features frequently in literature as the primary means of forming TEHV constructs. The technique is performed by locally heating two contacting surfaces and applying a pressure until the joint consolidates to form an irreversible bond. On a molecular scale, when thermal energy is applied so that the temperature of the polymer is raised above its melting temperature (T_m), the polymers chains at the surface of one component become sufficiently mobile to entangle with the chains in the adjoining component, resulting in a weld³³⁷.

For this study, all heat bonding procedures were performed using a digital soldering iron with a 1.6 mm flat tip attachment (MAGNUM® 2005, Magnum Products). The tool was operated at temperatures ranging between 160–178 °C depending on the scaffold thickness and strut size. The stent was handled over a diffused light source to assist the bonding procedure by illuminating the stent from beneath the scaffold.

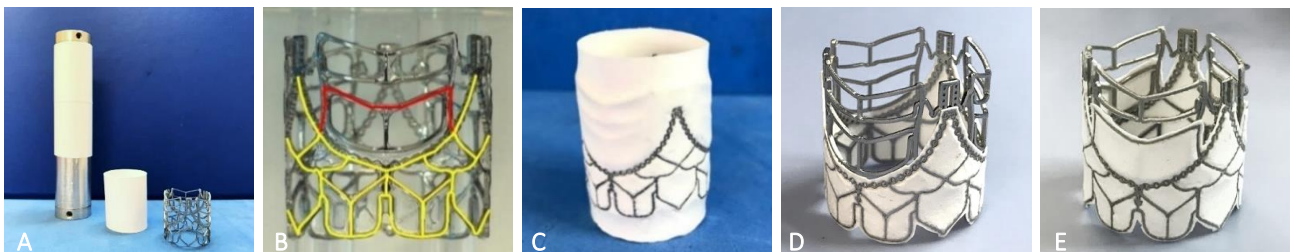


Figure 3.19: Skirt heat bonding method. A) Collector with electrospun scaffold segment and stent. B) Bonding pattern. C-D) First skirt layer bonded to stent before and after cutting. E) Second skirt layer bonded and cut.

To form the skirt and ensure sufficient paravalvular sealing, particularly in the region corresponding to the aorto-ventricular junction, two scaffold tubes, each $\sim 150\ \mu\text{m}$ in thickness, were bonded to the stent in succession. With reference to Figure 3.19B, the bonding pattern applied to the first layer is indicated in yellow, and the pattern applied to the second layer includes both yellow and red lines. The excess scaffold was trimmed along the top and bottom boundaries after bonding each layer.

Interface fusion

The nature of this technique relies primarily on the affinity of electrospun fibers to cohere to the surface on which they are spun. The adhesive and cohesive behaviour of fibers varies greatly depending on the material properties and surface topology of the structure on which they land, in which case these parameters be tuned to provide surfaces that promote good fiber adhesion. With good fiber adhesion, sandwich-like structures can be produced by electrospinning one material directly onto another (referred to as “sandwich-spinning”). The major advantage of this technique is in its potential for automation and minimising the adverse implications associated with material handling, such as compromised sterility and handling damage.

3.2.3.2 Leaflet attachment techniques

Suturing

Surgical sutures are one of the most common medical devices used in surgery and have an extensive history of effectively joining biological tissues to facilitate the closure and functional restoration of wounds. Sutures also feature prominently in biomedical device manufacturing as tools to construct biomaterial implants. Particularly in bioprosthetic valve manufacturing and heart valve tissue engineering, suturing is of the most commonly used techniques for attaching flexible valve materials to a stent or conduit wall to form a functional trileaflet structure. Suturing was therefore identified as a promising technique to explore.

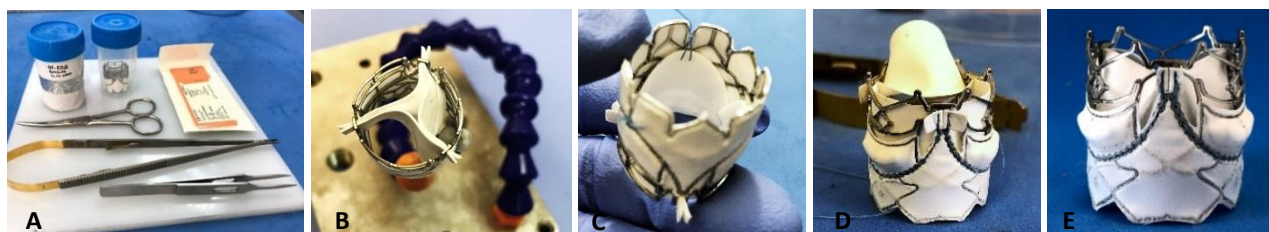


Figure 3.20: Leaflet suturing method. A) Suturing materials. B) Leaflet ears pulled through the commissure post slots. C) Place-holder stitch to secure leaflet position. D) Attachment of leaflets in continuous succession. E) Finished valve in a neutral closed position.

The materials and tools used to suture the valves included Ti-Cron™ 5-0 braided polyester sutures (90 cm), a micro-grip needle holder, atraumatic and splinter forceps, dissecting scissors, and a third-hand clamp stand. The suturing protocol involved: positioning the leaflets inside the stent by drawing the commissure ears through the commissure post slots, flattening the scaffolds against the stent frame, and aligning the leaflet attachment curves with the contour of the scallops. Prior to suturing, the leaflets were secured with a place-holder stitch at the inflection point of the scallop and along the commissure posts. Thereafter, a saddle running stitch was performed using a single thread to integrate all three leaflets in a continuous loop along the circumference of the stent. Each suture joint was secured by applying a set amount of tension to both sides of the thread before

proceeding to the next hole. The loop was finished off with six successive slip knots in alternating directions to permanently link the first and final stitch. The advantage of this stitching technique is that it utilises each hole along the scallop, and as the thread sequence is mirrored continuously along the scallops, suture failures are prevented from propagating.

Heat bonding

The leaflet bonding procedure developed for this study involved three main steps. Firstly, the leaflets were arranged into position by pulling the ears through the commissure post slots, flattening the scaffolds against the inside of the stent, and aligning the attachment region with the scallop of the stent. Once positioned, the leaflets were successively bonded to the luminal surface of the scallop, after which the commissure ears were bonded to the abluminal surface of the stent posts.

3.3 Concept generation

3.3.1 Concept ideation

Table 3.3 below summarises the different approaches that were considered for each aspect of manufacturing, including fiber collector geometry, scaffold fabrication, trileaflet configuration, and valve component assembly. Following this format, a concept diagram was created which illustrates the various combinations of operations that were conceptualised in the ideation phase (see Figure 3.21). From this diagram, six sequences of operations were selected for prototyping based on their fundamental practicality, resources requirements, and time implications.

Table 3.3: Manufacturing design concept summary

Fiber collector geometry	Scaffold fabrication and trileaflet configuration	Valve component assembly
1 Cylindrical	1 Die-cut leaflets from an electrospun sheet	1 Skirt attachment
2 Trileaflet	2 Preform electrospinning	a Heat bonding
3 Inverted-trileaflet	3 Preform electrospinning with thermoforming	b Interface fusion
4 Trileaflet-submould assembly	4 Preform submould assembly spinning	

The next section follows a slightly unconventional approach to reporting concept ideation and design development. As designing a valve manufacturing process involved formulating a sequence of operations from a selection of common methods, it was necessary to first identify and specify appropriate materials and manufacturing techniques in order to facilitate the concept ideation phase. With the materials and methods already established, the following section briefly describes each ideated concept, after which concept prototyping, prototype evaluation, and concept selection are discussed, respectively.

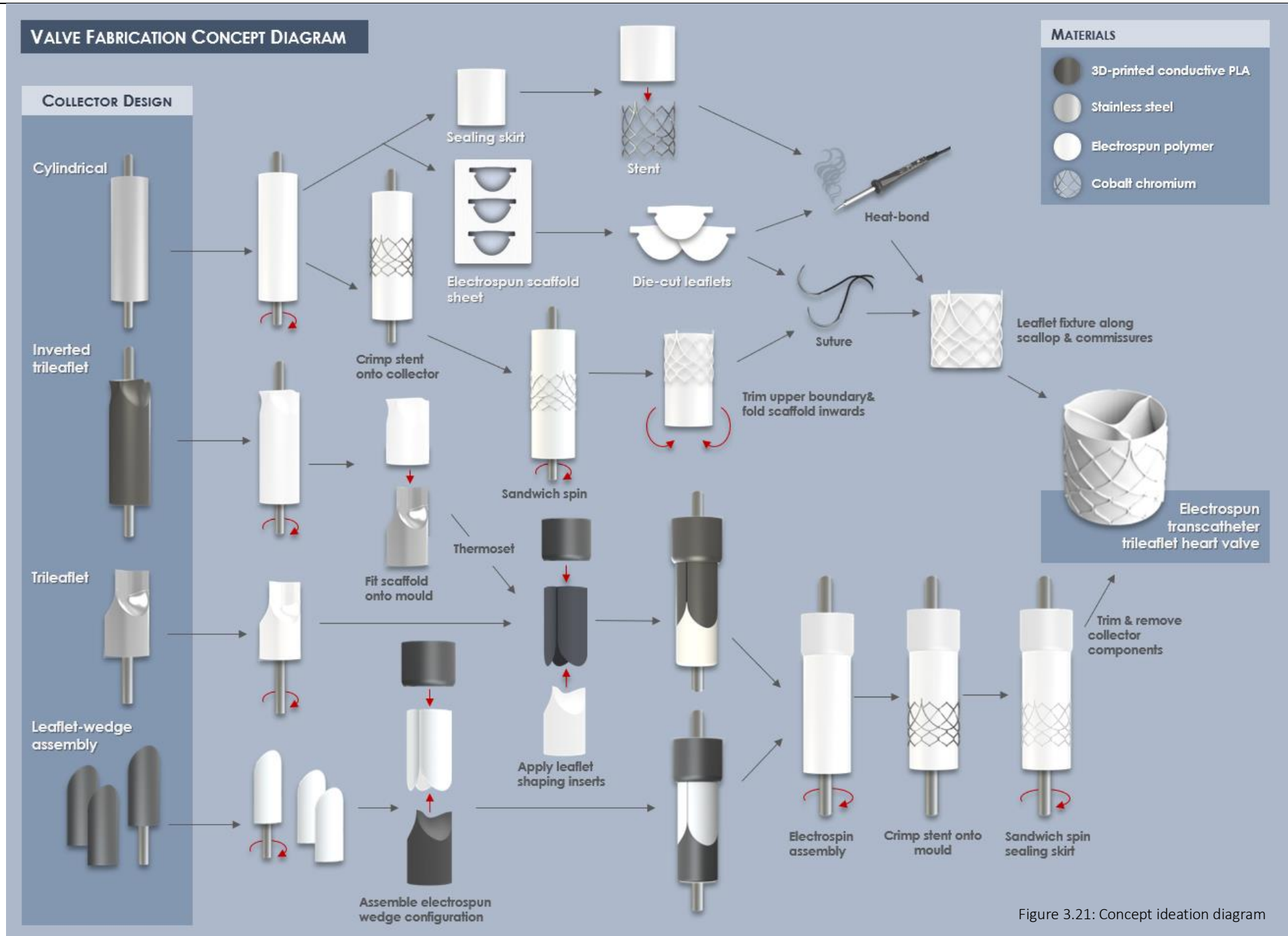


Figure 3.21: Concept ideation diagram

3.3.2 Concept descriptions

3.3.2.1 Concept 1: Pre-cut leaflet suturing process

As shown in Figure 3.22, concept 1 proposes the employment of conventional electrospinning system, where a cylindrical fiber collector shall be used to fabricate electrospun scaffold tubes to serve as both skirt and leaflet materials (1). The skirts shall be cut from the scaffolds into tube segments (2), whereafter they shall be fit onto the stent, heat bonded (3), and trimmed of excess material (4). Thereafter, pre-cut leaflets shall be fabricated using a die-cutting technique (5) and sutured onto the stent (6) to form the desired trileaflet configuration (7).

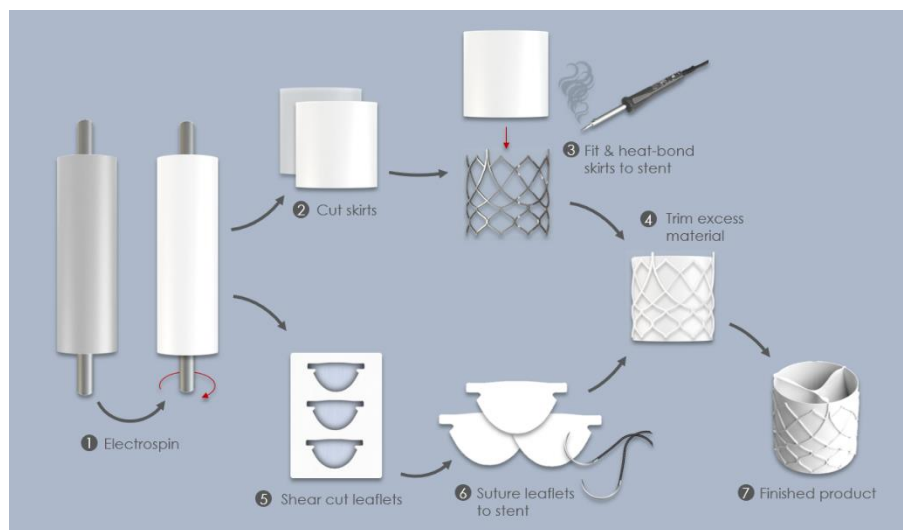


Figure 3.22: Illustration of the sequence of operations proposed in Concept 1

3.3.2.2 Concept 2: Pre-cut leaflet heat bonding process

As illustrated in Figure 3.23, concept 2 proposes the same first steps as concept 1A up to the leaflet attachment process, whereafter instead of suturing, the leaflets shall be heat bonded to the stent (6) to form the trileaflet structure (7).

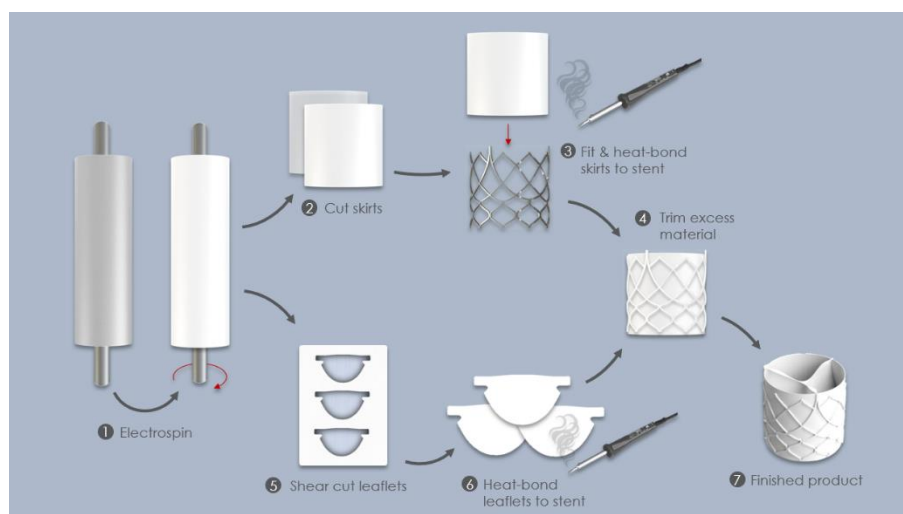


Figure 3.23: Illustration of the sequence of operations proposed in Concept 2

3.3.2.3 Concept 3: Sandwich-spun tube shaping process

Concept 3 explores the application of interface fusion as a means to attach the skirt to the stent, as illustrated in Figure 3.24. For this, a scaffold shall be spun onto a cylindrical collector (1), after which a stent shall be mounted on the collector and lightly crimped to secure its position (2). Thereafter, the assembly shall be electrospun (3) so that the fibers land over the stent and subsequently adhere to the underlying scaffold so as to encapsulate the stent. In this way, the stent shall become sandwiched between the two bonded layers which together shall form the sealing skirt. After removing the sandwich structure from the collector, the scaffold shall be trimmed along the upper perimeter of the stent, while preserving the lower section for its incorporation as valve leaflets. The leaflets shall be shaped by folding the scaffold into the stent (4) and suturing the scaffold along the scallop to secure the trileaflet configuration (5).

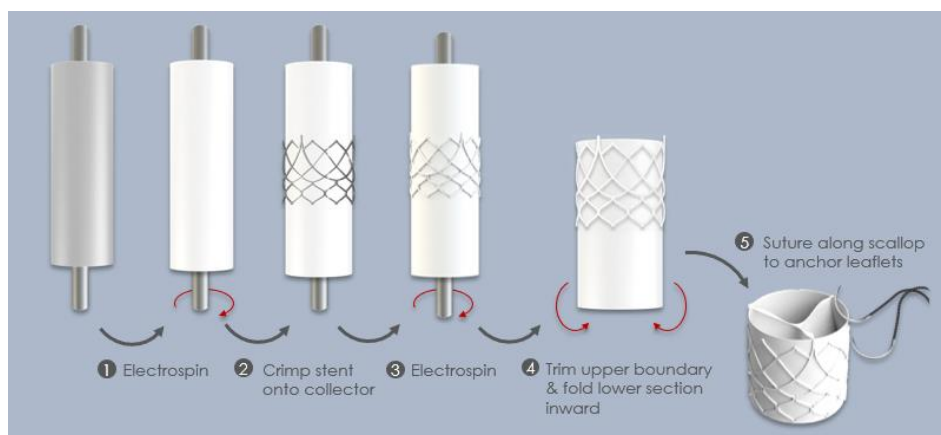


Figure 3.24: Illustration of the sequence of operations proposed in Concept 3

3.3.2.4 Concept 4: Trileaflet preform production process

Concept 4 presents the preform electrospinning process, where scaffolds shall be spun directly onto the trileaflet-shaped collector after which (1), the scaffold shall be removed and trimmed along the leaflet free-edge while preserving the lower tube section (2). The scaffold preform shall be positioned inside the stent so that the leaflet attachment edge aligns with the scallop, after which the lower section shall be folded outward over the stent to form the skirt (3). The skirt shall be heat bonded to the outside of the stent, while the leaflets shall be heat bonded to the inner surface of the stent along the scallop (4).

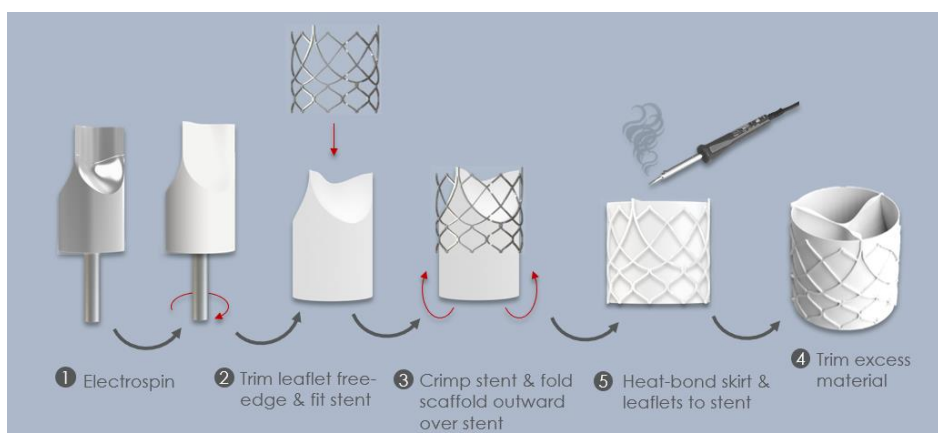


Figure 3.25: Illustration of the sequence of operations proposed in Concept 4

3.3.2.5 Concept 5: Inverted trileaflet thermoforming process

As illustrated in Figure 3.26 below, concept 5 proposes the process of electrospinning an inverted trileaflet-shaped preform (1) which shall be subsequently fit onto a trileaflet-shaped mould and (2) thermoformed into the closed trileaflet configuration by applying leaflet-shape submoulds (3) and heating the construct in an oven at 60° for 20 minutes (4). Thereafter, the scaffold shall be removed from the mould and trimmed along the leaflet free-edge (5). The scaffold shall then be placed inside the stent and with leaflet attachment edges in line with the scallops, the lower section of the scaffold shall be folded back over the stent to form the skirt (6). The skirt shall then be fixed to the stent by heat bonding (7) and the leaflets secured using sutures (8).

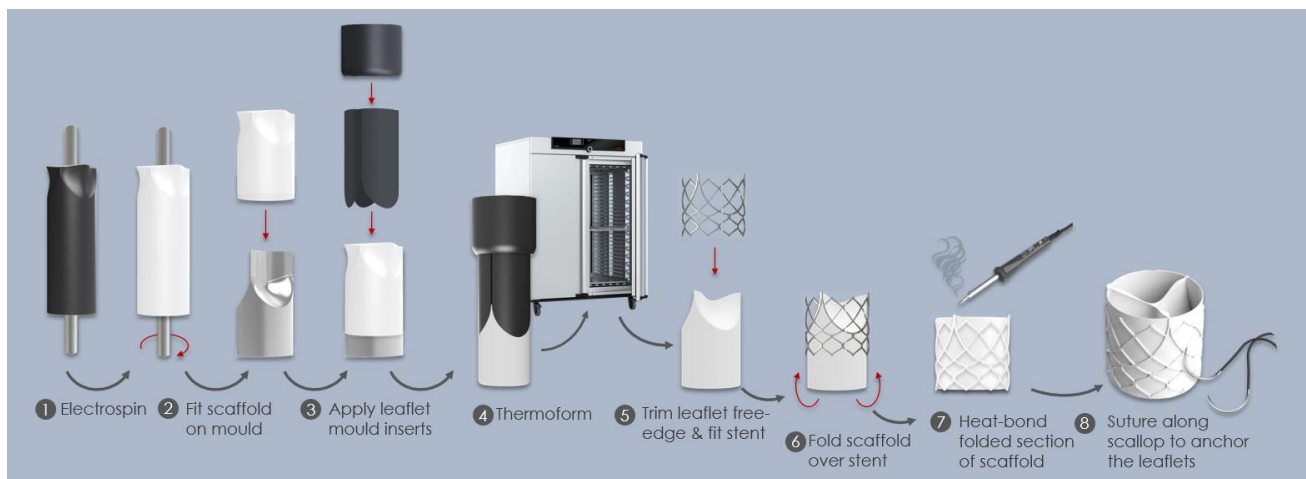


Figure 3.26: Illustration of the sequence of operations proposed in Concept 5

3.3.2.6 Concept 6: Trileaflet submould assembly process

Concept 5 presents the preform submould assembly procedure where leaflet-shaped submoulds shall be electrospun individually (1), followed by assembling the submoulds (2) and electrospinning the assembly as a whole (3). Thereafter, a stent shall be crimped onto the collector (4), and the construct shall be electrospun as a whole to form the outer layer of the skirt (5). Finally, the submoulds shall be removed from the scaffold construct, and excess material shall be trimmed, especially along the free-edges of the leaflets (6).

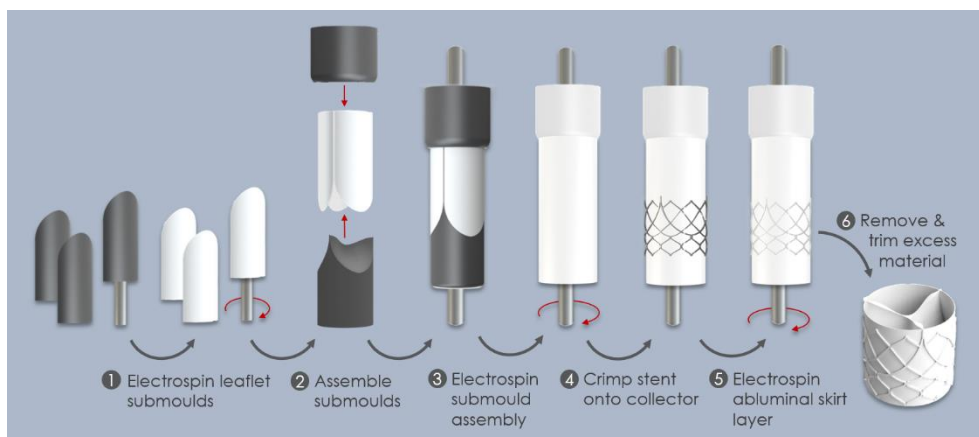


Figure 3.27: Illustration of the sequence of operations proposed in Concept 6

3.4 Concept prototyping

Prototyping is considered one of the most integral phases of concept exploration in engineering design³³⁸. In general, prototypes are crude mock-ups modelled from accessible materials that can be easily assembled for early assessments. For this study, the goal was to translate ideated solutions into rudimentary physical or working forms to assess the core feasibility of each concept. Through prototype evaluation and screening, the inherently flawed concepts could be easily identified and subsequently eliminated from further investigation. As such, only the most promising concepts underwent more comprehensive assessment.

3.4.1 Concept 1

Electrospun tubes were spun using the cylindrical collector (Figure 3.28A) and cut into segments to form the skirts. The skirts were effectively bonded to the stent using the technique described in section 3.2.3.1 and excess material was trimmed before attaching the leaflets (Figure 3.27BC). The pre-cut leaflets were fabricated using the die-cutting method described in section 3.2.1.3 (Figure 3.27D), and sutured to the stent using the technique described in section 3.2.3.2 (Figure 3.27E). With this approach, a trileaflet valve structure was successfully configured (Figure 3.27F), which from initial observations, appeared to be structurally robust.

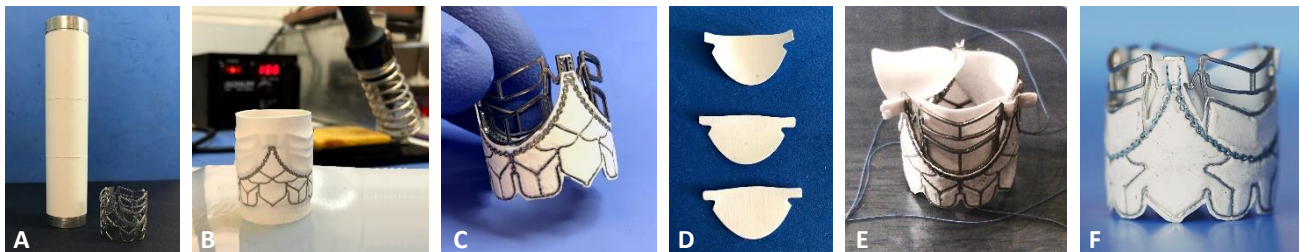


Figure 3.28: Pre-cut leaflet suturing process. A) Electrospun scaffold on collector and stent. B) Bonding first skirt layer. C) Bonded and trimmed skirt. D) Pre-cut leaflets. E) Leaflet suturing. F) Complete valve prototype.

3.4.2 Concept 2

An electrospun skirt and leaflet materials were fabricated using the same procedure as concept 1. The electrospun skirt (shown in Figure 3.29A) was attached to the stent using the same heat bonding technique applied in concept 1 (Figure 3.29CD). Thereafter, the pre-cut leaflets were heat bonded to the stent along the scallop and commissure posts using the technique described in section 3.2.3.2. As can be seen in Figure 3.29E, a trileaflet valve structure was successfully constructed using this procedure.

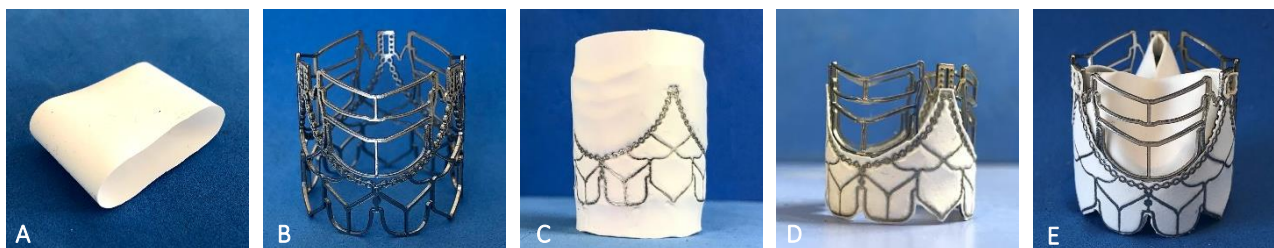


Figure 3.29: Pre-cut leaflet heat bonding process. A) Scaffold tube segment. B) Pre-coated stent. C) Skirt heat bonding. D) Bonded and trimmed skirt. E) Complete valve prototype with heat bonded leaflets.

3.4.3 Concept 3

To perform the sandwich-spinning process, the first scaffold layer was electrospun using a cylindrical collector, after which a stent was mounted onto the collector (Figure 3.30AB). For rapid prototyping, instead of crimping the stent onto the collector to prevent relative displacement during spinning, a section of the underlying scaffold was folded over the lower end of the stent, forming an alternative mechanism of attachment between the stent and collector (Figure 3.30B). The assembly was then electrospun to produce a sandwich-like structure with the stent enveloped between two scaffold layers that appeared to fuse at interface. To form the leaflets, the top section of the scaffold was folded inward and secured along the scallop by heat bonding. As shown in Figure 3.30DE, this procedure was effective in producing a trileaflet valve structure, however, based on initial observations, the valve appeared to be considerably more bulky than concept 1 and 2, and the free-edge of the leaflets were highly irregular.

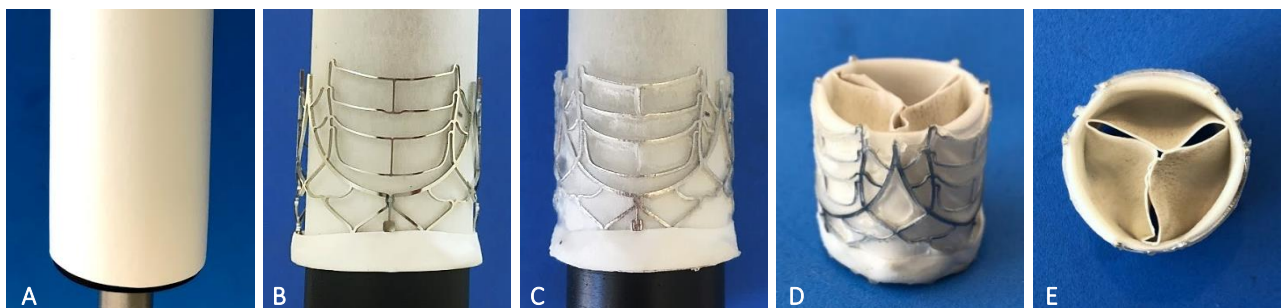


Figure 3.30: Sandwich-spinning investigation. A) Electrospun scaffold on cylindrical collector. B) Stent mounted onto the collector. C) Stent encapsulated by electrospun fibers forming the outer layer of the skirt. D--E) Leaflets folded and bonded to the stent.

3.4.4 Concept 4

The process of electrospinning directly onto a trileaflet-shaped collector to produce a complete electrospun trileaflet structure was attempted. However, as can be seen in Figure 3.31A, the experiments were ultimately unsuccessful. As shown in Figure 3.31B, fiber landing was not confined to the collector, but rather extended across the length of translating unit to form a dense web-like structure that spanned between the free-edge of the collector and frame endwall. Due to the rotation of the collector, the scaffold at the free-edge demonstrated excessive twisting, which led to tearing of the scaffold along the leaflet margin (Figure 3.31D).

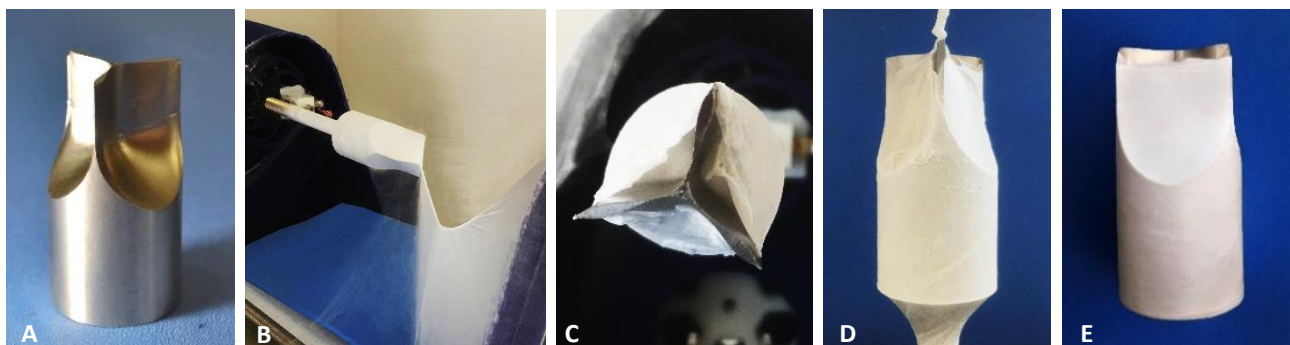


Figure 3.31: Trileaflet preform spinning. A) Metal trileaflet-HV mould mounted to mandrel axis. B) Cantilever electrospinning system. C) Significant spanning of electrospun scaffolds across cusp geometry. D--E) Significant spanning of electrospun scaffolds across cusp geometry.

Moreover, instead of conforming to the surface geometry of the leaflets, fibers were found to span across the concave features and deposit unevenly on the commissures. Several attempts were made to avert these issues by applying different processing parameters, but none were successful. Similar observations have been reported in studies that investigate this approach to constructing electrospun THVs. To overcome these issues, various adaptations to this approach have been developed by different research groups, such as double-component deposition and automated rotary jet spinning. However, to explore these strategies, fundamental modifications would need to be made to the electrospinning system in use, which was unfeasible for this project. To this end, this approach was determined to be ineffective and was subsequently eliminated from further investigation.

3.4.5 Concept 5

A smooth, uniform scaffold was produced by electrospinning onto the inverted trileaflet collector, as shown in Figure 3.32A. The scaffold was then removed from the collector and fit onto a metal trileaflet-shaped mould which served as the positive mould for the thermoforming process. The negative mould was fabricated from silicone using a casting method. Prior to applying the silicone mould, the scaffold was positioned so that the commissure and scallop features were aligned with those of the positive mould. In doing so, the shape of the preform leaflets mirrored those of the mould, as shown in Figure 3.32C. To revert the inverted leaflets into a closed position, the silicone mould was applied to the scaffold and secured before placing in an oven at 60°C for 10-15 minutes. Upon removing the moulds, the scaffold revealed to retain the set shape (Figure 3.32EF). Once shaped, the scaffold was sutured to the stent along the scallops and commissures. The suturing procedure was adapted from the protocol described in section 3.2.3.2 to accommodate the different structure of the leaflets compared to the pre-cut leaflets. To form the skirt, the lower section of the scaffold preform (which extended past the bottom of the stent) was fold outward to fit over the stent. The skirt was temporarily fixed to the stent using sutures and an improvised clipping mechanism incorporating a feature on the stent.

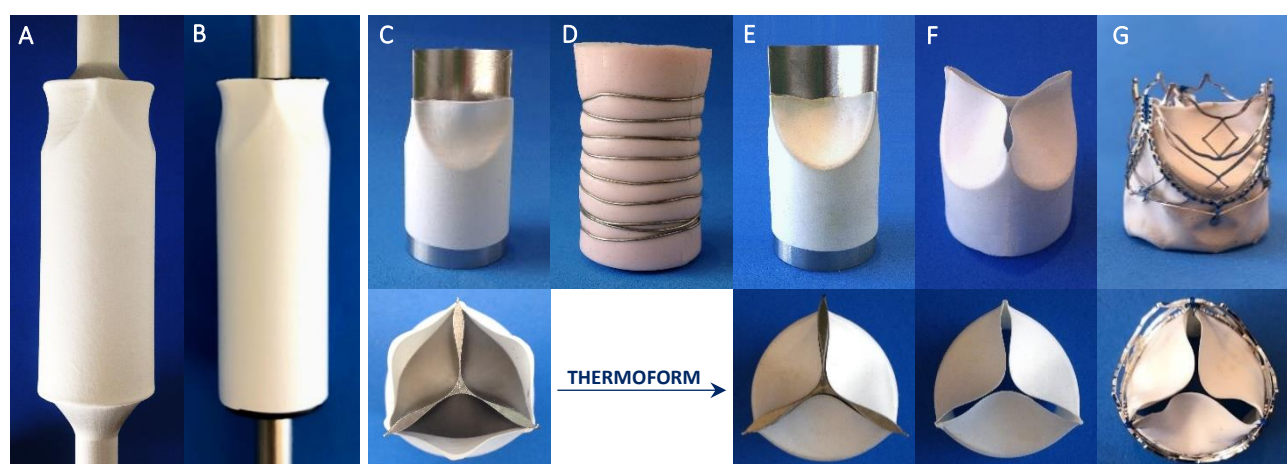


Figure 3.32: Inverted leaflet thermoforming and suture attachment procedure. A) A scaffold was electrospun onto the inverted trileaflet collector, and (B) cut along either end prior to removal. C) The scaffold preform was fit onto a metal trileaflet mould (positive), (D) pressed against a silicone mould (negative) and placed in an oven at 60°C for 10 minutes. E) Removing the silicone mould revealed that the leaflets had set into the appropriate shape, which (F) retained its shape when removing the metal mould. G) Thereafter, the scaffold was sutured to the stent to form a transcatheter trileaflet valve.

3.4.6 Concept 6

The electrospinning rig was modified into a cantilever system to electrospin the leaflet-shaped submoulds. Numerous attempts were made using a range of different processing parameters to electrospin onto the submoulds and obtain uniform fiber deposition across the collector, however none were successful. In general, the scaffolds were significantly thicker along the edges of the collector and exhibited highly irregular layering profiles, as is notable in Figure 3.35CD. Furthermore, scaffolds were prone to splitting upon removal from the collector (see Figure 3.35E). For these reasons, and due to time constraints for the rapid prototyping and system optimisation phase, this concept was regarded unfeasible for application in this study, and was subsequently eliminated from further investigation.

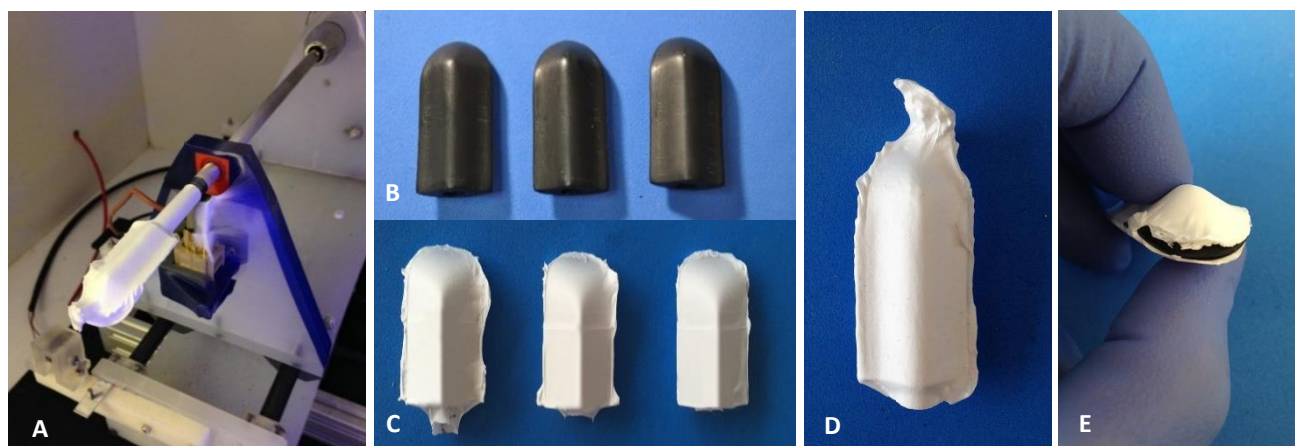


Figure 3.33: Trileaflet-submould assembly components and outcomes. A) Modified electrospinning rig setup. B) 3D printed leaflet-shaped submould collectors. C-D) Electrospun leaflet submould showing nonuniform scaffold layering specifically along the edges. E) Scaffold split along edge upon removal.

3.5 Concept evaluation

3.5.1 Prototype evaluation methods

Qualitative evaluation

Qualitative evaluations of the prototypes involved visual and physical inspections as well as weighing up the practical aspects of the manufacturing process based on insights gained from performing the process and findings reported in studies that explore similar manufacturing processes.

Quantitative evaluation

Based on qualitative evaluations, the valve prototypes that appeared to be feasible for functional assessment were subject to hydrodynamic testing. All pertinent information on the hydrodynamic assessment procedure is described in detail in the following chapter (Chapter 4), which focuses specifically on valve performance testing and evaluation. Briefly, the valve prototype was operated under conditions representative of a normotensive aortic environment, in compliance with ISO 5840 (peak differential pressure = 100 mmHg, cardiac output (CO) = 5 l/min, and heart rate = 70 bpm).

3.5.2 Prototype evaluations

3.5.2.1 Concept 1

The sutured valve prototype was tested in a pulse duplicator system to assess its functionality, quantify its hydrodynamic properties, and thereby determine the efficacy of the manufacturing procedure. As shown in Figure 3.34AB, the valve demonstrated good opening and closing behaviour; excellent hydrodynamic parameters, with a mean pressure gradient (ΔP) of 6.7 mmHg, regurgitant fraction of 16.1%, and EOA of 1.85 cm²; and withstood the applied pulsatile loading regime without suffering any notable structural or mechanical damage (see Figure 3.34CD). Due to these favourable outcomes, the concept proved to be a promising candidate for further development and more thorough evaluation.

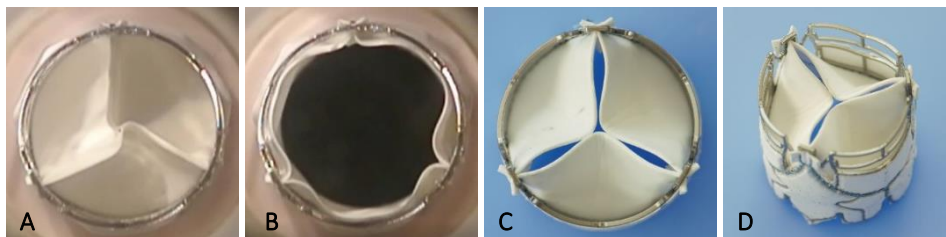


Figure 3.34: Hydrodynamic performance testing. A-B) Closed and open position during pulsatile testing. C-D) Trileaflet structure intact after hydrodynamic testing.

3.5.2.2 Concept 2

In terms of manufacturing, the heat bonding process was found to be more time efficient and less manually intricate than the suturing technique. However, in contrast to the impressive hydrodynamic performance demonstrated by the sutured valve, the heat bonded valves suffered complete structural collapse during hydrodynamic testing due to acute bond failure along the scallops (see Figure 3.35). This was considered to be a result of suboptimal precoating thickness on the luminal surface of the stent.



Figure 3.35: Hydrodynamic performance testing and failure mode inspection

The quality and strength of thermal bonds are known to be highly dependent on the thickness of the precoating. Studies have shown that bond strength increases with an increase in precoating thickness, until an optimum thickness is reached, beyond which the bond strength decreases^{335,339}. On this account, when the thickness of the precoating layer varies across the surface of a component, disparities in bond strength between different regions of the component will arise. This, in turn, would culminate in an uneven distribution of stress across the precoating layer and joint surfaces, rendering these aspects vulnerable to cracking, peeling, or debonding³⁴⁰. To this end, the main limitation with this method was that to achieve sufficiently strong and consistent bonds throughout the construct, the thickness of the precoating layer would need to be uniform across the entire stent, and particularly on the luminal surface. For this, a more sophisticated, automated

precoating system would need be employed, which due to manufacturing resource constraints, was not feasible for this study. For these reasons, this concept was regarded as unsuitable for further investigation in this study.

3.5.2.3 Concept 3

Although the sandwich-spinning approach was effective in encapsulating the stent between two adjoining scaffolds to form the sealing skirt, the interlayer bond strength between the cohered scaffold surfaces proved to be mechanically insufficient. This was assessed by manually peeling the scaffolds apart, which required minimal force to separate the layers, suggesting low resistance to delamination under operational loads. Furthermore, instead of tightly encapsulating the stent struts, fibers exhibited slight spanning across the strut edges. This indicated that scaffold layers had not completely cohered along the strut edges, rendering the interfacial bonds along these regions vulnerable to delamination. In addition, this approach relies on the stent to be sandwiched between two layers, which introduces a limitation resulting in an inherently bulky sealing skirt which is an unfavourable attribute for transcatheter delivery and counters delivery profile optimisation. Moreover, by covering the whole stent with material, there will be an increased risk of coronary occlusion³⁴¹. A number of studies have investigated this technique for fabricating transcatheter TEHV, of which most reported similar suboptimal outcomes, such as incomplete bonding, insufficient interlayer bond strength, and low resistance to delamination^{342,343}. Based on the inherent limitations associated with this approach, this concept proved to be an inadequate solution, and was subsequently eliminated from further investigation.

3.5.2.4 Concept 5

As shown in Figure 3.36B, the electrospun trileaflet scaffold obtained from thermoforming the inverted trileaflet preform exhibited a remarkable improvement in leaflet symmetry and thickness uniformity, compared to the preform obtained from electrospinning directly onto the trileaflet-shaped collector. Thus, it can be said that the thermoforming technique was successful in producing a trileaflet structure composed of uniform leaflets that retained a neutral closed position. However, attaching the scaffold to the stent using sutures was found to be problematic, specifically along the commissure posts. As the commissures of the scaffold comprised of a single sharp edge, a limited area of material was available for manipulating the commissures into the correct position and suturing the commissures to the stent posts (Figure 3.36DE). As a result, the commissure hinge features obtained using this technique were significantly distorted, nonsymmetric, and tore after applying pressure to the leaflets during initial inspections. To overcome this issue, an alternative method of attachment would need to be investigated. Leaflet heat bonding was considered as a potential alternative to suturing for this approach, but after obtaining adverse outcomes from the heat bonded valve prototype, it was not pursued further.

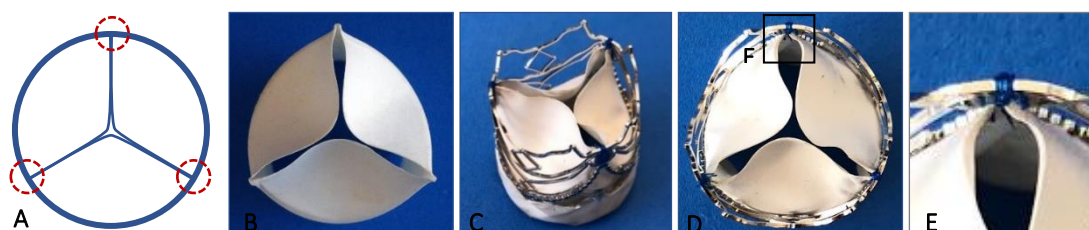


Figure 3.36: Qualitative analysis of concept 5 prototype. A) Schematic showing common stress concentration sites for trileaflet heart valve prosthesis. B) Trileaflet scaffold preform prior to suturing to stent. C-D) Image of valve prototype showing problematic commissure hinge fixations. E) Region likely to fail from point-mass loading at the commissure hinge due to the fixation technique.

3.5.3 Concept screening

A concept selection matrix was created to screen and compare prototypes concepts. The selection criteria were based on project-specific objectives. The concepts were rated on a scale between 3–0, where 3 = high, 2 = moderate, 1 = low, 0 = very low.

Table 3.4: Concept screening matrix

Production process		Concept 1	Concept 2	Concept 3	Concept 4	Concept 5	Concept 6
Collector geometry		Cylindrical	Cylindrical	Cylindrical	Trileaflet	Inverted trileaflet	Submould assembly
Leaflet production		Die-cut	Die-cut	Tube shaping	Direct preform	Thermoformed preform	Submould preform
Skirt attachment method		Heat bond	Heat bond	Interface fusion	Heat bond	Heat bond	Interface fusion
Leaflet attachment		Suture	Heat bond	Heat bond	Suture	Suture	Interface fusion
General criteria	Impact						
Controllability	+	3	3	2	0	2	0
Scaffold uniformity							
<i>Skirt</i>	+	3	3	1	1	3	0
<i>Leaflets</i>	+	3	3	3	0	2	0
Attachment integrity							
<i>Skirt</i>	+	3	3	0	NA	3	NA
<i>Leaflets</i>	+	3	1	1	NA	1	NA
Reproducibility	+	3	2	2	0	2	0
Automation potential	+	0	1	1	2	0	1
Time efficiency	+	1	1	2	1	1	1
Labour intensity	–	3	2	1	1	2	2
Skilled labour	–	3	2	2	2	3	2
Touch labour	–	3	3	3	2	3	2
Manufacturing complexity	–	2	2	2	2	2	2
Total		8	8	4	-3	4	-6
Relative Feasibility		High	High	Low	Low	Low	Lowest
Qualitative screening		✓	✓	✗	✗	✗	✗
Hydrodynamic assessment		✓	✗				
Most feasible concept		✓					

3.6 Concept selection

Based on outcomes from the prototype analyses and concept selection matrix, the most promising concept proved to be Method 1, defined as the pre-cut leaflet suturing method. In support of this selection, the efficacy of employing sutures to manufacture tissue engineered heart valves has been widely reported in literature^{132,203,344}, and owed to its long history of application in the medical field, it was an attractive technique to explore in this study.

4 Valve Evaluation

4.1 Introduction

4.1.1 Regulatory guidelines for evaluating tissue engineered heart valves

The regulatory guidelines that outline mandatory evaluation procedures for transcatheter heart valve (THV) substitute development are defined by the International Organisation for Standardisation (ISO) in Standard 5840-3³⁴⁵. The standard was recently published with an updated set of operational and performance requirements for cardiac valve substitutes³⁴⁶, which were adhered to for the *in vitro* hydrodynamic performance testing conducted in this project.

The evaluation protocols and minimum performance outcomes required for THV substitutes to achieve ISO compliance is naturally extensive and rigorous. Central to THV device evaluation is the assessment of functionality, biocompatibility, thrombogenicity, calcification susceptibility, crimping and deployment integrity, and long-term durability³⁴⁷. However, due to these specifications pertaining exclusively to non-degradable materials for long-term implantation, the performance outcomes pertinent to HVTE strategies are precluded³⁴⁸. Consequently, ISO standards remain largely insufficient for the substantially more intricate, multifactorial evaluation of TEHVs.

To date, no universal regulatory regime exists for the assessment of TEHVs in both preclinical and clinical contexts¹²⁸. For this reason, it has been widely adopted as common practise to supplement standard ISO evaluations for cardiac valve prostheses with augmented protocols that encompass tissue engineering criteria³⁴⁸. However, the methods and regulatory guidelines employed for TEHV evaluation remain largely uncoordinated among research groups across the globe, rendering TEHV evaluation unstandardised and vulnerable to subjectivity¹²⁸.

4.1.2 Valve functionality testing

Pulsatile testing facilitates a practical evaluation of the functional performance of heart valve substitutes by subjecting them to hydrodynamic conditions that simulate the native physiological environment³⁴⁹. For THVs, this assessment achieves a quantitative characterisation of the valve's flow and pressure behaviour in the deployed state³⁴⁷. Traditionally, these assessments are conducted with Pulse Duplicators (PDs) following protocols outlined by ISO. Standard ISO conformant PDs typically comprise of a left ventricle, atrium, and aortic chamber, each featuring pressure transducer ports; a functional mitral valve; and a silicon rubber annular seal mounted in a synthetic aortic root conduit for installing the test valve (illustrated Figure 4.1).

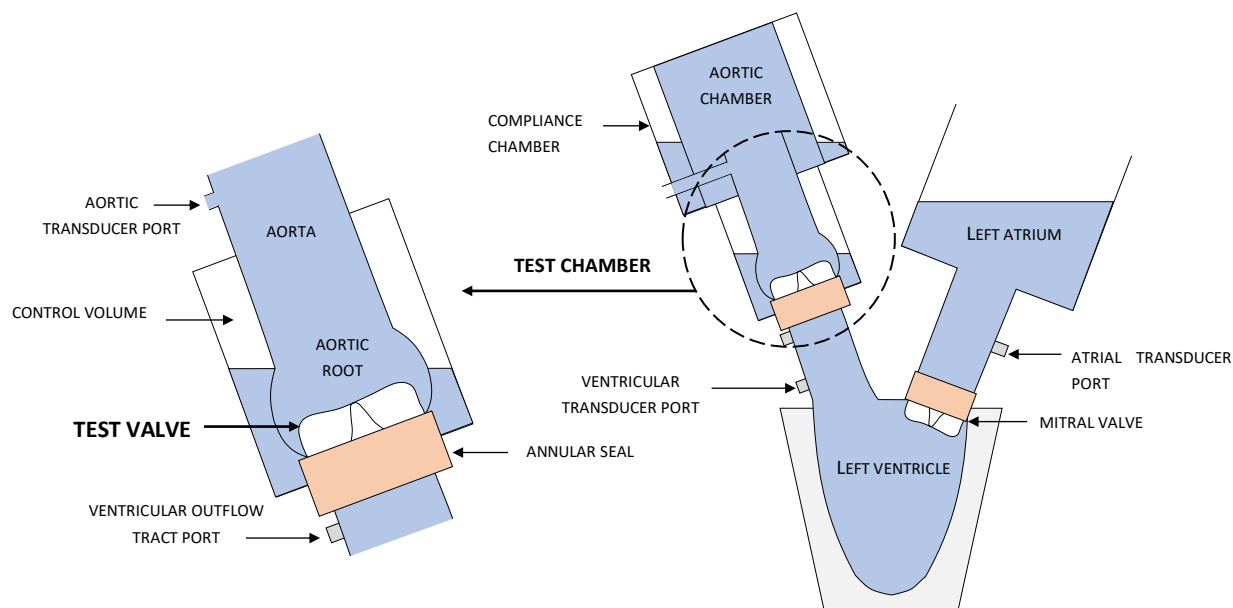


Figure 4.1: Schematic representation of pulse duplicator system.

4.1.2.1 Valve hydrodynamics

Typical hydrodynamic performance parameters reported for THV evaluations include mean transvalvular pressure gradient (ΔP), regurgitant fraction (RF), effective orifice area (EOA), and transvalvular energy losses³⁴⁷.

Mean pressure gradient

In general, pressure gradient (or pressure drop) in a fluid stream is defined as the pressure difference between two points where the downstream pressure is relatively higher than the upstream pressure³⁵⁰. This can be mathematically described by the Bernoulli equation, previously discussed in Chapter 3. With respect to the heart, positive pressure drops result from sudden fluctuations in chamber volume which drives the opening behaviour of heart valves and facilitates transvalvular fluid flow. Similarly, the transvalvular pressure gradient is defined as the pressure drop across the outflow and inflow points of the valve during forward flow.

The natural pressure drop phenomenon is disrupted when a pressure overload in the outflow region develops. Extreme or unstable pressure drops significantly affect the flow behaviour of the cardiac cycle and impact leaflet integrity. An abnormal pressure gradient can be indicative of valvular dysfunction, such as stenosis, and would likely manifest as cumulative regurgitation and subsequent progressive heart failure³⁵⁰. Evaluating the pressure drop is thus essential to establishing the stenotic potential and functional integrity of heart valve substitutes.

Regurgitant fraction

The regurgitant volume refers to the total volume of fluid that flows in the reverse direction through the valve during one cycle. It equates to the sum of the closing and leakage volumes (illustrated in Figure 4.2) and is generally expressed as a percentage of the forward flow volume known as the regurgitant fraction (RF). The closing volume is indicative of the valve dynamics during closure in a single cycle. It can be described as the volume of fluid required to close the valve, and which is governed by primarily by the geometry and flexural

properties of the configured leaflets. The leakage volume is defined as the amount of fluid that leaks through the valve during the closing phase and is the summation of the transvalvular and paravalvular leakage volumes.

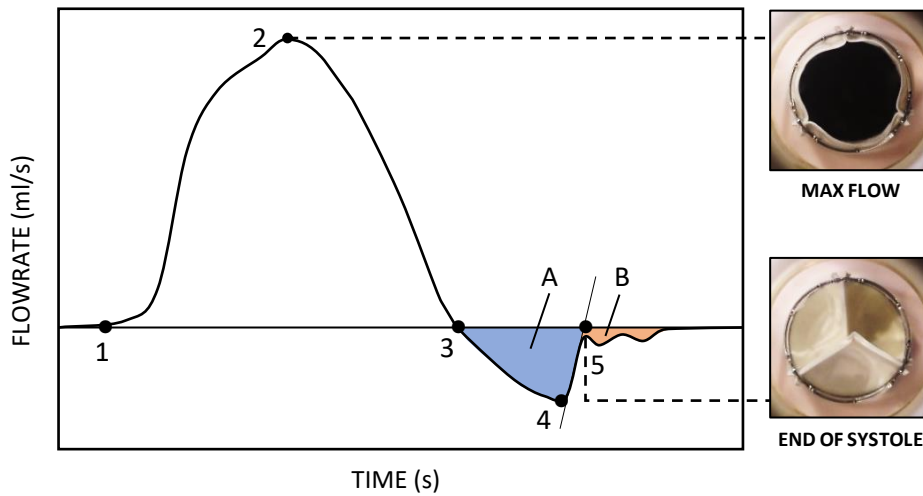


Figure 4.2: Typical pulsatile flow waveform over one cardiac cycle. Primary flow characteristics include: 1) start of forward flow, 2) peak forward flow, 3) end of forward flow, 4) peak reverse flow, 5) end of closing flow (a linear extrapolation of the highest slope is used in cases where no zero crossing is exhibited). The total regurgitant volume, indicated by the area of reverse flow curve, is comprised of (A) closing volume and (B) leakage volume for a single cycle.

Effective orifice area

The effective orifice area (EOA) is an estimation of the opening area of a valve during peak systole and is derived from the valves flow and pressure characteristics³⁴⁷. Conventionally, EOA is computed using a simplified Bernoulli equation known as the Gorlin formula, which was formulated to approximate the hydraulic area of cardiac valves³⁵¹ and was subsequently revised to give a generalised formula defines as:

$$EOA = \frac{Q_{RMS}}{51.6 \sqrt{\frac{\Delta P}{\rho}}} \quad (14)$$

where Q_{RMS} is the root mean square volumetric forward flow (ml/s), ΔP is the mean pressure drop (mmHg) over the positive differential pressure period, and ρ is the test fluid density (g/cm³).

4.1.2.2 Leaflet kinematics

Geometric Orifice Area

The geometric orifice area (GOA) is defined as the area between the free edges of the valve leaflets in the open position³⁴⁹. It is a measure of the opening capacity of the leaflet configuration and quantifies the area theoretically available for forward flow³⁵². Moreover, the evolution of the GOA over a pulse cycle has been used as metric for the evaluation of valve kinematics³⁵³. The GOA is also used to determine the valve's coefficient of orifice contraction, calculated as the ratio of EOA to GOA. Typical contraction coefficients vary between 0.7 and 0.9, with the optimal value of highest contraction being 1. Small contraction coefficients have been associated with elevated peak pressure gradients, increased energy losses due to flow turbulence around the leaflets, and reduced pressure recovery³⁵⁴. The contraction coefficient is therefore indicative of valve function efficacy and can be improved through leaflet geometry optimisation³⁵⁴.

Pinwheeling Index

Pinwheeling refers to the twisting action of leaflet free-edges during valve closure due to redundant leaflet material that convolutes in the coaptation region³⁴⁵. When leaflets are bent to excessively high curvatures during pinwheeling, the individual fibers within the leaflet material experience excessive stresses which manifest as fiber breakage and local degradation³⁰⁸. Localised bending, also known as buckling, correlates closely to premature degradation of leaflet materials³⁰⁸. For this reason, pinwheeling is undesirable and should be minimised.

4.1.3 Valve durability testing

Accelerated wear testing is widely considered the gold standard for evaluating *in vitro* durability of heart valve constructs. The continuous subjection of repetitive cyclic stress in the form of strain, flexure and shear *in vivo* is known to initiate mechanical degradation modes such as buckling and delamination of layered leaflet structures^{53,355}. This in turn leads to leaflet calcification, progressive deterioration, and early valve dysfunction and failure³⁰⁸. The assessment of mechanical fatigue is thus essential to characterising the long-term structural and mechanical properties of valvular constructs.

In the context of heart valve tissue engineering, defining the fatigue characteristics of valvular constructs is considerably more challenging due to the highly complex, unpredictable, and inherently variable interplay between scaffold degradation and tissue formation over time³⁵⁶. To this end, great disparity exists between the limited number of studies that report on the *in vivo* fatigue properties of TEHV constructs¹³¹. Moreover, investigations on the *in vivo* application of TEHVs have been largely unsuccessful and yielded perplexing results that challenge long-standing scientific conventions¹³². For this reason, evaluating the *in vitro* fatigue behaviour of TEHV constructs is imperative to valve development, and a necessary prerequisite to conducting *in vivo* investigations.

While accelerated fatigue assessments are elemental to TEHV development, it is important to consider that the high-frequency pulsatile loading induced during wear testing is not physiologic. This is especially significant for flexible material structures that demonstrate viscoelastic behaviour. The strain rate dependence of viscoelastic materials renders them vulnerable to excessive deformation under high loading rates³⁵⁷. Consequently, the wear mechanisms sustained during fatigue testing are greatly exacerbated and depend largely on the pulse frequency conditions. Furthermore, recent reviews on HVTE applications have proposed that fatigue outcomes from accelerated testing of TEHV constructs are diagnostically irrelevant³⁵⁸. This notion is based on the fact that cell behaviour is highly sensitive to the conditioning environment, which at high-frequency stimulation over prolonged periods, would greatly influence cellular activity and potentially impede the growth and remodelling of functional tissues³⁵⁸.

4.2 Materials and Methods

The valve evaluation procedures performed for this study were derived from relevant experimental studies^{194,213,344,347} and cross-referenced with ISO guidelines³⁴⁵. Overall, four main aspects of valve development were addressed, including (1) valve production; (2) valve hydrodynamics assessment; (3) valve kinematics analysis; and (4) valve durability testing.

4.2.1 Valve manufacturing

4.2.1.1 Leaflet scaffold fabrication

A total of 25 valves were fabricated for this study, of which 10 comprised of electrospun PEL leaflets (PEL valves) and 15 comprised of electrospun DP leaflets (DP valves). PEL valve leaflets were fabricated using the electrospinning parameters given in Table 4.1. Two sets of DP valve leaflets were produced over the course of scaffold development. The first set (DPVI) was produced in the early phase of scaffold development with initial electrospinning process parameters, while the second set (DPVII) was produced in the final stage of development with optimised parameters. The electrospinning parameters corresponding to each scaffold set of are summarised in Table 4.1. Operating protocols described in section 3.3.1 were employed for the electrospinning procedure.

Table 4.1: Electrospinning parameters employed for fabrication of PEL and DP valve scaffold materials

Set	wt%	Needle (G)	RH (%)	v_{rot} (rpm)	FR (ml/hr)	ΔV (kV)	TCD (cm)	n
PELV	16	19	45	1000	3	20	28	10
DPVI	18	19	40	1000	2	25	35	3
DPVII	15	19	35	1000	1	28	28	11

SC – solution concentration; RH, v_{rot} – rotational speed; FR – flowrate; TCD – tip-to-collector distance

4.2.1.2 Leaflet scaffold characterisation

In the absence of concrete specifications for TEHV scaffold design, evaluating the suitability of scaffold properties for their application as leaflet materials required a study specific approach³⁵⁹. The aim of this investigation was to establish suitable scaffold parameters for PEL and DP valve leaflets that demonstrate good hydrodynamic function and sufficient durability under pulsatile loading conditions. The parameters selected for leaflet scaffold characterisations included (1) scaffold microstructure, (2) mechanical properties, (3) hydraulic properties, and (4) leaflet thickness.

Microstructural, mechanical, and hydraulic characterisation

The microstructural (fiber diameter, pore size, porosity, and orientation) and mechanical properties (UTS, elastic modulus, percent elongation, and mechanical anisotropy) of the respective scaffold groups were quantified using the methods described in section 3.3.3 and 3.3.4, respectively. Hydraulic properties, including surface wettability and scaffold permeability, were characterised using the methods described in section 2.1.3.5

and 2.1.3.6, respectively. Where applicable, the correlation between scaffold parameters and valve performance was qualitatively or quantitatively analysed.

Leaflet thickness measurement

The average leaflet thickness for each valve was determined by measuring the scaffold thickness at twelve points across each of the three leaflets using a digital thickness gauge (Mitutoyo Corporation, Japan) (Figure 4.3). For each PEL and DP electrospinning experiment the spinning time, solution volume, and resultant scaffold thickness were recorded and used to derive a formula for predicting the resultant leaflet thickness as a function of flow rate and solution volume. With this, predefined leaflet thicknesses could be obtained by tuning the respective parameter variables relative to the flow rate. Four groups of PEL valves were fabricated with average leaflet thicknesses of 300 ($n = 2$), 420 ($n = 3$), 540 ($n = 3$), and 630 μm ($n = 2$). For the DP valves, the first set of DPV was manufactured with an average leaflet thickness of 480 μm ($n = 3$); and for the second set, four groups with averages of 340 ($n = 2$), 480 ($n = 3$), 620 ($n = 3$) and 720 μm ($n = 3$) were produced.

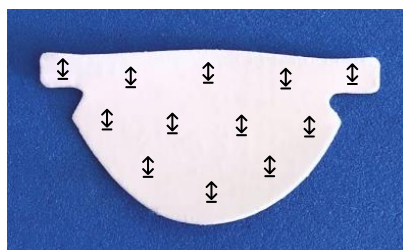


Figure 4.3: Leaflet thickness measurement points.

4.2.1.3 Valve manufacturing

All the valves were constructed using the same technique, as previously described in Section 4.3. In summary, the valves were comprised of three primary components, including a CoCr stent, an electrospun paravalvular sealing skirt, and electrospun leaflets. The sealing skirts for all the valves were fabricated from electrospun PEL tubes which were fitted onto the stents and integrated by heat-bonding. Subsequently, either electrospun PEL or DP scaffolds were cut into leaflets with a die cutter and joined to the stent using a suturing technique. All electrospinning, bonding, and suturing procedures involved in valve production were performed by the same person for all the valves to minimise manufacturing variability.



Figure 4.4: Sample batch of DP valves.

4.2.2 Valve functionality testing

4.2.2.1 Equipment setup and data acquisition

Hydrodynamic tests were performed using the ViVitro Labs Pulse Duplicator System (ViVitro Labs Inc., BC, Canada) in compliance with ISO 5840-3 Annex N ³⁴⁵. A summary of the operating procedure conducted for each test is described in Appendix C. The raw data acquired from each test was imported into an Excel® template whereby key performance parameters, including the mean transvalvular pressure gradient (ΔP), effective orifice area (EOA), regurgitation fraction (RF), and transvalvular energy losses (E_{loss}), were extracted for analysis.

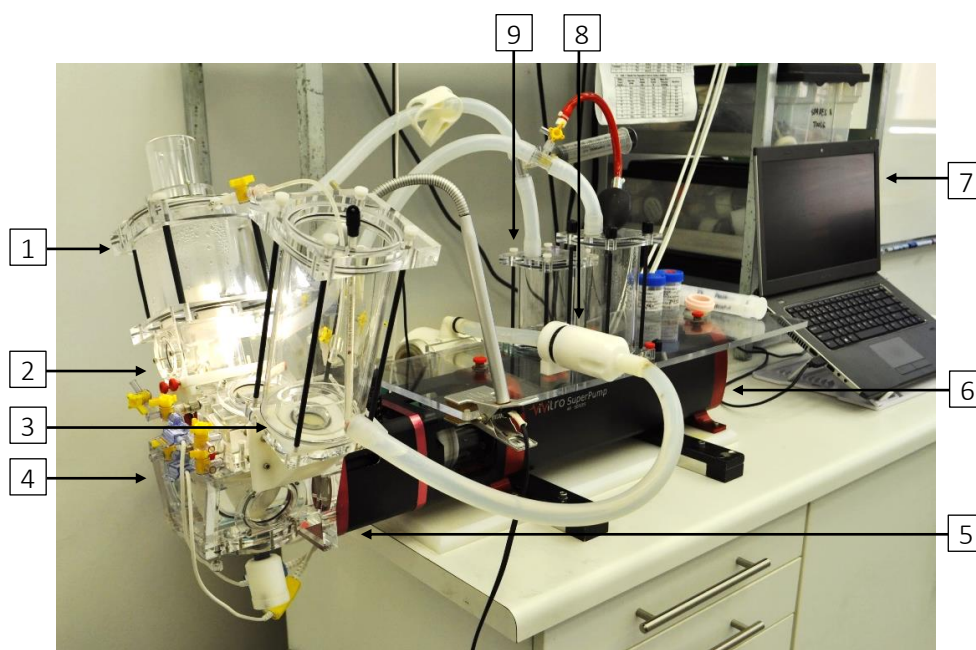


Figure 4.5: ViVitro Pulse Duplicator System. component assembly including: 1) aortic chamber, 2) aortic standoff, 3) atrium chamber, 4) ventricular chamber, 5) viscoelastic impedance adapter, 6) SuperPump, 7) computer, 8) peripheral resistance controller, and 9) compliance chambers. Below: Still series captured from video footage displaying the opening and closing behaviour of DP valve.

4.2.2.2 Operating environment feasibility assessment

Hydrodynamic test trials were conducted for the PEL and DP valves to evaluate their respective loading capacities and thereby establish feasible operating parameters for each category ($n = 1$ per valve type). The valves were developed with the aim of functioning as aortic THVs so were initially subjected to aortic normotensive pressure and flow conditions. The PEL valves exhibited good functionality, synchronous leaflet opening, complete coaptation, and proved to withstand hydrodynamic loading at both cardiac outputs of 3.5 and 5 l/min for the entire test duration (30 minutes). Conversely, the DP valve leaflets suffered acute rupture at the commissures within 10 minutes of dynamic loading under simulated aortic conditions, which subsequently led to total valve failure. On this account, the DP valves were considered more suitable for study under the milder conditions equivalent to the pulmonary environment, whereas the PEL valves were deemed sufficient for assessment under simulated aortic conditions.

The final operating parameters employed for the respective tests are summarised in Table 4.2. As the two valve types were operated at distinctly different pulsatile flow conditions, the hydrodynamic parameters were not comparable between the two categories. For this reason, the focus of this study was adapted to instead investigate the relative effect of leaflet thickness on the hydrodynamic functionality and durability of the respective valve types.

Table 4.2: Operational environment specifications for hydrodynamic valve performance tests

Parameter	Condition		
Test fluid	Isotonic saline solution (0.9% NaCl)		
Temperature	37°C		
Cycle rate	70 bpm		
Cardiac output	3.5 l/min and 5 l/min		
Systolic duration	35%		
Resultant pressure loads	<i>Peak systolic</i>	<i>End diastolic</i>	<i>Mean transvalvular</i>
Normotensive aortic (mmHg)	120	80	100
Normotensive pulmonary (mmHg)	18 – 35	8 – 15	13 – 25

4.2.3 Valve kinematics evaluation

4.2.3.1 Visual Documentation

High-speed videos were recorded during pulse duplicator testing to visually document the motion of the leaflets under dynamic loading. A high-speed digital camera (Nikon 1 J1 Camera, AF-S DX Micro NIKKOR f/2.8G lens) was employed to obtain real-time footage (1920 x 1080/60 fps) and slow-motion capture (640 x 240/400 fps). The footage was analysed both qualitatively and quantitatively to define the opening and closing characteristics of the leaflets. Qualitative analysis was performed by capturing still images at 7 key timepoints over the recording of a single cardiac cycle. The still images were compiled to provide visual insight into the general behaviour of the leaflets during pulsatile flow testing. With this, notable distinctions in the valve's open and closing behaviour, such as asynchronous opening, coaptation overlap, immobilised leaflets and leaflet fluttering, could be identified. Quantitative analysis of leaflet kinematics involved computation of the geometric orifice area (GOA) and pinwheeling index (PI). First-hand observations were also made during the tests and documented as references for analytical discussion.

4.2.3.2 Geometric Orifice Area

Video Processing

An open-source video editing software (Avidemux 2.6.1) was used to transcode the footage from .MOV to .AVI file format to achieve compatibility with ImageJ for further processing. Videos were split into segments that captured valve dynamics over 10-12 cardiac cycles for mean computation. For processing with ImageJ, videos were first transformed to a virtual stack (0.03 s per frame) and reduced to greyscale, prior to being cropped and cleared around the outer diameter of the stent. The measurement scale was set by drawing a line across the external diameter of the stent and equating the measured pixel distance to the known stent diameter

(~23mm). To enhance the definition of the valve leaflet edges relative to the orifice area, histogram equalisation was applied to the slice series. The Huang threshold was utilised to demarcate the orifice area in each frame for binary processing. Particle analysis was conducted to obtain area measurements of the fluctuation of particles over 10 cycles. The threshold image and mask outline of each frame was documented as respective videos as references for analysis. Particle area results were exported to excel for processing and analysis.

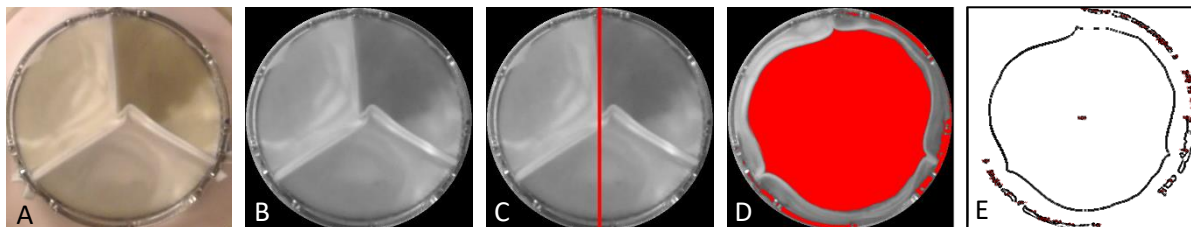


Figure 4.6: GOA measurement process. A) *En face* view. B) Crop and greyscale. C) Set scale. D) Threshold adjusting. E) Particle analysis.

Analytical Method

An Excel template was produced to expedite the extraction of relevant values for the computation of the mean geometric orifice area for each valve. Particle area results were plotted for every captured frame. The highest particle area in each cycle was identified as the GOA at peak forward flow (depicted in Figure 4.7). The mean GOA was computed as the average of the maximum area readings over 10 cycles, at respective cardiac outputs. The coefficient of orifice contraction was subsequently calculated using the standard formula EOA/GOA . The influence of stroke volume on the resultant GOA was assessed for valves tested at different cardiac outputs.

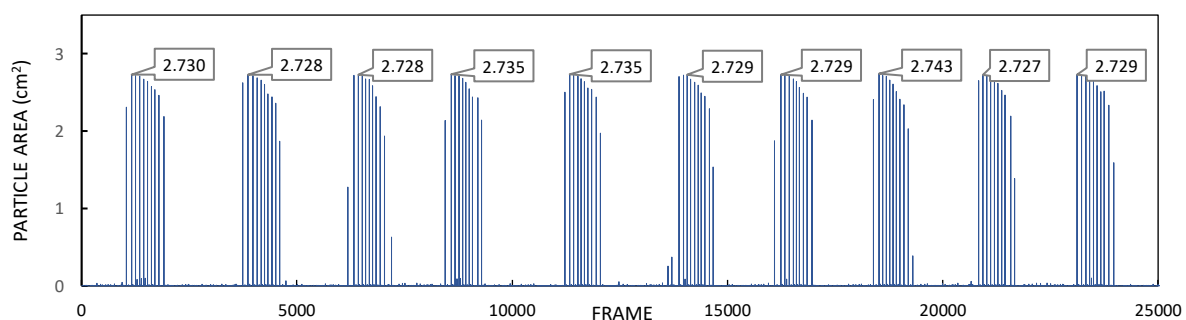


Figure 4.7: Particle area results plotted as a function of frame capture.

4.2.3.3 Pinwheeling Index

Pinwheeling was quantified using the technique described by Midha *et al.*³⁶⁰ and utilising ImageJ application software. Briefly, video footage captured from pulse duplicator tests were used to generate still images of the valves in closed position over 6 cardiac cycles. The length of the leaflet free-edge exhibiting the highest curvature was traced and measured relative to the ideal configuration. The degree of pinwheeling was quantified as a pinwheeling index (PI), mathematically expressed as:

$$PI = \frac{L_{actual} - L_{ideal}}{L_{ideal}} \quad (15)$$

where L_{actual} is the length of the leaflet free-edge from the internal perimeter of the stent to the centre of coaptation, and L_{ideal} is the length of the line running straight between those points. The values were computed as a percentage.

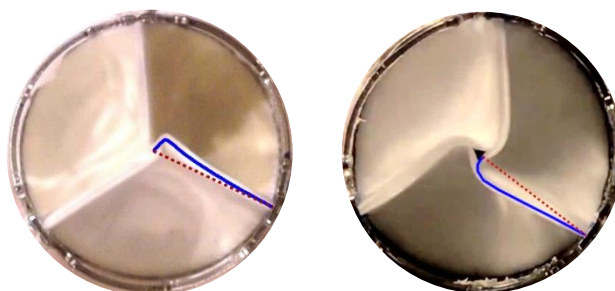


Figure 4.8: Pinwheeling index quantification method. Example of PEL and DP valves (left to right) showing different degrees of pinwheeling, where the solid line follows the actual length (L_{actual}) of the leaflet margin and the dashed line represents the ideal length (L_{ideal}).

4.2.3.4 Opening and closing phase analysis

The pressure and flow waveforms obtained from the hydrodynamic tests were evaluated in accordance with ISO compliant protocols reported by Retta *et al.*³⁶¹. This involved the assessment of key waveform attributes defined in Table 4.3 and illustrated in Figure 4.9, including the flow acceleration period (1 – 2), forward flow period (1 – 3), ventricular contraction period (6 – 10), and positive differential period (7 – 9). Furthermore, the waveform data was used to compute additional opening and closing attributes, including the rapid valve-opening time (RVOT) (Figure 4.9B (T1–T0)), the rapid valve-closing time (RVCT) (Figure 4.9C (T3–T2)), and the closing flow period (CFP) (Figure 4.9C (T4–T2)). With this, the rapid valve opening velocity index (RVOVI) and valve closing velocity index (VCVI), given by the formula $GOA/RVOT$ and GOA/CFP , respectively, were computed. The RVOVI and VCVI parameters represent a nondimensional speed for the opening and closing of the valve leaflets, which have been used in previous studies to investigate relative differences in valve kinematics^{353,362}.

Table 4.3: Waveform timepoints used for characteristic quantification

Timepoint	Time point description
1 Start of forward flow	First zero crossing
2 Peak forward flow	Peak flow velocity
3 End of forward flow	Second zero crossing
4 Peak reverse flow	Minimum flow timepoint
5 End of reverse stroke	First zero crossing of flow after peak reverse flow
6 Start of ventricular contraction	Inflection point indicating start of ventricular pressure acceleration
7 Start of positive differential pressure (initial valve opening)	Minimum aortic pressure and first pressure curve intersection
8 Maximum valve opening area	Sharp inflection of ventricular pressure curve during forward flow
9 End of positive pressure differential	Second pressure curve intersection
10 End of ventricular contraction	Start of steep ventricular pressure deceleration

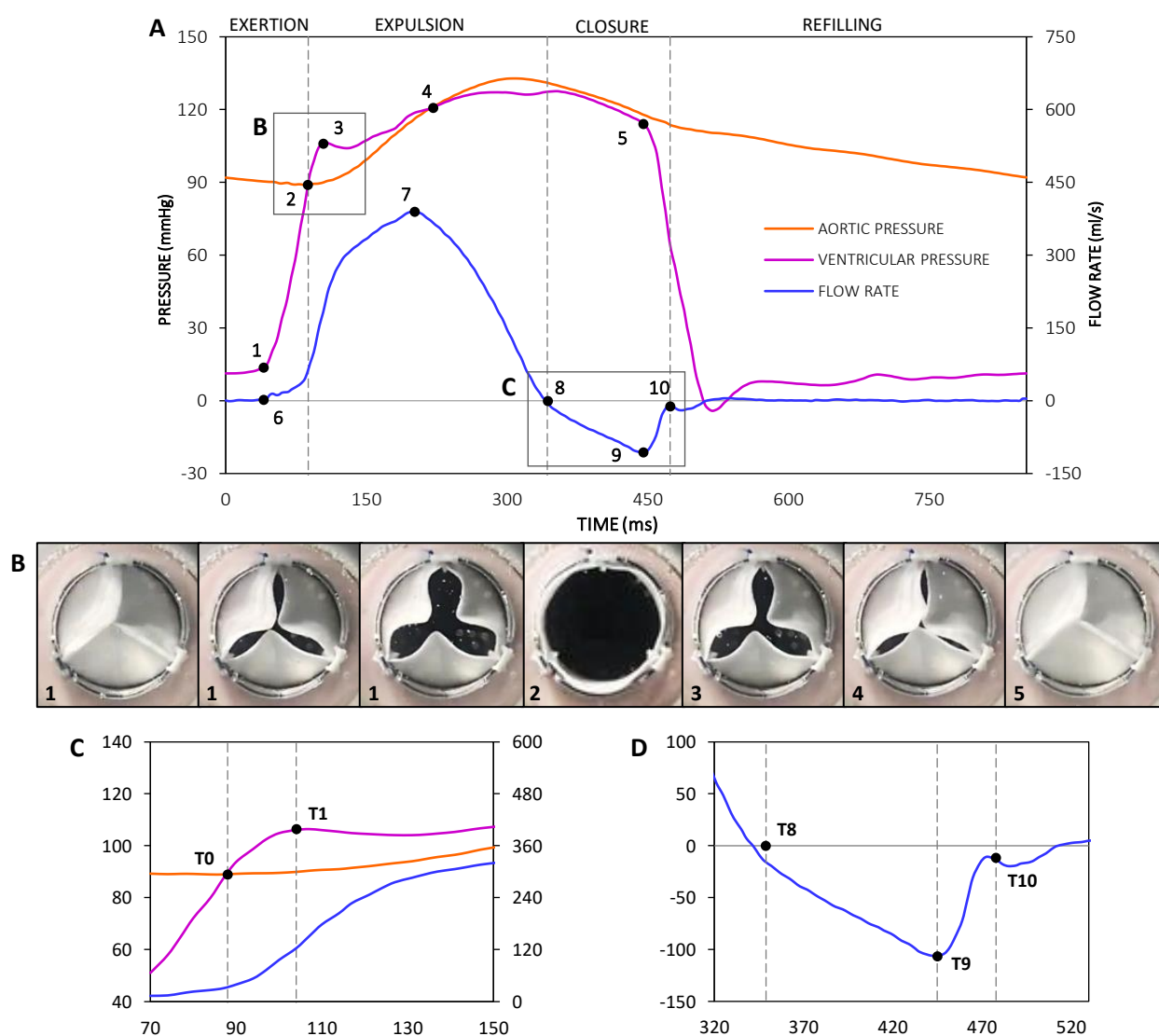


Figure 4.9: Pressure and flow waveform interpretation. A) Typical pressure and flow waveforms over one cardiac cycle of 0.854 s obtained from *in vitro* hydrodynamic tests. Significant timepoints used to quantify waveform characteristics are indicated numerically and defined in Table 4.3. B) A montage of images showing leaflet kinematics corresponding to key timepoints. C-D) Extracts indicating timepoints used to compute opening and closing attributes, including B) rapid valve-opening time (RVOT) ($T1-T0$); C) rapid valve-closing time (RVCT) ($T3-T2$); and closing flow period (CFP) ($T4-T2$).

4.2.4 Valve Durability Testing

4.2.4.1 Accelerated wear testing

Accelerated wear testing was conducted using the ViVitro Labs HiCycle System (ViVitro Labs Inc., BC, Canada) displayed in Figure 4.10AB. The tests were conducted to evaluate the fatigue life of each valve, analyse the wear behaviour of the valve materials, and assess the influence of long-term fatigue loading on the valve hydrodynamics. The equipment was operated first-hand with close reference to standard protocols detailed Appendix C. In brief, the valves were cycled at a frequency of 10Hz under mean differential pressures of 100 mmHg. In order to achieve balanced loading on the module shaker, the valves had to be installed in the

chambers in a symmetric configuration, which limited testing to a minimum of two and maximum of six valve samples per batch (Figure 4.10C).

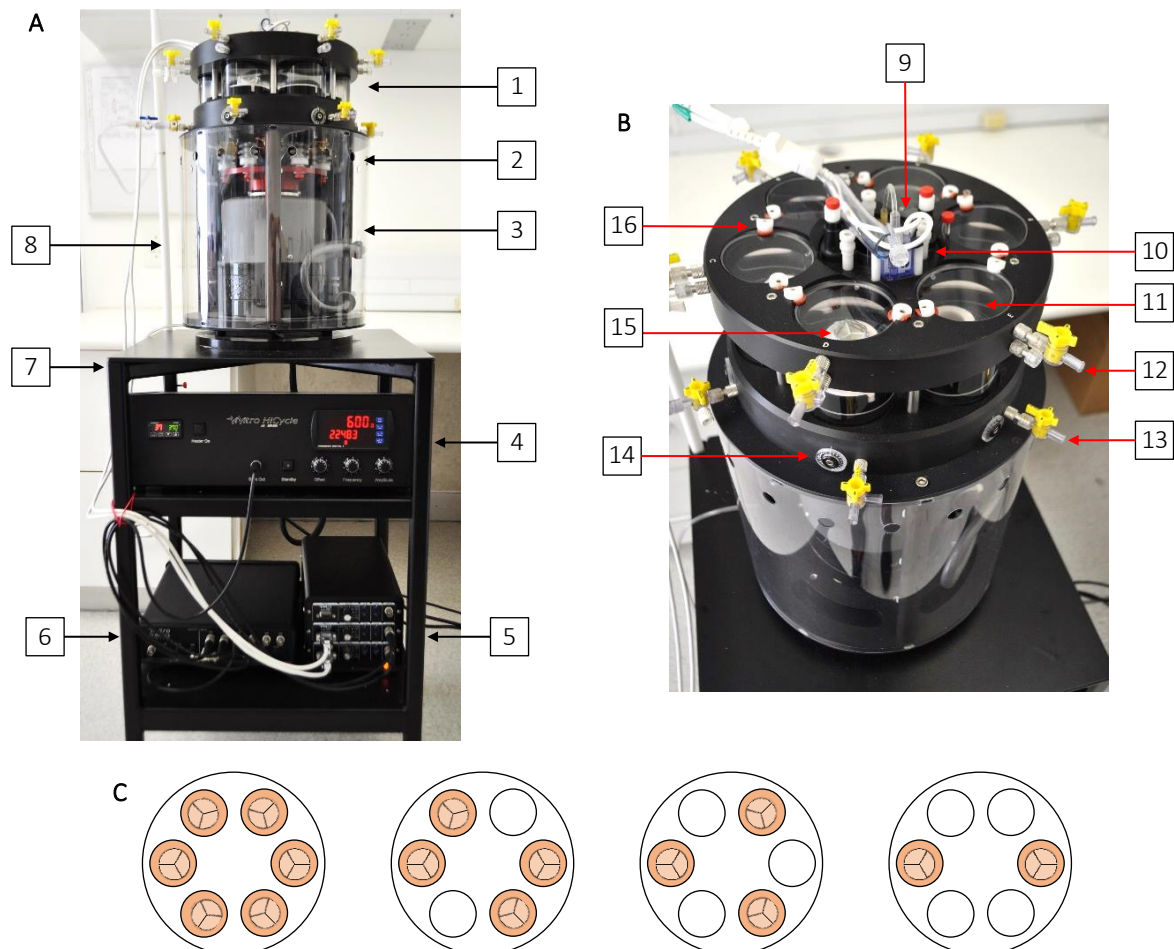


Figure 4.10: ViVitro HiCycle System Assembly. A) HiCycle Module comprised of (1) six test chambers, (2) an electro-dynamic shaker, and (3) electronic controller; (4) the HiCycle Controller Unit; (5) the AmPack Pressure Measuring System; (6) the I/O Module; (7) a rigid frame roller cart; and the (8) Pressure Head Assembly. B) Key components constituting the HiCycle Module included the (9) inflow and outflow pressure transducers, (10) Scan Valve, (11) chamber windows, (12) top and (13) bottom drain port, (14) chamber resistance dial, (15) test valve site, and (16) chamber window retention clamps. C) Valve mounting configurations options for to obtain balanced loading on the shaker plater.

4.2.4.2 Fatigue characterisation

The fatigue characteristics, failure mode, and number of survived cycles was recorded for each valve over the course of accelerated wear testing. Every test valve's fatigue response was evaluated both qualitatively and quantitatively, where applicable.

Macroscopic analysis

Gross valve inspections were performed on a daily basis over the fatigue testing period. Over the first week of testing (equivalent to approximately 200,000 cycles) valves were inspected every 4–6 hours to assess for signs of premature failure. Thereafter, wear characteristics were monitored every 12–18 hours. Inspections were performed with the valves in static and dynamic modes. In static mode, valves were inspected through the chamber windows and walls for signs of wear or failure. With the system running, the valves were inspected

with a stroboscope to assess their opening and closing behaviours. Valves demonstrating severe fatigue or failure characteristics were removed from the system. The remaining valves were subsequently rearranged into a symmetric configuration, after which the chambers were debubbled, transducers recalibrated, and chamber pressures retuned to achieve the desired operating conditions.

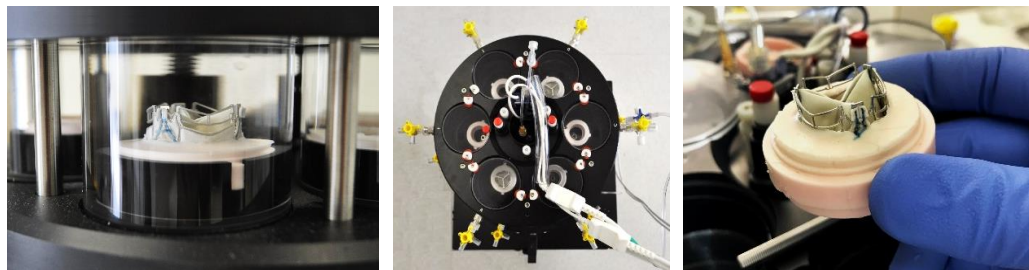


Figure 4.11: Valve inspection methods

Microscopic analysis

Scanning electron microscopy was conducted to examine the microstructural fatigue characteristics and failure modes of the leaflets. In this way, greater insight into the functional properties of electrospun scaffolds could be obtained. SEM imaging and acquisition of the fatigued scaffold samples was performed according to the procedure described section 3.3.3. Samples were excised from regions where stress concentrations in substitute heart valve leaflets are commonly reported, such as the (A) free-edge apex, (B) commissure hinge region, (C) commissure post attachment site, (D) belly region, (E) scallop base, and (F) attachment line^{132,202,363}.

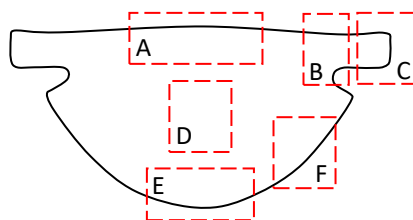


Figure 4.12: Regions of interest for SEM examination of scaffold fatigue. A) free-edge apex, B) commissure hinge region, C) commissure -post attachment site, D) belly region, E) scallop base, and F) sloped suture line

Evaluation criteria and failure ratings

The fatigue mechanisms most frequently reported in literature were used as a benchmark against which the degree of fatigue and risk of failure for each test valve was assessed. The fatigue mechanisms identified as being pertinent to this study include tears, holes, delamination, compression strain, permanent deformation, and kinematic dysfunction. A failure rating chart, presented in Table 4.4, was compiled to define the relative degrees of damage for each fatigue mode, and thereby provide a measurable definition for valve failure. The values assigned to the fatigue mechanisms and failure modes were derived from those reported in associated studies^{202,357}. Valves that demonstrated a fatigue rating of greater than 5 were considered to have failed, and subsequently removed from the fatigue tester. For the valves that withstood over 30 million cycles and underwent repeated hydrodynamic assessments, valve failure was defined as demonstrating a three-fold

increase in mean transvalvular pressure drop, regurgitation fraction, and/or energy loss relative to the pre-fatigue results.

Table 4.4: Fatigue criteria and failure ratings

	Holes	Tears	Fraying	Delamination	Excessive Deformation	Leaflet Dysfunction
0	None	None	None	None	None	None
1	None	None	Slight fraying of leaflet margins	None	None	Leaflet-stent contact
2	Small pinholes	Small notches along leaflet margins	Distinct fraying at the free-edge	Local delamination on a single leaflet ($\geq 5\%$ of the leaflet margin length)	None	Asymmetric leaflet motion
3	Enlarged pinholes	Small, trivial tears	Luminal surface fraying	Local delamination on more than one leaflets	Slight leaflet buckling or ballooning of the belly region	Single leaflet immobilised in closed position
4	Medium, localised holes	Large, localised tears	Degradation of leaflet thickness	Local delamination at the commissure posts	Buckling or ballooning resulting in restricted leaflet motion	Restricted motion of more than one leaflet
5	Large, expanding holes	Large propagating tears causing leaflet dislocation	Excessive wear resulting in leaflet rupture	Delamination at commissures or spanned across 90% of >1 leaflet margin	Severe buckling or regional prolapse causing incomplete coaptation and valve dysfunction	Impeded valve opening or incomplete coaption leading to complete loss of function

4.2.4.3 Valve hydrodynamics revaluation

For the valves that survived over 100 million cycles underwent hydrodynamic testing at 30 million ($n=3$) and 100 million ($n=2$) to evaluate the influence of fatigue on the valve's performance over time. The hydrodynamic tests were conducted using the same protocols described in section 4.2.2.1 and operated under the same conditions as the pre-fatigue tests. The test data was processed following the analytical methods described in section 4.2.2.1, and the results were compared against the pre-fatigue performance outcomes.

4.3 Results and Discussion

4.3.1 Valve characterisation

4.3.1.1 Leaflet scaffold microstructure

Representative SEM images from each leaflet group are displayed in Figure 4.13 and corresponding mean microstructural properties are presented graphically in Figure 4.14 and numerically in Appendix B, Table B1. Under SEM observation, the scaffolds were highly porous on both luminal and abluminal surfaces for all valve groups. Fiber structures were observed as being smooth, continuous, and notably uniform. Furthermore, bulk networks were absent of excessive interfiber fusion, fiber bundling and other anomalies that may affect the functional properties of the scaffolds.

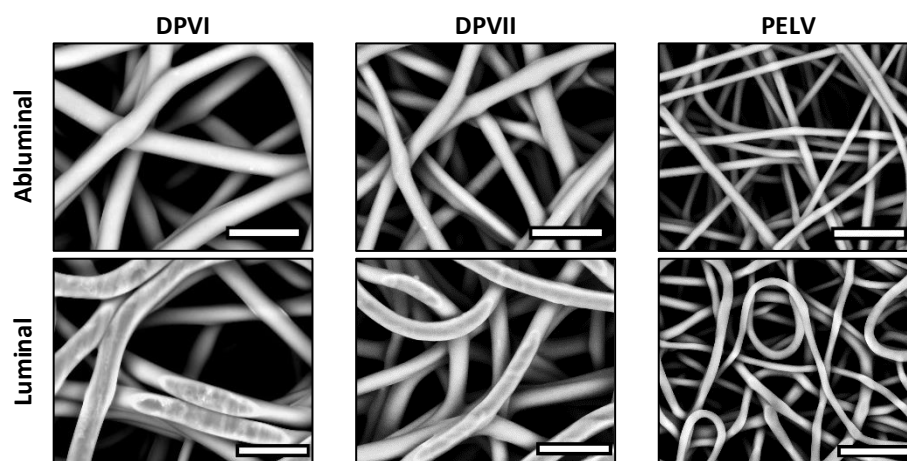


Figure 4.13: SEM images of electrospun valve leaflet scaffolds. Abluminal (left), luminal (middle), and x3600 A) DPVI, B) DPVII corresponding PEL and DP scaffolds. Scale bars indicate 20 μm .

Briefly, a mean fiber diameter of $1.5 \pm 0.4 \mu\text{m}$ was measured for the PELV leaflet scaffolds, and mean diameters of 4.1 ± 0.9 and $7.5 \pm 1.2 \mu\text{m}$ were measured for the DPVI and DPVII scaffolds, respectively. Average pore sizes varied significantly among each scaffold group, with the smallest pores attributed to the PELV scaffolds ($11.9 \pm 2.1 \mu\text{m}$), followed by the DPVI scaffolds ($17.2 \pm 4.5 \mu\text{m}$), while the DPVI scaffolds had the largest pores which were approximately twice the size of the PELV leaflets ($23.8 \pm 4.8 \mu\text{m}$). No differences were found in scaffold porosity between the leaflet groups, which averaged at $77.3 \pm 6.4\%$, and all scaffolds were random in orientation.

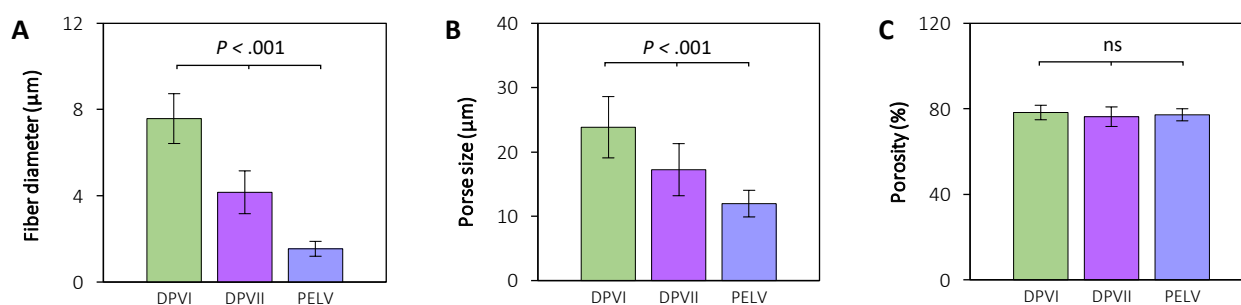


Figure 4.14: Leaflet scaffold microstructure characteristics. A) fiber diameter, B) pore size, and C) porosity.

4.3.1.2 Leaflet scaffold mechanical properties

The mechanical properties obtained for the electrospun PELV and DPV leaflets are provided Table 4.5, and the average tensile stress-strain and hysteresis curves for each group are presented in Figure 4.15. The stress-strain curves demonstrate a clear difference in tensile and viscoelastic behaviour between the PELV and DPV leaflets.

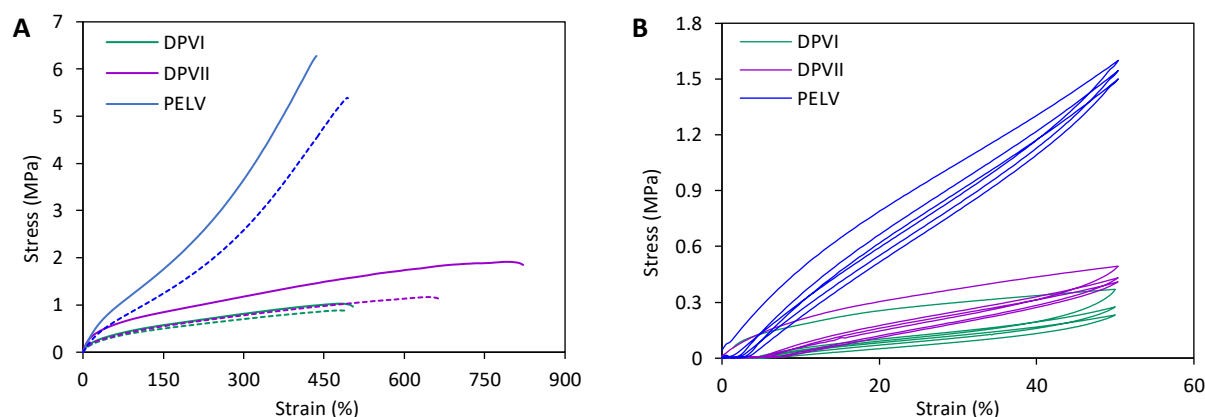


Figure 4.15: Average stress-strain curves obtained for PEL and DP valve leaflet scaffolds. A) Average tensile stress-strain curves obtained for the PEL and DPV scaffolds (solid lines represent the circumferential direction and dashed lines represent the longitudinal direction). B) Average hysteresis curves obtained from cyclic tensile tests showing the 1st, 20th, and 50th loading cycle.

As shown in Figure 4.16A

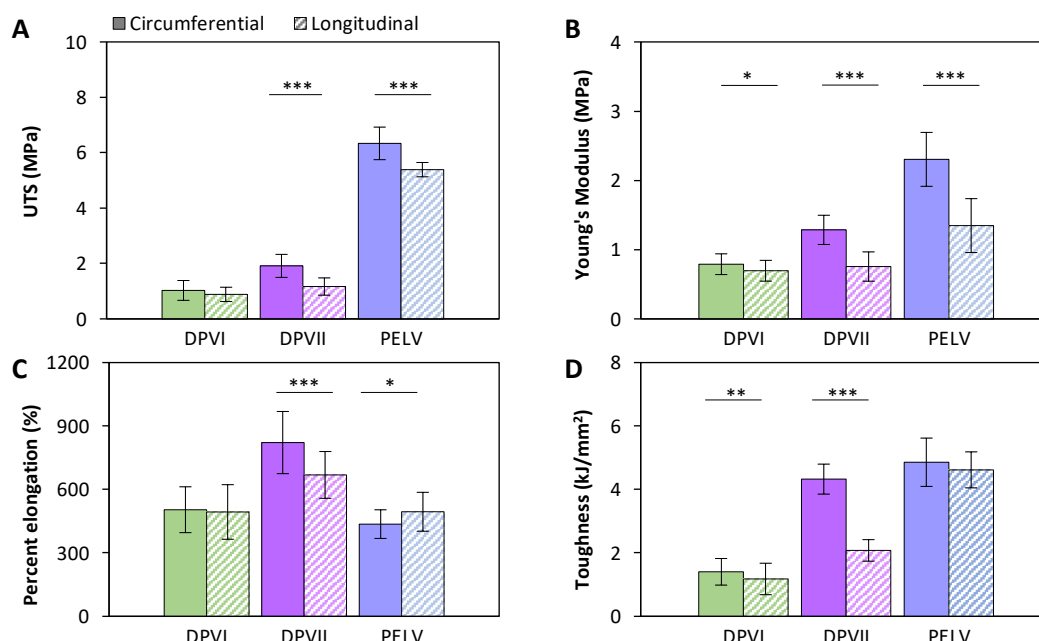


Figure 4.16, the UTS obtained for the DPV groups was considerably lower. For instance, in the circumferential direction, DPVI demonstrated a mean UTS approximately six times smaller than PELV leaflet (1.0 ± 0.4 MPa vs 6.3 ± 0.6 , $P < .0001$), while the UTS for DPVII was approximately three times smaller at 1.91 ± 0.4 MPa ($P < .0001$). In Figure 4.16C, it can be seen that the DPVII scaffolds performed the largest elongation of 820% in the circumferential direction, which was almost two times higher than the DPV scaffolds (440%, $P < .0001$) and approximately 40% higher than the PELV scaffold at 504% ($P < .0001$). Despite demonstrating significantly lower strength, the DPVII scaffolds demonstrated a comparable mean toughness to the PELV scaffolds (4.3 ± 0.5 vs

$4.9 \pm 0.8 \text{ kJ/mm}^2$) (Figure 4.16D) which could be credited to the superior extensibility demonstrated by the DPVII scaffold. Both PELV and DPVII leaflet scaffolds demonstrated stiffness anisotropy of 0.6, whereas the DPVI scaffolds were essentially isotropic, with an AR of 0.9.

The viscoelastic behaviour of all three groups manifested as hysteresis loops which appeared to shift down with each cycle (Figure 4.15B). This behaviour was especially prominent for the DPV groups and indicated that the contribution of the elastic component relative to the viscous component diminished over the course of cyclic loading. This phenomenon could be attributed to the change in fiber structure and matrix configuration that typically occurs when fibrous tissues are subjected to cyclic loading³⁰⁵. Another explanation is that during dynamic loading, the relative motion of fibers can cause rupturing of interfiber bonds, which would lead to a loss of structural stability and likely culminate in permanent deformation and significant mechanical weakening³⁶⁴. The PELV group demonstrated a similar trend but to a lesser degree which could be attributed to their superior elastic properties (specified in Table 4.5).

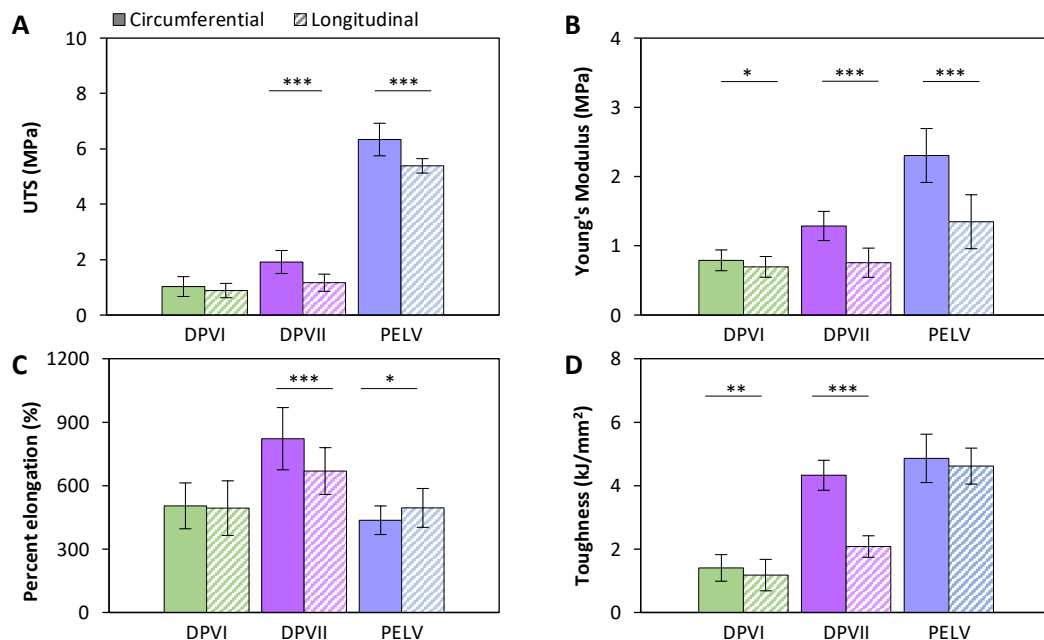


Figure 4.16: Mechanical properties of electrospun PEL and DP leaflet scaffolds. A) Ultimate tensile stress, B) Young's Modulus, C) percent elongation, and D) fracture toughness. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4.5: Mechanical properties of PEL and DPV leaflet scaffolds

	UTS (MPa)	E (MPa)	ϵ_{max} (%)	κ ($\times 10^3 \text{ MJ/m}^3$)	AR (E)
DPVI	1.03 ± 0.36	0.79 ± 0.15	504.06 ± 108.40	1.40 ± 0.42	0.88 ± 0.21
DPVII	1.91 ± 0.42	1.29 ± 0.21	821.71 ± 147.12	4.33 ± 0.47	0.59 ± 0.19
PELV	6.34 ± 0.59	2.30 ± 0.39	436.01 ± 67.60	4.86 ± 0.76	0.58 ± 0.11

As shown in Figure 4.17E, the variation in peak reaction force (R_c) for the DPVI scaffolds reached approximately 30% in the circumferential direction and 50% in the longitudinal direction after 50 cycles, which can be interpreted as a 30 and 50% decline in resistance to deformation, respectively. Overall, the DPVII scaffolds demonstrated a 20–25% decline in deformation resistance after 50 cycles, and the PELV LEAFLET scaffolds

demonstrated the lowest decline in deformation resistance of 10–12% and which appeared to have stabilised after approximately 30 cycles.

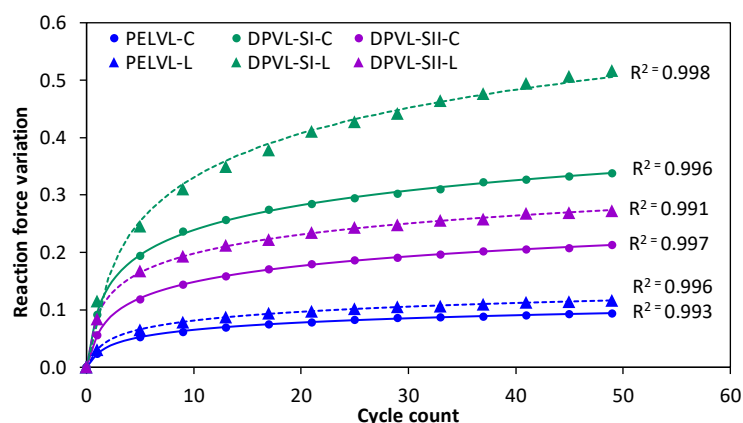


Figure 4.17: Normalised variation in maximum reaction force as a function of cycle count obtained from the cyclic tensile tests. The solid lines represent the curve fit for the circumferential direction and dashed lines represent the curve fit for the longitudinal direction (only every fourth cycle is presented).

4.3.1.3 Leaflet scaffold hydraulic properties

Surface wettability

The water contact angles on cast film and electrospun PELV and DPV leaflet scaffolds were measured, respectively, to influence of scaffold architecture on wettability. The results are given in Figure 4.18, and exemplary images of the contact angles captured for each scaffold are provided in Figure 4.18.

There was no effective difference in water contact angle measurements between the PEL and DP films, which averaged at $74.3 \pm 2.6^\circ$ and $78.6 \pm 3.0^\circ$, respectively (Figure 4.18C and D). For medical TPUs, contact angles between $35\text{--}80^\circ$ are considered hydrophilic and moderately wettable³⁶⁵. These values correspond well with prior literature which report contact angles for PEL films to be between $60\text{--}84^\circ$ ³⁶⁵⁻³⁶⁷ and for DP films to be between $65\text{--}79^\circ$ ³⁶⁸.

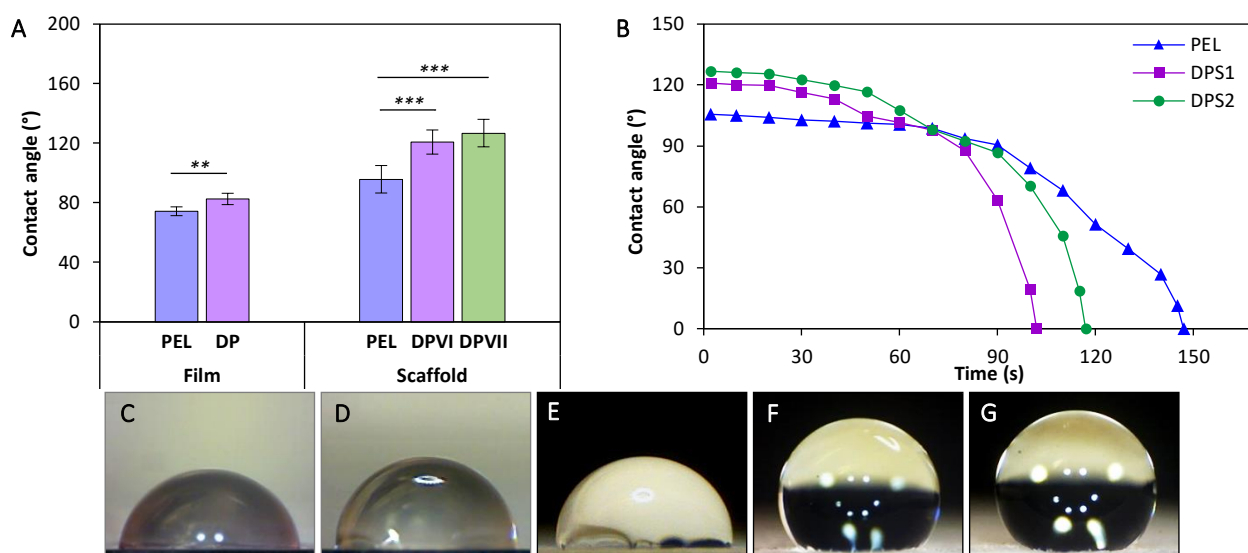


Figure 4.18: Dynamic wetting behaviour of PEL and DP scaffolds. A) Contact angle measurements for film (static) and electrospun scaffolds (starting angle). B) Variation in contact angle on electrospun PEL and DP scaffolds plotted as a function of time. Static contact angle images of PEL (A) and DP (B) films. Starting contact angle of electrospun PEL (E), DPV1 (F), DPV2 (G) DPV2 scaffolds

Contrastingly, the electrospun PELV and DPV leaflet scaffolds demonstrated dynamic wetting behaviour, where the droplet absorbed into the scaffold leading to a decrease in contact angle over time, as shown in Figure 4.18B. Upon first contact with water, all the scaffolds exhibited hydrophobic behaviour for about 60s, after which the surface became hydrophilic as the droplet proceeded to permeate into the scaffold until fully depleting. The PELV leaflet scaffolds, which demonstrated a starting contact angle of $110.6 \pm 7.2^\circ$, were slightly hydrophobic than the DPV1 and DPV2 scaffolds, which averaged a contact angle of $127.4 \pm 91.5^\circ$. It has been found that increasing the average fiber diameter of electrospun scaffolds leads to increased surface roughness³⁶⁹, which in turn causes an increase hydrophobicity due to minimised solid-liquid contact area³⁷⁰. This could explain the trend observed between the microstructure and initial contact angle of the scaffolds, where smaller fiber diameters were associated with lower contact angles, as shown Figure 4.18.

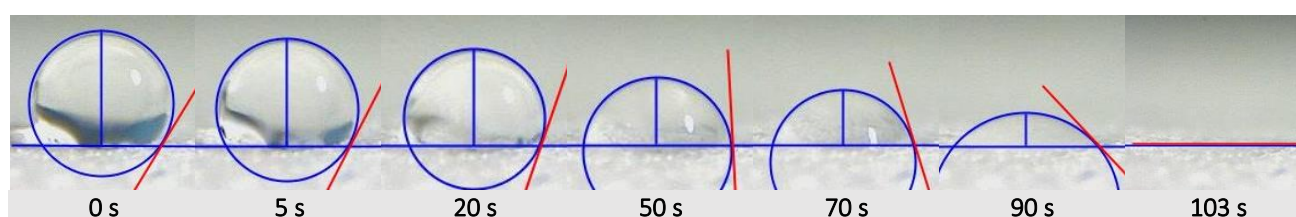


Figure 4.19: Example montage of images showing the dynamic wetting behaviour of a DP scaffold, illustrating the variation in contact angle and drop height over time.

However, despite being the least hydrophobic upon first contact with water, the PELV scaffolds demonstrated longer absorption times than both DPV leaflet scaffolds, as shown in Figure 4.18B. Research on droplet absorption dynamics on porous substrates have shown that liquid penetration is largely influenced by pore diameter and permeability of the absorbing substrate, among other factors³⁷¹. In general, materials with larger pores and higher permeabilities are more rapidly absorbing of fluids, which could explain the fact that the DPV scaffolds, which had significantly larger pores and higher permeabilities than the PELV scaffolds, demonstrated faster absorption times despite being more hydrophobic at first.

Although the hydrophilic quality of a material is widely considered to be favourable for biological applications, for tissue engineered substrates, the hydrophobic aspect also contributes positively to its biocompatibility. This is demonstrated in numerous studies which show that moderately hydrophilic scaffolds ($CA = 48\text{--}62^\circ$) demonstrate improved biocompatibility compared to highly hydrophilic ($CA < 35^\circ$) and hydrophobic ($CA > 80^\circ$) scaffolds^{372,373}. This can be attributed to the fact that excessively hydrophobic surfaces have enhanced cell affinity but reduced biocompatibility, whereas highly hydrophilic surfaces have enhanced fluid affinity, but prevent cell-substrate and cell-cell interactions, which are essential for tissue growth and regeneration³⁷⁴. For this reason, scaffolds for TE applications require a balance of hydrophilic and hydrophobic surface entities¹⁸¹. As the DP scaffolds presented a combination of hydrophobic and hydrophilic qualities, their wetting behaviour was considered to be favourable for facilitating essential cell behaviours^{368,369,375}.

Scaffold permeability

Figure 4.20A shows the permeability values measured for each group plotted as a function of time over 30 mins. The permeabilities of all the DP scaffolds were found to decrease exponentially within the first 15–25 minutes of fluid flow, after which the permeability became relatively stable. This phenomenon could be attributed to the applied pressure of the fluid on the scaffold, which would generate large compressive strains across the scaffold, leading to the collapse of open pore channels, fiber compaction, and a subsequent reduction in pore size, which in turn would lead to reduced permeability³⁷⁶. In contrast, the PEL scaffolds demonstrated a slight increase in permeability within the first 3–5 minutes of testing, after which it remained fairly consistent for the rest of the test.

As shown in Figure 4.20B, the PEL scaffolds were significantly less permeable than the DP scaffolds, demonstrating an average permeability coefficient nearly four times smaller than the DPS2 scaffolds ($5.5 \pm 1.6 \times 10^{-8}$ vs $1.9 \pm 0.4 \times 10^{-7} \text{ m}^4/\text{Ns}$, $P < .0001$) and over two times smaller than the DPS1 scaffolds ($1.3 \pm 0.3 \times 10^{-7}$, $P < .0001$). Since the PEL scaffolds had the smallest pores and the DPS2 scaffolds had the largest pores, these findings point to there being a positive correlation between pore size and permeability, which is consistent with outcomes reported in associated studies that investigate the effect of pore size on the permeability of porous scaffolds³⁷⁶⁻³⁷⁸.

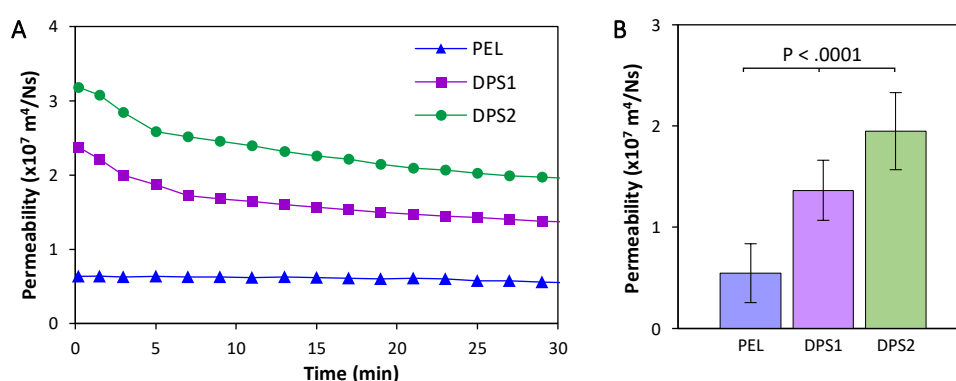


Figure 4.20: Permeability test results. A) Graph of average permeability of PEL and DP scaffolds as a function of time for the first 35 minutes of permeability testing. B) Mean permeability results obtained after 30 minutes of pressurised fluid flow through the scaffolds.

4.3.1.4 Leaflet scaffold thickness

The distributions of average leaflet thickness measured for each group are depicted in Figure 4.21, and corresponding means are provided in Appendix B, Table B3. Pairwise comparisons indicated that valves produced in any particular group showed no significant difference in mean thickness compared to valves in the same group, whereas differences in thickness between each group were statistically discernible ($P < .0001$).

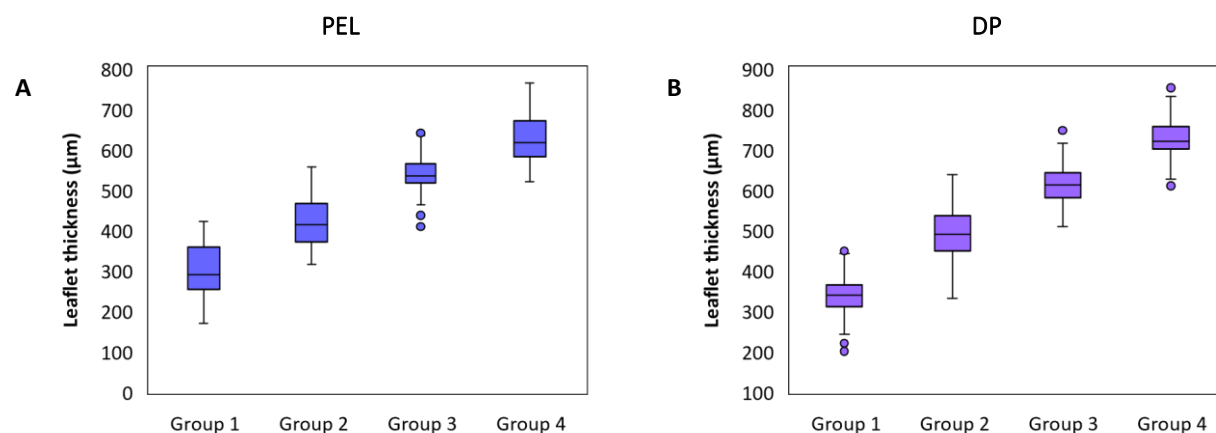


Figure 4.21: Leaflet thickness distributions for PEL and DP valve scaffolds (A and B, respectively).

4.3.1.5 Valve component assembly

The suturing method used to attach the leaflets to the stent worked effectively for both PEL and DP scaffolds; and the heat bonding process employed to secure the PEL skirts to the stents provided sufficient attachment integrity. Macroscopic inspections performed on each valve confirmed that neither the cutting nor integration of the skirt and leaflets to the stent caused any notable damage to the scaffolds that would influence valve function. Images of one valve from each group are presented in Figure 4.22.



Figure 4.22: Photo montage of an exemplary valve from each sample group. A) DPVI, B) DPVII, and C) PELV.

4.3.2 Valve hydrodynamics

4.3.2.1 Non-degradable valve hydrodynamics

Mean pressure gradient and effective orifice area

At a cardiac output of 3.5 l/min, the PEL300 and PEL420 valves obtained equally low mean pressure drops of 4.9 ± 0.2 and 4.9 ± 0.7 mmHg, respectively ($P=0.57$). Likewise, pressure drops obtained for the PEL540 and PEL630 valves were not statistically different (5.7 ± 0.5 vs. 5.7 ± 0.5 mmHg, $P=0.67$), but both differed significantly compared to the former two groups ($P<.0001$). At 5 l/min CO, the PEL300 and PEL420 valves maintained significantly lower pressure gradients compared to the PEL540 and PEL630 valves, as depicted in Figure 4.23A. Naturally, the trend was recapitulated with the data pooled across both COs, where average gradients came to 6.0 ± 1.1 , 6 ± 0.9 , 6.7 ± 0.8 , and 6.6 ± 0.8 mmHg for the PEL300, 420, 540 and 630 valves, respectively. Overall, the increase in leaflet thickness led to a slight linear increase in pressure drop but with marginal effect ($\tau=0.18$, $P<.0001$) (Figure 4.23C).

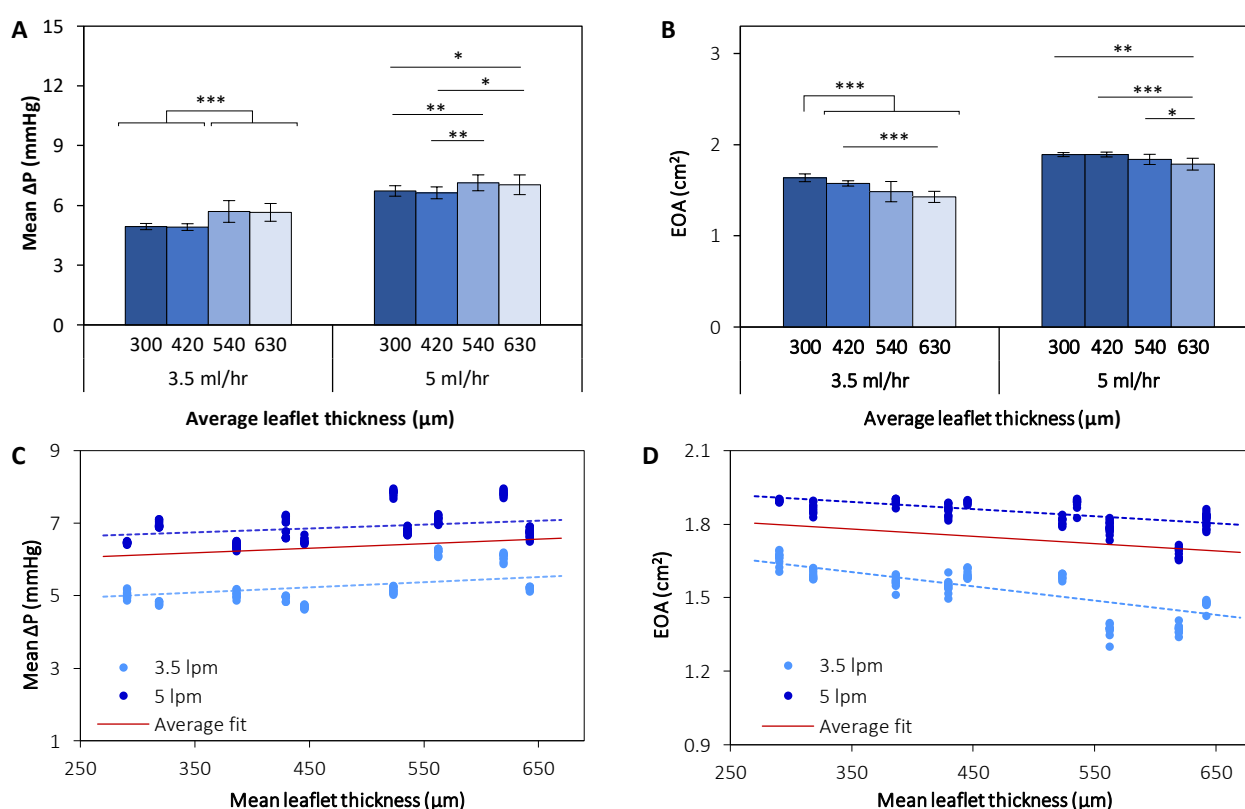


Figure 4.23: Mean ΔP and EOA results obtained for PEL valves. (A) Mean ΔP and (B) EOA plotted against group average leaflet thickness. (C) Mean ΔP and (D) EOA plotted as a function of mean valve leaflet thickness. *** $P<0.0001$, ** $P<0.01$, * $P<0.05$.

The EOA outcomes obtained for the valves are presented in Figure 4.23B and D as bar plots for representation of the group comparisons and a scatter plot for illustration of the regression analyses. In general, the effective orifice area showed to decrease significantly with increasing average leaflet thickness, where on average the largest EOA was achieved by the 300 μm valves and the smallest EOA was demonstrated by the 630 μm valves

($1.8 \pm 0.1 \text{ cm}^2$ vs. $1.7 \pm 0.8 \text{ cm}^2$, $P < .0001$). As depicted in Figure 4.23, the effect of leaflet thickness on EOA demonstrated a slightly higher impact compared to its effect on mean ΔP ($m_{TS} = -5.8 \times 10^{-3}$, $\tau = -0.54$, $P < .0001$ vs. $m_{TS} = -2.9 \times 10^{-3}$, $\tau = -0.44$, $P < .0001$, respectively). Overall, the PELVs obtained minimum and maximum pressure drops of $5.6 \pm 0.9 \text{ mmHg}$ and $7.2 \pm 0.4 \text{ mmHg}$, respectively, and the mean of the sample population came to $6.5 \pm 1.0 \text{ mmHg}$, which was well under the minimum ISO requirement of $\leq 10 \text{ mmHg}$. Furthermore, the mean EOA obtained for each valve exceeded the minimum ISO requirement of $> 1.25 \text{ cm}^2$.

Regurgitation

The amount of regurgitation, which is the sum of the closing and leakage volumes, was determined in volume and percentage of the total stroke volume. Results are presented graphically in Figure 4.25, and mean regurgitation parameters for each group are given in Appendix C in Table C2.

As expected, the PEL300s demonstrated the highest RF of $24.2 \pm 4.6\%$, of which the greatest percentage was attributed to leakage ($13.9 \pm 2.5\%$). Conversely, the PEL630s achieved the lowest RF ($10.5 \pm 3.7\%$), of which 90% was attributed to the closing fraction, and which overall demonstrated the smallest leakage fraction of $0.9 \pm 0.6\%$. Mean RF comparisons revealed significant differences between each paired group across both flowrates, as indicated in Figure 4.25A. Evidently, RF demonstrated a strong negative association with leaflet thickness at both flow rates ($\tau = -0.74$ and $\tau = -0.68$ ($P < .0001$) for 3.5 and 5 l/min, respectively) (Figure 4.25B).

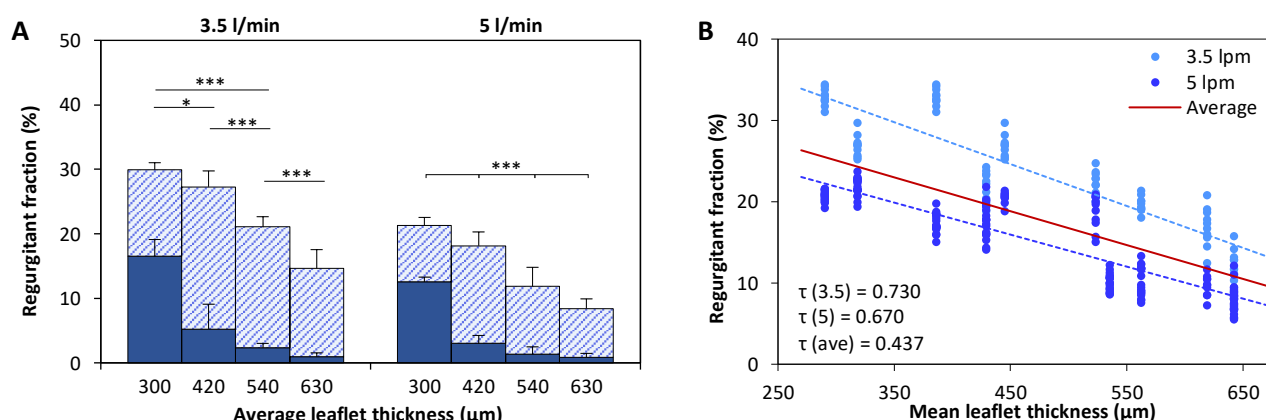


Figure 4.24: Regurgitant fraction results obtained for the PELVs. A) Closing and leakage components of the regurgitant fraction measured in relation to average leaflet thickness at 3.5 and 5 l/min, respectively. B) Total regurgitant fraction data points plotted as a function of mean valve leaflet thickness. *** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$.

In accordance with the RF, over both flowrates the total regurgitant volume decreased significantly with increasing leaflet thickness ($\tau = -0.67$, $P < .0001$), where between thicknesses of 300 and 630 μm , the amount of regurgitation decreased by over 50% (mean difference = 7.0 ml, $P < .0001$). As presented in Figure 4.25C, the closing and leakage fractions plotted as functions of mean leaflet thickness demonstrated distinct nonlinear trends. Quadratic power regression was performed to model the relationship between closing fraction and leaflet thickness, which demonstrated a good fit based on the normality of the residuals plot (Figure 4.25E), and high R^2 value ($R^2 = 0.70$, $P < .0001$). To model the relationship between leakage volume and leaflet thickness, a squared reciprocal transformation was applied ($R^2 = 0.85$, $P < .0001$). The plots demonstrate that valves with leaflets below 310 μm in thickness experienced higher leakage volumes than valves with thicker leaflets, across

both flow rates, and that leakage accounted for a greater fraction of the total regurgitation compared to the closing volume. Between 310 and 450 μm , the closing volume demonstrated a positive association with leaflet thickness and inverse relationship with the leakage fraction, but above 450 μm , an unusual phenomenon was observed where the closing volume began to decrease with increasing leaflet thickness. This could be explained by the fact that the leaflets were manufactured to adopt a neutral closed position, indicating that they would be more prone to staying in a closed position, which would lead to a faster closing time, and hence, a decrease in closing volume. This deduction was corroborated by evaluating the GOA relative to the closing flow period of the cardiac cycle for the different thickness groups which indicated that the valves with thicker leaflets performed significantly faster closing motions than the valves with moderate thicknesses (see section 4.2.3.4 to follow)

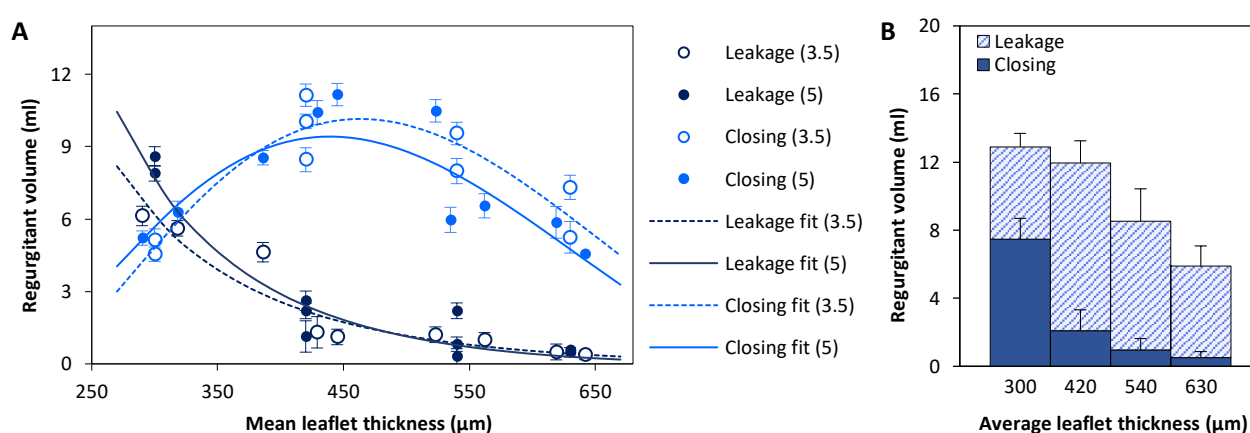


Figure 4.25: Regurgitant volume results obtained for PEL valves. B) Closing and leakage components of the mean regurgitant volume measured as a function of average leaflet thickness.

Transvalvular energy loss

The transvalvular energy loss parameters, including forward, closing, and leakage energy losses, were obtained in metric units (mJ) and as a percentage of the energy imparted on the fluid by the ventricle over one cardiac cycle (%). The parameters quantified for each group are presented graphically in Figure 4.26. As shown in Figure 4.26, across both cardiac outputs the 300 μm valves suffered substantially higher total energy losses compared to the rest of the valve groups in both amount and fraction of the ventricular energy ($P < .0001$). By demonstrating a total loss of 126.6 ± 23.5 mJ, the 300 μm valves dissipated approximately 17% of the energy generated by the ventricle. Interestingly, the 630 μm valves demonstrated the least amount of energy loss, which at 52.6 ± 12.2 mJ was approximately 60% less than the loss incurred by the 300 μm valves (mean difference = 74.0 mJ, $P < .0001$), and which accounted for only 6.7 ± 1.0 % of the ventricular workload.

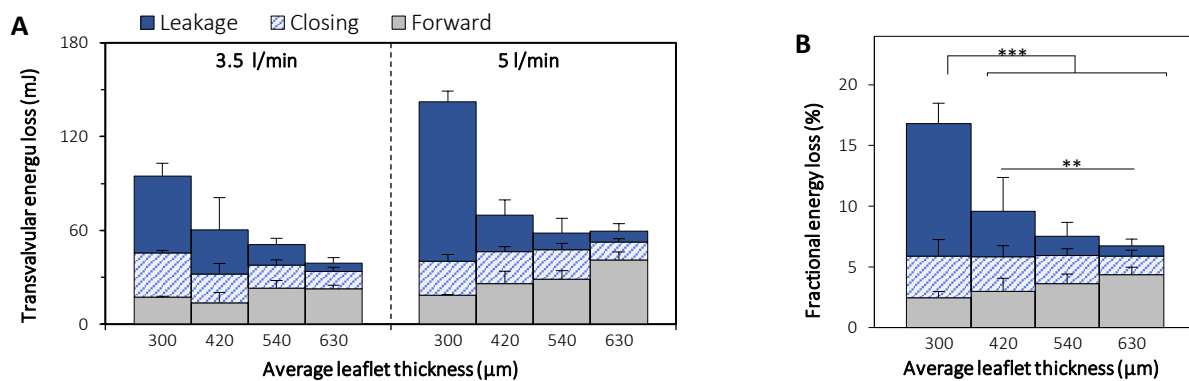


Figure 4.26: Transvalvular energy loss results for the PELVs. A) Forward, closing and leakage energy losses measured at A) 3.5 and 5 l/min, respectively, and B) determined as a fraction of the ventricular workload across both flowrates.

Figure 4.27A shows the total energy loss readings obtained for each valve over both cardiac cycles plotted as a function of leaflet thickness. A good fit was found for each plot by applying a reciprocal power transformation, which in each case indicated a significant effect ($R^2 = 0.65$ at 3.5 l/min, $R^2 = 0.76$, at 5 l/min and $R^2 = 0.65$ overall, $P < .0001$). The trend between the energy losses obtained during systole (forward losses) and diastole (closing and leakage losses) are presented in Figure 4.27B as functions of leaflet thickness.

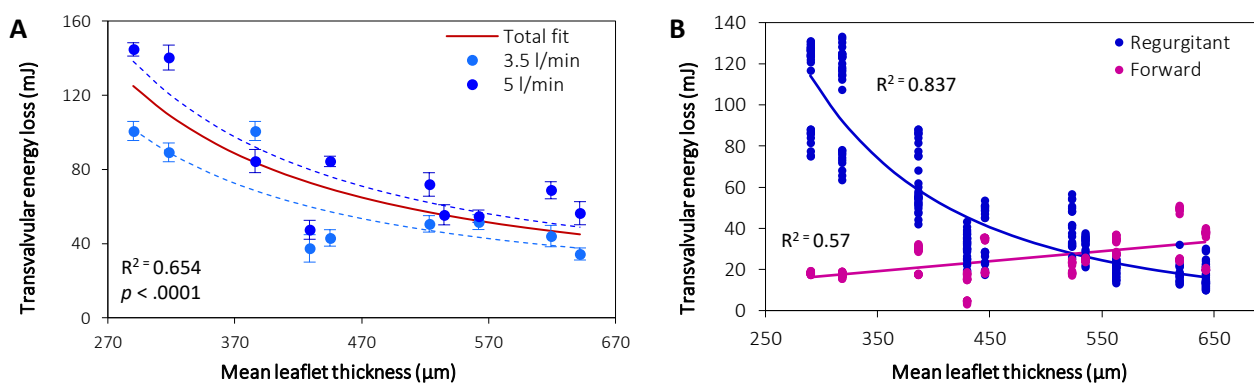


Figure 4.27: Transvalvular energy losses plotted as a function of leaflet thickness. (A) Transvalvular energy losses for each valve over both COs. The dashed lines represent the curve fits for the respective COs, and solid line represent overall fit, to which the statistical values correspond. (B) Systolic, regurgitant and total energy losses plotted as a function of average leaflet thickness.

On average, forward energy losses increased linearly with increasing leaflet thickness ($m_s = 0.007$, $\tau = 0.58$, $P < .0001$), while regurgitant energy losses corresponded with a nonlinear inverse trend. The relationship was modelled with reciprocal power regression which confirmed the significance of the association ($R^2 = 0.79$, $P < .0001$). While overall total energy losses decreased with increasing leaflet thickness, the systolic function of the valves appeared to become compromised at greater thicknesses, which was indicated by the relative increase in forward energy losses. However, the superior diastolic efficiency demonstrated by valves with thicker leaflets (indicated by the distinctly low closing and leakage energy losses) counteracted the increase in forward energy loss such that the total energy loss remained remarkably low.

4.3.2.2 Degradable valve hydrodynamics

4.3.2.3 Effect of material properties on valve hydrodynamics

The hydrodynamic parameters for both DPV groups are given in Table 4.6, and graphically depicted in Figure 4.28. The mean gradient obtained for DPVII was slightly lower compared to DPVI (4.4 ± 0.3 vs. 4.9 ± 0.8 mmHg; $P=0.01$), however no significant difference in EOA was observed between the two groups. On the other hand, DPVI demonstrated a regurgitant fraction of $34.7 \pm 2.9\%$, which was significantly higher in comparison to DPVII, which achieved an RF of $13.2 \pm 1.1\%$ – approximately one third of DPVI ($P<.0001$). Across both groups, the closing volume constituted the largest portion of the regurgitant volume compared to leakage. DPVI demonstrated a closing volume of 19.8 ± 2.5 ml and leakage volume of 2.2 ± 0.9 ml, while DPVII achieved significantly lower closing and leakage volumes of 5.6 ± 0.8 ml and 1.3 ± 0.4 ml ($P<.0001$ and $P=0.0005$, respectively). Likewise, the total transvalvular energy loss, which was evaluated as a fraction of the total ventricular energy, was significantly lower for DPVI compared to DPVII ($22.8 \pm 2.0\%$ vs. $13.0 \pm 1.4\%$; $P<.0001$). Across both groups, the greatest portion of ventricular energy was lost during forward flow, which in both cases accounted for 60% of the total loss. With regard to the reverse flow, the ratio of the energy losses derived from the closing and leakage flows was proportional to the relationship between the corresponding aspects comprising the regurgitant fraction.

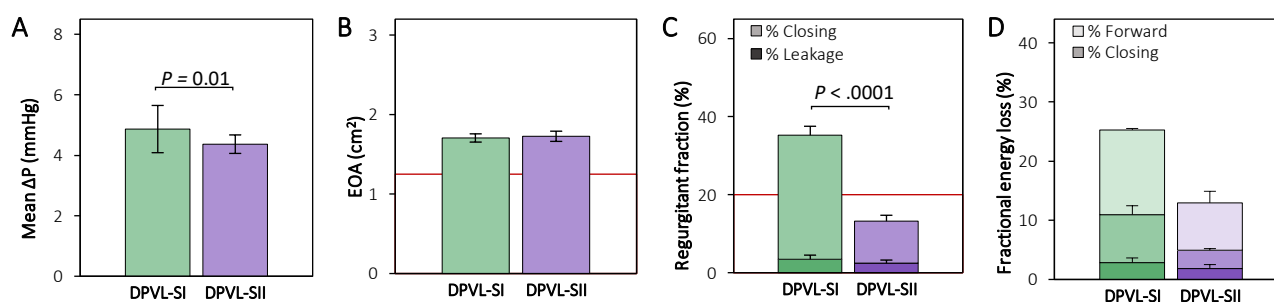


Figure 4.28: Comparison in hydrodynamics between DPVI and DPVII. A) Mean pressure gradient (ΔP). B) Effective orifice area (EOA). C) Regurgitant fraction comprised of closing and leakage fraction components. D) Forward, closing and leakage energy losses fractions.

Table 4.6: Hydrodynamic parameters of DPV

	ΔP (mmHg)	EOA (cm^2)	GOA (cm^2)	RF (%)	Energy loss (%)
DPVI	4.87 ± 0.78	1.71 ± 0.05	2.33 ± 0.14	34.68 ± 2.87	22.76 ± 1.97
DPVII	4.37 ± 0.31	1.73 ± 0.06	2.51 ± 0.04	13.15 ± 1.12	13.04 ± 1.35
p-value	0.0112	0.2603, NS	< .0001	< .0001	< .0001

4.3.2.4 Effect of leaflet thickness on hydrodynamics

Mean pressure gradient and EOA

Depicted in Figure 4.31A, the pressure drops obtained for the DPVs demonstrated a strong correlation with leaflet thickness ($\tau=0.72$, $P<.0001$), where an increase in leaflet thickness from $340 \mu\text{m}$ to $710 \mu\text{m}$ led to a significant increase in pressure gradient from 3.9 ± 0.2 mmHg to 5.3 ± 0.4 mmHg, respectively ($P<.0001$). A good fit was found with regression analyses which defined the relationship as $\Delta P = 0.004t_L + 2.41$ ($\tau = 0.72$, $P<.0001$).

EOA demonstrated a strong inverse correlation with leaflet thickness, defined by the linear relationship $EOA = -0.0007t_L + 2.09$ ($\tau = -0.63$, $P < .0001$) (Figure 4.31). Pairwise comparisons using the Wilcoxon rank-sum test indicated significant differences in EOA between all four group levels except between 620 μm and 710 μm valves ($P = 0.19$). As expected, the group set with the thinnest leaflets achieved the largest EOA of $1.8 \pm 0.1 \text{ cm}^2$, while the group set with the thickest leaflets obtained the smallest EOA of $1.6 \pm 0.1 \text{ cm}^2$. Regardless, the EOA outcomes obtained for each group exceeded the minimum ISO requirement of $>1.25 \text{ cm}^2$, and the corresponding pressure drops were well below the standard of $<10 \text{ mmHg}$.

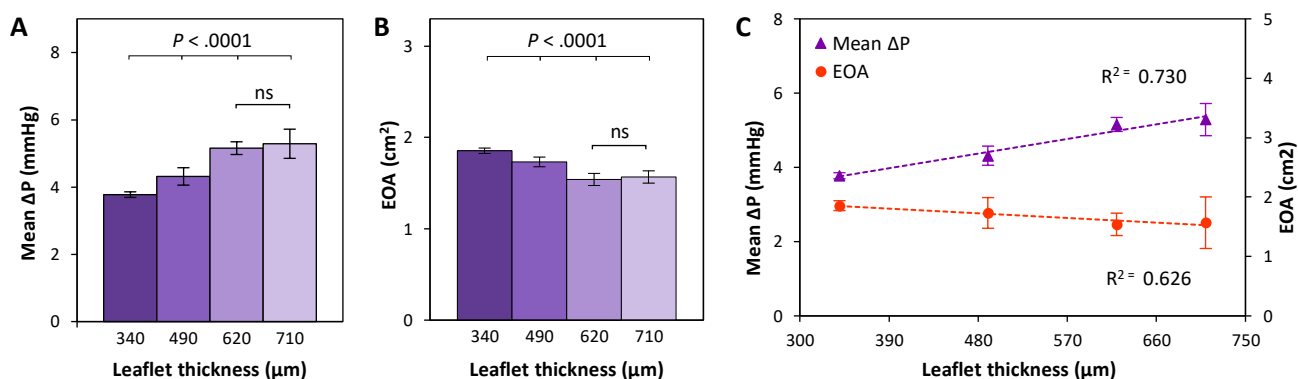


Figure 4.29: Mean ΔP and EOA results obtained for DP valves. A) Mean pressure gradient and B) EOA results obtained for the DPSII valves. C) Scatter plot of mean ΔP and EOA as a function of average leaflet thickness (ns = not significant).

Regurgitation

The amount of regurgitation, derived from the sum of the closing and leakage volume, was obtained in volume of each stroke volume, and as a percentage of the forward flow volume, known as the regurgitant fraction (RF) (Figure 4.30A). Overall, the regurgitation fraction revealed a strong correlation with leaflet thickness ($\tau = 0.445$, $P < .0001$). Between average thicknesses of 340 μm and 620 μm , the RF decreased by 5% from $15.4 \pm 3.2\%$ to $10.1 \pm 1.7\%$ ($P < .0001$). Across all groups, the closing volume accounted for the greatest portion of the regurgitant volume decreased with an increase in leaflet thickness, but with weak correlation ($\tau = 0.11$, $P = 0.021$). In contrast, the leakage volume varied distinctly in relation to leaflet thickness and was the main factor effecting the variation in regurgitation. As such, a strong correlation was found between leakage volume and leaflet thickness ($\tau = 0.75$, $P < .0001$). The lowest leakage volume and regurgitant fraction was achieved by the DP620 valves, which measured at $0.7 \pm 0.2 \text{ ml}$ and $10.1 \pm 1.7\%$, respectively. On the other hand, the DP340 valves suffered the largest leakage volume of 2.4 ml , and regurgitant fraction of $15.4 \pm 3.2\%$. Overall, as shown in Figure 4.30A, all DP valve met the minimum ISO performance requirement of $\leq 20\%$ RF.

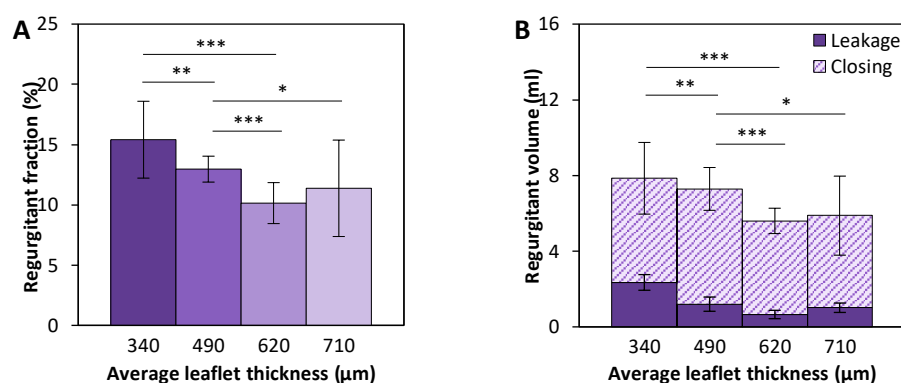


Figure 4.30: Regurgitation results obtained for DP valves. (A) Total regurgitant fraction. (B) Regurgitant volume performed in closing and leakage volumes (* $P=0.05$, ** $P=0.001$, *** $P<0.0001$).

Transvalvular energy loss

The transvalvular energy losses obtained for each group, including forward, closing and leakage losses, are presented graphically in Figure 4.31 as fractions of the left ventricular workload. Total transvalvular energy losses demonstrated a significant correlation with leaflet thickness ($R^2 = 0.76$, $P < .0001$), where an increase in leaflet thickness from 370 μm to 740 μm led to a 40% increase in total energy loss ($P < .0001$). Across all valve groups, forward energy losses accounted for the largest percentage of the total loss and had the greatest influence on the variation in total fractional losses. On average, the valves with the thinnest leaflets of 370 μm demonstrated the least amount of forward energy loss but suffered the largest closing and leakage losses. On the other hand, valves with thicker leaflets suffered larger forward losses but revealed lower closing and leakage losses. Overall, the least amount of energy was lost by the 460 and 550 μm valves, which on average incurred a total loss of 23.2 ± 1.8 mJ (equivalent to 13% of the ventricular energy).

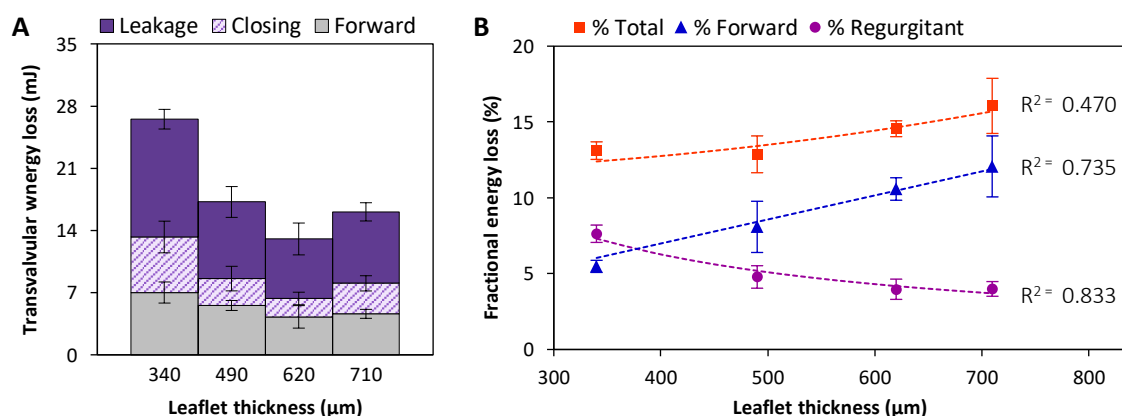


Figure 4.31: Transvalvular energy loss results for the DP valves. A) Average transvalvular energy losses corresponding to forward, closing, and leakage performances plotted as a function of leaflet thickness. B) Fractional energy losses plotted as a function of leaflet thickness.

The relationships between leaflet thickness and the total, forward, and regurgitant fractional energy loss (determined as the sum of the closing and leakage loss fractions) were model with power ($R^2 = 0.48$, $P < .0001$), linear regression ($R^2 = 0.74$, $P < .0001$), and reciprocal regression ($R^2 = 0.83$, $P < .0001$), respectively (Figure 4.31). These outcomes indicate that optimising leaflet thickness for minimised systolic (forward) energy losses leads to a trade-off for higher regurgitant losses.

4.3.3 Valve kinematics

4.3.3.1 Geometric orifice area

For both PEL and DPVs, the group with the thinnest leaflets presented distinctly larger GOAs at peak systole compared to the set with the thickest leaflets, which is visually presented in Figure 4.32. This was likely due to the higher bending stiffness inherent to the thicker leaflets, which led to significant leaflet buckling and evidently smaller opening areas. Average GOA quantifications are presented graphically in Figure 4.33, which clearly demonstrate the negative trend observed between GOA and leaflet thickness for both PEL and DPVs. Averaged over both flow rates, the GOA for PEL300 was approximately 15% larger than the PEL630s (mean difference = 0.4 cm^2 , $P < .0001$). Likewise, a significant decrease in GOA was determined between the DP340 and DP710 valve groups, which with respective estimates of 2.7 ± 0.1 to $2.0 \pm 0.2 \text{ cm}^2$ equated to a 25% drop ($P < .0001$).

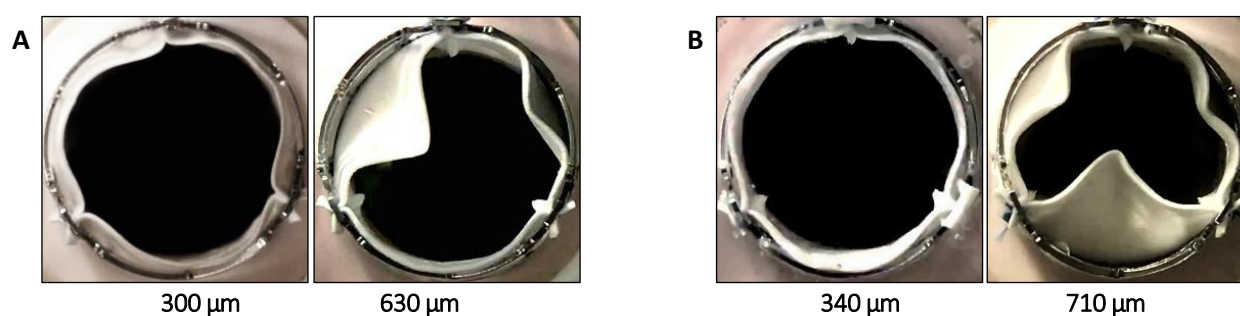


Figure 4.32: Visual comparison of GOAs performed by PEL and DP valves. (A) PEL and (B) DPVs demonstrating the distinct difference in opening area at peak systole between the groups with the thinnest and thickest leaflet.

Regression analyses indicated the relationship between GOA and leaflet thickness was different for the PELVs and DPVs, where the association strength was greater for the DPVs ($\tau = -0.84$, $P < .0001$ vs. $\tau = -0.60$, $P < .0001$, respectively). As shown in Figure 4.33C, the DPV GOAs decreased significantly across each level, whereas no discernible difference in GOA was found between the PEL300, 420, and 540s, with only the PEL630s demonstrating a notably smaller GOA (2.6 ± 0.1 vs. 2.3 ± 0.1 , $P < .0001$).

On the contrary, the coefficient of orifice contraction (C_c), which is considered indicative of pressure and flow efficiency, demonstrated a positive correlation with leaflet thickness for both PEL and DPVs despite the negative association observed with GOA ($\tau = 0.32$ and $\tau = 0.30$, $P < .0001$, respectively). On average, the PEL300s demonstrated a C_c of 0.7, which was significantly smaller than the PEL630s with an estimate of 0.74 ($P < .0001$). Similarly, between the DP340s and DP710s the C_c increased from 0.7 to 0.8 ($P < .0001$). This outcome suggests that even though the valves with thinner leaflets presented significantly larger GOAs, which is generally considered optimal, since they demonstrated smaller ratios between EOA and GOA, they may be vulnerable to increased energy losses due to flow turbulence around the leaflets, and reduced pressure recovery which would limit valve durability. In support of this finding, as previously described in section 4.3.2.1-2, on average the PEL and DPVs with the thinnest leaflets experienced significantly higher energy losses compared to the groups with thicker leaflets.

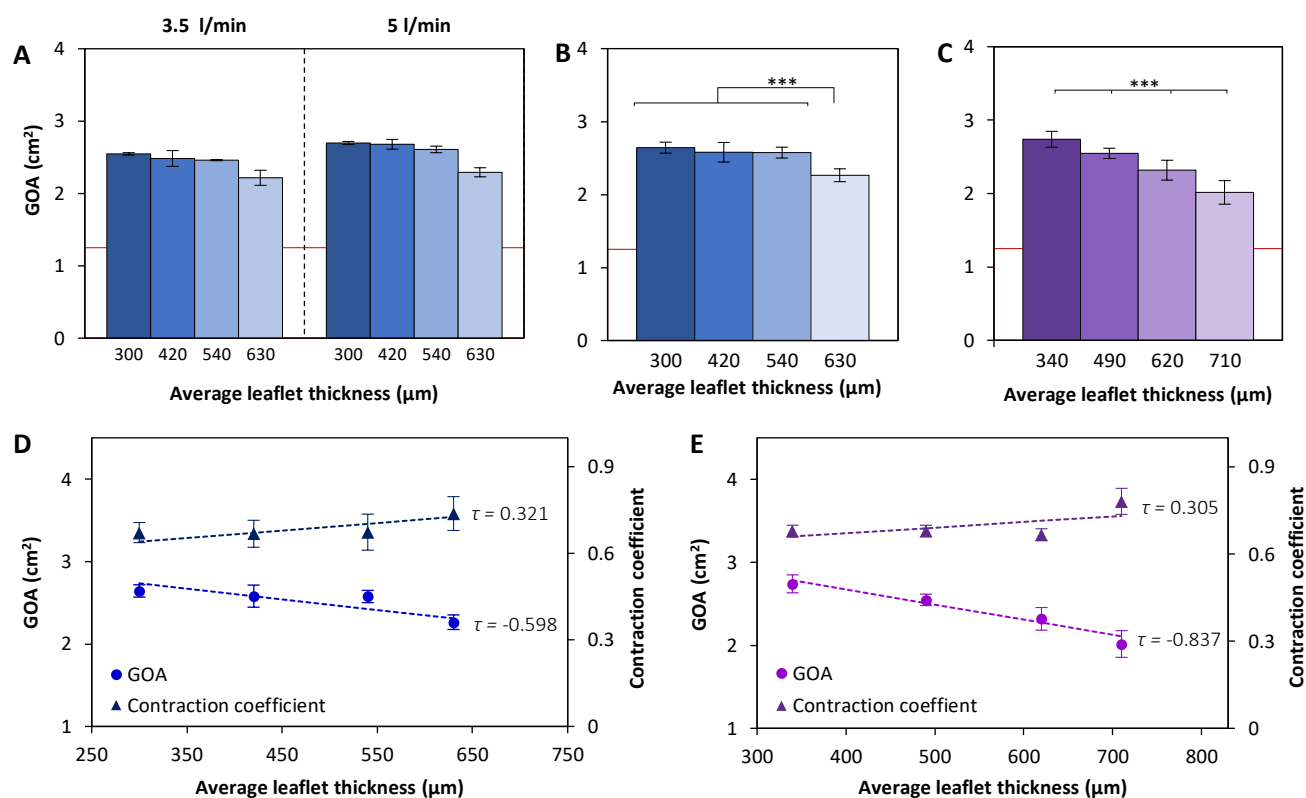


Figure 4.33: Geometric orifice area results as a function of leaflet thickness. A) Mean GOA results for PEL valves. B) PEL GOA results average across flow rates. C) DP GOA results as a function of leaflet thickness. D-E). PEL and DP GOA and contraction coefficient results plotted as a function of leaflet thickness.

4.3.3.2 Pinwheeling

The pinwheeling index (PI) was measured for each valve in the closed position during diastole and assessed relative to average leaflet thickness. For the PELVs, the degree of pinwheeling increased with increasing leaflet thickness (Figure 4.34) but with weak effect ($m_s = 0.01\%/\mu\text{m}$, $\tau = 0.38$, $P = 0.01$). Pairwise comparisons indicated that differences in average PI between groups 300 to 540 μm were not significant, whereas the 630 μm valves demonstrated more severe pinwheeling than the 300 and 420 μm valves (8.6 ± 3.1 vs. 4.6 ± 3.0 , $P = 0.02$; and $5.1 \pm 1.8\%$, $P = 0.03$, respectively) (Figure 4.34D). Likewise, the DPVs demonstrated a slight increase in PI with increasing leaflet thickness, where between 460 and 720 μm the average PI increased from 9.7 ± 2.5 to $11.9 \pm 4.5\%$ ($P = 0.43$), however there was no discernible difference between either of the groups, so the overall effect of leaflet thickness on PI was negligible ($\tau = 0.12$, $P = 0.39$).

Interestingly, despite the fact that the PELVs were exposed to more severe hydrodynamic loads than the DPVs, on average they showed significantly smaller degrees of pinwheeling (5.9 ± 2.7 vs. $10.3 \pm 3.5\%$, $P < .0001$). This phenomenon could be attributed to the higher stiffness and strength demonstrated by the PEL scaffolds compared to DP. With these attributes, the PEL leaflets showed to withstand high degrees of flexion without suffering notable structural deterioration or acute mechanical degradation, which likely facilitated smaller degrees of pinwheeling. In contrast, the significantly lower yield strength and higher plastic strain energy of the DP scaffolds would have rendered it vulnerable to more severe stretching, leading to higher bending stress and permanent deformation under cyclic loading, and thus resulting in a greater degree of pinwheeling. The high

localised bending stresses induced by pinwheeling are considered greatly detrimental to valve functionality as they have been linked to high rates of structural deterioration, elevated fatigue and accelerated failure of heart valve prostheses³⁷⁹. For this reason, according to ISO 5840 guidelines, large degrees of pinwheeling should be avoided and bending stresses minimised³⁶⁰.

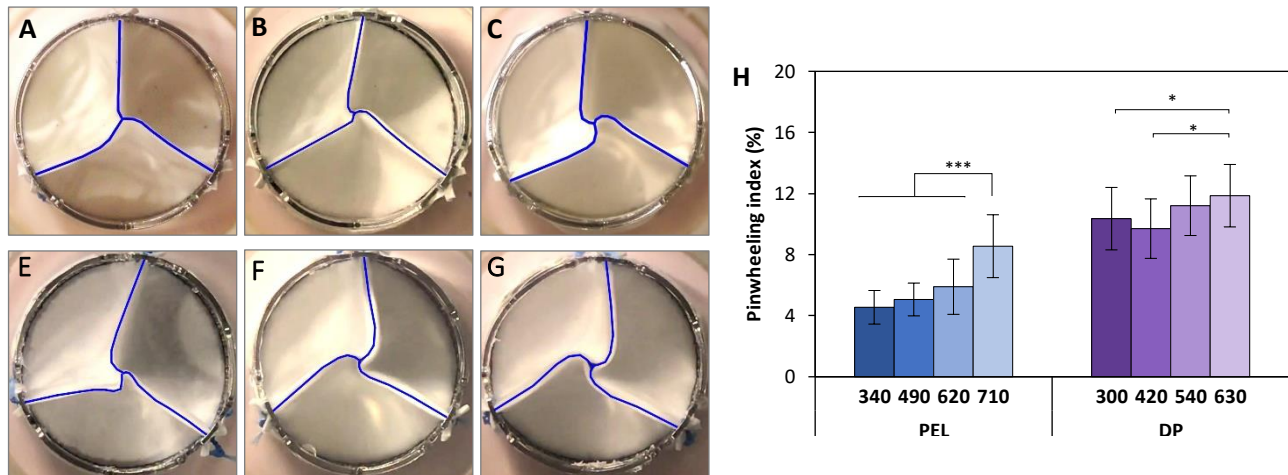


Figure 4.34: Pinwheeling index measurements. *En face* images of PEL (A-C) and DP (E-G) valves showing difference in pinwheeling behaviour at different leaflet thickness. H) Average pinwheeling indices plotted as a function of leaflet thickness for PEL and DP valves.

4.3.3.3 Leaflet motion behaviour

The leaflet motions over one cardiac cycle for each valve is presented Figure 4.35 as a montage of images representing forward flow (t_0 – t_3), peak systole (t_3), retrograde flow (t_4 – t_6), and diastole (t_6 – t_0). The qualitative kinematic aspects, such as leaflet synchronisation and geometric symmetry were visually assessed, while the quantitative aspects, such as geometric opening area, degree of pinwheeling, and opening and closing intervals were quantified. Video analyses of the leaflet kinematics revealed that 80% of the PELVs opened synchronously, whereas less than half of the DPVs achieved synchronous opening. Unsynchronised opening occurred for at least one DPV from each thickness group, but overall, the incidence and grade of asynchronous behaviour increased markedly as the leaflet thickness increased. Asynchronous leaflet closure was observed for all the valves but was especially prominent for valves with thicker leaflets.

In particular, leaflets of over 550 μm thickness demonstrated the most asynchronous closing behaviour, where leaflets were observed to close in distinct succession, rather than moving simultaneously. In general, unsynchronised leaflet motions are indicative of disproportionate stress distributions between valve leaflets, which is considered detrimental to valve durability and can result in severe hydrodynamic inefficiencies. Achieving synchronous opening and closing behaviour is thus an important consideration for valve design optimisation. Based on the deformation geometries demonstrated during acceleration, valves with thinner leaflets were prone to deforming asymmetrically, and leaflet margins commonly featured small wrinkles or sharp kinks. With increased thickness, leaflets maintained more axisymmetric configurations throughout the acceleration period, and typically adopted smooth uniform contours. The kinematic characteristics of valves with thicker leaflets was poor compared to thinner leaflet. In general, regions of sharp leaflet curvature produce

high stress concentrations in individual fibers, rendering them susceptible to mechanical fatigue and fiber breakage when subject to cyclic loading. For this reason, optimising for synchronous leaflet motion and reducing the incidence of sharp wrinkles is essential.

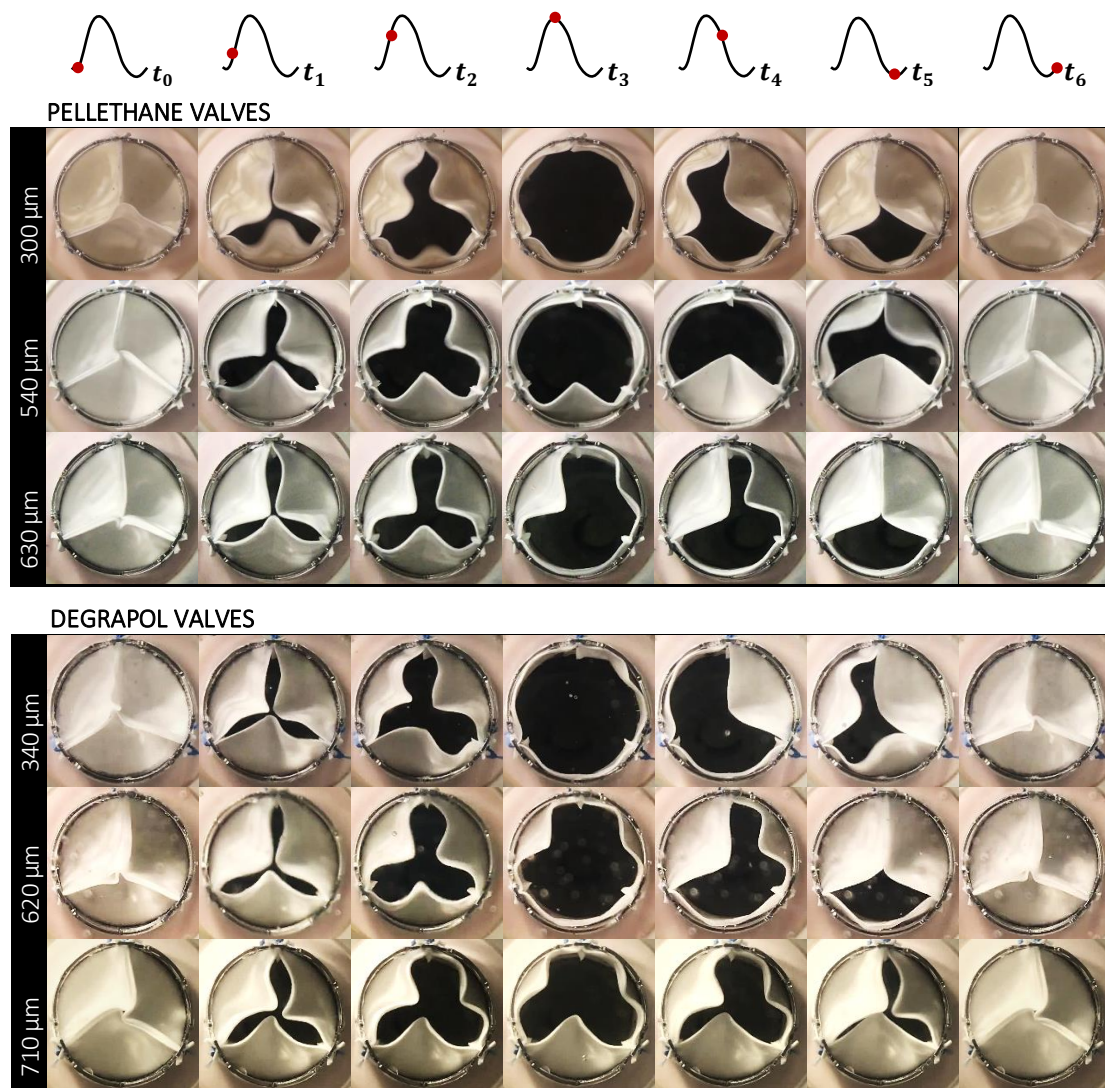


Figure 4.35: Image sequence of opening and closing behaviour of PEL and DP valves over one cardiac cycle.

4.3.3.4 Opening and closing phase characteristics

The pressure and flow waveforms acquired from the pulsatile tests were used to estimate the opening and closing characteristics of each valve. Figure 4.36 presents the mean results plotted in bar and Figure 4.37 presents examples of the pressure and flow waveforms of the thinnest and thickest leaflet groups from each valve category. Regarding the opening phase, for both categories the valves with thinnest leaflets performed the shortest rapid valve opening times (RVOTs), while the valves with the thickest leaflets demonstrated the lowest RVOT (14.5 ± 2.0 vs. 18.5 ± 2.2 ms between PEL300 and PEL630; and 21.5 ± 2.1 vs. 26.0 ± 1.7 ms between DP340 and DP710). Although there was no discernible difference between the group means for either category (Figure 4.36A and D), in both cases, regression analyses indicated a strong linear trend between RVOT and leaflet thickness ($\tau = 0.61$, $P = 0.03$ for PELV, and $\tau = 0.64$, $P = 0.01$ for DPV).

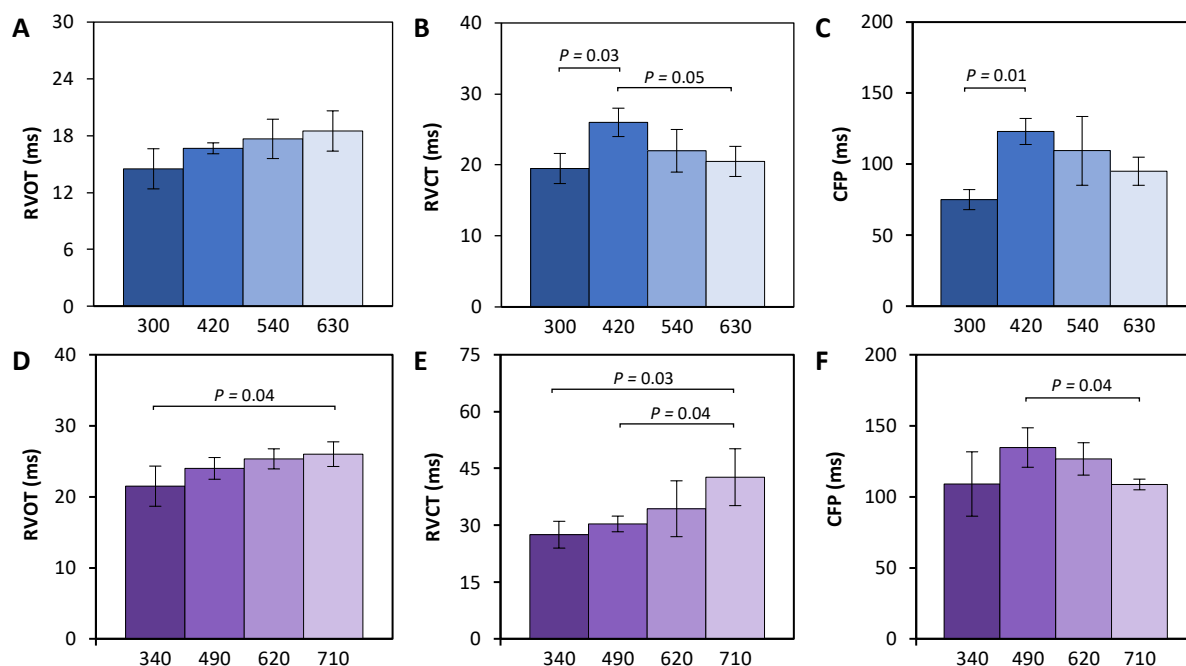


Figure 4.36: Opening and closing phase quantifications. Rapid valve opening time (RVOT), rapid valve closing time (RVCT) and closing flow period (CFP) plotted for the PEL (A–C) and DPSII valve groups (D–F), respectively.

For the closing phase, the PELVs and DPVs presented distinctly different behaviours. For the DPVs, average rapid valve closing times (RVCTs) increased significantly with increasing leaflet thickness (Figure 4.36E), where between 340 and 710 μ m, the RVCT increased by a factor of 1.5 ($P=0.03$) following a power trend (0.62, $P=0.01$). Conversely, no clear trend was observed between LT and RVCT for the PELVs (Figure 4.36B), where differences between PEL300, PEL540, and PEL630 were not significant.

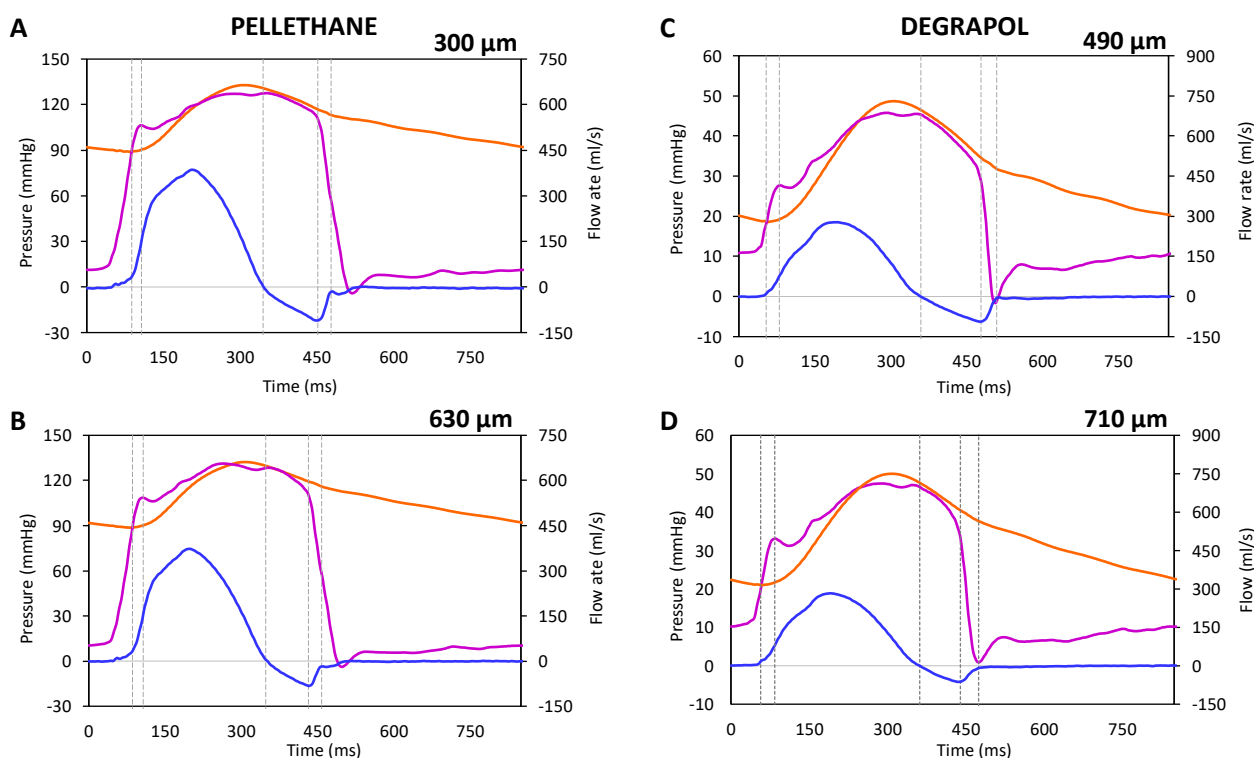


Figure 4.37: Pressure and flow waveforms obtained for PEL and DP valves. Average pressure and flow waveforms for PEL valve at (A) 300 and (B) 630 μ m in thickness, and DP valves at (C) 490 and (D) 710 μ m in thickness.

Curiously, the PEL420 valves were associated with the longest RVCT of 26 ± 2 ms, which was significantly slower than the PEL300 and PEL630 valves which performed the fastest RVCTs of 19.5 ± 2 ($P=0.03$) and 20.5 ± 2 ms ($P=0.05$), respectively. Despite exhibiting significantly different RVCT trends, a similar non-linear trend between leaflet thickness and closing flow period (CFP) was observed for both valve categories (Figure 4.36C and F). Regarding the PELVs, on average the thinnest leaflet group demonstrated the shortest CFP of 75 ± 7 ms, which contrasted significantly to the $420 \mu\text{m}$ valves which performed the longest closing time of 127 ± 9 ms ($P=0.01$). Between 420 and $710 \mu\text{m}$, the average valve closure times dropped again to 95 ± 10 ms ($P=0.04$). Similarly, DPV closure times increased between 340 and $490 \mu\text{m}$, but without a discernible difference (mean difference = 26 ms, $P=0.06$), and then decreased again between 490 and $710 \mu\text{m}$ (mean difference = 26 ms, $P=0.04$). Considering the GOA was distinctly smaller for valves with thicker leaflets, theoretically the leaflets would need to travel over a shorter distance to achieve maximum opening and complete closure, and hence, require less time. This notion was reflected in the quantifications of the rapid valve-opening and closing velocity indices (RVOVI and VCVI, respectively) which expresses the leaflet speed as a ratio between GOA and the respective phase times.

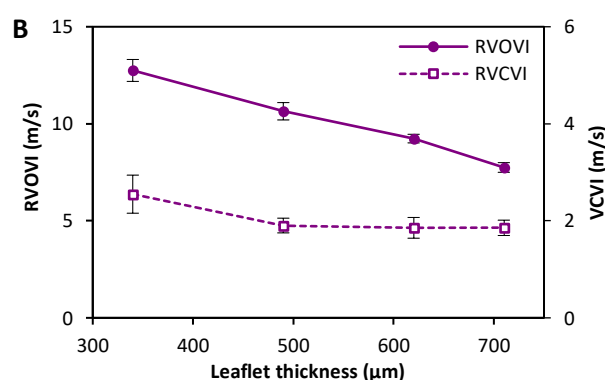


Figure 4.38: Valve opening and closing velocity indices as a function of leaflet thickness for DP valves

4.3.4 Valve durability

4.3.4.1 Evolution of valve hydrodynamics

The hydrodynamic parameters of the valves that survived accelerated wear testing (AWT) were reevaluated in the pulse duplicator after 30 and 100 million fatigue cycles ($n=3$), which is equivalent to 1 and 2.5 years respectively. As shown in Figure 4.39A, at both flow rates the valves pressure drop increased with respect to cycle count ($P<.0001$). Although no significant difference in the average pressure drop was demonstrated between the first and second timepoint (6.4 ± 0.9 vs. 6.5 ± 1.0 , $P=0.44$), after 100 million cycles the pressure drop increased significantly to 7.4 ± 1.1 mmHg ($P=0.003$) (Figure 4.40C).

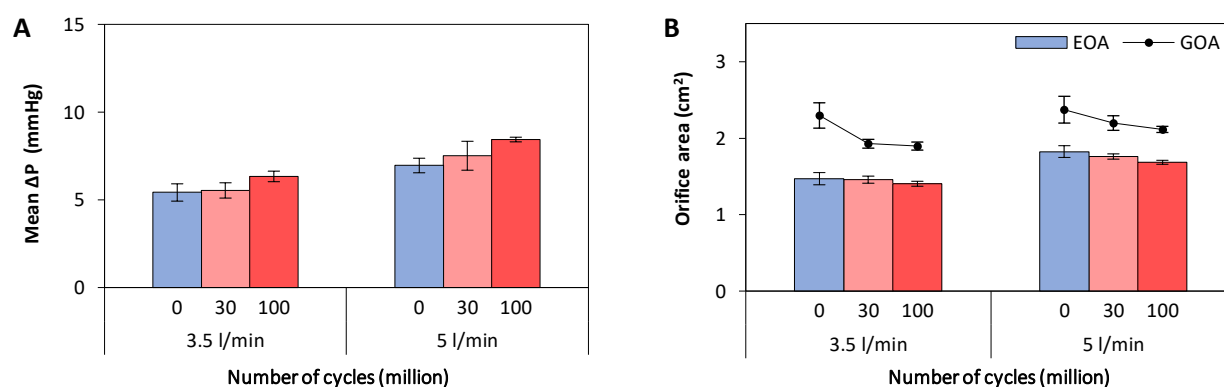


Figure 4.39: Effect of accelerated fatigue on valve hydrodynamics. (A) Mean pressure gradient and (B) EOA and GOA quantifications.

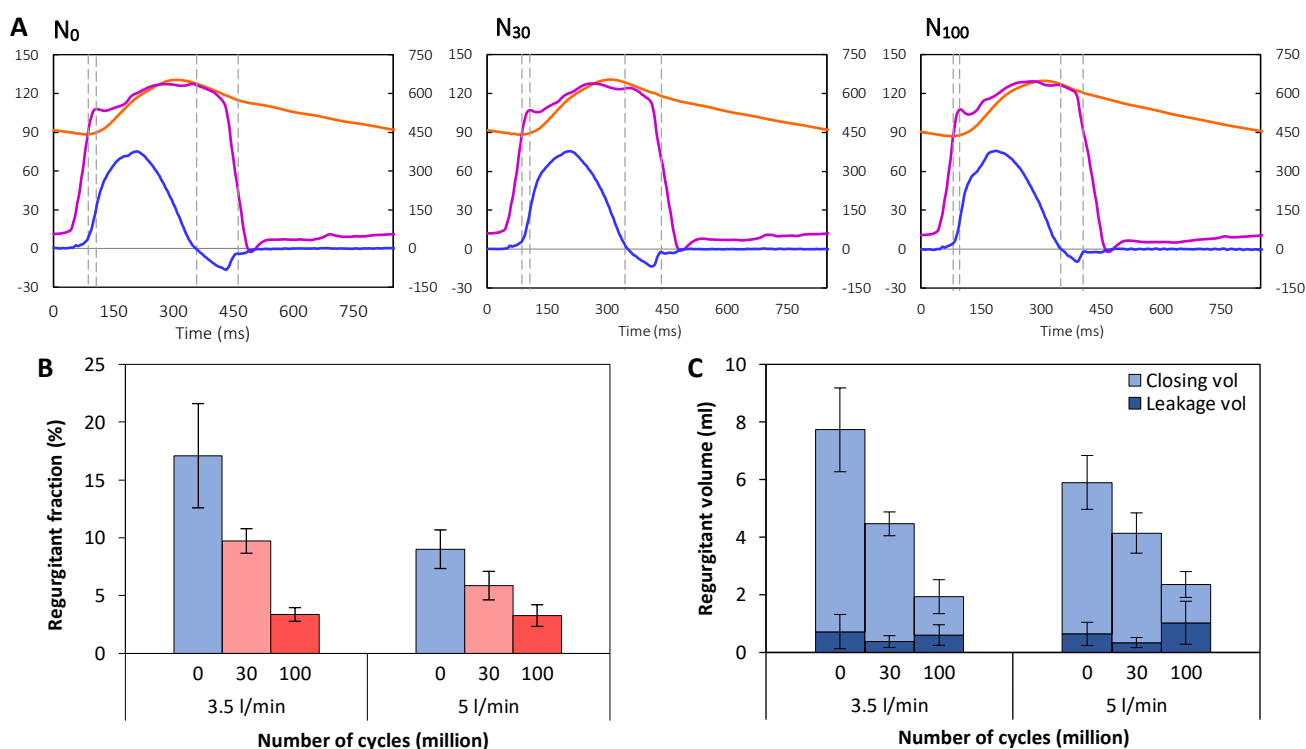


Figure 4.40: Evolution of PEL valve hydrodynamics. A) Average pressure and flow waveforms obtained at 0, 30, and 100 million fatigue cycles. B) Mean regurgitant fraction and (C) closing and leakage volumes plotted as a function of cycle count.

As shown in Figure 4.39, the EOA demonstrated a slight decrease with increasing cycle count, where between 0 and 100 million cycles, it decreased by only 0.2 cm² from 1.7 ± 0.2 to 1.5 ± 0.2 cm² ($P < .0001$). Similarly, the GOA decreased significantly with AWT, as shown in Figure 7.30, but was more severely affected after 30 million cycles than the EOA. This was indicated by the 12% decrease in GOA after 30 million fatigue cycles (from 2.3 ± 0.2 cm² to 2.06 ± 0.16 cm², $P < .0001$) compared to the 6% decrease in EOA between the same timepoints. However, beyond 30 million cycles average GOA outcomes remained relatively constant such that the percentage decrease in GOA and EOA reconciled after 100 million cycles (~10%, respectively). Despite the overall decline in systolic performance, on average the performance parameters obtained at each evaluation timepoint, including the pressure drop, EOA, GOA maintained accordance with the minimum ISO specifications.

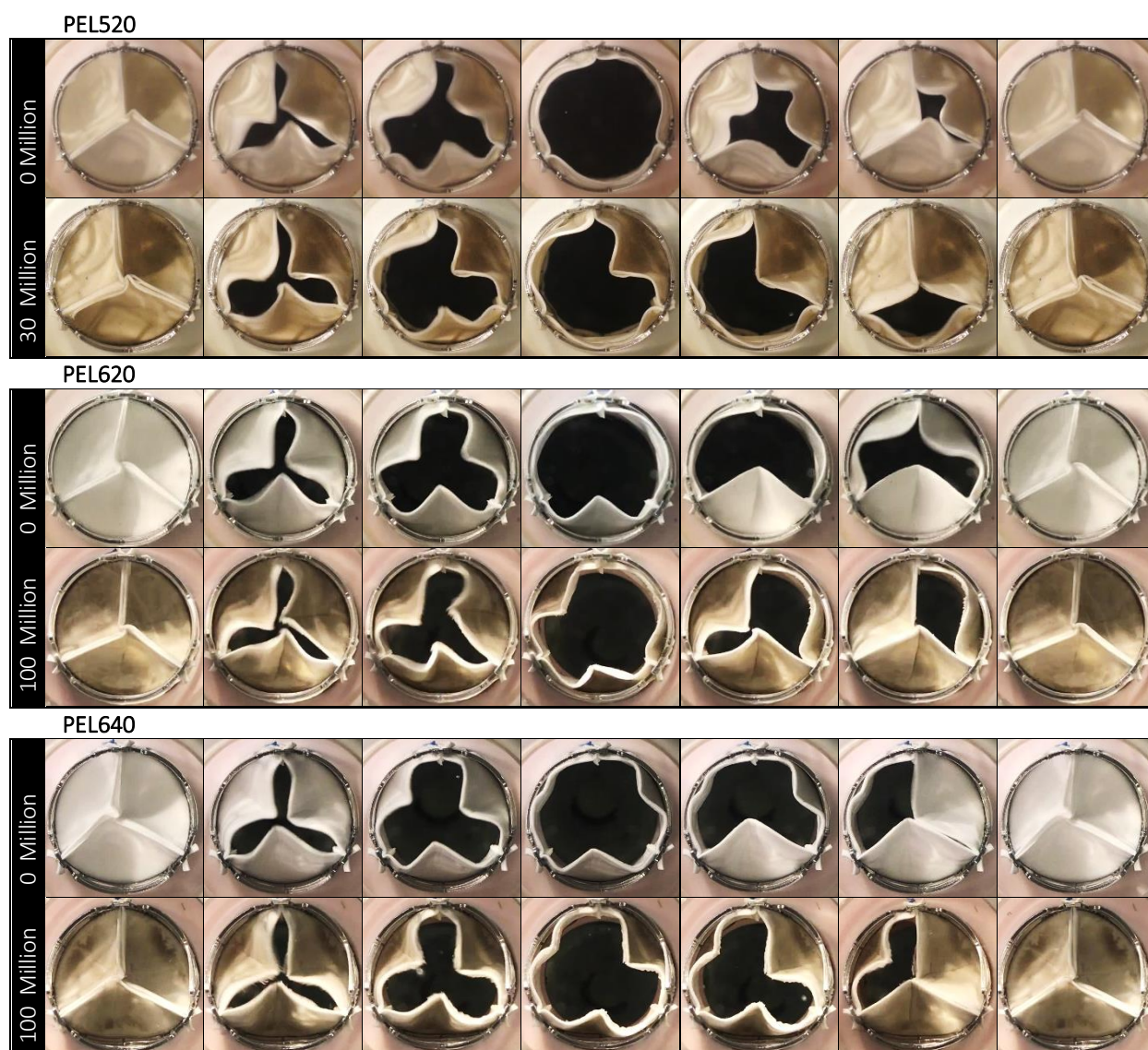


Figure 4.41: Photo montage of the opening and closing characteristics of fatigued valves tested at 0 and 100 million cycles.

Remarkably, accelerated fatigue loading had a significantly positive impact on the valves' overall diastolic performances, which is indicated in Figure 4.40A by the decrease in closing flow period in conjunction with the reduction in negative area bounded by the flow curve between each time point. On average, between initial testing and 100 million loading cycles the RF decreased by approximately 70% (11.7 ± 4.8 vs. $3.3 \pm 0.8\%$, $P < .0001$) which correlated proportionately to the considerable decrease in closing volume from 5.9 ± 1.4 to 1.3 ± 0.5 ml ($P < .0001$) (Figure 4.40B and C). Even though the valves demonstrated an overall increase in leakage volume after 100 million cycles, the difference was without significance ($P = 0.37$) and the effect was of minimal impact on the total regurgitant volume ($R^2 = 0.04$, $P = 0.01$). Considering that after 100 million fatigue cycles the valve hydrodynamics met standard requirements, they were considered suitable for further AWT.

Table 4.7: Hydrodynamic parameters obtained after 40 and 100 million accelerated loading cycles

Cycle count	ΔP (mmHg)	EOA (cm ²)	GOA (cm ²)	RF (%)	Closing (ml)	Leakage (ml)
0	6.44 ± 0.85	1.71 ± 0.19	2.34 ± 0.17	11.74 ± 4.83	5.85 ± 1.40	0.67 ± 0.47
30 million	6.53 ± 1.02	1.61 ± 0.16	2.06 ± 0.16	7.80 ± 2.28	3.94 ± 0.58	0.36 ± 0.19
100 million	7.38 ± 1.09	1.54 ± 0.15	2.03 ± 0.10	3.32 ± 0.77	1.33 ± 0.52	0.82 ± 0.62

Fatigue life

The results from the accelerated wear tests for each valve are provided in Table 4.8, with a brief description of their failure modes. In Figure 6.32 the fatigue life demonstrated by each valve is plotted as a function of mean leaflet thickness. The most durable valve, which had a leaflet thickness of $\sim 620\ \mu\text{m}$, withstood 262 million accelerated fatigue cycles, followed by the valve with a leaflet thickness of $640\ \mu\text{m}$. Eight of the ten PELVs sustained leaflet tears at the commissure hinge, each of which transpired over different timescales. The two valves with average leaflet thickness of 535 and $619\ \mu\text{m}$, that did not acquire tears, were eventually failed due to excessive delamination, which had propagated circumferentially along the free-edge and radially along the coapting surface. Although the delaminated regions did not appear to influence the opening and closing behaviour, the high degree of structural deterioration indicated a critical loss in mechanical compliance which was regarded as valve insufficiency.

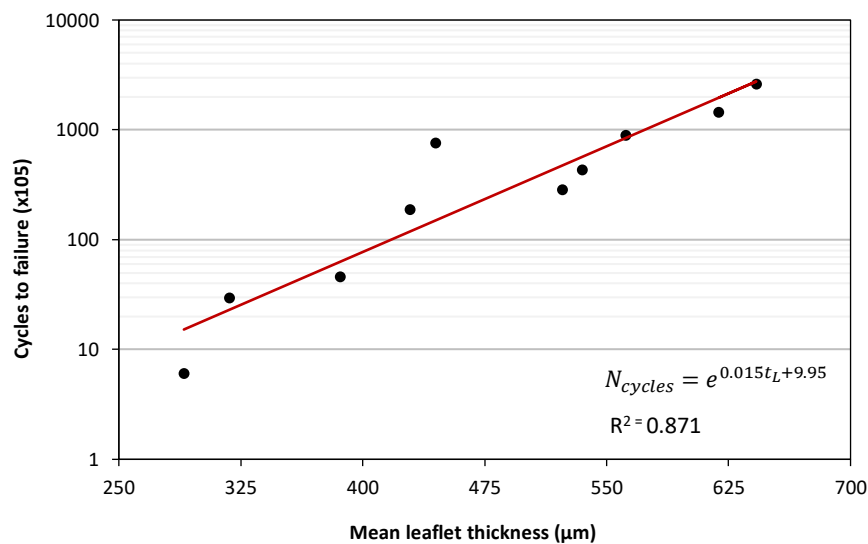


Figure 4.42: Fatigue life as a function of mean valve leaflet thickness

Table 4.8: Accelerated wear testing results summary

Valve ID	Mean LT (μm)	Failure mode	Life cycles (million)
PEL290A	290	Commissure tear	0.6
PEL320A	318	Commissure tear	2.9
PEL390B	386	Commissure tear	4.6
PEL430B	429	Commissure tear	18.8
PEL450C	445	Commissure tear	75.9
PEL520C	523	Commissure tear	28.5
PEL540D	535	Delamination buckling and fracture	43
PEL560D	562	Commissure tear	88.6
PEL620E	619	Delamination buckling and fracture	145
PEL640E	642	Commissure tear	262

4.3.4.2 Wear characteristics and failure modes

In most cases, macroscopic failure of polymer constructs is the culmination of stress-induced dissipative and disruptive processes that occur on a microscopic scale in localised regions throughout the structure. However, the initiation and development of such processes are often masked by deformational processes that manifest uniformly across the construct. Consequently, wear mechanisms and failure processes typically go unnoticed until catastrophic crack propagation or other instabilities manifest³⁸⁰. This highlights the importance of performing microscopic examinations on valve leaflets over the course of fatigue testing to credibly characterise the wear behaviour and more accurately detect the onset of failure. The main wear processes and failure modes observed between the PEL and DP valve are listed in Table 4.9 below. In general, each valve demonstrated a unique combination of wear mechanisms which acted in concert with a range in severity.

Table 4.9: Summary of observed scaffold wear mechanisms and failure modes

Leaflet scaffold wear mechanisms

- | | |
|---------------------|--------------------------------|
| ▪ tearing | ▪ compaction and consolidation |
| ▪ delamination wear | ▪ adhesive wear |
| ▪ transfer wear | ▪ abrasive wear |
| ▪ buckling | ▪ surface fatigue |

Tearing

Scaffold tearing only occurred at the leaflet commissures, suggesting these regions experience the highest stress concentrations. This inference corresponds with reports from associated studies that evaluate the distribution of stress across trileaflet valve structures, all of which confirm that the highest stresses are in fact experienced at the commissure hinge^{175,281,381,382}.

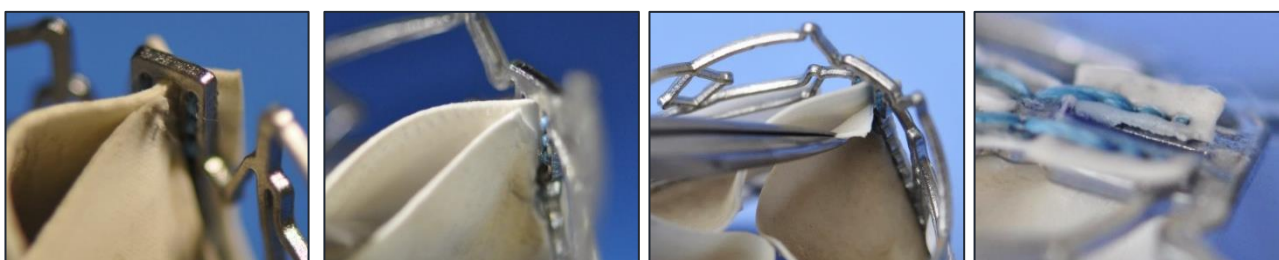


Figure 4.43: Sample images of PEL valve leaflets torn at the commissure hinge

Delamination wear

Delamination was of the most prominent wear mechanisms observed across the PELVs. Most of the valves presented mild degrees of delamination, especially in the central coaptation region, but did not appear to effect valve function. In two cases, however, the delaminated zones gradually propagated until the scaffold suffered acute transverse rupture. For one valve this occurred at the commissure post (Figure 4.44A), and for the other it occurred at the central curve of the leaflet free-edge (Figure 4.44B).

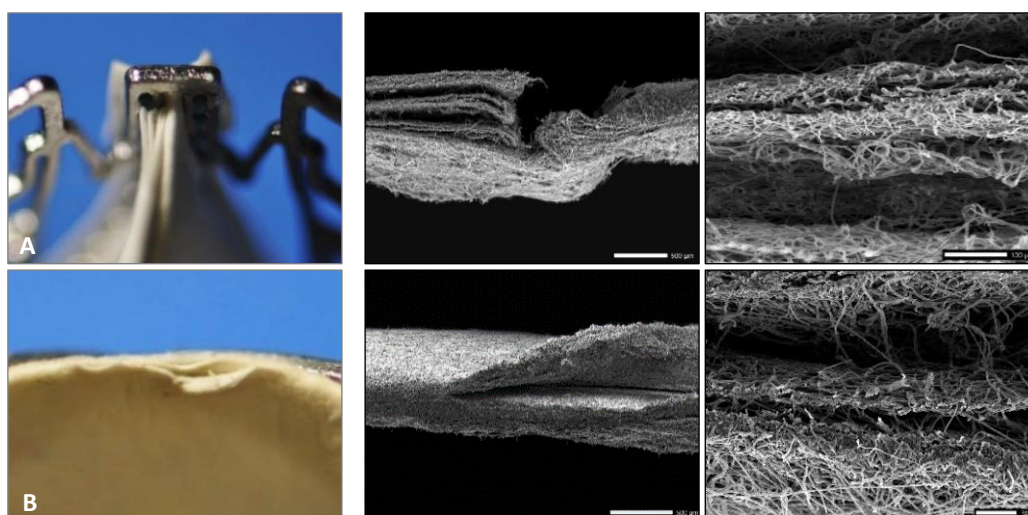


Figure 4.44: Macroscopic and microscopic images of delamination at (A) the commissure hinge and (B) leaflet free-edge.

Delamination can be characterised as the splitting, cracking and/or separation of laminar structures along a plane parallel to the surface³⁸³. For electrospun scaffolds, which are essentially composed of hundreds of micro/nanofibrous layers, the process of delamination is governed by the interlaminar bond strength of the construct, which relies largely on the cohesive strength between intersecting fibers and the strength of the individual fibers^{384,385}. Multidirectional matrix structures are known to be especially vulnerable to interlaminar failures, owing to the successive layers presenting distinct mechanical behaviours which lead to stress coupling phenomena³⁸⁶. As a result, out-of-plane stresses develop which facilitate the initiation and growth of interlaminar cracks and reduce buckling strength³⁶⁴. Due to the lower lateral stability of the outer scaffold layers, delaminations are prone to initiating at the outmost layers, as shown in Figure 4.44C–D. As interlaminar cracks propagate and coalesce, sublaminae form which subsequently act as independent load-bearing entities³⁸⁷ (demonstrated in Figure 4.44B–D). Due to the reduced structural and mechanical integrity of these structures, they are more susceptible to splitting and buckling, which promotes delamination growth. As this process of delamination progresses, the individual layers eventually suffer transverse fractures, as shown in Figure 4.44B.

Numerous studies report delamination to be greatly detrimental to the *in vivo* functionality of electrospun constructs³⁸⁸. This is because the culmination of interlaminar failures yields structurally unstable and mechanically insufficient environments for cellular activity and effective tissue ingrowth. As delamination was the most prominent wear mechanism for the PELVs, establishing techniques to improve the delamination resistance of electrospun valves leaflets for long-term durability and *in vivo* feasibility should be a key consideration for future study.

Buckling

When leaflets bend during valve opening, compressive stresses develop on the inner surface while counteracting tensile stresses develop on the outer surface, and vice versa during valve closure. The magnitude of these stresses correlates proportionately to the flexural modulus of the leaflet material³⁰⁸. As such, for stiff leaflets that cannot conform easily to curvature fluctuations, the stresses generated will be exceedingly high, which among other distortion failures, can lead to compressive buckling.

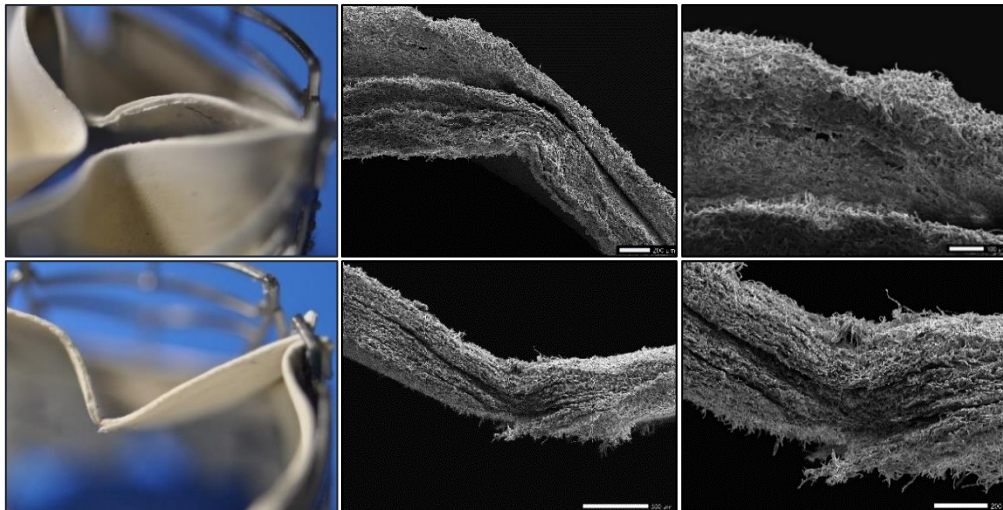


Figure 4.45: Example images of multimode failure by leaflet buckling and delamination.

Compressive buckling manifested as sharp kinks and crinkling of the surface inside the bend, as demonstrated in Figure 4.45D–E. It generally arises when materials are subjected to large compressive stresses that cause excessive strain deformations resulting in localised structural collapse. Buckling is commonly observed in situations where materials are bent to extreme curvatures³⁰⁸. Owing to the complex bending regimes that heart valve leaflets must endure, studies on cardiac valve devices often report the occurrence of leaflet buckling and its adverse effects on valve performance^{125,389–391}. For electrospun scaffold leaflets, buckling is the culmination of the deformation, crushing and breakage of individual fibers in response to excessive compressive loads. An additional governing factor is the ability of the scaffold fibers to internally rearrange to accommodate the induced bending stresses without suffering structural or mechanical breakdown. This behaviour is governed by a multitude of factors, which can essentially be reduced to the mechanical properties of the individual polymer fibers and the interfacial bond strength between the intersecting fibers throughout the scaffold matrix.

Surface fatigue

Surface fatigue can be characterised as the formation and development of cracks on a material surface as a result of cyclic loading. Wear phenomena occur when these cracks propagate and intersect to form wear debris that dislocates from the surface, leading to a loss in material³⁰⁹. Fatigue wear differs from abrasive wear in that its governing mechanism relies on elastic deformation as opposed to plastic deformation. On this account, surface fatigue is generally more prevalent in low modulus polymers and elastomers³⁰⁹.

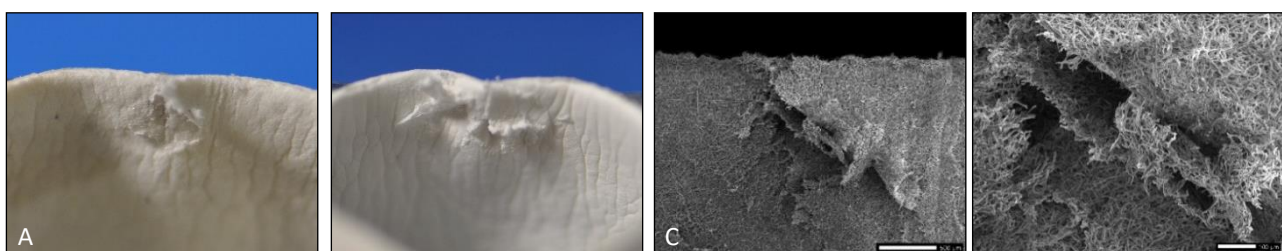


Figure 4.46: Surface fatigue at central coapting region of leaflets. Example images from (A) macroscopic and (B) microscopic inspections.

4.3.4.3 DegraPol valve durability

All fourteen DPVs suffered acute premature failure within 5–10 minutes of hydrodynamic loading under nominal pulmonary conditions. Thus, none of the DP valves were eligible for accelerated fatigue testing. The primary mode of failure across all DP valves was tearing of the leaflets at the commissures (Figure 4.47). For three of the valves, the tears propagated rapidly from the commissures down the leaflet attachment line, where within minutes two of the three leaflets had fully detached from the scallop. For the remaining valves, the tears propagated more gradually, but the difference was minor, and bore no clear relationship with leaflet thickness. Thus, unlike the PEL valves, no correlation was found between DP valve leaflet thickness and leaflet durability.

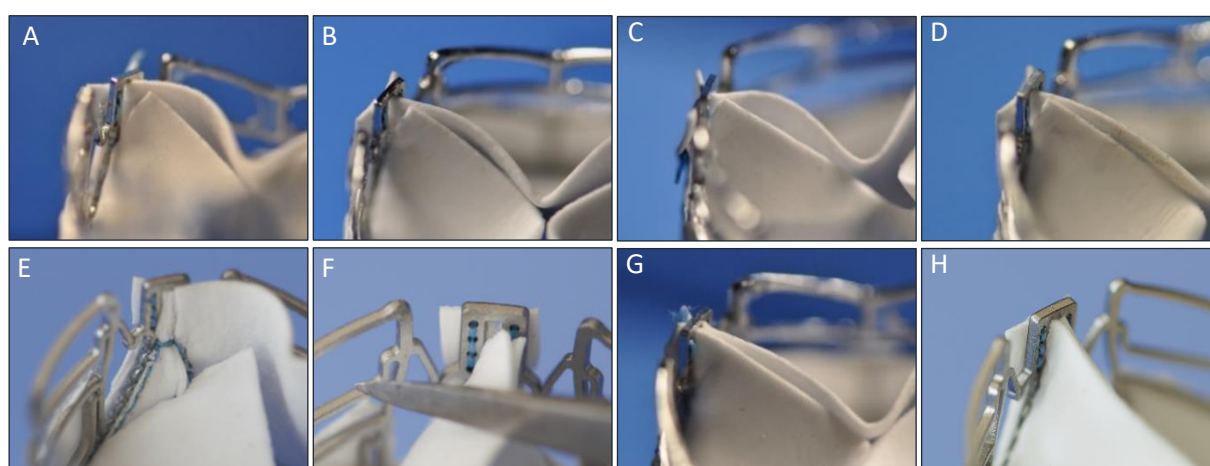


Figure 4.47: Visual inspection of DPV leaflets after pulsatile testing. A-F) Tearing at commissure hinge. G-H) Commissure hinge intact

SEM analysis of the fatigued DP leaflets revealed that the scaffolds suffered severe microstructural deterioration in the regions of rupture, as shown in Figure 4.48. Across all DPV leaflets, the mechanism of failure manifested primarily as a combination of adhesive wear, compressive strain, and compaction. This was likely caused by the complex fluctuation of stresses imposed on the fibers during hydrodynamic loading, which appeared to provoke excessive internal friction, and subsequently elicit a viscous flow response accompanied with permanent deformation^{392,393}. In addition, the viscous drag generated during interstitial fluid flow through porous substrates can cause fiber compaction which, depending on the applied load, can lead to scaffold rupture¹⁹². Porous structures are specifically prone to compaction as large hydrodynamic forces can drive the pores to compress until adjacent fibers completely fuse. In this way, excessive scaffold compaction can permanently eliminate open pore channels and lead to large volumes of densely packed fibers. This would not only weaken the scaffold's mechanical integrity, but would also suppress cellular activity, as cells would be prevented from infiltrating the consolidated regions and migrating freely through the scaffold, leading to incomplete tissue ingrowth (Wang et al., 2010). Scaffold compaction and consolidation also featured prominently along the suture lines (Figure 4.48B) and leaflet free-edge (Figure 4.48CD). Evidently, these areas experienced large compression stresses which could be to do with the compressive forces applied by the sutures (that are in a permanent state of tension to effectively secure the leaflet to the stent); and the application of the die-cutting tool to shape the leaflets. This suggests that the DP scaffolds possessed unsuitable compression properties for the manufacturing techniques employed in this study.

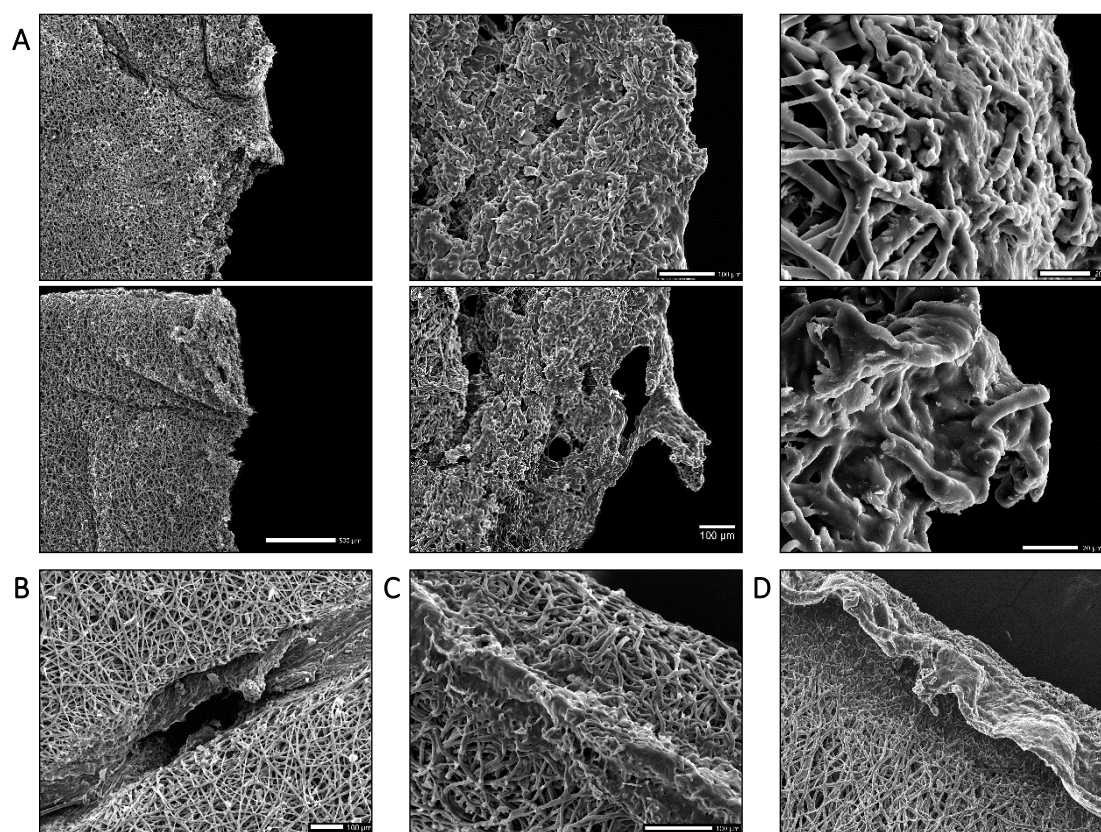


Figure 4.48: Compaction and consolidation of DP leaflet scaffold in commissure hinge tear region.

4.3.5 Results summary

- PEL valves were tested under simulated aortic conditions, while DP valves were test under simulated pulmonary conditions due to insufficient strength to withstand aortic pressures.
- Both PEL and DP valves demonstrated good hydrodynamic function under the respective loading regimes and were found to perform appropriate opening and closing leaflet motions. Both types of valves demonstrated ISO compliant hydrodynamic parameters ($\Delta P < 10$ mmHg, $EOA > 1.25$ cm², and $RF < 20\%$).
- Despite demonstrating a positive correlation between forward energy losses and leaflet thickness, total transvalvular energy losses were found to decrease with increase in leaflet thickness, which was attributed to the corresponding exponential decrease in regurgitant energy loss with increasing leaflet thickness.
- PEL valves reached up to 260 million of accelerated fatigue cycles, whereas DP valves were not eligible for durability testing due to insufficient mechanical properties.
- A positive correlation was found between leaflet thickness and valve durability for the PEL valve group.
- The most common mode of valve failure across all test valves was tearing of leaflets at the commissures.
- The most common wear mechanism exhibited by the PEL valve leaflets was delamination and surface fatigue along the central and peripheral coaptation regions.

5 Conclusion and Recommendations

This chapter presents the conclusions and recommendations that were formulated based on the findings encountered in this study, which culminated in the production and characterisation of nondegradable and degradable electrospun polymer transcatheter heart valves.

5.1 Conclusions

5.1.1 Summary

The overall objective to develop and hydrodynamically assess biostable and biodegradable electrospun trileaflet THV constructs was accomplished. Effective parameters for fabricating electrospun PEL and DP scaffolds were established, and a suitable procedure for assembling the scaffolds onto a stent frame to form a trileaflet structure were developed.

Hydrodynamic testing demonstrated proof of concept using electrospun PEL scaffolds as valve leaflets that could withstand simulated *in vivo* aortic conditions. Furthermore, durability testing showed that the PEL valves could be cycled for up to 260 million repetitions while maintaining appropriate pressure differentials. Conversely, although the DP valves demonstrated good hydrodynamic characteristics under simulated *in vivo* pulmonary conditions, DP scaffold leaflets suffered acute failure in less than five minutes of operational loading, irrespective of average leaflet thickness. Microscopic examinations revealed that the DP leaflets experienced severe deformation instabilities, which was attributed to their subpar mechanical properties. The insufficient durability demonstrated by the DP scaffold leaflets indicated that their application as materials for HVTE in the current embodiment was unfeasible.

On the other hand, the excellent hydrodynamic properties and remarkable durability demonstrated by the electrospun PEL scaffolds showed that along with their material properties, the manufacturing procedure employed to construct the valve samples was highly effective. Moreover, although the nondegradable nature of Pellethane renders it inappropriate for traditional HVTE applications, the promising outcomes demonstrated by these valves begs the question as to whether a nondegradable electrospun THV that can facilitate tissue ingrowth while providing lifelong durability could be a superior alternative to current heart valve replacement prostheses.

5.1.2 Detailed

The conclusions drawn from the findings obtained for each phase of research and development are as follows:

Scaffold development

- A humidity control system was designed, and successfully integrated into the electrospinning rig which enabled the effective electrospinning of DP scaffolds and overall, enhanced the controllability of the electrospinning experiments which facilitated the production of more repeatable scaffold parameters.
- The effect of solution and process parameters on the physical properties of electrospun PEL and DP scaffolds was established. For PEL scaffolds, the solution flow rate and rotational collecting speed were found to be influential in tuning the scaffold architecture and enhancing the mechanical properties. For DP scaffolds, relative humidity, solution concentration, flow rate, and collecting speed proved to be key parameters in controlling and enhancing resultant the architectural and mechanical aspects.
- For both PEL and DP scaffolds, a variation in flow rate provoked the most significant change in fiber diameter, while a variation in rotational speed provoked the most significant change in mechanical behaviour. Specifically for DP scaffolds, relative humidity played a critical role in controlling fiber diameter distribution, average pore size, and porosity.
- From the respective parametric investigations, optimal conditions for electrospinning PEL and DP scaffolds were established. Suitable parameters for electrospinning 16 wt% PEL solutions in DMF/THF (1:1) using a 19G needle were identified as: a relative humidity of 50%, solution flow rate of 3 ml/hr, collecting speed of 1000 rpm, an applied voltage of +17 kV supplied to the needle and -3 kV to the collector, and a working distance of 25 cm. Suitable parameters for electrospinning DP solutions were identified as: a solution concentration of 15 wt% DP in chloroform, 19-gauge needle, relative humidity of 35–40%, solution flow rate of 1 ml/hr, collecting speed of 1000 rpm, an applied voltage of +23 kV supplied to the needle and -2 kV to the collector, and a working distance of 28 cm.
- PEL and DP scaffolds demonstrated considerably different mechanical characteristics. On average, DP scaffolds demonstrated a quarter of the strength and half the stiffness of the PEL scaffolds but were approximately two times more extensible.
- Interestingly, the mechanical anisotropy of PEL and DP scaffolds responded differently to a change in rotational velocity. While DP scaffolds demonstrated an increase in anisotropy with an increase in collecting speed, the PEL scaffolds decreased in anisotropy with an increase in collecting speed, despite exhibiting an increase in fiber alignment.
- Viscoelastic behaviour was characterised for PEL and DP scaffolds, respectively. Under the same loading conditions, DP scaffolds reached peak stresses three times lower than PEL scaffolds, manifested double the degree of hysteresis, and demonstrated a greater vulnerability to cyclic softening.
- On average, DP scaffolds were three times more permeable than PEL scaffolds, which was attributed to their larger pores which were overall double in size. Scaffold permeability correlated positively with average pore

size. Regardless, both scaffold groups demonstrated permeabilities that, according to literature, was regarded as suitable for HVTE applications.

- PEL scaffolds exhibited greater hydrophilicity upon first contact with water, however DP scaffolds exhibited a higher water absorption rate. Nevertheless, the surface wettability exhibited by both scaffold groups was ultimately hydrophilic and could therefore be considered as suitable for HVTE applications.

Valve manufacturing design

- A systems analysis on the development process of an electrospun TETHV was conducted by means of an Entity Relations Diagram, which gave insight into the multifactorial complexity of this field of research.
- With the knowledge gained from the systems analysis, key requirements and criteria for developing an ideal TETHV were compiled and categorised as physical, functional, performance, manufacturing, and biological requirements. From this, the requirements pertinent to this study were identified.
- A set of distinct electrospinning systems, fiber collector geometries, and material joining techniques were identified from literature or innovated by design to selectively incorporate into a valve manufacturing procedure. In this way, different sequences of operations were formulated to manufacture an electrospun trileaflet THV, from which the most feasible concepts were selected for prototyping.
- Six unique concepts were prototyped, of which two were only partially completed due to inherent shortfalls, and in the end, only two of the four complete valve prototypes were viable for hydrodynamic evaluation.
- Overall, the most uniform scaffolds, in thickness and microstructure, were achieved by electrospinning onto a standard cylindrical fiber collector. Employing collectors that featured sharp edges and/or deep concave surfaces resulted in grossly irregular scaffold deposition, which is a highly unfavourable attribute in HVTE. Of the material-joining techniques, interface fusion was the least effective technique to secure the skirt to the stent due to insufficient bond strength between the apposing scaffold layers. On the other hand, heat bonding was identified as a superior method of attachment in terms of bond strength and reproducibility.
- The hydrodynamic testing and subsequent screening of the valve prototypes revealed the most effective valve manufacturing procedure to be the pre-cut leaflet suturing procedure. This method involved electrospinning scaffold tubes, using a standard cylindrical collector, from which tubular segments were cut to form the skirts, and individual leaflets were die-cut to form pre-shaped leaflets. Subsequently, the skirts were heat bonded to the stent, and thereafter, three pre-cut leaflets were individually sutured to the stent to form a trileaflet configuration in a neutral closed position.

Valve evaluation

- The valve manufacturing procedure developed for this study was successfully employed to produce trileaflet THV constructs composed of electrospun PEL or DP scaffold leaflets integrated onto a metal stent.
- Initial hydrodynamic testing of the PEL and DP valve samples under simulated *in vivo* aortic conditions revealed that the PEL valve leaflets were mechanically sufficient to withstand the loading regime and demonstrated impressive hydrodynamic characteristics. On the other, DP valves suffered catastrophic

failure under simulated aortic conditions, for which reason they were considered to be more suited to hydrodynamic test conditions representative of the *in vivo* pulmonary environment.

- The influence of leaflet thickness on valve hydrodynamics for both PEL and DP valves was significant. Overall, superior hydrodynamic characteristics were achieved by valves with thicker leaflets. For the PEL valves, the optimal leaflet thickness was between 560–640 μm , and for the DP valves, the optimal leaflet thickness was between 620–710 μm . Leaflet thickness was also observed to have a considerable effect on leaflet kinematics, where thinner leaflets demonstrated superior kinematic characteristics compared to thicker leaflets, such as larger maximum opening areas, faster opening and closing times, and lower degrees of pinwheeling. The resultant compromise between hydrodynamic and kinematic performance with respect to leaflet thickness was deemed likely to affect the long-term durability of the valves as suboptimal kinematics is generally indicative of functional inefficiency^{353,394}.
- Overall, all the PEL valves successfully withstood hydrodynamic testing and were therefore eligible for durability testing. Conversely, the DP valves suffered catastrophic failure within 5–10 minutes of hydrodynamic testing under simulated pulmonary conditions and were therefore not eligible for durability testing.
- Durability test performed on the PEL valves revealed the relationship between leaflet thickness and durability to have a strong positive correlation. The durability of the valves with the thinnest leaflets was limited to approximately 5 million accelerated fatigue cycles, whereas the valves with the thickest leaflets were the most durable, reaching up to 260 million fatigue cycles.
- Hydrodynamic tests performed on the valves between 0–100 million cycles demonstrated a slight increase in mean pressure, a negligible decrease in EOA, and a remarkable decrease in regurgitation. However, the kinematic characteristics and structural quality of the valves deteriorated considerably over the course of fatigue testing, which among a few other factors, was attributed to the suboptimal kinematic behaviour demonstrated by these valves prior to durability testing.
- Leaflet commissure tears were the principal mode of failure across all valves. The most common wear mechanisms exhibited by PEL valves in response to accelerated fatigue testing were free-edge delamination and surface wear on the coapting surfaces of the leaflets, particularly in the central region.
- In the short time that the DP valves were subject to hydrodynamic loading, the leaflet scaffolds suffered severe fiber compaction and consolidation, which manifested as a viscous flow response caused by the dynamic frictional forces acting on the scaffold fibers.

5.2 Recommendations

As this research is still in its infancy and addresses a small portion of the process toward developing a tissue engineered heart valve, there is much work to be done to optimise the scaffold properties and manufacturing procedure of the electrospun trileaflet THV construct for it to be eligible to undergo tissue processing and subsequently be introduced to an *in vivo* environment. For each phase of the process explored in this project, there are aspects that must be investigated and further refined in order to make this a viable approach for future application in a clinical setting.

Scaffold development

- Alternative biodegradable polymers with mechanical characteristics comparable to the PEL scaffolds developed in this project should be investigated for their application in this valve manufacturing approach.
- Mechanical characterisations of biomaterial scaffolds should focus on utilising dynamic mechanical testing systems, such as cyclic tension tests, cyclic compression test, and biaxial testing procedures. This will assist in better predicting the mechanical response of the leaflet scaffolds to applied hydrodynamic loads, such that redundant verification testing due to ineffective prototype screening processes can be avoided.
- Scaffold development should focus on enhancing the compression characteristics of the scaffolds to minimise the susceptibility of scaffold fibers to undergo compaction and consolidation, in order to avoid the development of nonporous zones throughout the leaflet scaffolds, which would likely lead to incomplete tissue ingrowth and thereby compromise the functional and biological outcomes of the valves.
- Further improvement of control over the electrospinning working environment, such as temperature and air flow parameters, would be beneficial for improved repeatability of the scaffold production process.

Valve manufacturing design

- Material joining techniques that are less reliant on skilled labour should be investigated to reduce production cost, minimise impact of human error, and limit physical handling of materials, which presents a safety risk regarding material sterility.
- Different techniques to automate aspects of the manufacturing procedure should be investigated to improve reproducibility, repeatability, and standardisation of the processes and outcomes.
- Alternative valve geometries and/or methods of securing the leaflet commissures to the stent should be investigated to minimise stress concentration from developing at the commissure hinge and thereby improve valve durability.
- Techniques to avoid pinching at the leaflet free-edge should be investigated by exploring different cutting mechanisms or minimising the thickness and maximising sharpness of the die-cutter blade.
- Stent precoating procedures should be optimised to obtain even coating thicknesses across the stent, and thereby improve bond integrity of the skirts to the stent.

Valve evaluation

- The influence of leaflet thickness in conjunction with mechanical properties on valve hydrodynamics and durability should be more comprehensively investigated.
- The effects of crimping and balloon expansion on the structural and mechanical integrity of the leaflets should be characterised
- The impact of valve crimping on hydrodynamic and kinematic performance of the electrospun valve leaflets should be investigated.
- To overcome durability issues encountered with the degradable DP valves, the mechanical properties of the respective scaffolds could be improved by reinforcing the scaffolds with nondegradable PEL fibers by utilising a co-electrospinning system to form hybrid scaffolds.
- Regarding biodegradable scaffold leaflets, the influence of degradation behaviour on valve functionality and durability should be characterised.
- The long-term biocompatibility and tissue processing potential of the electrospun PEL scaffolds should be characterised to establish its eligibility for application as a nondegradable polymer THV replacement.

Appendix A

Table A1: Primary polymer properties

Polymer	Grade	PU Class	Degradation	MW (kDa)	ρ (g/m ³)	T _g (°C)	T _{Vicat} (°C)	T _m (°C)
Pellethane	2362-80AE	Polyether	EU Stable	~ 54 ^a	1.12 ^b	-47 ^b	81.70 ^b	188–204 ^b
DegraPol	DP15	Polyester	AU Hydrolytic	~ 110 ^c	1.15 ^d	-30 ^e	NA	120–136 ^f

Obtained from (a) Seidler *et al.*³⁹⁵, (b) Lubrizol Corp.³⁹⁶, (c) Buschmann *et al.*²⁵⁷, (d) Simonet *et al.*²⁵⁹, (e) Stoll³⁹⁷, (f) Lendlein *et al.*²⁵⁴.

Table A2: Electrospinning solution solvent properties

Solvent	Abrev.	Formula	MW (kDa)	P (g/mL)	ϵ	T _b (°C)	V _p (mmHg)
Chloroform		CHCl ₃	119.4	1.492	4.81	60.5–61.5	160.0
N,N-Dimethylacetamide	DMAc	CH ₃ CON(CH ₃) ₂	87.1	0.937	37.80	164.5–166.0	2.0
N,N-Dimethylformamide	DMF	C ₃ H ₇ NO	73.1	0.944	36.71	153.0	2.7
Ethyl alcohol		C ₂ H ₆ O	46.1	0.785	24.55	78.0	71.3
Tetrahydrofuran	THF	C ₄ H ₈ O	72.1	0.886	7.58	67.0	143.0

Table A3: Standard bulk mechanical properties for PEL and DP

	UTS (MPa)	E (MPa)	ϵ_{max} (%)
PEL (a)	29.9	4.8	650
DP (b-c)	1-6	6.0	277

Obtained from (a) Lubrizol Corp.³⁹⁶, (b) Buschmann *et al.*²⁵⁷, and (c) Stoll³⁹⁷.

Electrospinning operating procedure

The electrospinning procedure was performed as follows:

1. Firstly, the electrospinning environment conditions were established by tuning the RH parameter settings and allowing roughly one hour for the environment to equilibrate.
2. The solution feed apparatus was prepared by mounting the spinneret to the bracket, connecting the power cable, and securing the syringe to the pump located outside the rig housing box.
3. The electrospinning processing parameters, including the rotational velocity (rpm); tip-to-collector distance (mm); translational speed (mm/s); and translating distance (mm), were configured on the Arduino software, and the test controller was initiated.
4. Once the collector was operational and in working range, the syringe pump and power supplies were turned on to start the experiment.
5. The duration of the spinning process was calculated relative to the solution feed rate, feed volume, and desired scaffold thickness, which typically ranged between 1 – 4 hours.
6. Once the spinning process was complete, the polymer scaffold was removed from the collector by trimming either end with a scalpel blade and carefully rolling it off to preserve the tube form.
7. Electrospun PEL samples were stored in airtight test tubes, while DP samples were stored in a covered desiccator to prevent both hydrolytic degradation and photodegradation.

Appendix B

Table B1: Average fiber diameter, pore size and porosity of DP scaffolds electrospun at different relative humidities

	RH (%)	Fiber diameter (μm)	Pore size (μm)	Porosity (μm)
PEL	30	5.53 $\pm$ 0.70	10.94 $\pm$ 1.97	68.27 $\pm$ 0.81
	35	5.02 $\pm$ 0.54	16.73 $\pm$ 4.15	75.71 $\pm$ 1.50
	40	3.54 $\pm$ 0.54	15.62 $\pm$ 3.61	77.94 $\pm$ 2.06
	45	4.41 $\pm$ 1.09	8.87 $\pm$ 3.28	64.25 $\pm$ 3.64
DP	25	7.83 $\pm$ 0.82	23.07 $\pm$ 2.73	78.51 $\pm$ 2.50
	30	9.57 $\pm$ 0.43	21.31 $\pm$ 3.82	79.34 $\pm$ 1.57
	35	10.29 $\pm$ 1.58	14.46 $\pm$ 5.05	65.62 $\pm$ 5.21

Table B2: Tensile test results for electrospun PEL and DP scaffold

	RPM	UTS (MPa)	E _s (MPa)	E _t (MPa)	Percent elongation (%)	Toughness (MJ/m ³)
PEL	500	4.89 $\pm$ 0.34	1.41 $\pm$ 0.05	1.31 $\pm$ 0.09	474.47 $\pm$ 13.75	4131.10 $\pm$ 388.10
	1000	6.36 $\pm$ 0.90	1.70 $\pm$ 0.15	1.62 $\pm$ 0.09	492.44 $\pm$ 17.91	5203.48 $\pm$ 322.27
	1500	6.90 $\pm$ 0.66	1.76 $\pm$ 0.14	1.75 $\pm$ 0.18	463.83 $\pm$ 15.46	5377.88 $\pm$ 717.49
DP	500	1.41 $\pm$ 0.20	0.90 $\pm$ 0.09	0.19 $\pm$ 0.08	665.48 $\pm$ 137.71	2706.45 $\pm$ 700.10
	1000	1.65 $\pm$ 0.17	1.01 $\pm$ 0.11	0.20 $\pm$ 0.07	744.47 $\pm$ 86.05	3422.67 $\pm$ 577.00
	1500	1.77 $\pm$ 0.31	1.09 $\pm$ 0.17	0.22 $\pm$ 0.04	763.45 $\pm$ 123.42	3695.17 $\pm$ 1010.83

Table B3: Leaflet thickness measurements obtained for the PEL and DPVs

Scaffold	Set	Group	Average	Minimum	Maximum	Mean $\pm$ SD (μm)	n
PELV		1	300	169	422	304 $\pm$ 68	60
		2	420	315	556	422 $\pm$ 62	60
		3	540	409	644	539 $\pm$ 57	60
		4	630	521	764	627 $\pm$ 62	60
DPV	I	1	480	357	578	481 $\pm$ 65	90
		1	340	200	458	343 $\pm$ 62	60
	II	2	480	331	637	483 $\pm$ 74	90
		3	620	508	748	615 $\pm$ 55	90
		4	720	610	852	724 $\pm$ 55	90
		4	720	610	852	724 $\pm$ 55	90

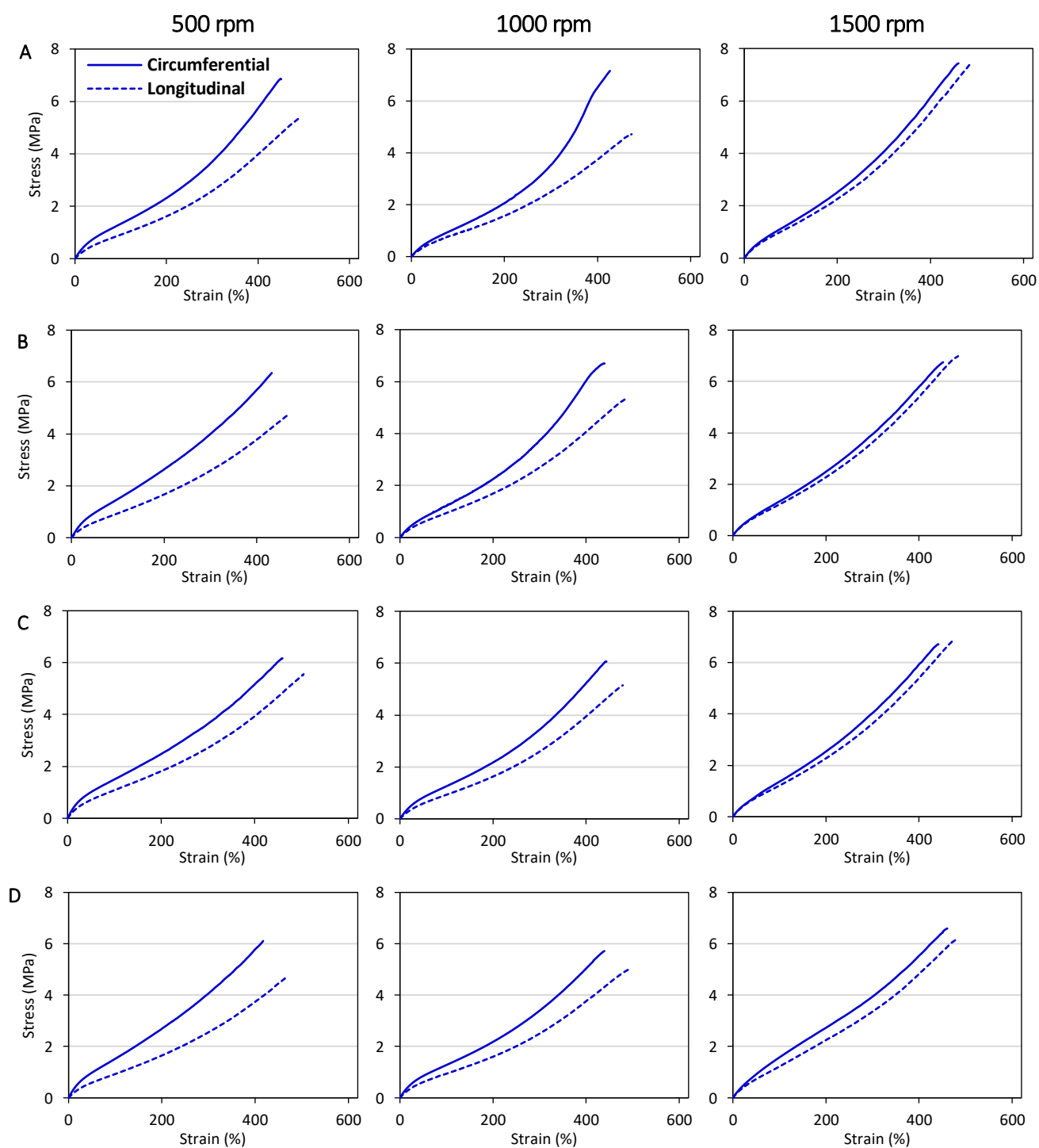


Figure B1: Average stress strain curves for PEL scaffolds electrospun at different process parameters. PEL scaffolds spun at (A) 3, (B) 4, (C) 5, and (D) 6 ml/hr at 500, 1000 and 1500 rpm (left to right), respectively.

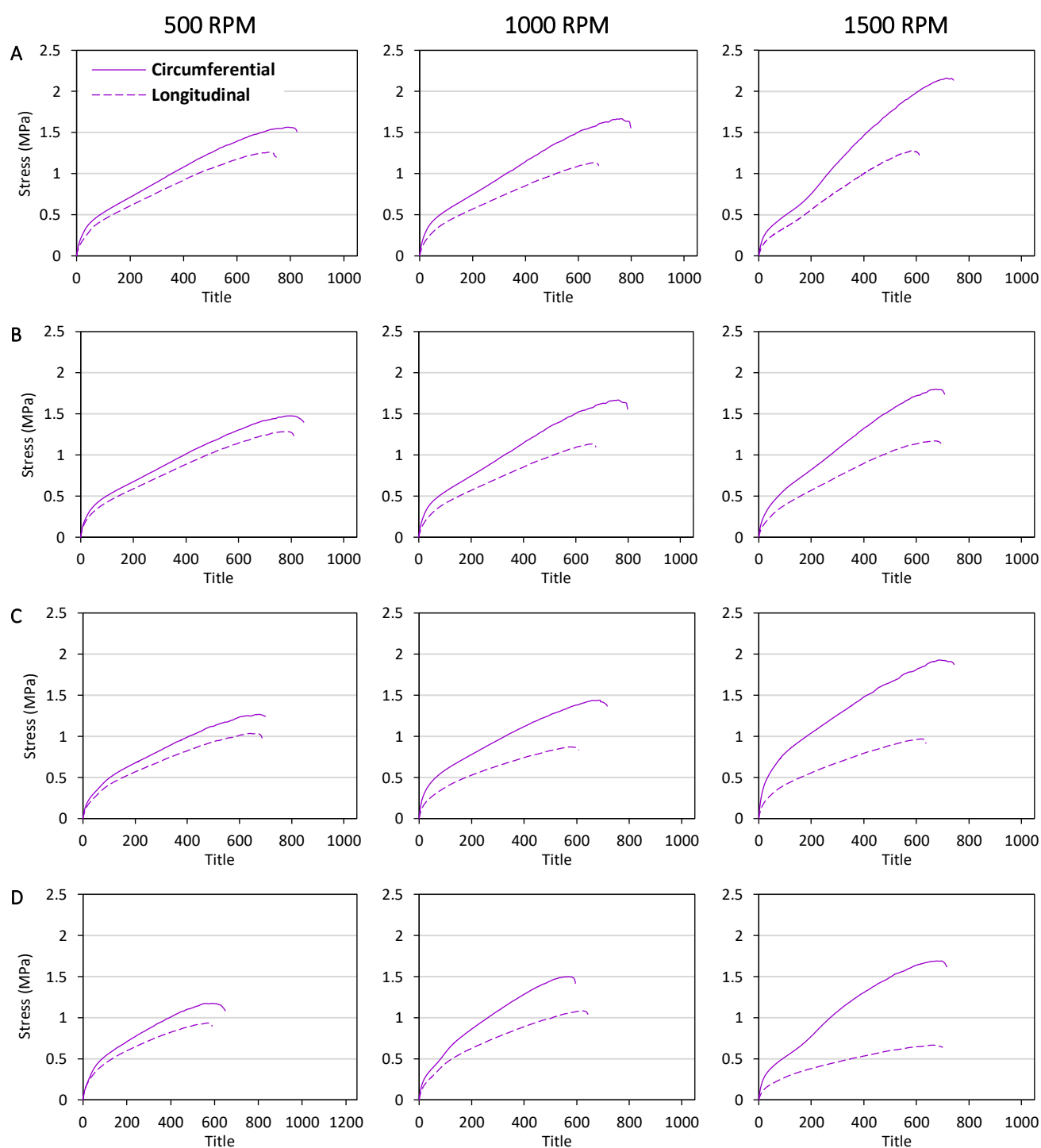


Figure B2: Average stress strain curves for DP scaffolds electrospun at flow rates of (A) 3, (B) 4, (C) 5, and (D) 6 ml/hr at 500, 1000 and 1500 rpm (left to right) respectively.

Appendix C

Table C1: Leaflet scaffold microstructure results summary

Scaffold	Fiber diameter (μm)	Pore size (μm)	Porosity (%)
DPVI	7.58 $\pm$ 1.15	23.84 $\pm$ 4.77	78.27 $\pm$ 3.40
DPVII	4.16 $\pm$ 0.99	17.24 $\pm$ 4.05	76.31 $\pm$ 4.56
PELV	1.54 $\pm$ 0.35	11.96 $\pm$ 2.08	77.19 $\pm$ 2.83

Table C2: Regurgitation results for PEL and DP valve sample groups

Valve	Group	Closing vol (ml)	Leakage vol (ml)	Closing fraction (%)	Leakage fraction (%)	RF (%)
PEL	300	5.44 $\pm$ 0.79	7.45 $\pm$ 1.24	10.32 $\pm$ 2.52	13.86 $\pm$ 2.48	24.18 $\pm$ 4.59
	420	9.84 $\pm$ 1.32	2.09 $\pm$ 1.23	17.74 $\pm$ 4.03	3.85 $\pm$ 2.75	21.59 $\pm$ 5.51
	540	7.58 $\pm$ 1.90	0.95 $\pm$ 0.69	12.89 $\pm$ 4.53	1.61 $\pm$ 1.14	14.51 $\pm$ 5.34
	630	5.36 $\pm$ 1.19	0.51 $\pm$ 0.35	9.65 $\pm$ 3.57	0.89 $\pm$ 0.60	10.54 $\pm$ 3.72
DP	340	5.51 $\pm$ 1.89	2.35 $\pm$ 0.41	10.81 $\pm$ 3.75	4.60 $\pm$ 0.80	15.41 $\pm$ 3.19
	490	6.09 $\pm$ 1.13	1.20 $\pm$ 0.38	10.75 $\pm$ 1.36	2.18 $\pm$ 0.82	12.97 $\pm$ 1.07
	620	4.95 $\pm$ 0.67	0.65 $\pm$ 0.22	9.19 $\pm$ 1.82	1.18 $\pm$ 0.32	10.14 $\pm$ 1.70
	710	4.87 $\pm$ 2.09	1.01 $\pm$ 0.25	9.42 $\pm$ 4.09	1.96 $\pm$ 0.48	11.38 $\pm$ 4.00

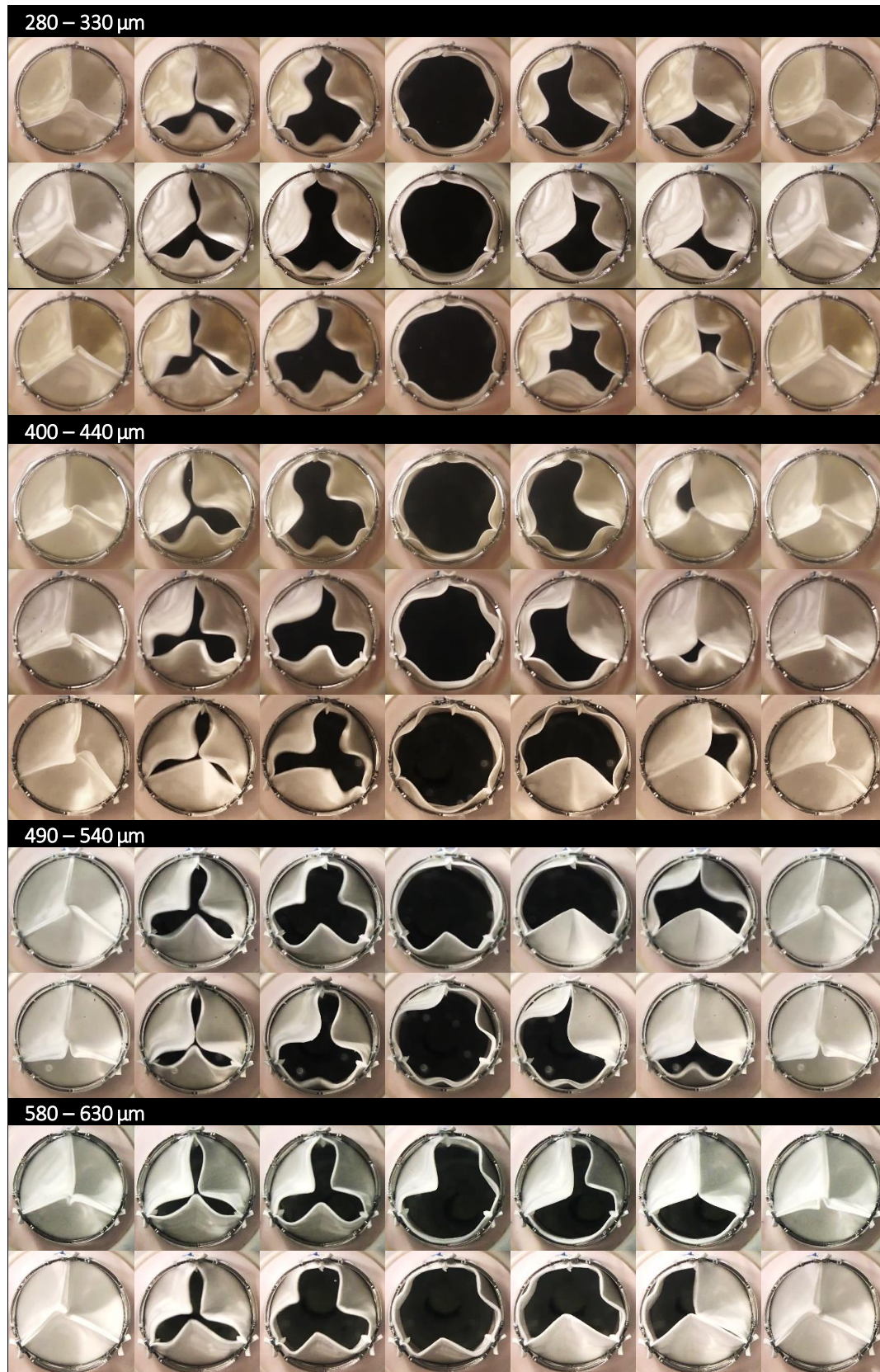
PELLETHANE VALVES

Figure C1: Photo montage of PEL valve leaflet motions over one cardiac cycle.

DEGRAPOL VALVES

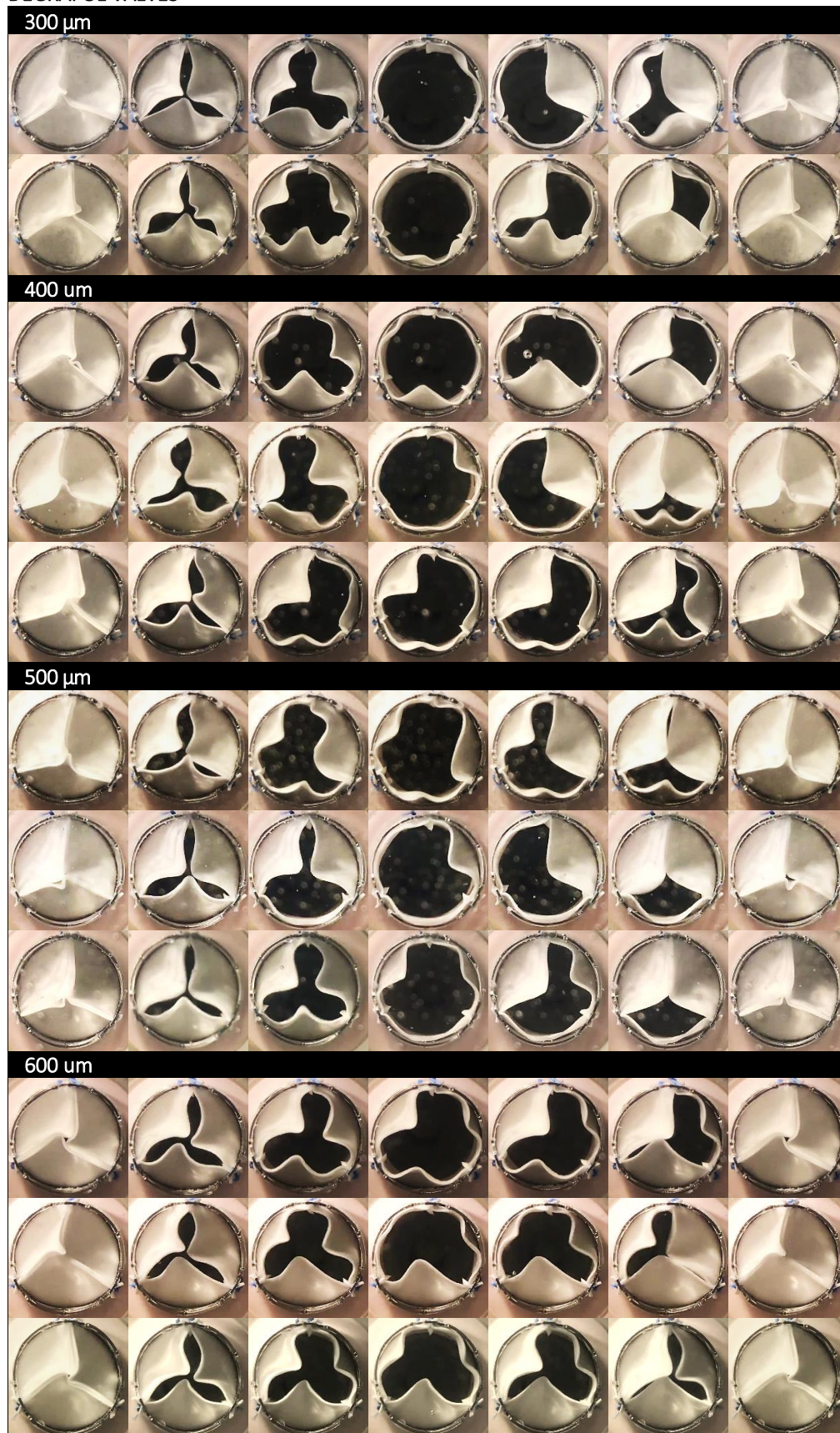


Figure C2: Photo montage of DP valve leaflet motions over one cardiac cycle.

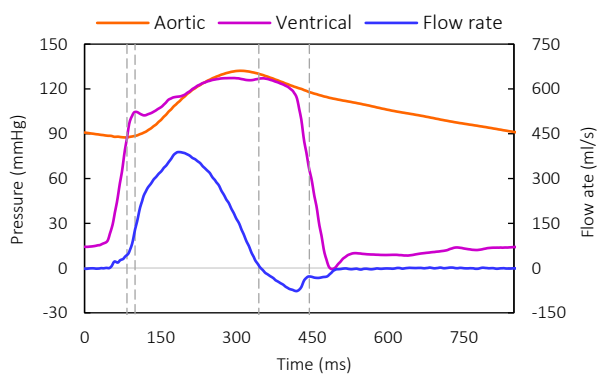
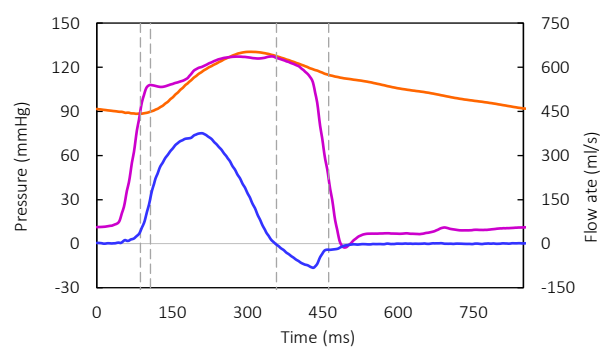
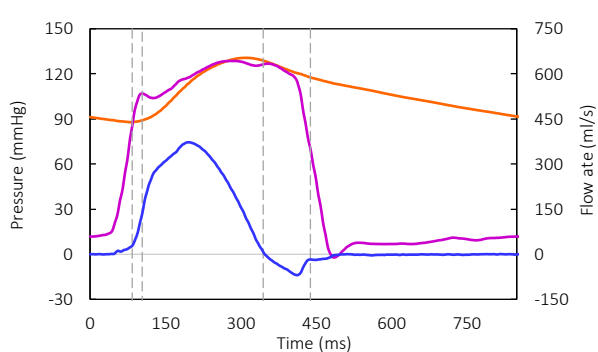
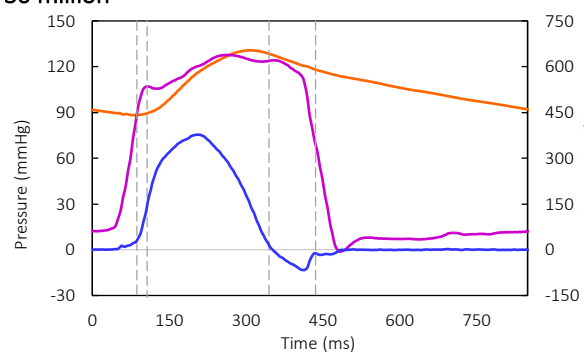
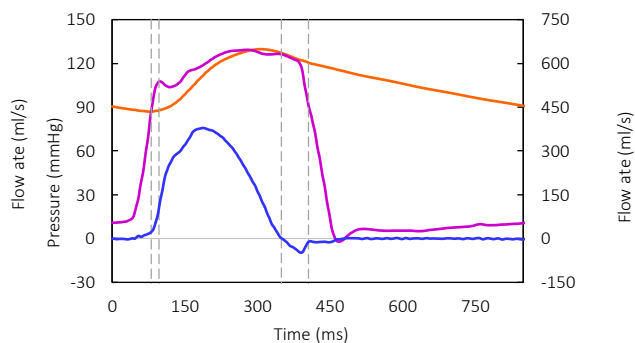
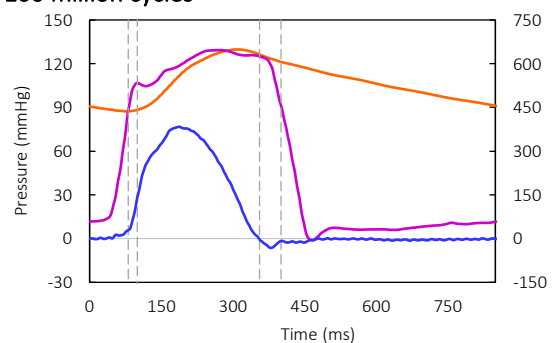
0cycles**30 million****100 million cycles**

Figure C3: Pressure and flow waveforms of PEL valves pre- and post-fatigue testing up to 100 million cycles.

Pulse duplicator SOP

The following pulsatile testing protocol was conducted using the ViVitro Labs Pulse Duplicator System (ViVitro Labs Inc., BC, Canada) with reference to ISO 5840-3 guidelines stipulated in Annex N ³⁴⁵.

1. The system equipment was setup as a permanent installation and had been assembled in accordance with standard protocols specified by the product supplier (ViVitro Labs Inc.).
2. Prior to testing, the flow probe was mounted in the site designated for aortic valve testing.
3. The system was filled with test fluid (saline solution with density $1\,010\text{ kg/m}^3$) and debubbled prior to setting the heat bath to a target temperature of 37°C .
4. System calibration was conducted prior to each experiment and at key intervals throughout testing. The calibration procedure involved: zeroing sensor signals to obtain transducer readings relative to zero voltage; calibrating the pressure transducer sensors (aortic, atrial, ventricular) and static head compensation; as well as flowmeter and pump calibration.
5. Once the system was calibrated, test valves were mounted in a silicone annular sealing ring and inserted in the aortic test site.
6. Test fluid was added to the system and the SuperPump was initiated.
7. Any air collected in the chambers or entrapped in the transducers was removed.
8. The fluid level was monitored and the air volume in the compliance chambers adjusted until an appropriate fluid level was reached.
9. With the pump stopped, the SuperPump control settings were set. A sinusoidal waveform and standard beat rate of 70 bpm were applied for all experiments. The stroke volume was adjusted
10. Peripheral resistance was adjusted to achieve a mean pressure of $100 \pm 1\text{ mmHg}$ for testing PEL valves ($n=10$) under aortic conditions, and $24 \pm 5\text{ mmHg}$ for testing DP valves ($n=12$) under pulmonary conditions.
11. Aortic compliance was tuned to obtain pressures between 120 ± 2 and $80 \pm 2\text{ mmHg}$ for PEL valve tests, and 40 ± 4 and 11 ± 5 for DP valve tests. The applied operating environments were based on standard normotensive aortic and pulmonary conditions, respectively.
12. Viscoelastic impedance compliance (VIA) was adjusted for final tuning, and the temperature of the test fluid was assessed for a stable reading.
13. Once stable operating conditions were obtained, valves were dynamically conditioned for 10 mins prior to data capture. This allowed valves to adopt a stable seating position which facilitated the consistency of captured cycle readings.
14. Performance data was captured over 10 cardiac cycles, at a frequency of 256 sampled per cycle.
15. Two data sets were acquired from each capture, including (1) the pressure and flow waveform readings, and (2) the computed performance results summary. Raw data was exported as .CSV files for processing in Excel.

High-speed video recordings were performed for each test at 2-3 min intervals during conditioning and data capture. Videos were later processed for qualitative and quantitative analysis.

Accelerated wear testing SOP

The equipment was operated first-hand with close reference to standard protocols detailed in ³⁹⁸. The following procedure was performed for each batch of test valves prior to initiating the cycle count:

1. With the Module initially empty, individual chambers were filled to half capacity with working fluid comprised of 0.05% biocide (ProClin™ 300, SigmaAldrich) in deionised water.
2. The Module Controller, AmPack and heater were switched on to allow sufficient time to reach operating temperatures. A preset value of 37°C was maintained throughout the testing procedure to emulate normal physiological temperatures.
3. Prior to installation, the valves were catalogued according to their lot number and material specifications.
4. Valves were then mounted in a silicone ring mould and inserted in the test chambers in a symmetrically spaced configuration. Chamber windows were fastened with screws of uniform tightness. Valve positions were recorded by referencing the alphabetic inscription on each chamber.
5. Chambers were filled with test fluid fed from a syringe attached to the bottom drain port.
6. The pressure head assembly was connected to the assigned chamber, the reservoir filled with 60 ml of test fluid, and the stopcock opened to the system. A few minutes were allowed for the pressure head to bleed into the rest of the chambers, after which the fluid level was replenished.
7. Prior to debubbling the chambers, the Scan Valve was positioned between chambers to allow open flow throughout the system.
8. The spring compensation was adjusted to ensure the shaker plate was positioned mid-stroke to obtain a symmetric pulsatile amplitude.
9. Starting at rest, the amplitude was gradually increased until slight pulsing was observed. Thereafter, the frequency was cycled through a broad range to agitate and effectively dislodge any air bubbles trapped in the system. The amplitude was kept minimal during high frequency tuning to avoid excessive loading on the leaflets and prevent the onset of valve closure.
10. With the system on standby and the pressure head stopcock closed, the chambers were systematically debubbled which involved flushing the bubbles out of the top drain port by feeding test fluid into the chamber from the bottom port with a syringe.
11. Steps 9 and 10 were repeated several times until each chamber was clear of bubbles.
12. The pressure transducers were subsequently calibrated using the Calibration Wizard featured in the HiTest software. The calibration procedure involved pressure transducer calibration and sensor static head zeroing. Re-zeroing of transducers and re-calibration of the pressure measuring system was performed prior to acquiring data over the course of testing.
13. With the HiTest program open and in Live Running mode, the differential pressures for inflow, outflow and transvalve were confirmed to display zero readings.

14. Prior to chamber pressure tuning, the flow restrictors were opened (set to 0), the amplitude set to 0, and the Scan Valve turned to align with first chamber.
15. The drive frequency was set to 600 bpm (10 Hz) and the amplitude was gradually increased until an adequate opening and closing leaflet motion was observed using a stroboscope.
16. By referring the live pressure readings, the flow restrictor was adjusted until a mean pressure differential of 100 mmHg was achieved for the 5% and 95% rule.
17. After adjusting the chamber resistance, the valve opening performance was re-evaluated and amplitude adjusted if necessary.
18. Steps 16 and 17 were repeated until the pressure requirements and valve opening behaviour was adequate.
19. The tuning procedure (steps 14 – 18) was repeated for the remaining chambers until a full-circle check indicated that all chambers were at desired conditions and no further tuning was required.
20. Data was captured for each test chamber and slow-motion videos were recorded for each valve.
21. After resetting the counter and recording the controller settings and chamber resistances, the test was started, and cycle count commenced.

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