

Mathematical Modelling of Growth Factor Induced Cell Migration in 3D Engineered Matrices



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Declaration

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Abstract

Cells mechanically interact with their environment to sense, for example, topography, elasticity, mechanical cues from other cells, and chemical signals. Multi-signalling stimuli have profound effects on cellular behaviour, including migration. The current study aims to develop a mathematical model for chemo-mechanically induced migration of individual cells in a collective in three-dimensional engineered extracellular matrices governed by the mechanical properties of the matrix and a growth factor gradient.

In the developed model, each cell is assumed to transmit a traction force that locally deforms a planar elastic substrate, resulting in spatially varying strain energy density gradients. The magnitude and direction of strain energy density gradients define cell migration. Cell-substrate adhesion and partial random motion are included. Further, the Green function and Duhamel principle are used to solve the diffusion equation to describe the presence of a growth factor and represent chemo-mechanically induced deterministic collective cell migration on planar elastic substrates. Finally, three-dimensional strain energy density gradients due to local matrix deformation by embedded cells are obtained using finite element methods and implemented in the model to describe chemo-mechanically induced collective cell migration in extracellular matrices.

Deterministic and random migration of up to 50 cells on planar substrates and three-dimensional extracellular matrices with spatially uniform and varying stiffness is predicted. The effect of varying growth factor productivity and diffusivity is explored for cell migration on a planar substrate induced by a growth factor only and combined with mechanical cues. The model predicts that the maximum velocity of a cell migrating towards the growth factor source increases with increasing productivity and decreasing diffusivity of the growth factor. Collective cell migration due to mechanical cell interactions in the extracellular matrix is studied with sequential and non-sequential changes in matrix stiffness. The overall migration is directed towards the stiffest region for the sequential stiffness change and the softest region for the non-sequential stiffness change in the matrix. The chemo-mechanically induced cell migration is presented in three sequential

extracellular matrices with an overall migration direction towards the growth factor in the softest region.

The mathematical models can adequately simulate the chemo-mechanically induced collective cell migration in elastic planar substrates and three-dimensional extracellular matrices. The models provide qualitative results demonstrating collective cell migration in complex environments with several cues increasing the potential and capabilities to replace *in vitro* and *in vivo* experiments with *in silico* simulations in, for example, wound healing, cancer treatment, and regenerative medicine.

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Nomenclature

ECM	Extracellular matrix
PDEs	Partial differential equations
Integro-PDEs	Integral terms in the partial differential equations
2D	Two-dimensional
3D	Three-dimensional
t	Time
Δt	Time step
$\hbar$	Time step size of the numerical integration
$t_{\hbar}$	Discrete time points
$\mathbf{c}_i(t), \mathbf{c}_i$	Centre position of cell i on the substrate (Chapter 2)
$\mathbf{C}_i(t), \mathbf{C}_i$	Centre position of cell i in the ECM (Chapter 4)
$\mathbf{c}_j(t), \mathbf{c}_j$	Centre position of cell j on the substrate (Chapter 2)
$\mathbf{C}_j(t), \mathbf{C}_j$	Centre position of cell j in the ECM (Chapter 4)
ω	Substrate deformation
R	Cell radius
p	Cell traction force distributed on the substrate (Chapter 2)
$\mathcal{F}$	Cell traction force in the ECM (Chapter 4)
r	Distance between centres of two cells
ν	Substrate Poisson ratio
h	Substrate thickness
k	Substrate stiffness
E	Elastic modulus of the substrate (Chapter 2)
E_m	Elastic modulus of the ECM (Chapter 4)
E_c	Elastic modulus of the cell
V	Deformed volume of the substrate

A	Area between two cells on the substrate (Chapter 2)
$\mathcal{A}$	Cell surface area in the ECM (Chapter 4)
S_i	Strain energy density induced by cell i on the substrate (Chapter 2)
S_j	Strain energy densities from cells j on the substrate (Chapter 2)
Ψ^0_i	Strain energy density around cell i in the ECM (Chapter 4)
Ψ^0_j	Strain energy density from cells j in the ECM (Chapter 4)
ψ	Total strain energy density on the substrate (Chapter 2)
Ψ	Total strain energy density in the ECM (Chapter 4)
$\mathbf{v}_i$	Migration direction of cell i on the substrate (Chapter 2)
$\mathbf{v}_i$	Migration direction of cell i in the ECM (Chapter 4)
$\mathbf{u}_{ij}$	Unit vector connecting cell i to cell j on the substrate (Chapter 2)
$\mathbf{u}_{ij}$	Unit vector connecting cell i to cell j in the ECM (Chapter 4)
N	Total number of cell receptors
$\rho(X), \rho$	Friction coefficient of the cell-substrate adhesion (Chapter 2)
$\beta(\Lambda), \beta$	Friction coefficient of the cell-ECM adhesion (Chapter 4)
B	Cell-ECM interaction parameter (Chapter 4)
k_s	Cell-substrate interaction parameter (Chapter 2)
X	Position of a point in the cell that varies in the interval occupied by the cell on the substrate (Chapter 2)
Λ	Position of a point in the cell that varies in the interval occupied by the cell in the ECM (Chapter 4)
ρ_0	Cell-substrate adhesion strength (Chapter 2)
β_0	Cell-ECM adhesion strength (Chapter 4)
n_b	Density of bounded integrins to the substrate (Chapter 2)
n_0	Density of the ECM ligands (Chapter 4)
n_b	Concentration of bounded integrins to the ECM ligands (Chapter 4)
n_f	Free integrins concentration on the substrate (Chapter 2)
n_f	Free integrins concentration in the ECM (Chapter 4)
n_s	Substrate ligands concentration (Chapter 2)
n_s	ECM ligands concentration (Chapter 4)
n_{fi}	Diffusion of free integrin receptors in the ECM (Chapter 4)
k_f	Formation rate of bounded integrins to the substrate (Chapter 2)
K_f	Formation rate of bounded integrins to the ECM (Chapter 4)

$k_r g_r(\mathbf{X})$	Dissociation rate varies along with the position in the cell on the substrate (Chapter 2)
$K_r f_r(\Lambda)$	Dissociation rate varies along with the position in the cell in the ECM (Chapter 4)
φ_r	Integrins dissociation rate on the substrate (Chapter 2)
Υ_r	Integrins dissociation rate in the ECM (Chapter 4)
$\Delta \mathbf{B}$	Standard Brownian vector on 2D (Chapter 2)
$\Delta \mathbf{W}$	Standard Brownian vector in 3D (Chapter 4)
ϱ	Possible changes in cell motion due to substrate uncertainties (Chapter 2)
ℓ	Possible changes in cell motion due to ECM uncertainties (Chapter 4)
P_i	Probability of cell i death or division
$\mathfrak{h}_i$	Probability rate of a cellular event
p_i	Probability rate of cell i death
q_i	Probability rate of cell i division
$\mathcal{H}(x, y), \mathcal{H}$	Growth factor source position on the substrate (Chapter 3)
$\mathbf{H}(x, y, z), \mathbf{H}$	Growth factor source position in the ECM (Chapter 4)
$g(t, \mathcal{H}), g$	Growth factor concentration on the substrate (Chapter 3)
$G(t, \mathbf{H}), G$	Growth factor concentration in the ECM (Chapter 4)
α	Growth factor production rate on the substrate (Chapter 3)
Γ	Growth factor production rate in the ECM (Chapter 4)
D	Growth factor diffusion rate on the substrate (Chapter 3)
$\mathcal{D}$	Growth factor diffusion rate in the ECM (Chapter 4)
ζ	Cell mobility to the growth factor concentration on the substrate (Chapter 3)
ξ	Growth factor signalling rate in the ECM (Chapter 4)
Δ	Laplace operator
δ	Dirac Delta function
ε	ECM strain at the cell surface
$\mathbf{N}_{ij}$	Surface distance between cell i and cell j
γ	Mechanical signal attenuation ratio in the ECM
Ω	Computation domain

1 Introduction and background

1.1 Problem statement

Cell migration is an essential feature of living cells in which a cell moves from one location to another (Kurosaka and Kashina 2008) and without which many biological processes cannot occur, including organ development and growth, wound healing, and the normal immune response to infection. Cell migration is a complex and periodic process underlying physiological development and diseases. Understanding mechanisms of cell migration is vital in the interpretation of emerging areas such as cellular transportation, tumour growth, the manufacture of artificial tissues in biotechnology, and regenerative medicine, which facilitate the *in silico* design of tissue engineering and regeneration (Horwitz and Webb 2003, Koulikovska *et al.* 2015, Maddaluno *et al.* 2017).

It is considered that cell migration utilises two different mechanisms: (i) swimming through a liquid, such as sperm cells and bacteria, or (ii) crawling across solid surfaces, such as epithelial cells, where the cell extends protrusions of the leading edge, adheres the leading edge to the environment, and pulls the cell body forward using cytoskeletal contraction. Crawling motion is the type of locomotion considered in this study.

Cell migration depends on various factors such as cell type, single or collective locomotion, substrate and extracellular matrix (ECM), adhesion strength, and organisation of the cellular cytoskeleton. Physical properties of the cellular environment, such as mechanical and chemical signals, matrix topography, geometry, viscosity, and stiffness, can alter cell behaviour, tissue organisation, and cell-generated forces. Understanding mechanisms affecting cell behaviour can elucidate the movement of motile cells to either encourage or impede the direction of motion (Flaherty *et al.* 2007) and can also prevent or control mistargeted migration.

Understanding the sensing mechanisms of cells is crucially important for different biological processes, for example, to test various drug combination strategies and improve drug therapies. *In*

in vivo studies provide accurate descriptions of complex interactions and responses that occur within living organisms. However, ethical considerations, costs, resource constraints, and time complexity are challenges associated with these studies. *In vitro* experiments have become more sophisticated and complex and can generate accurate findings similar to *in vivo* studies. However, certain interactions or responses cannot be captured *in vitro*. Mathematical models and computational simulations can generate fast, reproducible, and controlled experiments at significantly lower costs and in line with 3R principles in research involving animals by predicting cell behaviours and guiding experimental design. Further, these models and simulations can provide valuable information and insights that are difficult to obtain experimentally. Combinations of computational modelling and *in vitro* experiments have opened new opportunities for researchers to directly obtain additional information on specific factors on individual and collective migration that can be evaluated only indirectly from *in vitro* experiments (Kim M-C *et al.* 2018, Merino-Casallo *et al.* 2018, Mousavi *et al.* 2013, Staddon *et al.* 2022).

Mathematical models can be developed on different scales ranging from population-based macroscopic to individual-based mesoscopic models. Microscopic cell-based models can be characterised according to (i) the description of cell migration, i.e., single or collective migration, (ii) the numerical approach, i.e., discrete, continuous, or hybrid, and (iii) the type of processes and cues, i.e., mechanical, chemical, or chemo-mechanical.

Although several mathematical models for the migration of collective cells have been developed, the mechanical and chemical properties of the cellular environment guiding the migration of individual cells in a collective are not fully understood.

1.2 Cell migration

Cells migrate in different modes depending on the cell type and function (Figure 1.1). Amoeboid and mesenchymal are the two most characterised migration modes. The amoeboid mode is mainly for individual migration, where cells migrate with weak adhesion and pulling forces, and is used by immune cells, such as leukocytes and neutrophils (Sixt and Lämmermann 2011, Yamada and Sixt 2019, Zhelev *et al.* 2004). The amoeboid mode allows cells to move quickly and efficiently as (i) cells do not establish an organised detachment of adhesions and (ii) the pulling force sufficiently accelerates the cell body to overcome the environmental viscous drag. The mesenchymal mode can be used for individual and collective migration as some cells change their migration mode as they navigate the environment, such as fibroblasts and some cancer cells (Bear and Haugh 2014,

Duchez *et al.* 2019, Merino-Casallo *et al.* 2018). Mesenchymal cells adhere tightly to the substrate or ECM and exert pulling force while pushing their body forward.

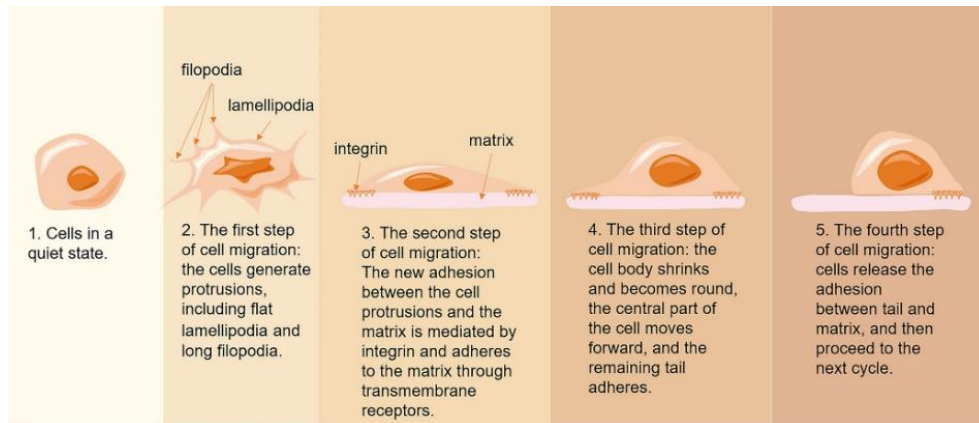


Figure 1.1. Cell migration step cycles. From Lou *et al.* (2021) under the open access Creative Commons Attributions Licence (CC-BY).

Collective mesenchymal migration is characterised by the weak connection among cells in the group, and cells may move individually in the group. However, migration of other crawling cells usually involves stronger connection among the cells, polarisation of individual cells, and leading cells (Alert and Trepap 2020, González-Valverde and García-Aznar 2018, Plou *et al.* 2018). The leading cells direct other cells in the group to respond to the environmental cues (Figure 1.2). Crawling movement is the most common mechanism utilised by cells (Ananthakrishnan and Ehrlicher 2007, Tanimoto and Sano 2014, Trepap *et al.* 2009). Collectively crawling cells respond to mechanical and chemical cues in their surrounding environment. Cells in a collective interact mechanically with neighbouring cells in their environment and influence their migration behaviour.

One of the mechanical processes that influence collective cell migration is cell-cell communication. Cells adhere and apply mechanical traction forces to the environment, i.e., substrate or ECM, through the focal adhesions that deform the surrounding cellular environment. The traction force is used to measure the cell-substrate or cell-ECM traction field to understand physiological and pathological incidents, such as inflammation, angiogenesis, and wound healing. This traction force diffuses in its surrounding environment and can be sensed by neighbouring cells (Reinhart-King *et al.* 2008), which can control the direction of migration according to a variety of stimuli.

There are different approaches to measuring the cell traction forces on the substrate and in the ECM. Some approaches include the visualisation of traction force generation through the wrinkling of elastic silicon (Burton and Taylor 1997, Harris *et al.* 1980) along with the measurement of these forces through traction force microscopy (Brask *et al.* 2015, Brockman *et al.* 2018, C ndor

et al. 2017, Hur *et al.* 2020, Schwarz US and Soiné 2015, Steinwachs *et al.* 2016, Style *et al.* 2014, Tang X *et al.* 2014), or microfabricated techniques (Marelli *et al.* 2014, Nerger *et al.* 2017, Oakes Patrick w *et al.* 2014, Polacheck and Chen 2016, Soman *et al.* 2012).

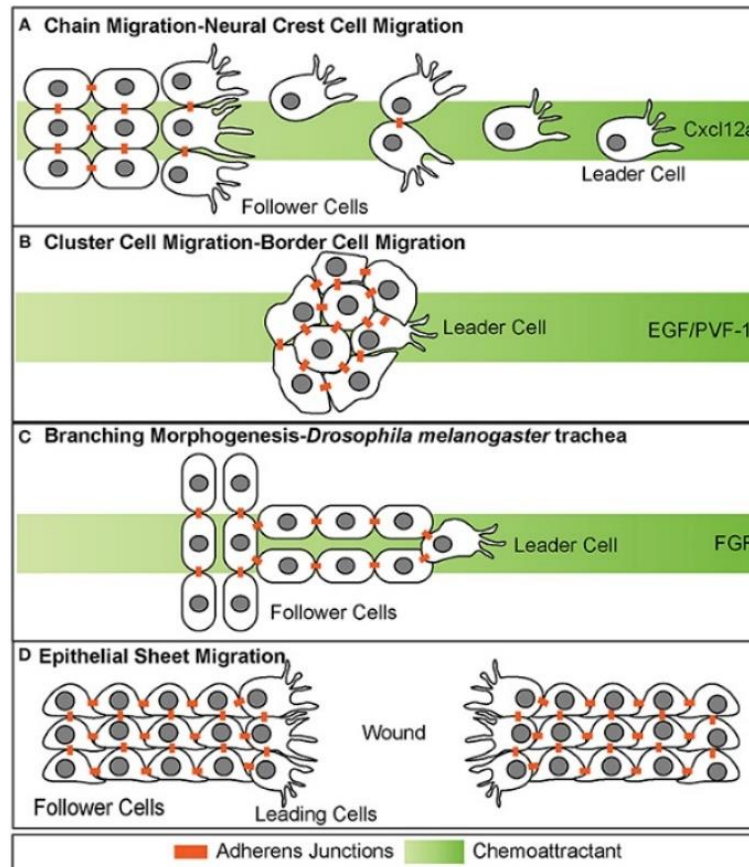


Figure 1.2. Different modes of collective cell migration. A) Chain migration of neural crest cells. Cells migrate from the neural cluster towards the chemoattractant source (Cxcl12a). B) Migration of border cells in *Drosophila melanogaster*. Cells migrate as adherent clusters towards the chemoattractant source (EGF/PVF-1) with the leading cell exhibiting extensive protrusion behaviour. C) A leading cell of *Drosophila melanogaster* trachea migrates towards the chemoattractant source (FGF) while trailing cells form a tube-like structure. D) Leading epithelial cells migrate towards each other to close the wound. From Olson and Nechiporuk (2018) under the open access Creative Commons Attributions Licence (CC-BY).

Another mechanical property influencing the migration of cells is the stiffness of the environment. Stiffness changes in the substrate and ECM guide and control cell migration. Different experimental studies showed that cells prefer to migrate towards stiffer regions in the substrate and ECM (Duchez *et al.* 2019, Gupton and Waterman-Storer 2006, Hadjipanayi *et al.* 2009, Lo *et al.* 2000, Tusan *et al.* 2018). Increasing intracellular forces or substrate stiffness induces more stable cell-matrix adhesions, which explains the cell's tendency to move toward stiffer or more fixed regions (Chiquet *et al.* 2009).

Chemical gradients can be found in biomolecules (such as chemokines, proteins, and hormones) and growth factors (such as epidermal growth factor and vascular epidermal growth factor). Diffusion of a chemical through the environment, i.e., substrate or ECM, also influences cell migration. The migration of cells due to the attraction or repulsion action of chemical gradients depends on the chemotactic signal type. Chemoattractant agents, secretion of lactates due to the presence of bacteria, and cancerous tumour growth direct the migration towards or away from the chemical sources. Many experimental studies have shown that an immediate migration is a response to chemical variations depending on the diffusion speed and distance between chemical sources and cells (Shellard and Mayor 2020, Stock *et al.* 2014, Whitehead *et al.* 2016, You *et al.* 2012).

1.3 Models for single and collective cell migration

1.3.1 Models for a single-cell migration

Modelling single-cell motion is essential in observing and quantifying cell behaviours and incorporating this information at a large-scale level. Several models simulate the influence of environmental cues on one or more intercellular processes of a single cell, such as protrusion, adhesion, and contraction (Borau Carlos *et al.* 2014, Campbell and Bagchi 2018, Danuser *et al.* 2013, Kim M-C *et al.* 2015, Merino-Casallo *et al.* 2018, Wang T *et al.* 2017, Zhu and Mogilner 2016). Other mathematical models consider the migration of a single cell holistically. These models incorporate the whole cell movement cycle responding to its environment in a single mode (Campbell and Bagchi 2018, Gracheva and Othmer 2004, Hodge and Papadopoulos 2012, Mehrayin *et al.* 2012, Raynaud *et al.* 2016, Sakamoto *et al.* 2011).

Models for cellular mechano-sensing for a single cell have been developed to understand the mechanisms with which a cell senses its environment based on the force that the cell transmits to the environment (Marzban *et al.* 2019, Schlüter *et al.* 2012, Voloshin 2016, Zeng and Li 2011). Different researchers model cell movement in response to its environment based on stochastic movement (Kim M-C *et al.* 2018, Malik Adam A. and Gerlee 2019, Malik A. A. *et al.* 2020). These types of models consider the cell's ability to sense its environment in different ways through random changes in a cell's position or velocity. It is observed that a single cell senses mechanical differences in the surrounding environment, i.e., stiffness, through the deformation magnitude by applying different forces depending on the local environmental stiffness. The cell-environment interaction is studied using different approaches, e.g. viscoelastic models (Dokukina and Gracheva 2010, Mofrad *et al.* 2006,

Sakamoto *et al.* 2011), cellular Potts models (Allena *et al.* 2016), and finite element models (Hodge 2011, Stefanoni *et al.* 2011). The mechanical cell-cell interaction has not been incorporated in these models.

The response of a single cell to a chemoattractant agent in its environment is modelled using reaction-diffusion equations. The partial differential equations (PDEs) are solved using the finite element method (Macdonald *et al.* 2016, Mackenzie *et al.* 2021). The cell's response to a chemical gradient can dominate the mechano-sensing process even for a moving chemoattractant source by generating local chemoattractant gradients. Also, the chemoattractant source and mechanical properties of the environment activate intracellular signalling molecules which increases cell polarisation and stimulates spatial local protrusion activities. (Macdonald *et al.* 2016, Mackenzie *et al.* 2021).

Some researchers couple biochemical processes with mechanical properties associated with cell polarisation by incorporating moving finite element boundary problems to simulate diffusion of proteins in cell interior which play an important role in cell migration (Barreira *et al.* 2011, Cusseddu D. *et al.* 2019, Cusseddu Davide and Madzvamuse 2022, Elliott *et al.* 2012, George U *et al.* 2013, Madzvamuse and Kiplangat 2020, Murphy and Madzvamuse 2020, Yang *et al.* 2017). Neilson *et al.* (2011b) and Neilson *et al.* (2011a) incorporate chemical diffusion and mechanical forces exerted on the cell boundary using PDEs to investigate changes in cell shape during cell movement in crawling cells.

The migration behaviour of individual cells in a collective in response to environmental stimuli is different from single-cell migration as cell-cell interaction has to be considered.

1.3.2 Models for collective cell migration

Analytical solutions for simplified models and numerical methods for complicated models have been developed for collective migration. The movement of individual cells in a cell collective described in cell-based models can be (i) assigned to a rigid grid or lattice points in the space (lattice-based) where each point in lattice point has certain properties or (ii) freely in space (lattice-free based) (Figure 1.3).

Lattice-based models use structured (Tang J *et al.* 2011) or unstructured meshes (Van Liedekerke *et al.* 2015) to describe movement of individual cells along the lattice points where (i) many cells occupy one lattice point (Radszuweit *et al.* 2009), (ii) an individual cell occupies a single lattice point (Block *et al.* 2007, Tang J *et al.* 2011), or (iii) a single cell occupies many lattice points (Szabó and Merks 2013, Voss-Böhme 2012). Lattice-based models can be divided mainly into two categories: cellular automata and cellular Potts models. Cellular automaton models describe the movement of individual cells in a

collective by updating the individual positions synchronously or asynchronously, where each cell can only occupy an empty lattice point (Tang J *et al.* 2011, Van Liedekerke *et al.* 2015). Lattice-gas automaton models are specific types of cellular automata that track the motion of multiple cells through a single lattice point. These types of models simulate large cell populations over time using statistical mechanics theories (Boghossian 1999, Ghaemi and Shahrokhi 2006). This provides methods that model the dynamics of a cell population with velocity as a variable instead of tracking the positions of individual cells (Nava-Sedeño *et al.* 2020, Van Liedekerke *et al.* 2015). Cellular Potts models use energy functionals generalised from the Potts models to describe the dynamics and interactions of individual cells by assigning each cell to multiple lattice points (Szabó and Merks 2013, Voss-Böhme 2012). The cellular Potts models simulate cell morphologies and mechanics that cannot be described in the cellular automata (Marée *et al.* 2007, Van Liedekerke *et al.* 2015) by determining the probability of lattice point state based on a Hamiltonian function; however, Potts models are computationally intensive, and the physical calibration time of Monte Carlo steps can be challenging.

Lattice-free models can be divided into two approaches: (i) boundary-based models and (ii) centre-based models. Boundary-based models consider cell boundaries by focusing on forces that act on cells. These types of models can be divided according to the approach used to calculate the forces acting on cells: vertex-based methods and front-tracking methods. Vertex-based methods compute the forces that act on cell vertices by considering the cell shape as polygon and polyhedral on substrate and ECM, respectively (Fletcher *et al.* 2014). Front-tracking methods track the movement of cell boundaries by solving different equations, such as partial differential equations (PDEs) for fluid flow between and inside cells (Rejniak 2007). Boundary-based models are useful in simulating the biomechanics of cells coupling with fluid diffusion and tissue.

Lattice-free centre-based models consider the volume or mass of individual cell centres in one or more clusters where adhesion, locomotion, repulsion, and drag forces are formulated between centres to compute the new positions of cells or clusters (Metzcar *et al.* 2019, Van Liedekerke *et al.* 2015). Cell morphologies can be incorporated in the centre-based models by breaking individual cells into subcellular elements (each cell is described by multiple interacting centre-based particles) (Milde *et al.* 2014, Newman 2005), but it can be computationally expensive when considering cell mechanics. The centre-based models take into account the complex interactions between cells or with their environment. The cell-based model approaches for collective cells with no consideration of cell morphologies can be classified into discrete, continuum, and hybrid according to whether spatial domain or time are considered continuous or discrete variables.

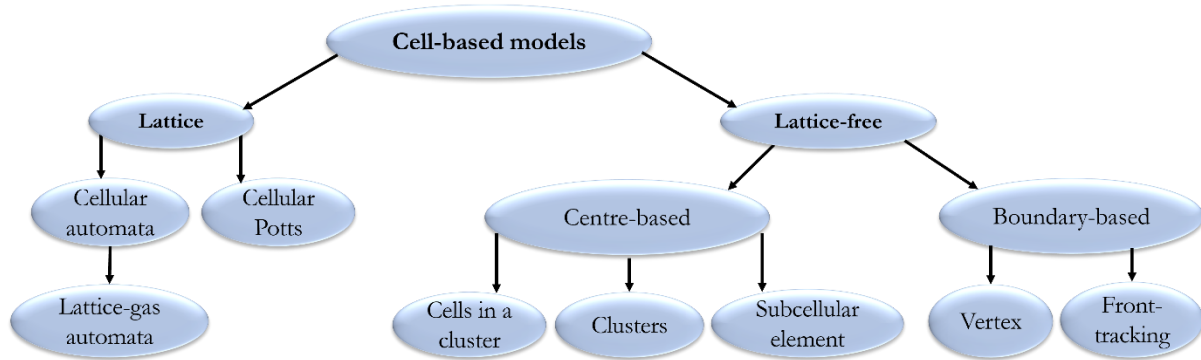


Figure 1.3. A schematic cell-based models' approach.

1.4 Discrete, continuum, and hybrid models for cell migration

1.4.1 Discrete models

Spatially discrete approaches may involve discrete or continuous time steps. All lattice-based models and lattice-free centre-based models are spatially discrete approaches. Lattice-free centre-based models consider individual cells in a cluster as particles with fixed or variable geometries during migration due to environmental cues, i.e., chemical, mechanical, or electrical cues. The most widely centre-based approach is particle models, which can describe the interaction between cells in a collective through cell boundaries that are connected to cell central points (Mousavi and Doweidar 2016, Mousavi *et al.* 2014b, Vermolen 2015). These types of models are efficient in tracking the positions of individual cells and have different applications where cell-cell interactions are considered, e.g., growth of cell colonies with proliferation (Dudaie *et al.* 2015, Mousavi and Doweidar 2015, 2016, Vermolen and Gefen 2012) and immune system response to simulate engulfment of pathogens (Vermolen 2015, Vermolen and Gefen 2013b, Vermolen *et al.* 2014).

Other cell-centre discrete approaches consider the simulation of multiple interacting cell clusters as particles that are affected by environmental cues (Gefen 2014, Ghaffarizadeh *et al.* 2018, Sottoriva *et al.* 2015, Sun *et al.* 2017). These types of models track the movement of clusters by considering energy functionals to compute adhesion, locomotion, repulsion, and drag forces between centres of clusters. Interacting clusters have great computational efficiency in incorporating heterogeneous details into cell clusters compared to particle models; however, individual cells are less tractable than continuum models.

1.4.2 Continuum models

Continuum models are formulated in terms of PDEs to simulate the migration of cell collectives as coarse-graining based on hydrodynamics principles. These types of models are useful in characterising the material properties of cell collectives using tissue scale parameters where velocity, polarisation, and cell densities describe the evolution of cell migration.

The most widely used PDE is the diffusion equation, where the random movement of individual cells induces the migration of cell collectives by deriving (i) the telegraph equation from a stochastic velocity jump process (Malik Adam A. and Gerlee 2019, Painter 2009) and (ii) stochastic lattice-based and lattice-free models where time is continuous for updating a cell position (Charteris and Khain 2014, Nava-Sedeño *et al.* 2020). PDE derivatives from discrete cell-based models provide analytical results for invading cell collectives by supporting travelling wave solutions for the long-time behaviour (Markham *et al.* 2015).

Some researchers describe the migration of cells in confined monolayers on a substrate as a continuum by assuming that the exerted cellular forces on the substrate are opposite to the cell polarisation and the friction forces (Banerjee and Marchetti 2019, Blanch-Mercader and Casademunt 2017, Hakim and Silberzan 2017, Lee and Wolgemuth 2011). These types of models have been used to incorporate moving boundary problems to simulate the interface between different states, such as the interface between the undamaged tissue and a wound (Arciero *et al.* 2011, Javierre *et al.* 2009b) or tumour (Vianna Gallinaro *et al.* 2013).

Some of the PDE models used integral terms in PDEs (Integro-PDEs) to track interfaces between cells and tissues or between different cell types (Armstrong *et al.* 2006, Gerisch and Chaplain 2006). Chauviere *et al.* (2007a) and (Chauviere *et al.* 2007b) incorporate adhesion gradients, chemical diffusion, and interactions of cell-cell and cell-ECM in integro-PDE frameworks. These models provide insights into cellular behaviour in anisotropic and heterogeneous ECM. Other researchers apply integro-PDEs to investigate cell-cell and cell-ECM interactions in tumours (Andasari *et al.* 2011, Gerisch and Chaplain 2008, Szymańska *et al.* 2024).

Other PDE models simulate the transition between different states gradually, such as fibre orientations that regulate ECM mechanics and cell-ECM interactions on pattern tissue formation, where collagen, culture medium, and cells are modelled as a three-phase mixture for their respective densities (Dyson *et al.* 2016, Hillen 2006, Painter 2009).

Although continuum models are computationally less expensive than discrete models, continuum approaches require more assumptions compared to discrete models. Further, the migration rate and behaviour of individual cells cannot be captured.

1.4.3 Hybrid models

Most cell-based models combine continuous and discrete approaches (referred to as hybrid models) and are sometimes combined with *in silico* or *in vitro* experiments to produce more accurate representations of *in vivo* studies. Most hybrid models consider cells as discrete entities and other processes or signals as continuum approaches. In cell-based models, continuum approaches at the tissue level must be transformed into discrete formulations at the cellular level. There are several hybrid models with different cell migration applications, here we mention a few examples related to the study background.

An integro-PDEs framework for unspecialised cells (stalk) is combined with particle models for specialised cells (tip) to describe the movement of heterogeneous cell populations (Bookholt *et al.* 2016, Colombi *et al.* 2015). The model is useful in describing specific functions of an individual cell phenotype in a collective. Phenotypic variability in tumours using integro-PDEs and *in silico* models is described in other studies (Lorenzi *et al.* 2016, Lorenzi *et al.* 2018).

The mechanical behaviour of epithelial cells in tissue is studied for wound closure using a hybrid model where cells are described as discrete particles and tissue as a continuum material representing cells and a substrate with different spatial domains (González-Valverde and García-Aznar 2018). In this model, the finite element method is combined with particle approaches to solve generated mechanical forces at the tissue level.

The most widely used hybrid models use reaction-diffusion PDEs to simulate the transportation of oxygen, lactates, growth factors, chemokines, and drugs with centre-based formulations to describe cell motion (Chen J *et al.* 2018b, 2020, Ghaffarizadeh *et al.* 2015, Ghaffarizadeh *et al.* 2018, Peng and Vermolen 2020, Vermolen and Gefen 2013b, Vermolen *et al.* 2015).

1.5 Models for mechanically, chemically, and chemo-mechanically induced cell migration

1.5.1 Mechanically induced cell migration

Discrete and continuum models can represent mechanically induced cell migration by considering the cellular mechano-sensing mechanisms, i.e., forces that cells apply to their environment, cell-cell and cell-environment adhesions, or environmental stiffness.

Mechanical cellular forces that deform the surrounding substrates have been considered in previous models as perpendicular (Ben-David and Weihs 2021, Dudaie *et al.* 2015, Vermolen and Gefen 2012) or tangential (Alert and Trepap 2020, Danuser *et al.* 2013, Kim M *et al.* 2016, Staddon *et al.* 2022) to the substrate surfaces.

The mechanical communication among individual cells in a collective through deformation of the environment has been studied using discrete or hybrid models, for example, to track the motion of each cell towards other cells based on their applied forces (González-Valverde and García-Aznar 2018, Kim M *et al.* 2016, Mousavi *et al.* 2014b, Rey and Garcia-Aznar 2013, Vermolen and Gefen 2012). Other models additionally incorporate cell-cell interactions in cell aggregates for the prediction of collective cell migration (Alert and Trepap 2020, Banerjee and Marchetti 2019, Blanch-Mercader and Casademunt 2017, Löber *et al.* 2015, Peng and Vermolen 2020, Staddon *et al.* 2022).

Incorporating cell-environment adhesion in mathematical models is considered for a single-cell motion. Most mechanical models neglect cell-environment adhesion or consider the parameter implicitly for collective migration. Although a few models incorporate adhesion for collective cells, the adhesion parameter is assumed to be constant (Dudaie *et al.* 2015, Rey and Garcia-Aznar 2013, Vermolen and Gefen 2012).

The combination of mechanical cell-cell interactions and cell-environment adhesions in mathematical models allows for studying individual cells in a collective response to mechanical changes in the cellular environment. Hybrid models are used to study the migration behaviour of cells in different environmental stiffness (Duchez *et al.* 2019, Dudaie *et al.* 2015, Escribano *et al.* 2018, Van Oers *et al.* 2014, Voloshin 2016). These models indicate that individual cells prefer a movement direction due to several mechanical cues in the environment. Further, it is observed that cells move with different velocities depending on the environmental stiffness.

1.5.2 Chemically induced cell migration

Diffusion of chemical gradients through the environment has been incorporated into mathematical models using continuum approaches (Szymańska *et al.* 2024, Talkenberger *et al.* 2017). These continuum models focus on tracking the motion dynamics of cancerous cells towards chemotactic gradients. Other studies that combine chemotactic tools with statistical analysis have been developed to study the migration behaviour of individual cells in a collective up to chemical

gradient (Di Costanzo *et al.* 2016, Merino-Casallo *et al.* 2018, Plou *et al.* 2018, Schwarz J *et al.* 2016, Tomasova *et al.* 2019). These studies do not capture the effect of mechanical cues on cell migration.

1.5.3 Chemo-mechanically induced cell migration

Hybrid models are needed to study the effect of mechanical and chemical cues on the migration behaviour of collective cells. A few hybrid models focused on capturing the effect of chemical gradients while neighbouring cells mechanically interact with each other during wound healing (Asakura *et al.* 2021, Liebchen and Löwen 2018, Peng and Vermolen 2020, Vermolen 2015, Vermolen and Gefen 2015, Zorn *et al.* 2015), cancer tumour growth (Chen J *et al.* 2018b, 2020, Vermolen *et al.* 2015), and different collagen gel concentration (Del Amo *et al.* 2017, Merino-Casallo *et al.* 2022).

1.6 Limitations of the state-of-the-art

Even though many experimental studies have been undertaken to understand the mechanical properties and effect of the environment on cell migration, only a few mathematical and computational models for mechanically induced cell migration on substrates and in ECM have been developed (Escribano *et al.* 2018, González-Valverde and García-Aznar 2018, Kim M *et al.* 2016, Peng and Vermolen 2020, Staddon *et al.* 2022). One of the main limitations of these models is that they are (i) two-dimensional (2D) models, (ii) failed to properly balance the active movement forces generated by each cell affecting cell-cell interactions, and (iii) some of these models do not consider effective forces such as traction, drag and protrusion forces which act on a cell during migration.

Many experimental studies have been presented regarding chemotactic effects on single and multi-cell migration (Del Amo *et al.* 2017, Schwarz J *et al.* 2016, Whitehead *et al.* 2016), while only a very few computational models have been proposed to understand the stimuli involved and the response observed (Merino-Casallo *et al.* 2018, Szymańska *et al.* 2024, Talkenberger *et al.* 2017). There is limited information available on the effect of immobilised growth factors in experimental studies and less is known from mathematical and computational models. Therefore, it is important to study migration behaviour in the presence of an immobile chemical gradient and multi-signalling stimuli.

Collective cells deliberate over several external stimuli such as chemical and mechanical cues before migrating into their environment (Abuelba *et al.* 2015, Li YB *et al.* 2023, Stamm *et al.* 2016). Although several studies dealing with multi-cellular migration considered cell migration in

response to multi-signalling stimuli (Chen J *et al.* 2018b, 2020, Peng and Vermolen 2020), the migration of collective cells in multi-signalling stimuli is poorly understood. The approaches used (i) are simple 2D models, (ii) facilitate the interpretation of the interplay between a cell and substrate and the change in cell speed and direction in a basic mechanism, and (iii) neglect some of the mechanical cues, such as environment stiffness, that affect the migration behaviour. The migration behaviour of collective cells in an ECM with multi-signalling stimuli differs from 2D cell migration (Merino-Casallo *et al.* 2022).

In addition, the effects of an immobile growth factor and ECM stiffness need to be incorporated into mathematical and computational models to provide comprehensive multi-signalling models. Such models will allow the prediction of migration behaviour under different conditions and environmental properties, such as the reaction of multi-cellular constructs (tissue and organs) to their environment.

1.7 Aim and objectives

The research aims to develop a mathematical model for chemo-mechanically induced collective cell migration in engineered extracellular matrices and demonstrate the predictive capabilities for the migration of individual cells in a collective based on the mechanical properties of the matrices and a growth factor gradient in extracellular matrices.

To achieve this aim, the objectives of this study are:

1. To develop a mathematical cellular mechano-sensing model on planar elastic substrates and demonstrate the predictive capabilities for the migration of cells in a collective on planar elastic substrates.
2. To extend the mathematical model for the mechanically induced cell migration on the planar elastic substrates (from objective 1) to capture the effect of a growth factor gradient on the migration of individual cells in a collective.
3. To develop a mathematical cellular mechano-sensing model based on mechanical interactions between a cell and its surroundings in three-dimensional (3D) engineered matrices and illustrate the predictive capabilities for the migration of cells in a collective in the extracellular matrix (ECM).
4. To establish a mathematical model to describe and predict the effect of a growth factor gradient on collective cell migration in the 3D microenvironments.

1.8 Thesis structure

The further chapters of this thesis comprise the following:

Chapter 2 describes collective cell migration on a planar elastic substrate induced by strain energy density gradients in the substrate originating from cellular traction forces. The mathematical approach describes individual cell locomotion due to mechanical cell-cell and cell-substrate interactions. The model considers the mechanical properties of the planar substrate, i.e., varying elasticity and thickness of the substrate.

Chapter 3 describes the extension of the mechanical model due to combined chemical and mechanical cues. The mathematical model investigates the effect of growth factor gradient and strain energy density gradients on cell migration behaviour on a planar elastic substrate. Effects of growth factor production rate and diffusion rate on migration of single cells in a colony are studied.

Chapter 4 describes the extension of the chemo-mechanically induced cell migration model to account for collective cell migration within a 3D extracellular matrix. The migration behaviour with varying elasticity of the extracellular matrix and in presence of the growth factor gradient is investigated.

Chapter 5 is the conclusion chapter, which summarises the main findings of this research and highlights the novel aspects of the research in relation to the developed models. Additionally, this chapter summarises the major recommendations for future research in modelling collective cell migration.

2 Modelling of mechanically induced migration of cells on planar elastic substrates

This chapter describes the investigation of cellular mechano-sensing on planar elastic substrates and the prediction for the migration of individual cells in a colony on planar substrates.

A manuscript of this study has been published as Ahmed RK, Abdalrahman T, Davies NH, Vermolen F, Franz T. Mathematical model of mechano-sensing and mechanically induced collective motility of cells on planar elastic substrates. *Biomech Model Mechanobiol*, 2023, 22(3), 809-24. The following sections are taken from this manuscript with minimal changes with permission of the University of Cape Town's Doctoral Degrees Board.

2.1 Introduction

Cell migration has been described for different cell types and environments (Ladoux and Mège 2017, Matte *et al.* 2019, Shellard and Mayor 2020, Tusan *et al.* 2018, Wang W *et al.* 2019, Yamada and Sixt 2019). It is considered that the migration of all cell types occurs by similar molecular mechanisms. Cells interact mechanically with the substrate through focal adhesions and apply forces that deform the surrounding substrate area. Neighbouring cells sense these forces and deformations (Angelini Thomas E *et al.* 2010, Gov 2009, Oakes PW 2018, Reinhart-King *et al.* 2008, Throm Quinlan *et al.* 2011, Treppe *et al.* 2009) and move in the direction of principal signals. Cells sense physical changes in the substrate, such as topographical traits and stiffness gradients (Hadjipanayi *et al.* 2009, Schwarz US and Safran 2013). Cells can identify substrate rigidity based on the strength of a mechanical signal they sense (Lo *et al.* 2000, Yip *et al.* 2013). Changes in the cellular environment guide cell migration, e.g., cells move towards stiffer substrate regions (Gupton and Waterman-Storer 2006, Hadjipanayi *et al.* 2009, Lo *et al.* 2000).

Cells move individually and collectively, the latter particularly in wound healing (Arciero *et al.* 2011, Liang *et al.* 2007), tissue regeneration (Angelini Thomas E. *et al.* 2011, Zorn *et al.* 2015) and cancerous tumour invasion (Mendoz and Lim 2011, Rørth 2009). In a collective, cells interact with the substrate and neighbouring cells (Reinhart-King *et al.* 2008). The cell-substrate interaction can affect the rate and directionality of the collective cell migration (Bloom and Zaman 2014, Borau C. *et al.* 2011, Liang *et al.* 2007).

Some computational models have incorporated stochastic processes such as cell proliferation (Mousavi and Doweidar 2015, 2016) and cell death (Chen J *et al.* 2018b, 2020), and have used the random walk to mimic cell migration (Safaeifard *et al.* 2018, Vermolen *et al.* 2015). However, most of these models did not consider the interactions between cells.

The mechanical communication of cells through substrate deformation due to traction forces has been considered previously in experimental and computational studies (Ben-David and Weihs 2021, Franck *et al.* 2011, Maskarinec *et al.* 2009, Nerger *et al.* 2017, Reinhart-King *et al.* 2008, Vermolen and Gefen 2012). These studies focused on capturing each cell's migration on the substrate. Neighbouring cells on the substrate attract and move toward each other based on their traction forces. Cell locomotion, propagation, and apoptosis are considered with a semi-random probability distribution.

2.2 Methods

2.2.1 Mechanically induced cell migration

Two or more cells (of the same cell type) are considered attached to and exerting traction forces on a planar substrate. Considering traction forces perpendicular to the substrate surface is inspired by the experimental work of several groups (Ben-David and Weihs 2021, Franck *et al.* 2011, Maskarinec *et al.* 2009, Nerger *et al.* 2017, Reinhart-King *et al.* 2008). The traction forces deform the substrate underneath and in the vicinity of each cell. Each cell senses the deformation gradient induced in the substrate by neighbouring cells. For simplicity, the planar substrate is assumed to be an elastic foundation attached to a rigid plate, and the cells are assumed to be rigid disks (Figure 2.1).

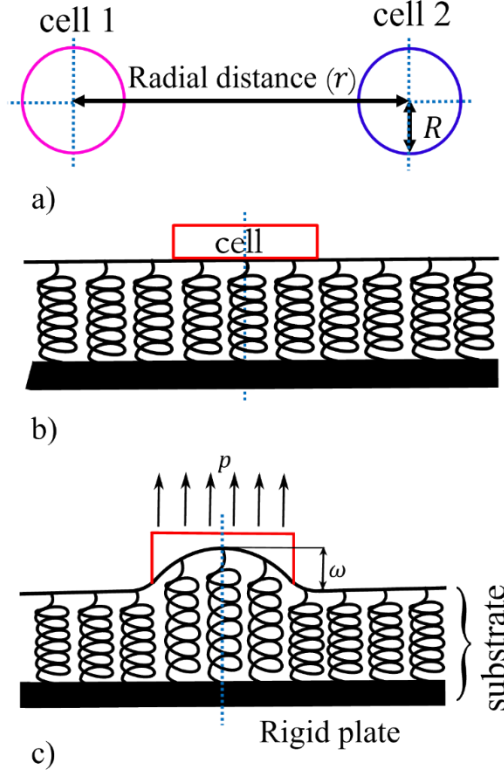


Figure 2.1. Schematic illustration of cells, planar substrate, and cell-substrate interaction. a) Top view of two cells with radius R at a distance r between their centres. b) Side view of a single cell (red) on an elastic substrate (black), represented by spring elements on a rigid plate. c) Deformation of the elastic substrate underneath and around a single cell due to cellular traction force p exerted on the substrate.

With $\mathbf{c}_i(t) = (x_i(t), y_i(t))$ as the centre position of the cell i at a time t , the substrate deformation, ω , underneath and around each cell i (Loof 1965) is:

$$\omega = \frac{p}{k} \begin{cases} (1 - e^{-sR}) \cosh(sr), & \|r - \mathbf{c}_i\| \leq R \\ \sinh(sR) e^{-sr}, & \|r - \mathbf{c}_j\| > R' \end{cases} \quad \text{for all } i, j = \{1, 2, \dots, n\}, \quad (2.1)$$

where R is the cell's radius, p is the traction force distributed on the substrate over a cell area, r is the distance between the centres of two cells, $\mathbf{c}_j(t)$ denotes the centre positions of cells j in the neighbourhood of cell i , and

$$s = \frac{(1 - \nu)^2}{0.156h(1 - 2\nu)},$$

with ν as the Poisson's ratio and h as the thickness of the substrate. For a uniformly distributed force, the substrate stiffness, k , (Loof 1965) is:

$$k = \frac{E}{h} \frac{1 - \nu}{(1 + \nu)(1 - 2\nu)}, \quad (2.2)$$

where E denotes the elastic modulus of the substrate.

With the strain energy obtained from the extension of the Pasternak foundation model (Selvadurai 1979), the strain energy density (i.e., strain energy per unit volume) can be expressed as a function of the substrate deformation:

$$S = \frac{1}{2V} \int k \omega^2 dA, \quad (2.3)$$

where V is the deformed volume of the substrate. This deformed substrate volume V is determined by the volume of a cylinder under each cell, i.e., $V = \pi R^2 h$ and a hollow cylinder from cell i to other cells j , i.e., $V = \pi(r^2 - R^2)h$ (Figure 2.2). Thus, the area, A , between every two cells on the substrate is considered an annulus or a ring shape:

$$A = \int_R^r 2\pi d\rho = \pi(r^2 - R^2). \quad (2.4)$$

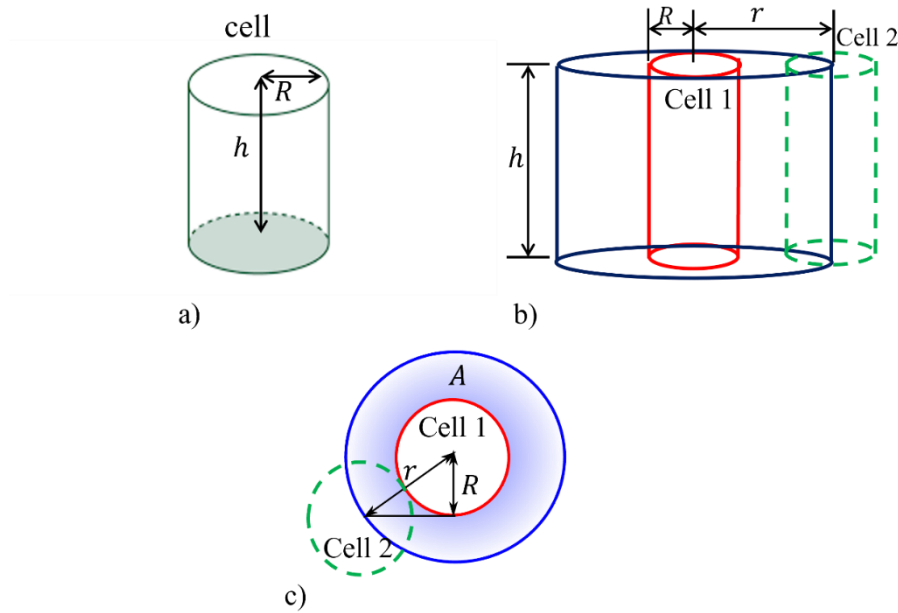


Figure 2.2. Schematic illustration of deformed volume and area of a planar elastic substrate underneath and around a single cell due to cellular traction force exerted on the substrate. a) Deformed substrate volume with thickness h underneath a single cell with radius R . b) Deformed substrate volume with thickness h between two cells with radius R at a distance r between their centres. c) Top view of a deformed area, A , between two cells with radius R at a distance r between their centres.

It is assumed that each cell is a source and receiver of strain energy density signals due to cellular traction forces on the substrate. The total strain energy density, ψ , of each cell i is the strain energy density due to the substrate deformation induced by cell i , and the strain energy densities from other cells j that cell i senses:

$$\psi = \begin{cases} S_i, & \|r - \mathbf{c}_i\| \leq R \\ S_j, & \|r - \mathbf{c}_j\| > R \end{cases}, \quad \text{for all } i, j = \{1, 2, \dots, n\}, i \neq j. \quad (2.5)$$

Since the strain energy density is additive, Eq. (2.5) can be rewritten as:

$$\psi(\mathbf{c}_i) = S_i + \sum_{\substack{j=1 \\ j \neq i}}^n S_j. \quad (2.6)$$

Equation (2.6) represents the sum of all strain energies. The first term represents the strain energy density under cell i based on the substrate deformation due to its traction force. The second term represents the sum of all strain energy densities under the same cell i resulting from substrate deformation caused by traction forces of other cells in the domain.

The migration direction of each cell i is the direction of the strain energy density resultant:

$$\mathbf{v}_i = \sum_{\substack{j=1 \\ j \neq i}}^n S_j(\mathbf{c}_i(t)) \mathbf{u}_{ij}(t), \quad \text{for all } i, j = \{1, 2, \dots, n\}, \quad (2.7)$$

where $\mathbf{u}_{ij}(t)$ denotes the unit vector connecting cell i to other cells j i.e.,

$$\mathbf{u}_{ij} = \frac{\mathbf{c}_j - \mathbf{c}_i}{\|\mathbf{c}_j - \mathbf{c}_i\|}. \quad (2.8)$$

The normalised form of the migration unit vector is:

$$\hat{\mathbf{v}}_i(t) = \frac{\mathbf{v}_i}{\|\mathbf{v}_i\|}. \quad (2.9)$$

The magnitude of the displacement is assumed to be proportional to the strength of the mechanical signal, i.e., the substrate deformation. Thus, the movement of cells towards each other is:

$$\mathbf{c}_i(t + \Delta t) - \mathbf{c}_i(t) = \frac{\Delta t \psi(\mathbf{c}_i) \hat{\mathbf{v}}_i}{2 \int_0^R \rho(X) dX}, \quad (2.10)$$

where $\rho(X)$ denotes the friction coefficient of the cell-substrate adhesion, and X denotes the position of a point in the cell that varies in the interval occupied by the cell, i.e., $X = [-R, R]$.

2.2.2 Cell-substrate interaction

Individual cells are considered homogenous rigid components. Suppose that the effective friction coefficient of the cell-substrate adhesion, $\rho(X)$, is linearly dependent on the concentration of integrins attached to substrate ligands. Therefore, the cell adhesion strength is:

$$\rho(X) = \rho_0 n_b(X), \quad (2.11)$$

where ρ_0 is the friction coefficient for a cell receptor binding to the substrate, and $n_b(X)$ is the density of the bounded integrins.

Suppose n_f is the concentration of free integrins and n_s is the concentration of the substrate ligands. The propagation of free integrins n_f , attachment of integrin receptors to substrate ligands, and detachment of integrin receptors from substrate ligands are assumed as instantaneous mechanisms compared to the time scale of cell migration. It is also assumed that the bond formation rate k_f is constant, the dissociation rate $k_r \varphi_r(X)$ varies with the position in a cell, and the density of substrate ligands n_s is uniform and constant. Ligand depletion can be neglected when the ligand number is saturated. Then, the concentration of free integrins n_f can be considered constant, and the density of the integrins is:

$$n_b(X) = \frac{k_f n_s}{k_r \varphi_r(X)} n_f. \quad (2.12)$$

To represent the cell adhesion dynamics, suppose that:

$$\varphi_r(X) = \varphi_r + (1 - \varphi_r) \frac{X}{2R}, \quad (2.13)$$

where $\varphi_r \geq 1$ denotes a decrement of the dissociation rate from the rear to the front of the cell.

The total number of integrin receptors is assumed to be constant over time and obtained from Gracheva and Othmer (2004) and Zaman *et al.* (2005). The concentration of integrin receptors and free integrins n_f are estimated from Dai *et al.* (2015). With parameter $k_s = k_f n_s / k_r$ representing the cell-substrate interaction, the friction coefficient of cell-substrate adhesion is:

$$\rho(X) = \rho_0 \frac{k_s n_f}{\varphi_r(X)}. \quad (2.14)$$

2.2.3 Stochastic processes in cell migration

2.2.3.1 Cell migration with randomness

Cell migration is a biological and partial random process. Thus, a certain degree of motion randomness is incorporated into the present model using the Brownian motion approach. The standard Brownian vector, $\Delta \mathbf{B}(t)$, has two independent entries that are normally distributed with zero mean and variance Δt , that is, the entries of $\Delta \mathbf{B}(t) \sim N(0, \Delta t)$. Hence, for cells with partial random motion:

$$\mathbf{c}_i(t + \Delta t) - \mathbf{c}_i(t) = \frac{\Delta t \psi(\mathbf{c}_i(t)) \hat{v}_i}{2 \int_0^R \rho(X) dX} + \varrho \Delta \mathbf{B}(t), \quad (2.15)$$

where ϱ considers probabilistic variations due to uncertainties such as in tissue composition, cell composition, and access to necessary chemicals. $\varrho \Delta \mathbf{B}(t)$ is commonly referred to as random walk. The definition of Brownian motion can be found, for instance, in Karatzas and Shreve (2012) and Bressloff (2014).

2.2.3.2 Cell death and division

Cell death and division can be represented as random processes since cell cycles differ from cell to cell (Kar *et al.* 2009). Here, cell death and division are modelled using stochastic principles described in Chen J *et al.* (2018b) and Chen J *et al.* (2020). For simplicity, each cell in the population is assumed to be either viable or dead, purposely disregarding that a cell may mutate or be injured and then recover or die. The probability P_i of a cellular event of cell i , i.e., death or division, within a time interval Δt is considered to follow an exponential distribution and calculated by:

$$P_i = 1 - \exp(-\mathfrak{h}_i \Delta t), \quad (2.16)$$

$$P_i \approx \mathfrak{h}_i \Delta t \quad \text{if} \quad \mathfrak{h}_i \Delta t \ll 1,$$

where $\mathfrak{h}_i$ is the probability rate of cellular event per second. At each time interval Δt , a cellular event $\mathfrak{z}$ is generated from the standard uniform distribution $U(0, 1)$, i.e., $\mathfrak{z} \sim U(0, 1)$, such that:

$$0 \leq \mathfrak{z} \leq 1 - \exp(-\mathfrak{h}_i \Delta t). \quad (2.17)$$

In this model, cell death and division are simulated using the probability rates $\mathfrak{p}_i$ and $\mathfrak{q}_i$, respectively. The probability rate for the death of cell i is assumed to be $\mathfrak{p}_i = 0.001 \text{ s}^{-1}$ (Vermolen *et al.* 2012). The probability rate of cell death is generally related to the cell's condition or environment, such as temperature and pH level. The probability rate that cell i dies within a time interval Δt does not depend on the size of the time step of the numerical solution. Dead cells are removed from the simulation immediately at each time frame Δt (Figure 2.3), and their vacated positions can be occupied by any viable cell.

The same probabilistic principles are applied to cell division. Let $\mathfrak{q}_i$ be the division probability rate of each cell i during a time interval Δt . The probability rate for cell i to divide is assumed to be $\mathfrak{q}_i = 0.05 \text{ s}^{-1}$ (Vermolen *et al.* 2012). For the division of a cell into two cells, the original cell is shifted by a distance R in a random direction and a new daughter cell is added to the model such that their edges resemble the centre position of the original cell (Figure 2.3). It is assumed that once the original cell and the daughter cell are in contact, they stay together until the migration force changes their polarisation orientation, after which they may separate. This is a simplifying essential assumption; hence, the authentic tendency is also a stochastic parameter.

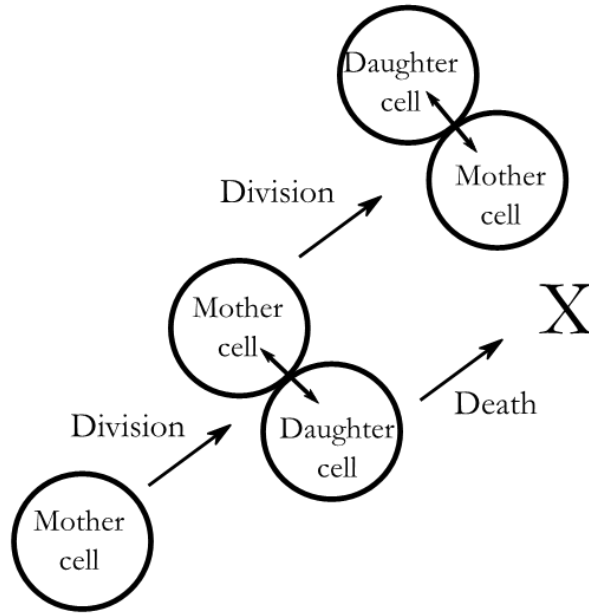


Figure 2.3. Schematic illustration of cell division and cell death in the model. The mother cell shifts in a random direction with a magnitude equal to the cell radius for a new cell to be generated. Edges of mother and daughter cells touch each other at the centre of the original cell. A dead cell is removed immediately from the simulation.

2.2.4 Cell migration simulations

The computational domain of the planar substrate is considered as $1,000 \mu\text{m} \times 1,000 \mu\text{m}$ for all cell migration simulations except those involving cell death and division, for which $1,500 \mu\text{m} \times 1,500 \mu\text{m}$ is used. The cells with a radius $R = 50 \mu\text{m}$ are considered. The simulations mimic hypothetical cases; hence, hypothetical values have been used for many input parameters. The values and their sources, if applicable, of the model parameters introduced in the above equations are listed in Table 2.1 unless stated otherwise.

Each simulation experiment runs with a time step of 1 s. It is worth noting that the time scale varied and depended on the migration velocity. Of course, there is a likelihood of a small displacement vector, i.e., close to zero, during one or more time steps; hence, a cell may not have migrated to a new location, and the cell velocity may be close to zero during these time steps.

First, the evolution of the 2D substrate deformation underneath a single cell is investigated for different values of the elastic modulus (E) and thickness (h) of the planar substrate. After that, the migration due to cellular mechanical interactions and the effect of substrate elasticity and thickness are studied. Finally, the model's capability of incorporating cell death and division in collective cell migration is demonstrated.

Cell deformation due to cell-cell contact (Peng and Vermolen 2020) is considered in the model by permitting cells to overlap up to 30%, after which repulsing of the cells is assumed. This condition is implemented by a negative attraction vector in Eq. (2.8) that corresponds to the strain energy density for 30% cell deformation. This penalty condition prevents 100% overlap of the cells.

Table 2.1. Parameters and values used in the model.

Parameter	Definition	Value and unit	Reference
p	Cellular traction force	1 kPa	(Dudaie <i>et al.</i> 2015, Vermolen and Gefen 2012).
R	Cell radius	$50 \mu\text{m}$	(Buxboim <i>et al.</i> 2010a).
E	Substrate elastic modulus	5 kPa	(Buxboim <i>et al.</i> 2010b, Tusan <i>et al.</i> 2018).
h	Substrate thickness	$100 \mu\text{m}$	(Tusan <i>et al.</i> 2018).
ν	Substrate Poisson ratio	0.3	(Akiyama and Yamada 1985, Ulrich <i>et al.</i> 2009).
ρ_0	Adhesion strength	$5 \times 10^{-15} \text{Ns}/\mu\text{m}^3$	(Mehrayin <i>et al.</i> 2012).

k_s	Cell-substrate interaction	10^{-3}	(Gracheva and Othmer 2004).
n_f	Concentration of free integrins	$5490 \mu\text{m}^{-1}$	(Dai <i>et al.</i> 2015).
φ_r	Dissociation rate of integrins	3.33	(Gracheva and Othmer 2004).
p_i	Probability rate of cell death	0.001 s^{-1}	(Vermolen <i>et al.</i> 2012).
q_i	Probability rate of cell division	0.05 s^{-1}	(Vermolen <i>et al.</i> 2012).
ϱ	Probability of cell motion variability due to uncertainties	$0.4472 - 4.4721$	
Δt	Time step	1 s	

The mathematical model has been implemented in MATLAB (The MathWorks, Inc., Natick, MA, USA).

2.3 Results

2.3.1 Substrate deformation by a single cell

The deformation of the substrate normal to its surface due to a uniformly distributed traction force p of a cell with radius $R = 50 \mu\text{m}$ is determined for different values of the substrate elastic modulus and thickness (Figure 2.4). The substrate deformation decays exponentially with distance from the cell centre, and it increases with decreasing elastic modulus and increasing thickness of the substrate.

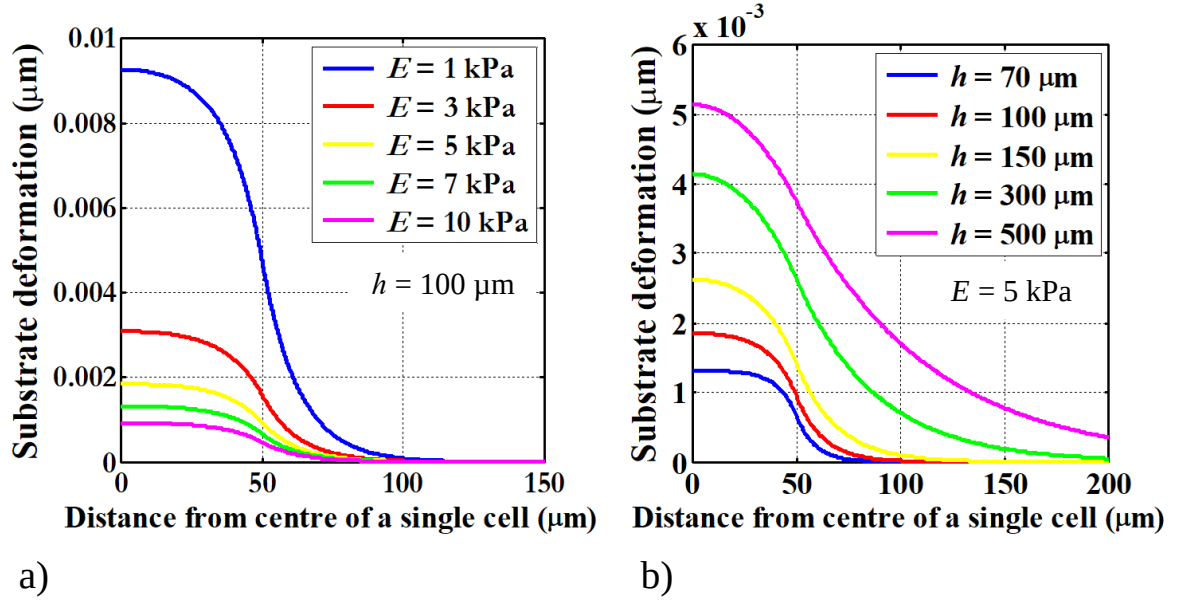


Figure 2.4. Substrate deformation normal to its surface by the distributed traction force p of a single cell with radius $R = 50 \mu\text{m}$ versus distance from cell centre for different values of the substrate elastic modulus E with constant substrate thickness $h = 100 \mu\text{m}$ (a) and thickness h with constant $E = 5 \text{ kPa}$ (b). The substrate deformation normal to the substrate plane decays exponentially from the cell centre and the magnitude of the substrate deformation increases with decreasing substrate elasticity E (a) and increasing substrate thickness h (b). (Model parameter values and sources are listed in Table 2.1 except for values above in Figure labels are estimated from Buxboim *et al.* (2010a) and Tusan *et al.* (2018)).

2.3.2 Effect of substrate elasticity and thickness on the migration velocity of two approaching cells

The movement of two cells with fully deterministic motion towards each other on a substrate is considered for different substrate elastic modulus and thickness values. The migration velocity of the cells increases with decreasing substrate elasticity (Figure 2.5a) and thickness (Figure 2.5b). The initial distance between the two cells is random, i.e., different for each case (variation of elastic modulus and thickness, respectively) but the same for the parametric simulations of each case. Cell viability is defined as a discrete parameter, and continued cell viability is ignored, implying that a cell is either fully viable or dead.

The final distance between the two cells is smaller than the cell diameter ($2R = 100 \mu\text{m}$), i.e., the cells impinged on each other since repulsive forces are not considered.

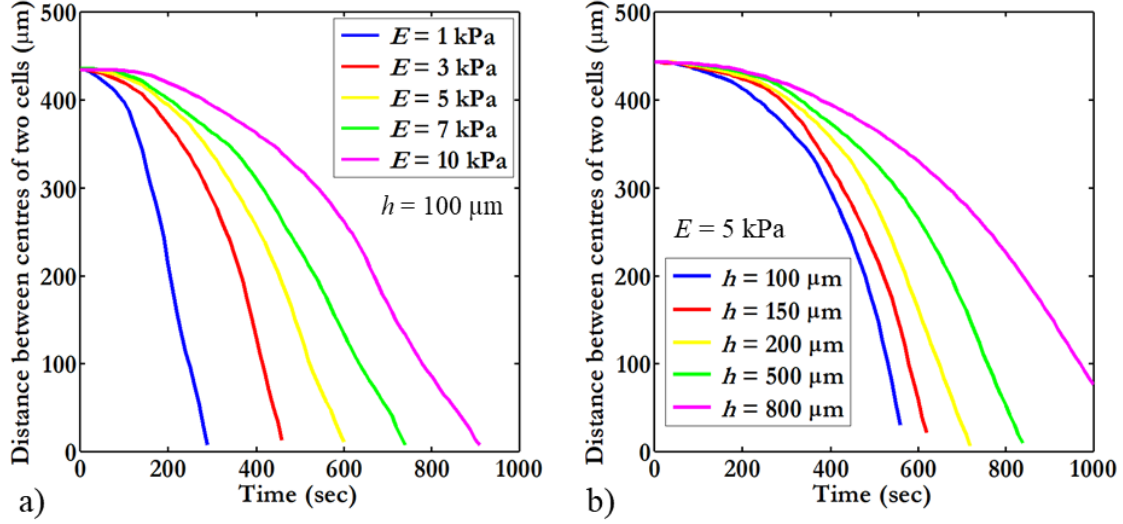


Figure 2.5. Distance versus time of two cells approaching each other in fully deterministic motion (i.e., absence of stochastic motion) on a planar substrate for different substrate elasticity and thickness. The migration velocity of the cells increases with decreasing substrate elasticity E (a, for $h = 100 \mu\text{m}$) and substrate thickness (b, for $E = 5 \text{ kPa}$). (Model parameter values and sources are listed in Table 2.1 except for values above in Figure labels are estimated from Buxboim *et al.* (2010a) and Tusan *et al.* (2018)).

2.3.3 Collective cell migration in wound healing

Collective cell migration due to mechanical cues on a planar substrate with uniform elastic modulus and thickness with and without standard Brownian motion (random movement) is investigated using 25 cells arranged randomly in two concentric rows of cells (with an outer radius of $400 \mu\text{m}$). The arrangement provides a central cell-free space mimicking a wound with a radius of approximately $200 \mu\text{m}$. The probabilities of cell death p_i and cell division q_i are assigned small values to emphasise the mechanically induced cellular motion, i.e., $p_i = 0.0001 \text{ s}^{-1}$ and $q_i = 0.001 \text{ s}^{-1}$. The p_i and q_i values are estimated from Vermolen and Gefen (2015) and Dudaie *et al.* (2015).

With fully deterministic motion (Figure 2.6a), cells initially move towards neighbouring cells in the dominant direction of the resultant strain energy density gradient in the substrate ($t = 22 \text{ s}$). In addition, the cells in contact deform (shown as overlap) as inter-cellular forces are not considered in the model. The interaction between cells mainly depends on the initial cell positions. Once cells are in contact, they start migrating together into the central cell-free area ($t = 44 \text{ s}$). The cells fill the cell-free central area following further migration towards the centre regardless of the initial cell locations ($t = 66 \text{ s}$). Including stochastic perturbations in the model (Figure 2.6b) adds variability in the direction and magnitude of the cellular motion during each solution time step ($t = 0, 20,$

and 40 s). However, the stochastic perturbation does not change the final cluster arrangement ($t = 61$ s).

The evolution of locomotion paths of 10 cells arranged in a circle with fully deterministic and randomly perturbed motion illustrates the effect of Brownian motion, i.e., the deflection of the mechanically induced motion (see Figure 2.7).

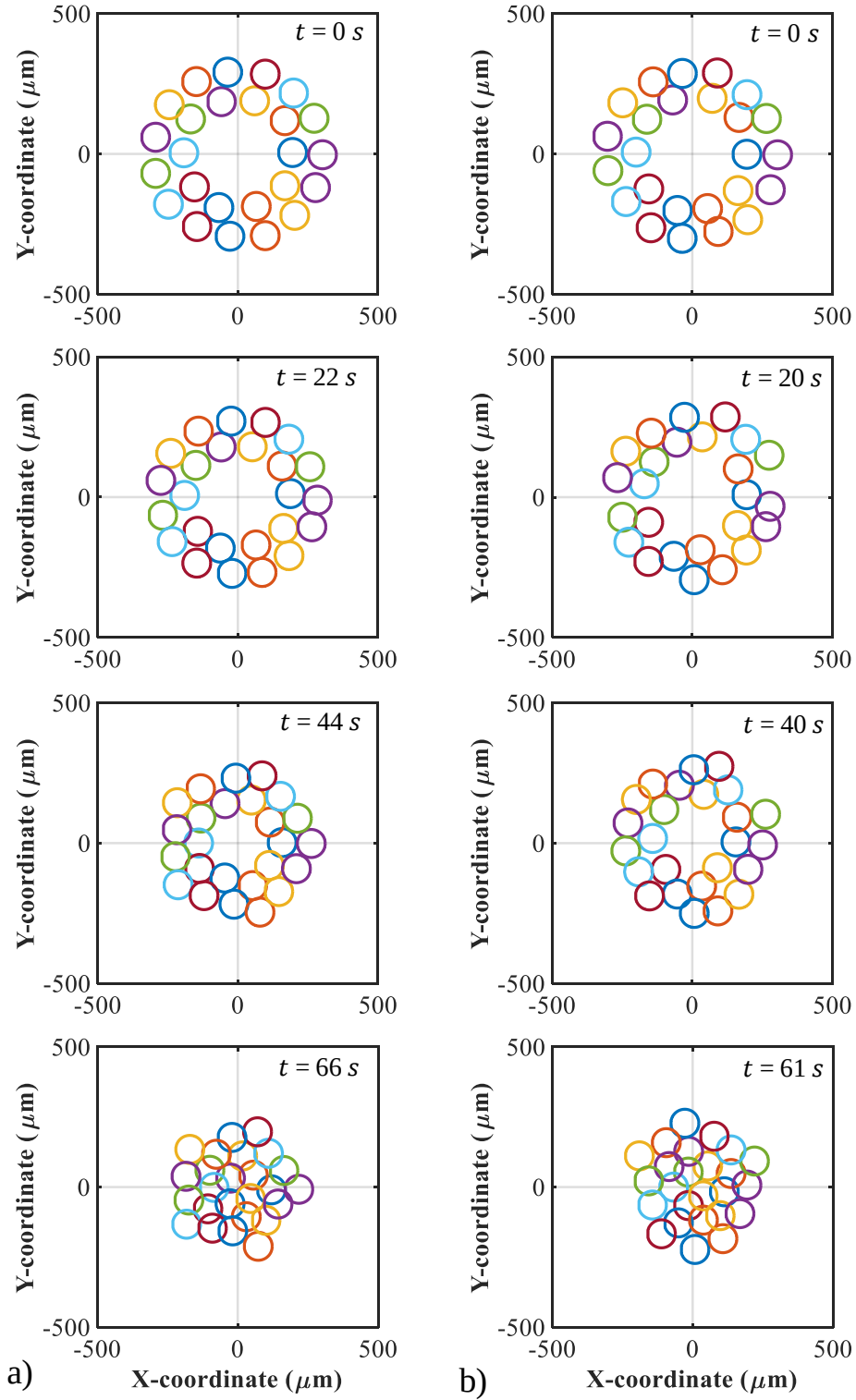


Figure 2.6. Positions of 25 cells on a planar substrate during collective migration with (a) fully deterministic and (b) randomly perturbed motion as a function of time. a) Initial positions of the cells at $t = 0$ s in two concentric rows (outer radius: $400\ \mu\text{m}$, inner radius: $200\ \mu\text{m}$) with a cell-free centre area. At $t = 22$ s, neighbouring cells move towards each other, directed by the resultant strain energy density gradient (see also supplemental video V2.6A). The cells contact each other and start deforming (represented by overlap) in the absence of repulsive inter-cellular forces. At $t = 44$ s, cells start forming clusters and moving into the cell-free central area (wound). At $t = 66$ s,

cells collectively move into the centre and fill the cell-free central area. b) Initial positions of the cells at $t = 0$ s. At $t = 20$ s, neighbouring cells move towards each other following the resultant strain energy density gradient. At $t = 40$ s, cells start to form clusters and move toward the cell-free centre of the substrate. At $t = 61$ s, cells migrate into and close the cell-free central substrate area (see also supplemental video V2.6B). (Model parameter values and sources are listed in Table 2.1 except $p_i = 0.0001 \text{ s}^{-1}$ and $q_i = 0.001 \text{ s}^{-1}$ are estimated from Vermolen and Gefen (2015) and Dudaie *et al.* (2015)).

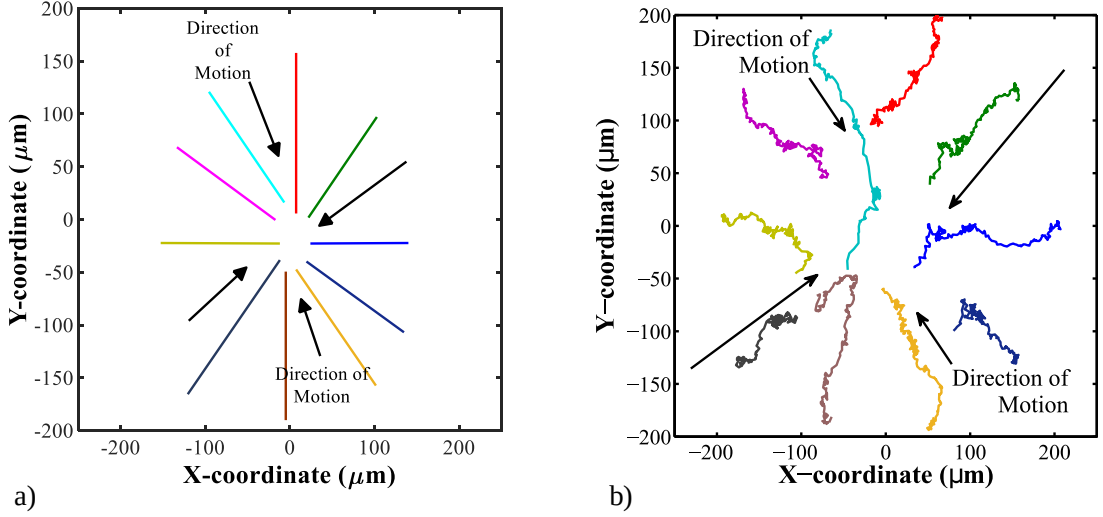


Figure 2.7. Trajectories of ten cells during collective migration for (a) fully deterministic and (b) randomly perturbed motion starting from an initial circular arrangement and moving for the same period. Coloured lines illustrate the movement paths of cell centre points. Black arrows indicate the overall direction of the cellular motion. For fully deterministic motion, cells move along straight paths towards the centre of the substrate (computational domain) and form a cluster. For perturbed motion, the trajectories show the randomness overlaying the mechanically induced motion of the cells. (Model parameter values and sources are listed in Table 2.1 except $p_i = 0.0001 \text{ s}^{-1}$ and $q_i = 0.001 \text{ s}^{-1}$ are estimated from Vermolen and Gefen (2015) and Dudaie *et al.* (2015)).

2.3.4 Cell migration for spatially varying substrate elasticity

To study the effect of substrate regions with different stiffness on cell migration, a planar substrate of $1,000 \mu\text{m} \times 1,000 \mu\text{m}$ with a constant thickness of $h = 100 \mu\text{m}$ is divided into a stiff ($E = 15 \text{ kPa}$ (Buxboim *et al.* 2010a)) and soft region ($E = 5 \text{ kPa}$). The elastic modulus is changed as a step at $X = 0 \mu\text{m}$, providing two regions with the dimensions of $500 \mu\text{m} \times 1,000 \mu\text{m}$ (Figure 2.8). Cell death and division are not considered; hence $p_i = 0 \text{ s}^{-1}$ and $q_i = 0 \text{ s}^{-1}$ are used.

The first case considers four cells, i.e., one cell in each substrate quadrant, with an equal distance between the two cells in the stiff and soft substrate region (Figure 2.8a). The two cells in each stiffness region of the substrate primarily move towards each other (i.e., towards $Y = 0$) with a minor movement component towards $X = 0$. The cells in the soft region move faster than in the

stiff region. Once the two cells in the soft region reach each other (at $Y \approx 0$), they move collectively in the X-direction towards the cells in the stiff region. Similarly, once the cells in the stiff region reach each other, they move collectively towards the cells in the soft region. The four cells finally form a cluster in the centre of the substrate, although slightly in the stiff region (see also supplemental video V2.8A).

For 15 cells in the ring-shaped arrangement (Figure 2.8b) on the same substrate as in the previous case, the cells initially move towards and connect with neighbouring cells. The cells in the stiff substrate region remain distributed in two- and three-cell clusters compared to the more connected cells in the soft substrate region ($t = 110$ s). The cell cluster in the soft substrate region joins two clusters in the stiff substrate region ($t = 220$ s). In the final configuration, all cells cluster slightly towards the stiff region in the substrate centre and fill the cell-free space ($t = 330$ s) (see also supplemental video V2.8B). The 15 cells fill the cell-free central substrate area after $t \approx 330$ s, whereas this takes $t \approx 420$ s for the four cells, indicating an increase in migration velocity with an increase in cell number.

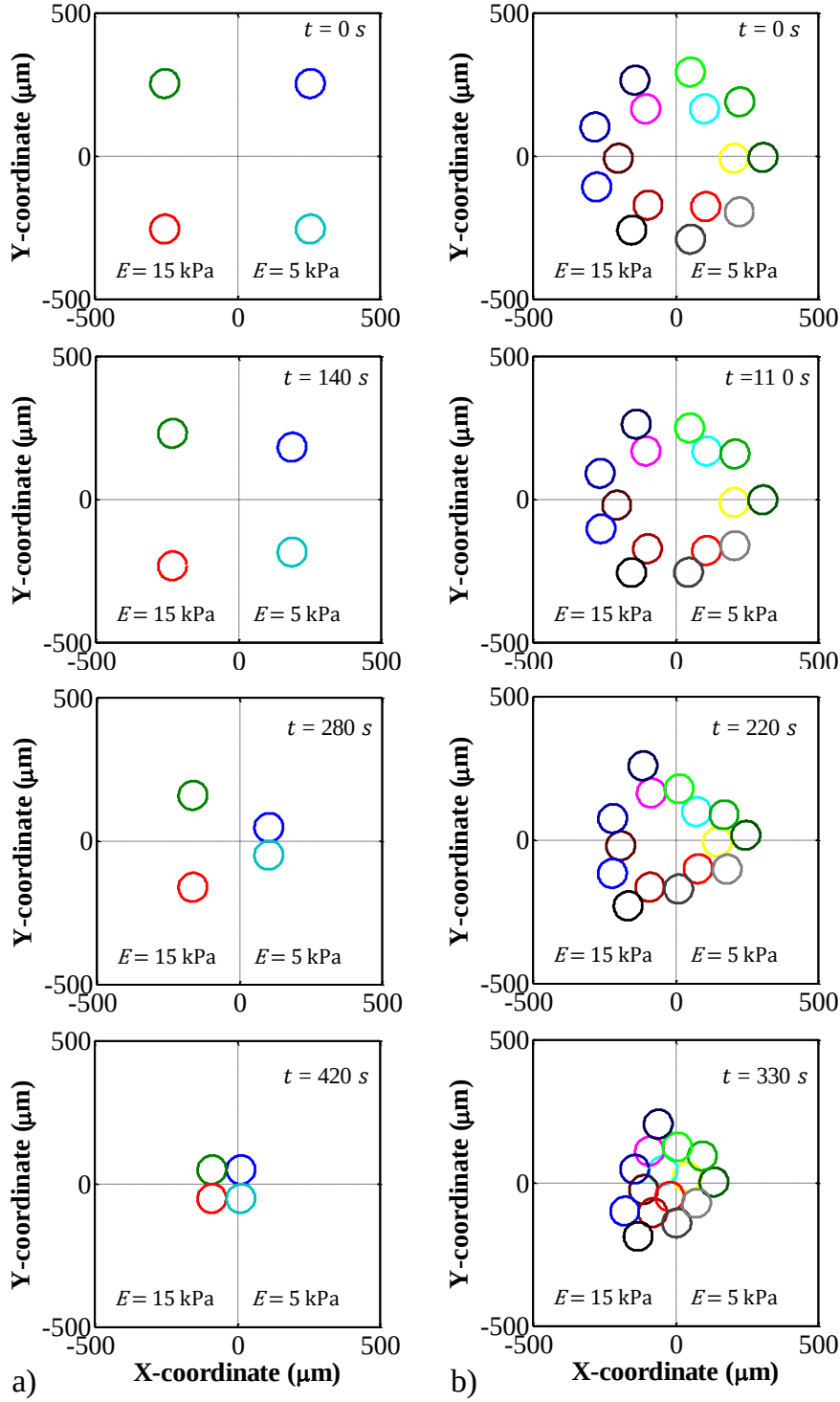


Figure 2.8. Positions of 4 (a) and 15 (b) cells migrating with fully deterministic motion on a planar substrate with varying elastic modulus E and constant thickness $h = 100 \mu\text{m}$ as a function of time. E changes at $X = 0 \mu\text{m}$ from $E = 15 \text{ kPa}$ in the substrate region with $X < 0$ (left, stiff region) to $E = 5 \text{ kPa}$ in the region with $X \geq 0$ (right, soft region). a) Initial positions of four cells at $t = 0 \text{ s}$, with two cells each on the stiff and soft substrate region. After $t = 140 \text{ s}$, the two cells in the left and right region, respectively, move towards each other (i.e., towards $Y = 0$) due to the mechanical cues. The cells migrate faster in the soft than the stiff region. At $t = 280 \text{ s}$, the cells on the soft region reach each other (at $Y \approx 0$) and continue moving collectively in the X -direction towards the

cells in the stiff substrate region (see also supplemental video V2.8A). At $t = 420$ s, the four cells cluster in the substrate centre, slightly towards the stiff substrate region. b) Initial ring-shaped arrangement of 15 cells at $t = 0$ s. At $t = 110$ s, the cells migrate towards each other on both stiff and soft substrate regions. At $t = 220$ s, the cells migrate farther and have larger velocities on the soft than the stiff region. At $t = 330$ s, the cells from both regions form a single cluster. Cells in the soft substrate region migrate more towards and into the stiff region, whereas cells in the stiff region migrate less towards the soft region (see also supplemental video V2.8B). (Model parameter values and sources are listed in Table 2.1 except for $E = 15$ kPa provided above in this caption is estimated from Buxboim *et al.* (2010a)).

2.3.5 Cell migration for spatially varying substrate thickness

Four cells are considered on a substrate with a step-wise change of thickness h along $X = 0$, from $h = 300$ μm (Tusan *et al.* 2018) for $X < 0$ to $h = 100$ μm for $X \geq 0$, and a constant elastic modulus of $E = 5$ kPa. Initially, two cells are located in the thick and thin regions, and the cells in each region have the same distance from each other (Figure 2.9a, $t = 0$ s). Cell death and division are not considered here, and $p_i = 0$ s^{-1} and $q_i = 0$ s^{-1} . Initially, the single cells preferentially move towards each other, faster in the thin than the thick region ($t = 106$ s), until they are in contact at $Y \approx 0$ in each region ($t = 213$ s). After that, the two two-cell clusters move towards each other and aggregate on the boundary between thin and thick substrate regions ($t = 320$ s). The cells keep moving around each other randomly and do not cross from the thick region to the thin region of the substrate (see also supplemental video V2.9A).

The collective migration of 15 cells (Figure 2.9b) from an initial circular arrangement on a substrate with two thickness regions (as in the previous case) starts with the movement of individual cells towards neighbouring cells. However, cells on the thin substrate region near the thickness change moved towards and into the thick substrate region, but not vice versa. The overall migration tendency of cells is towards the thick substrate region ($t = 90$ s). The cells move collectively towards and into the cell-free central substrate region, with similar migration speeds in the thick and thin substrate regions ($t = 180$ s). Finally, the cells fill the central cell-free substrate area and cluster slightly more in the thick substrate region ($t = 270$ s) (see also supplemental video V2.9B).

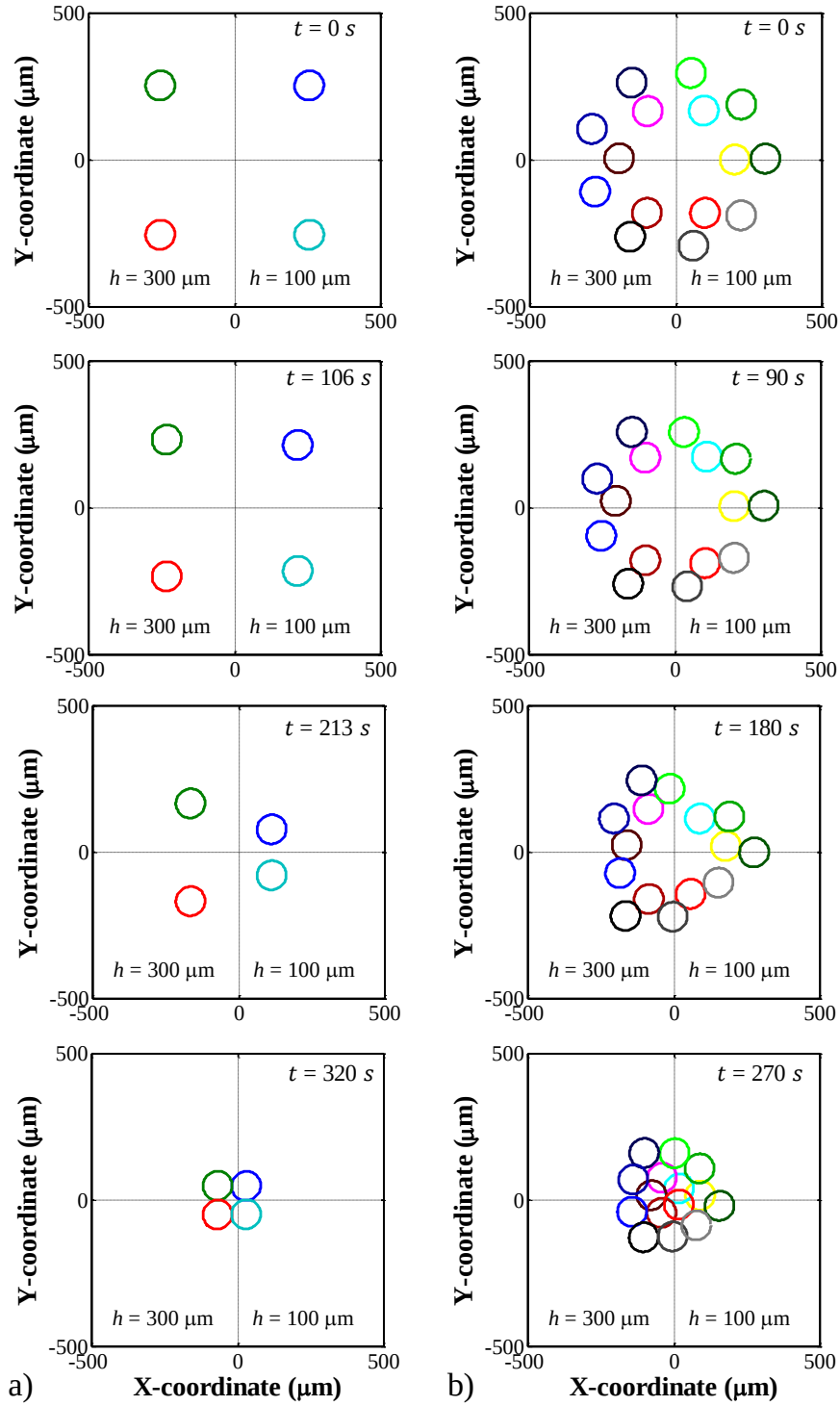


Figure 2.9. Positions of cells migrating with fully deterministic motion on a substrate with a step-wise change of thickness h along $X = 0$, from $h = 300 \mu\text{m}$ for $X < 0$ to $h = 100 \mu\text{m}$ for $X \geq 0$, and a constant elastic modulus of $E = 5 \text{ kPa}$. a) Initial configuration of four cells with the same distance between the two cells in the thin and thick substrate region at $t = 0 \text{ s}$. At $t = 106 \text{ s}$, the two cells in each substrate region preferentially move towards each other. Cells in the thin region migrate at a higher rate. At $t = 213 \text{ s}$, the two cells in each region join at $Y \approx 0$. At $t = 320 \text{ s}$, the two two-cell clusters move towards each other and aggregate at $X \approx 0$ slightly towards the thick substrate region (see also supplemental video V2.9A). b) The initial circular arrangement of 15

cells with a cell-free central substrate area at $t = 0$ s. At $t = 90$ s, cells move to neighbouring cells. Cells in the thin substrate region but close to the thickness interface at $X = 0$ μm move towards and into the thick substrate region. At $t = 180$ s, the cells move collectively towards the substrate centre into the cell-free region. At $t = 270$ s, the cells fill the cell-free central substrate area (see also supplemental video V2.9B). (Model parameter values and sources are listed in Table 2.1 except for $h = 300$ μm provided above in the caption is estimated from Tusan *et al.* (2018)).

2.3.6 Cell migration with cell death and division

The prediction of collective cell migration with cell death and division is demonstrated with 45 cells in two concentric rows with an inner and outer radius of 400 μm and 550 μm (Hettler *et al.* 2013, Lan *et al.* 2010), respectively, on a uniform substrate with an elastic modulus of $E = 5$ kPa and a thickness of $h = 100$ μm . The probabilities of cell death and division are set to $p_i \leq 0.001$ s^{-1} and $q_i \leq 0.05$ s^{-1} (Vermolen *et al.* 2012) as required for the different cases, i.e., collective migration with (i) cell death only, (ii) cell division only, and (iii) cell death and division. The larger number of 45 cells for these simulations compared to 15 and 25 cells in the previous cases are chosen based on the larger initial circular cell configuration.

For the case of migration with cell death only, the number of cells decreases during the collective migration into the central, cell-free substrate area from initially 45 cells to 40 cells after $t = 115$ s when the central wound area is closed (Figure 2.10a). For migration with cell division only, the cell number increases from 45 to 72 while the cells migrate into and close the central cell-free substrate area in 50 s (Figure 2.10b). For collective cell migration with cell death and division, the central cell-free substrate area is closed after $t = 60$ s while the cell number increases from 45 to 66 (Figure 2.10c).

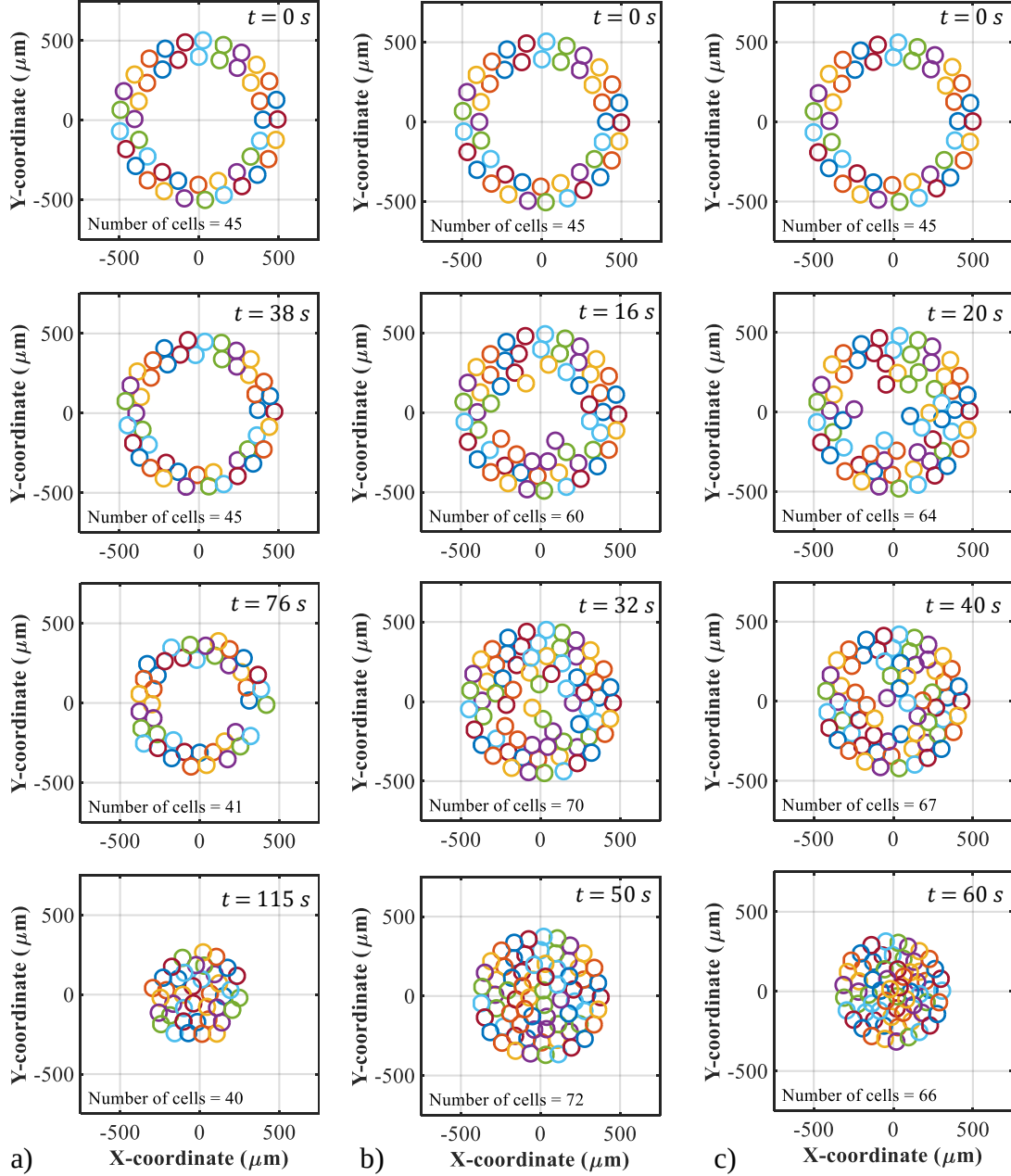


Figure 2.10. Positions of initially 45 cells on a planar substrate and changes in number and position of cells over time during collective migration with fully deterministic motion with (a) cell death only, (b) cell division only, and (c) cell death and division. The initial arrangement of 45 cells in two concentric rows (inner radius: $400 \mu\text{m}$, outer radius: $550 \mu\text{m}$) is the same for the three cases (at $t = 0 \text{ s}$). a) For cell death only, the cells close the cell-free central substrate area in $t = 115 \text{ s}$ and the number of cells decreases from 45 to 40. b) For cell division only, the central cell-free substrate area is closed in $t = 50 \text{ s}$, and the cell number increases from 45 to 72. c) For cell death and division, the central cell-free substrate area is closed after $t = 60 \text{ s}$, and the cell number increases from 45 to 66. (Please also see supplemental videos V2.10A to V2.10C for cases a to c. The supplemental video V2.10D shows cell migration with randomly perturbed motion with cell death and division not included above.) (Model parameter values and sources are listed in Table 2.1 except for cell death and division probabilities are estimated as follows: a) $p_i \leq 0.001 \text{ s}^{-1}$, $q_i = 0 \text{ s}^{-1}$, b) $p_i = 0 \text{ s}^{-1}$, $q_i \leq 0.05 \text{ s}^{-1}$, c) $p_i \leq 0.001 \text{ s}^{-1}$, $q_i \leq 0.05 \text{ s}^{-1}$).

2.4 Discussion

A two-dimensional (2D) model for the simulation of mechanically induced migration of cells on a planar substrate is developed. The chosen approach is different from those proposed in previous studies (Camley and Rappel 2017, George M *et al.* 2017, González-Valverde and García-Aznar 2018, Kim M *et al.* 2016, Zorn *et al.* 2015) in considering the mechanical properties of the cellular microenvironment and cell-substrate adhesion with the initial goal of analysing how mechanical changes of the substrate guide cell migration.

2.4.1 Single-cell substrate deformation and migration velocity for varying substrate elasticity and thickness

The developed model indicates that substrate elasticity and thickness are essential in cell migration. An increase in substrate deformation due to traction forces of a single cell is observed for decreasing stiffness and increasing substrate thickness (Figure 2.4). The predicted effect of substrate stiffness is consistent with reports (Vianna Gallinaro *et al.* 2013, Voloshin 2016, Yip *et al.* 2013). The increasing deformation restriction may explain the increase in substrate deformation with the increase in substrate thickness in an elastic layer with decreasing thickness connected to a rigid foundation. Similar changes in substrate mechanics with substrate thickness are observed experimentally for several cell types (Buxboim *et al.* 2010a, Buxboim *et al.* 2010b, Merkel *et al.* 2007).

The increasing migration velocity of two interacting cells predicted for a decreasing elastic modulus, i.e., softening, of the substrate (Figure 2.5a) correlates with the increasing substrate deformation (i.e., mechanotactic cue) by a single cell for decreasing substrate elastic modulus (Figure 2.4a) and agrees with experimental reports (Adlerz *et al.* 2016, Lo *et al.* 2000, Yip *et al.* 2013). However, the increase in migration velocity of two cells with a decrease in substrate thickness (Figure 2.5b) contrasts the decrease in substrate deformation with a decrease in substrate thickness for a single cell (Figure 2.4b). Increased Mechano-sensing of cell colonies compared to single cells (Tusan *et al.* 2018) and the slowing down of cells with increasing substrate strain (Van Oers *et al.* 2014) may explain the model prediction, at least in part.

2.4.2 Collective cell migration on a substrate with uniform elasticity and thickness

The model results reveal several differences between collective and single-cell migration on a planar substrate. Compared to the migration of two cells, the mechanical interactions between cells in colonies (i) delay their arrival at the final destination and (ii) increase the magnitude of the strain energy density and local velocity, which is also reported by Mousavi *et al.* (2014b). Furthermore, the stochastic movement is slightly larger for a cell colony than for a single cell, agreeing with the results from Mousavi *et al.* (2014a).

The dependence of early cell interactions and migration on the initial cell positions predicted by the model has been ascribed to the initial dominance of the mechanical signals exerted by the cells in a colony (Reinhart-King *et al.* 2008). However, the initial cell positions do not affect the final stage of the collective cell migration, i.e., closure of the cell-free central substrate area. This migration behaviour is qualitatively similar to experimental (Grasso *et al.* 2007) and numerical results on wound healing (Rey and Garcia-Aznar 2013, Vermolen *et al.* 2012).

Although each simulation run of the model gives a different cell position, the overall direction of the migration with random movement is the same for different simulations and consistent with *in vitro* experiments (Liang *et al.* 2007, Merks and Koolwijk 2009, Topman *et al.* 2012, Van Oers *et al.* 2014).

It is worth noting that the predicted cell aggregation at the centre of a substrate with uniform elasticity and thickness, respectively, agrees with previous computational studies (Chen J *et al.* 2020, González-Valverde and García-Aznar 2018, Vermolen *et al.* 2012, Vermolen *et al.* 2015) and is observed for different cell types (Palsson 2001).

2.4.3 Collective cell migration on substrates with varying elasticity and thickness

The collective cell migration on a substrate with two different elasticity regions is investigated. Whereas cells generally migrate towards stiffer substrate regions (Dokukina and Gracheva 2010, Kim M-C *et al.* 2018, Mousavi *et al.* 2014a, Mousavi *et al.* 2014b), the developed model indicates that cells in a collective might not do so instantly. Instead, cells in the same substrate region initially move towards each other (at a speed depending on the substrate elasticity) to form a cluster before moving towards the substrate region with a different elasticity (Figure 2.8). It is argued that the mechanical signal, i.e., substrate deformation gradient, of neighbouring cells in the same substrate region is stronger than the mechanical cue from the substrate elasticity change. These results are in qualitative agreement with several studies reporting that the mechanotactic cue from the

deformation of a substrate region between two neighbouring cells is stronger than the mechanical signal originating from another substrate region (Borau C. *et al.* 2011, Dudaie *et al.* 2015, Rey and Garcia-Aznar 2013).

The cell colonies aggregate in the substrate centre across the elasticity interface, favouring the stiff substrate region. The cell cluster originating in the soft substrate region migrates partially into the stiff substrate region. In contrast, cells in the stiff substrate region do not cross into the soft substrate region (Figure 2.8). The promotion of cellular interaction forces on soft substrates suggested by other studies (Guo *et al.* 2006, Reinhart-King *et al.* 2008) may explain this behaviour.

The substrate thickness variation causes a smaller difference in migration velocities of cells in the different substrate regions than the same three-fold variation in substrate elasticity. Some cells in the thin substrate region detach from the group and move to the thick substrate region (Figure 2.9). This is observed for cells near the thickness interface and can be ascribed to a weaker cell-cell interaction than the cue based on the thickness change, similar to reports from other studies (Reinhart-King *et al.* 2008, Tusan *et al.* 2018). Overall, cell migration is directed towards the thick substrate region. Cells migrate from the thin to the thick substrate region but not vice versa (Figure 2.9). This behaviour is based on a stronger mechanotactic cue from the substrate thickness change than the substrate deformation caused by neighbouring cells (Guo *et al.* 2006, Lo *et al.* 2000, Reinhart-King *et al.* 2008). Cell groups in the thin substrate region are attracted to the thick substrate region. In addition, the cells in a group are attached or packed, more closely in the thick than the thin substrate region, which is observed experimentally by Tusan *et al.* (2018).

Collective cell migration is faster on substrates with uniform than non-uniform elasticity and thickness. The cell interactions in each of the different substrate regions increase the time required to form a final cluster in the substrate centre, i.e., decreasing the overall migration velocity.

2.4.4 Collective cell migration with cell death and division

Collective cell migration with concurrent cell death leads to a substantially longer migration period of $t = 115$ s to close a central cell-free substrate area than for the migration with cell division only and with cell death and division ($t = 50$ and 60 s, respectively) (Figure 2.10). The slower migration in the case of only cell death compared to the cases including cell division is ascribed to the weaker mechanical cues. The weaker cues are associated with an 11% decrease in cell number in the case of cell death compared to increases in cell number of 60% for migration with cell division only and 47% for migration with cell division and death. However, it should be noted that the values

for cell death and division probabilities in the presented simulations are exaggerated to demonstrate the effects of cell death and division.

The final clustering can represent wound closure observed in *in vitro* cell migration assays and *in vivo*. The simulation results predict the migration of cells into the central cell-free space mimicking a wound with a radius of approximately 350 μm . However, the migration of cells outside the two concentric rows of cells has not been shown in the current model.

2.4.5 Limitations

Even though the model generated results that align qualitatively with experimental data for cell migration over complex environments, the model has some limitations.

The current model considers cellular traction forces perpendicular to the substrate surface. This is inspired by the work of several groups who report that such forces do play a role in cell migration (Ben-David and Weihs 2021, Franck *et al.* 2011, Maskarinec *et al.* 2009, Nerger *et al.* 2017). Loof's model was used, which is based on cell forces that act normally on the substrate surface. The formalism is generic in this sense since one can also incorporate tangential forces in the same kind of formalism using a different equation for displacement in future work.

The current model considers cell division with a constant probability while cells go through stages to proliferate. Several *in vivo* experiments showed that cells have different capabilities to proliferate, and the maximum number of cell divisions increases with increasing telomere length of the cells' DNA (Allsopp *et al.* 1992, Frenck *et al.* 1998, Lindsey *et al.* 1991).

In the presented model, the degradation of dead cells is ignored, and since only mechanical effects are considered, the chemical effect of dead cells is neglected. Moreover, any influence of dead cells on the migration of viable cells is ignored. It is further assumed that a cell's death is sudden and discrete and not affected by its environment.

The model includes some simplifying assumptions, such as a constant cell radius, mechanical isotropy of the substrate, the geometry of cell positions, and a simplified computational domain. However, these simplifications do not affect the conclusions of this work. The collective migration of cells in a multi-signalling substrate associated with different complex biological processes can be easily incorporated into the present framework. Other biological processes, such as cell differentiation (Deshpande RS and Spector 2017, Prokharau *et al.* 2014) should be considered when organ development is modelled. The nonlinearity of the problem needs an inner iteration to determine the cell positions, making an implicit method less attractive.

2.4.6 Recommendations

It is possible to include alternative cell shapes, corresponding to different cellular phenotypes. Extending the proposed model to include several cell types with different shapes is also possible. Further, the presented model can be extended to include the movement of different cell types that exert different traction forces on the substrate. Such extension may relate to the substrate deformation function (Loof's model) or by using different equations in future work.

The model formalism considers the adhesion friction coefficient of the cell-substrate interaction as a viscous force in a one-dimension. This is not suitable for describing the migration of all cell types on the substrate. It is important to consider the appropriate viscous properties of a cell type. The formalism is generic in this sense since the adhesion interaction can be incorporated into the model using different equations.

The model formalism uses a simple approach to describe cell-cell interactions mediated through the substrate. Cell-cell junctions and repulsive forces resulting from cells impinging on each other have not been considered, whereas cell deformation has been incorporated as partial overlapping. Reinhart-King *et al.* (2008) showed a combination of repulsion and overlapping of cells. We realise that repulsion is an important process for inclusion in our future studies.

Extending the mechano-sensing model to include substrate stiffness gradient is important for inclusion in future studies. The stiffness gradient is an important process that regulates biological processes such as wound healing, cancer invasion, blood vessel formation, and tissue regeneration. The stiffness gradient depends on the elasticity and thickness of the substrate. With some extension of the current model, gradually varying (gradient) elasticity and thickness of the substrate can be useful in creating complex environments similar to *in vitro* and *in vivo* experiments.

2.5 Conclusion

The developed model can adequately predict collective cell migration on planar elastic substrates induced by strain energy density gradients in the substrate originating from cellular traction forces. The model considers the mechanotactic cell-cell interactions through the substrate, the substrate's structural properties, the randomness of cell motion, and cell viability. The rate, orientation, and directionality of the cell migration depend on substrate elasticity and thickness. The model is suitable for extension to include other cell and substrate shapes and chemotactic migratory cues – increasing the potential and capabilities to replace *in vitro* and *in vivo* experiments with *in silico* simulations in, for example, wound healing, regenerative medicine, and cancer treatment.

3 Modelling of growth factor-induced migration of cells on a planar elastic substrate

This chapter presents a mathematical model for combined chemically and mechanically induced collective cell migration on planar substrates. The formulation uses an approach that considers individual cells in a colony similar to particle simulations typical in biochemical problems, such as representing flow problems at the molecular scale.

3.1 Introduction

The movement of cells in response to a chemical stimulus allows neutrophils to chase bacteria and amoebae to aggregate into multicellular masses. The migration of cells due to a chemical stimulus is a crucial mechanism by which tissues and organs become organised during development. Chemical stimulus is involved in, for example, embryonic development, wound healing, and cancer metastasis. It can be desirable to promote or inhibit chemically induced cell migration to support wound healing or prevent cancer progression.

Cells migrate towards or away from gradients of chemical sources, such as growth factors or bacterial agents, depending on the chemical type of the signal (Neilson *et al.* 2011b). Experimental studies illustrate that a gradient of a chemical signal orientates cells (Del Amo *et al.* 2017, Jin 2013, Schwarz J *et al.* 2016, Sixt and Lämmermann 2011, Whitehead *et al.* 2016, You *et al.* 2012). It has been suggested that the movement of a cell in response to a chemical stimulus is driven by a ‘compass’, processing the signal in an intracellular mechanism that drives the cell in the direction of the gradient (Swaney *et al.* 2010, Van Haastert 2010). However, other authors disregard this notion and propose that the cell orients itself by pseudopods (Neilson *et al.* 2011b).

Several computational and mathematical models have been developed to study the effects of chemical cues on single (Deshpande VS *et al.* 2006, Mousavi *et al.* 2013) and collective cell migration (Charteris and Khain 2014, Liebchen and Löwen 2018, Tan and Chiam 2018, Tomasova *et al.* 2019). Most of these studies did not consider the multi-signalling cues of chemical and mechanical signals, and the effects of mechanical cues on cell migration have been neglected. To combine chemical and mechanical cues, a mathematical description of the chemical stimulus requires the representation of the cell's ability to sense chemical and mechanical cues and account for chemo-mechanical interactions.

Many mathematical models use PDEs to describe biological processes like tumour growth or wound healing. Analytical solutions are developed for simplified models, whereas discretisation methods are used for more complicated modelling cases. The numerical solution of PDEs on evolving surfaces is required for several problems in biological sciences, for instance, pattern formation of organisms growing on surfaces (Leung and Berzins 2003) and diffusion-induced motion (Neilson *et al.* 2011a, Vermolen *et al.* 2006).

3.2 Methods

3.2.1 Chemically induced cell migration

The migration of cells on the 2D substrate, described in Chapter 2, in the presence of a growth factor gradient, is investigated. The diffusion of a growth factor source (chemoattractant) that attracts the cells is approximated as a point source; hence, the point source is considered to be small compared to the cell areas. The chemoattractant point source is assumed to be stationary, and the diffusion field that surrounds the point source is assumed to be instantaneous with time.

With $\mathcal{H}(x, y)$ as the position of the growth factor source, the diffusion of the growth factor that attracts the cells, with centre positions $\mathbf{c}_i(t) = (x_i(t), y_i(t))$ on the substrate and time t , is:

$$\begin{aligned} \frac{\partial g}{\partial t} - D\Delta g &= \alpha(t)\delta(\mathcal{H} - \mathbf{c}_i), \\ g(t, \mathcal{H}) &= 0, \quad t = 0, \end{aligned} \tag{3.1}$$

where $g(t, \mathcal{H})$ denotes the concentration of the growth factor, $\alpha(t)$ is the amount of growth factor produced by the source per time, i.e., the production rate of the growth factor, D is the diffusion coefficient of the growth factor and is considered as an isotropic and homogeneous, $\Delta = (\partial^2/\partial x^2$

$+ \partial^2/\partial y^2$) represents the Laplace operator, and $\delta(\mathcal{H} - \mathbf{c}_i)$ is the Dirac Delta function, which satisfies:

$$\begin{cases} \delta(\mathcal{H}, \mathbf{c}_i) = 0, & \text{if } (x, y) \neq 0 \\ \delta(\mathcal{H}, \mathbf{c}_i) = \infty, & \text{if } (x, y) = 0 \end{cases} \quad \text{with} \quad \int \delta(x, y) d\Omega = 1. \quad (3.2)$$

Initially, the growth factor concentration is assumed to be zero with additional boundary conditions so that the diffusion of the growth factor at its site creates a semi-bounded domain:

$$\begin{aligned} \frac{\partial g}{\partial t}(t, \mathcal{H}(x, y)) &= 0, \quad \text{where } x = \text{const}, \\ \text{const} \leq x \leq \infty, \quad & -\infty \leq y \leq \infty. \end{aligned} \quad (3.3)$$

Green's fundamental solution and Duhamel principle are used to solve Eq. (3.1) for the point source that triggers the release of the growth factor (Evans 1998).

$$g(t, \mathcal{H}) = \int_0^t \frac{\alpha(s)}{4\pi D(t-s)} \exp\left(-\frac{\|\mathcal{H} - \mathbf{c}_i(s)\|^2}{4D(t-s)}\right) ds. \quad (3.4)$$

The representation of Eq. (3.4) allows evaluation of the chemical concentration at any point on the substrate. However, it requires integration over an increasing time interval.

The diffusion of the growth factor results in a concentration gradient away from the site of the point source due to the particle movement from a region of high concentration to a region of low concentration. Therefore, cells sense the direction of the chemical gradient and migrate in the direction of the increasing gradient. The concentration gradient that diffuses through the substrate and attracts the cells is:

$$\nabla g(t, \mathbf{c}_i) = \int_0^t \frac{\alpha(s)(\mathcal{H} - \mathbf{c}_i(s))}{8\pi D^2(t-s)^2} \exp\left(-\frac{\|\mathbf{c}_i(s) - \mathcal{H}\|^2}{4D(t-s)}\right) ds. \quad (3.5)$$

The solution of the concentration gradient of the growth factor is obtained by using the Trapezoidal rule with the parameters listed in Table 3.1. The time is divided into a discrete set of points, $t_n = n\Delta t$, where Δt is the time step, and n indicates the size of each time step at which the numerical solution is computed. The time step for the implicit Trapezoidal rule is limited and fluctuates over the simulation time which makes the method more stable than any other explicit

method. Further, the numerical solution of the concentration gradient for long times is smooth and decays exponentially as the time step decreases down to zero.

3.2.2 Chemo-mechanically induced cell migration

The growth factor point source is assumed to be located in a certain region of the substrate, and all cells are on one side of the growth factor point source (Figure 3.1).

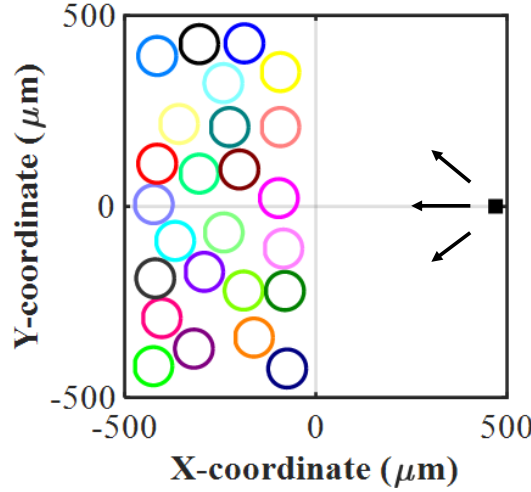


Figure 3.1. Initial positions of cells (open circles), and a growth factor point source (solid square). The growth factor diffuses through the 2D substrate and attracts the cells. The growth factor source is stationary and located at $\mathcal{H}(x, y) = (470, 0)$, from where it diffuses in the direction of the cells.

For the combination of the two migratory mechanisms (mechanical and chemical), Eqs. (2.16) and (3.5) are combined to describe the influence of the growth factor stimulus on the mechanically induced cell migration. This gives the time-dependent displacement of the cells in the presence of the growth factor stimulus and random walk as:

$$\mathbf{c}_i(t + \Delta t) - \mathbf{c}_i(t) = \frac{\Delta t \psi(\mathbf{c}_i(t)) \hat{v}_i}{2 \int_0^R \rho(X) dX} + \zeta \nabla g(t, \mathbf{c}_i(t)) \Delta t + \varrho \mathbf{B}(t), \quad (3.6)$$

where ζ denotes the mobility rate parameter of the cell's response to the concentration gradient of the growth factor. This cell mobility rate ζ can depend on spatial sensing over the cell body and its signalling rate. For simplicity, ζ is assumed as a constant in this model.

Each cell on the substrate is assumed to migrate due to sensing mechanical and chemical signals in Eq. (3.6). The reduction or obstruction of the chemical signal a cell receives by its neighbours

is not considered in the current model, i.e., the cells that are behind other cells still sense the same amount of chemical signal.

The current model allows us to predict cell migration behaviour under different conditions and environmental properties by incorporating the effects of the chemical gradient in mechanically induced cellular movement.

Table 3.1. Parameters and values used in the model.

Parameter	Description	Value and unit	Reference
α	Growth factor productivity (production rate)	8,000 mol/ $\mu\text{m}^2\text{s}$	(Savinell <i>et al.</i> 1989).
D	Growth factor diffusivity (diffusion rate)	179 $\mu\text{m}^2/\text{s}$	(Nauman <i>et al.</i> 2007).
ζ	Cell mobility to the growth factor concentration	1,000 $\mu\text{m}^4/\text{mols}$	(Vermolen and Gefen 2013a, Vermolen <i>et al.</i> 2014).
h	Time step size of the numerical integration	0.01 s	(Chen J <i>et al.</i> 2018a).
Δt	Time step	1 s	

3.2.3 Cell migration simulations

The computational domain of the planar substrate is considered as $1,000 \mu\text{m} \times 1,000 \mu\text{m}$ for all cell motility simulations. The cells are presented by open circles with a radius $R = 50 \mu\text{m}$, and the shape of the growth factor point source is indicated by a solid square with a length equal to $30 \mu\text{m}$. The cells are initially located on the negative side of the X-axis of the substrate with dimensions $500 \mu\text{m} \times 1,000 \mu\text{m}$ (Figure 3.1), where the chemical concentration is zero. Therefore, it is assumed that the release of the growth factor source that attracts the cells starts at $t = 1$ s. Further, it is assumed that the growth factor source is located at $\mathcal{H}(x, y) = (470, 0) \mu\text{m}$ that causes a point symmetric chemical gradient over the 2D space.

The simulations mimic hypothetical cases; hence, hypothetical values have been used for many input parameters. The values and their sources, if applicable, of the model parameters introduced in the above equations are listed in Table 3.1 unless stated otherwise.

Each simulation experiment runs with a time step of 1 s. It is worth noting that the cells move around the growth factor source randomly once they reach the source (at $X \approx 470$). Cells are

allowed to 100% overlap during migration induced by chemical cues. Cell-cell contact corresponding to the strain energy density is considered in the current model to prevent 100% cell overlap during cellular mechanical interactions. However, once the cells reach the growth factor source, they can 100% overlap around the growth factor source.

First, the evolution of the growth factor concentration gradient on a single is investigated for different values of the growth factor production rate (α) and diffusion rate (D). Secondly, the migration due to the chemical concentration gradient and the effects of the production rate and the diffusion rate of the growth factor source are demonstrated. Finally, the migration of cells as a response to the chemical concentration gradient and mechanical cellular interaction is studied.

The numerical results are produced using MATLAB (The MathWorks, Inc., Natick, MA, USA).

3.3 Results

3.3.1 Chemically induced single and collective cell migration

3.3.1.1 Effect of growth factor productivity and diffusivity on chemically induced migration velocity of a single cell

The migratory displacement of a single cell with fully deterministic motion towards a growth factor source on a substrate is considered for different growth factor production rates and diffusion rates. The time required for the cell to migrate to the growth factor source decreases with increasing the growth factor productivity (Figure 3.2a) and diffusivity (Figure 3.2b). The initial distance between the cell and the growth factor source is fixed and equal to 900 μm for each case. The final distance between the cell and the growth factor source is smaller than the cell radius ($R = 50 \mu\text{m}$), i.e., the cell reaches the growth factor source since repulsive force is not considered.

The migration velocity of a single cell with fully deterministic motion towards the growth factor source on a 2D substrate is determined for different growth factor production rates (Figure 3.2c) and diffusion rates (Figure 3.2d). The maximum velocity of the cell increases with increasing the growth factor production rate α (Figure 3.2c) and decreasing diffusion rate D (Figure 3.2d). The overall velocity of the cell shows similar behaviour and slight differences in values for different D values (Figure 3.2d).

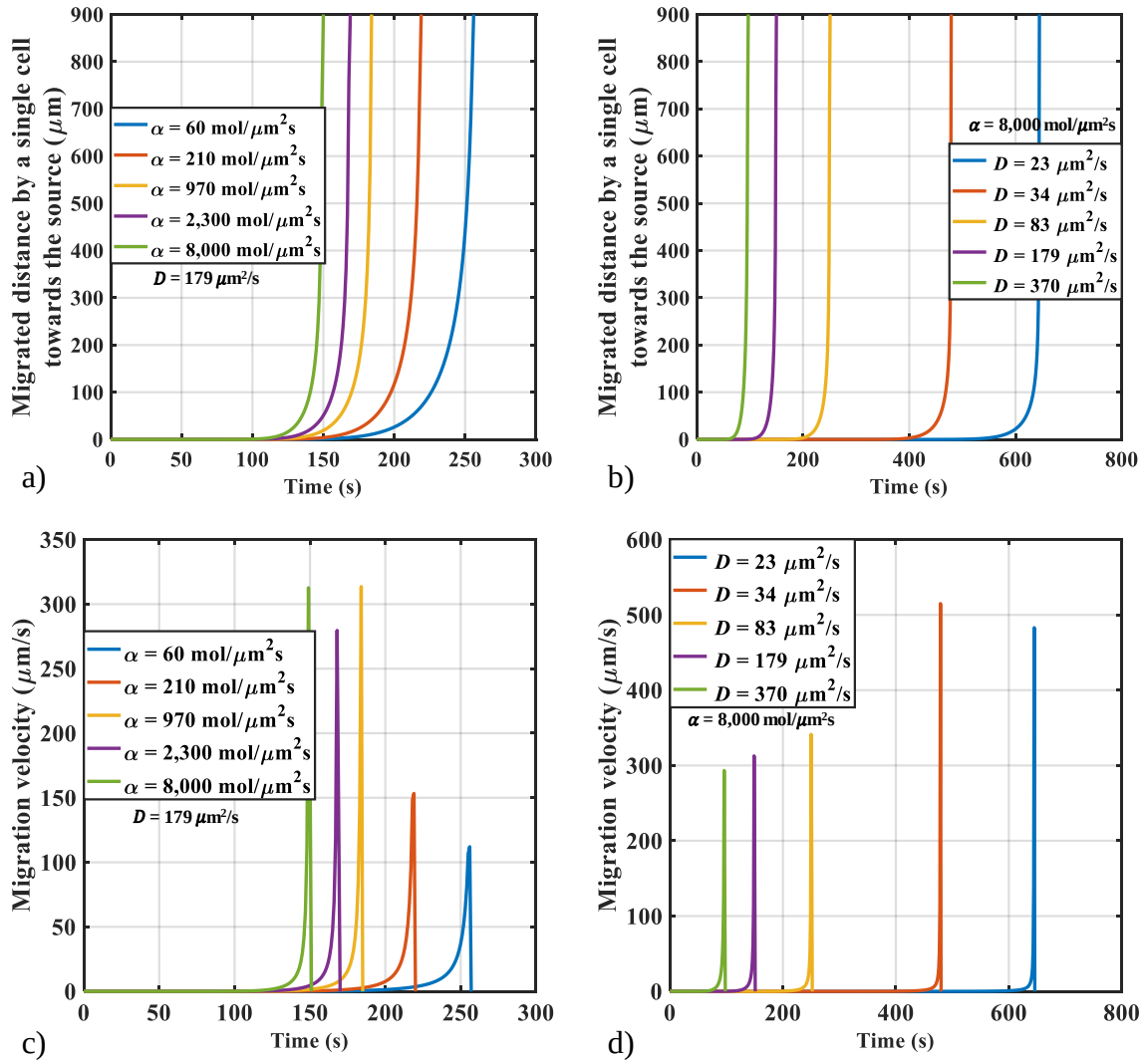


Figure 3.2. Chemically induced migration of a single cell. Distance between cell centre and growth factor source versus time of a cell centre reaching the growth factor source in fully deterministic motion (i.e., absence of stochastic motion) on a planar substrate for different values of the growth factor production rate (a) and diffusion rate (b). The time required for the cell to migrate to the growth factor source decreases with increasing the growth factor production rate α (a, for $D = 179 \mu\text{m}^2/\text{s}$) and growth factor diffusion rate D (b, for $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$). Velocity versus time of a cell centre migrating towards the growth factor source in fully deterministic motion (i.e., absence of stochastic motion) on a planar substrate for different values of (c) the growth factor production rate α with constant growth factor diffusion rate $D = 179 \mu\text{m}^2/\text{s}$ and (d) the diffusion rate D with constant $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$. The maximum velocity of the cell increases with increasing the growth factor production rate α (c) and decreasing the growth factor diffusion rate D (d). (Model parameter values and sources are listed in Table 3.1 except for α and D values provided above in Figure labels are estimated from Savinell *et al.* (1989) and Nauman *et al.* (2007), respectively).

3.3.1.2 Chemically induced collective cell migration for varying growth factor productivity

Twenty-five cells randomly located in the region $-500 < X < 0$ of the substrate with the dimensions of $1,000 \mu\text{m} \times 1,000 \mu\text{m}$ and a growth factor source with a constant diffusion rate of $D = 179 \mu\text{m}^2/\text{s}$ are used to study the effect of the growth factor production rate on cell migration. Fully deterministic motion (i.e., without random movement) is considered. Three different growth factor production rates are considered: $\alpha = 60, 970$, and $8,000 \text{ mol}/\mu\text{m}^2\text{s}$ (Savinell *et al.* 1989).

For the growth factor source with $\alpha = 60 \text{ mol}/\mu\text{m}^2\text{s}$ (Figure 3.3a), the cells closest to the growth factor source start migrating at $t = 70 \text{ s}$ after the growth factor release is started. The two cells initially closest to the growth factor source reach the growth factor source first ($t = 133 \text{ s}$). The cells with initial positions furthest from the growth factor source do not detect the chemical concentration gradient ($t = 200 \text{ s}$). Once the cells have reached the growth factor source, they stay close to the source and keep moving around it along the Y-axis. All cells reach the growth factor source at a later stage ($t = 294 \text{ s}$). The cells in contact deform (shown as overlap) as inter-cellular forces are not considered during chemical signal in the model (see also supplemental video V3.3A).

The growth factor source with $\alpha = 970$ and $8,000 \text{ mol}/\mu\text{m}^2\text{s}$ results in similar cell behaviour as the growth factor source with $\alpha = 60 \text{ mol}/\mu\text{m}^2\text{s}$. In the final stage, all cells reach the growth factor source at $t = 200 \text{ s}$ for $\alpha = 970 \text{ mol}/\mu\text{m}^2\text{s}$ (Figure 3.3b) and at $t = 176 \text{ s}$ for $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$ (Figure 3.3c), indicating an increase in migration velocity with an increase in the production rate α . The cells start migrating earlier toward the growth factor source and reach faster migration velocities for a high production rate α compared to a lower production rate (see also supplemental videos V3.3B and V3.3C).

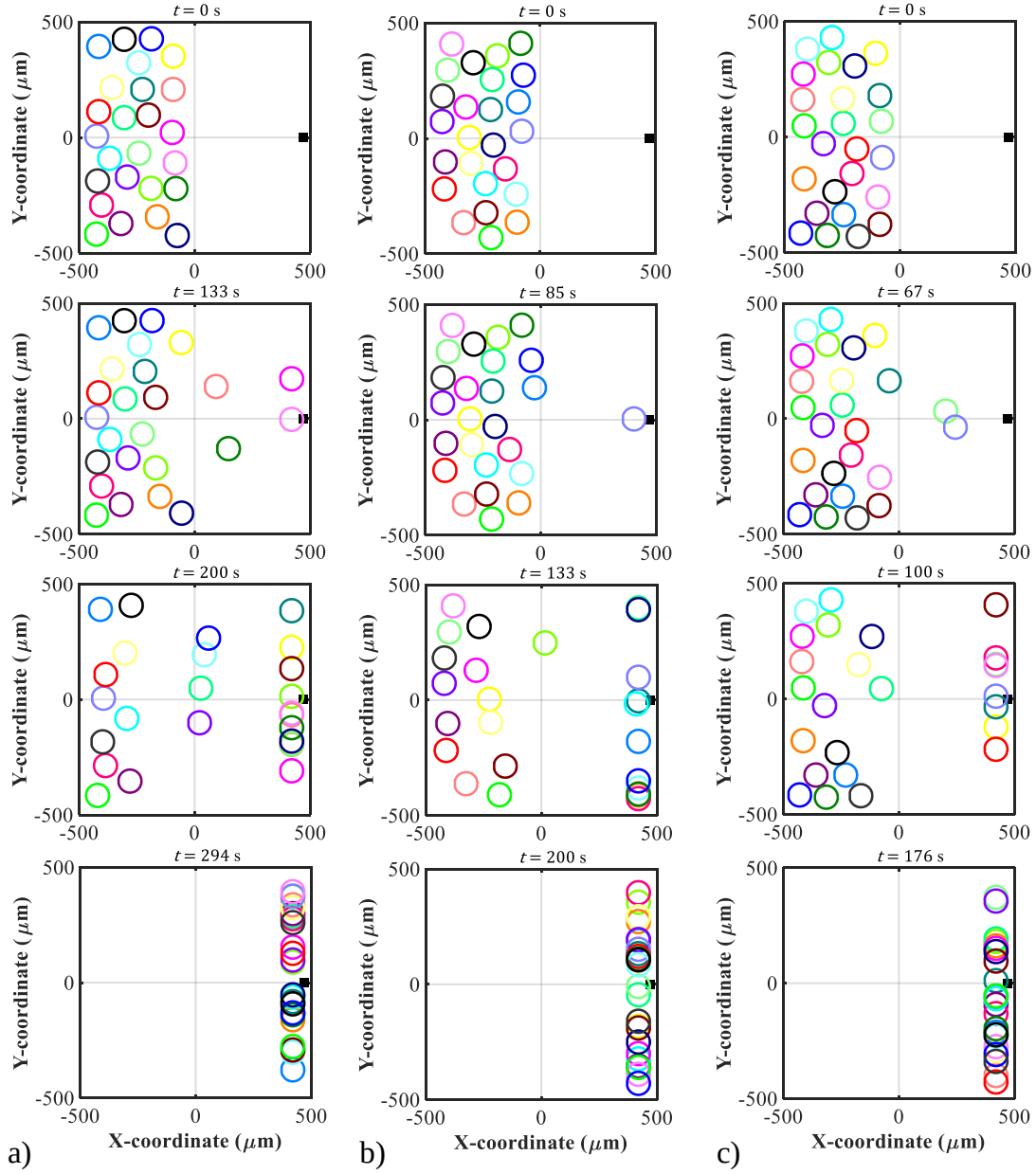


Figure 3.3. Chemically induced collective cell migration for varying growth factor productivity. Positions of 25 cells (open circles) migrating with fully deterministic motion on a 2D planar substrate towards a growth factor source (solid square) with a constant diffusion rate $D = 179 \mu\text{m}^2/\text{s}$ and different production rates of $\alpha = 60 \text{ mol}/\mu\text{m}^2\text{s}$ (a), $\alpha = 970 \text{ mol}/\mu\text{m}^2\text{s}$ (b), and $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$ (c). The growth factor source is located on the substrate region with $X > 0$ (right), and the initial arrangement of the cells on the substrate region with $X < 0$ (left) is the same for the three cases (at $t = 0$ s). a) For $\alpha = 60 \text{ mol}/\mu\text{m}^2\text{s}$, the closest cells to the growth factor source start migrating towards the source, and two cells arrive at the source first at $t = 133$ s. At $t = 200$ s, the cells furthest from the growth factor source do not migrate towards the growth factor source as they do not detect the chemical concentration gradient (also see supplemental video V3.3A). The cells overlap in the absence of repulsive cellular interactions. After the cells arrive at the growth factor source, they move around it randomly along the Y-axis. At $t = 294$ s, all cells arrive at the growth factor source. b) For $\alpha = 970 \text{ mol}/\mu\text{m}^2\text{s}$, one cell arrives at the growth factor source at $t = 58$ s. At $t = 133$ s, the closest cells arrive at the growth factor source and continue to move around it randomly along the Y-axis. The cells furthest from the growth factor source do not

migrate towards the source. The cells overlap in the absence of repulsive cellular interactions (see also supplemental video V3.3B). At $t = 200$ s, all cells arrive at the growth factor source. c) For $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$, the closest two cells arrive at the growth factor source at $t = 67$ s. At $t = 100$ s, the closest cells arrive at the source and move around it randomly along the Y-axis. The cells furthest from the growth factor source do not migrate towards the source (see also supplemental video V3.3C). The cells overlap in the absence of repulsive cellular interactions. At $t = 176$ s, all cells arrive at the growth factor source. (Model parameter values and sources are listed in Table 3.1 except for values provided above in this caption are estimated from Savinell *et al.* (1989)).

3.3.1.3 Chemically induced collective cell migration for varying growth factor diffusivity

Collective cell migration due to a growth factor source on a planar substrate with three different growth factor diffusion rates of $D = 23, 83,$ and 179 $\mu\text{m}^2/\text{s}$ (Nauman *et al.* 2007) and a constant growth factor production rate of $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$ is investigated using 25 cells arranged randomly in the region $X < 0$ of the substrate with the dimension of $1,000$ $\mu\text{m} \times 1,000$ μm . The random movement of the cells is not considered.

For $D = 23$ $\mu\text{m}^2/\text{s}$, cells start moving at $t = 240$ s after the growth factor release is started (Figure 3.4a). Only two cells move towards the growth factor source until the closest single cell reaches the growth factor source first ($t = 315$ s). After that, the closest cells to the growth factor source reach the source and continue to move along the Y-axis ($t = 485$ s). The cells contact each other and deform (represented by overlap) without repulsive inter-cellular forces (see also supplemental video V3.4A). All cells reach the growth factor source at $t = 836$ s.

For $D = 83$ $\mu\text{m}^2/\text{s}$ (Figure 3.4b), only two cells initially move towards the growth factor source, and one cell reaches the source first at $t = 140$ s. The closest cells to the growth factor source reach the source and keep moving around it along the Y-axis ($t = 200$ s). The cells around the growth factor contact each other and deform (represented by overlap) in the absence of repulsive inter-cellular forces. Finally, all cells migrate and reach the growth factor source after $t = 325$ s (see also supplemental video V3.4B).

For $D = 179$ $\mu\text{m}^2/\text{s}$ (Figure 3.4c), the cells closest to the growth factor source migrate first towards the source, and two cells reach the source first ($t = 68$ s). The cells furthest from the growth factor source do not move as they do not detect the chemical concentration gradient ($t = 115$ s). Once the cells reach the growth factor source, they keep moving around it randomly along the Y-axis (see also supplemental video V3.3C). All cells reach the growth factor source after $t \approx 176$ s,

whereas this takes $t \approx 325$ s for $D = 83 \mu\text{m}^2/\text{s}$ and $t \approx 836$ s for $D = 23 \mu\text{m}^2/\text{s}$, indicating an increase in migration velocity with an increase of growth factor diffusion rate.

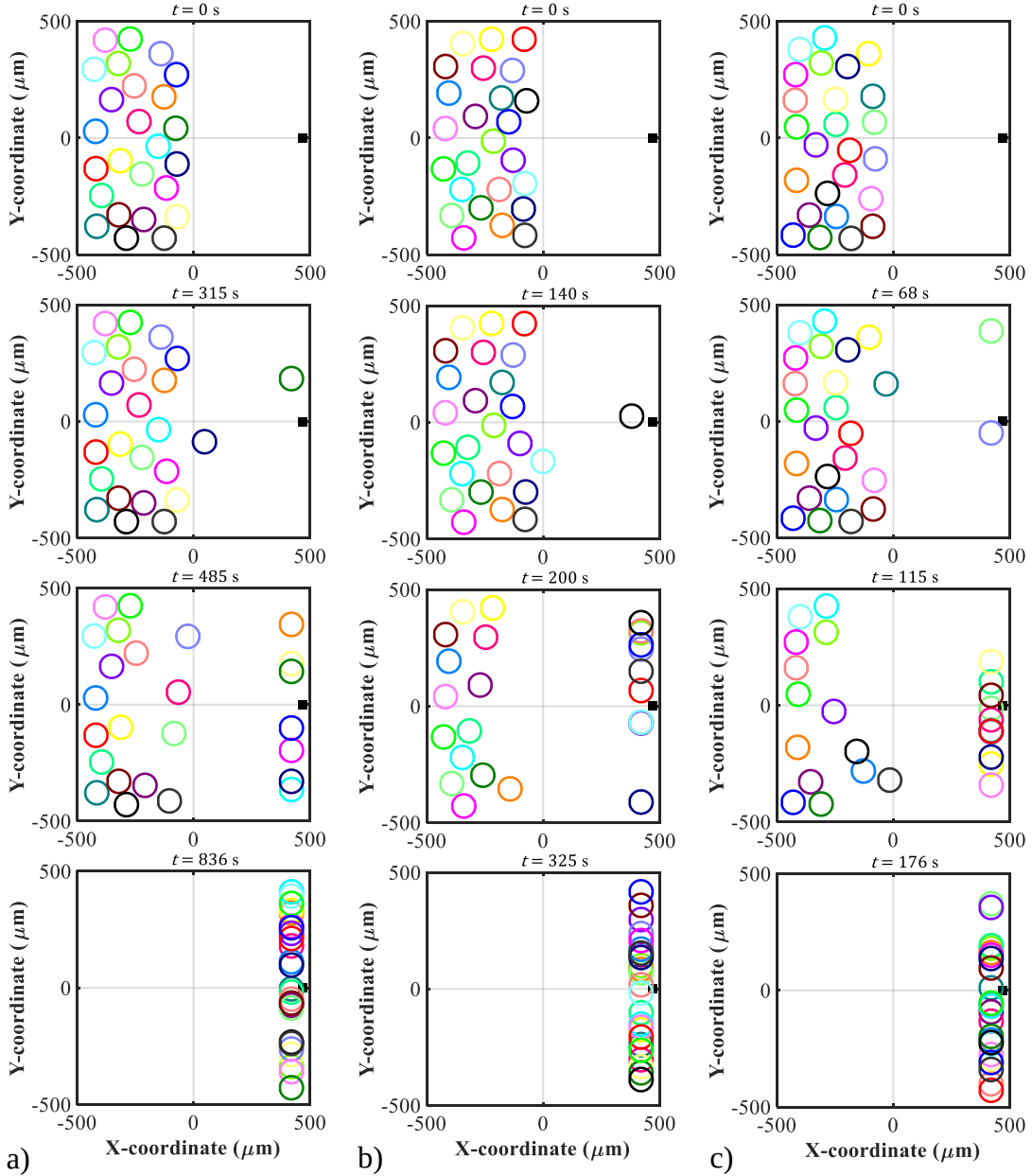


Figure 3.4. Chemically induced collective cell migration for varying growth factor diffusivity. Positions of 25 cells (open circles) migrating with fully deterministic motion on a 2D planar substrate towards a growth factor source (solid square) with a constant production rate $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$ and different diffusion rates of $D = 23 \mu\text{m}^2/\text{s}$ (a), $D = 83 \mu\text{m}^2/\text{s}$ (b), and $D = 179 \mu\text{m}^2/\text{s}$ (c). The growth factor source is located on the substrate region with $X > 0$ (right), and the initial arrangement of the cells on the substrate region with $X < 0$ (left) is the same for the three cases (at $t = 0$ s). a) For $D = 23 \mu\text{m}^2/\text{s}$, the closest two cells to the growth factor source start migrating towards the source, and one cell arrives at the growth factor source first at $t = 315$ s. At $t = 485$ s, the cells furthest from the growth factor source do not migrate towards the source as they do not detect the chemical concentration gradient (see also supplemental video V3.4A). After the cells arrive at the growth factor source, they move around it randomly along the Y-axis. The

cells overlap in the absence of repulsive cellular interactions. At $t = 836$ s, all cells arrive at the growth factor source. b) For $D = 83 \mu\text{m}^2/\text{s}$, only two cells migrate towards the growth factor source, and one cell arrives at the source at $t = 140$ s. At $t = 200$ s, the closest cells arrive at the growth factor source and continue moving around it randomly along the Y-axis (see also supplemental video V3.4B). The cells furthest from the growth factor source do not migrate towards the source. The cells overlap in the absence of repulsive cellular interactions. At $t = 325$ s, all cells arrive at the growth factor source. c) For $D = 179 \mu\text{m}^2/\text{s}$, the closest cells start migrating towards the growth factor source, and two cells arrive at the source at $t = 68$ s. At $t = 115$ s, the cells furthest from the growth factor source do not migrate to the source (see also supplemental video V3.3C). The cells overlap in the absence of repulsive cellular interactions. After $t = 176$ s, all cells arrive at the source and move around it randomly along the Y-axis. (Model parameter values and sources are listed in Table 3.1 except for values provided above in this caption are estimated from Nauman *et al.* (2007)).

3.3.2 Chemo-mechanically induced collective cell migration

3.3.2.1 Chemo-mechanically induced collective cell migration for varying growth factor productivity

Four cells migrating due to mechanical cues and a chemical signal are considered on a substrate with three different growth factor production rates of $\alpha = 60, 970$, and $8,000 \text{ mol}/\mu\text{m}^2\text{s}$ (Savinell *et al.* 1989) and a constant diffusion rate of $D = 179 \mu\text{m}^2/\text{s}$. Initially, the cells are located in a rhombus shape with a side length of $327 \mu\text{m}$, minor axis length of $260 \mu\text{m}$, and major axis length of $600 \mu\text{m}$ in the substrate region $X < 0$ (Figure 3.5, $t = 0$ s).

For $\alpha = 60 \text{ mol}/\mu\text{m}^2\text{s}$ (Figure 3.5a), the cells primarily move towards each other in the dominant direction of the resultant strain energy density gradient in the substrate until two cells are in contact at $Y = 0$ ($t = 145$ s). After that, the cell nearest to the growth factor source starts moving towards the source, and the other three cells continue moving towards each other and the growth factor source ($t = 169$ s). After the first cell reaches the growth factor source, the three other cells aggregate and move collectively towards the source until the chemical signal is more dominant than the mechanical cues ($t = 195$ s). Finally, the cells reach the growth factor source and impinge on each other since repulsive forces are not considered ($t \approx 202$ s) (see also supplemental video V3.5A).

For $\alpha = 970 \text{ mol}/\mu\text{m}^2\text{s}$, the cells move towards each other initially (Figure 3.5b, $t = 81$ s). The cells continue moving towards the growth factor source (see also supplemental video V3.5B); hence, the chemical signal is more dominant than the cellular mechanical interaction ($t = 110$ s).

The cells closest to the growth factor source move faster and reach the source first ($t = 150$ s). Finally, all four cells reach and cluster around the growth factor source ($t = 156$ s).

For $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$ (Figure 3.5c), the cells move slightly towards each other (see also supplemental video V3.5C) until the chemical signal is more dominant than the mechanical cues ($t = 85$ s). The front cell reaches the growth factor source first ($t = 115$ s) and the cells closer to the growth factor source move faster towards the source than the more distant cell ($t = 120$ s). The cells move randomly around the source along the Y-axis. The distant cell reaches the source last, and the cells cluster around the source ($t \approx 128$ s).

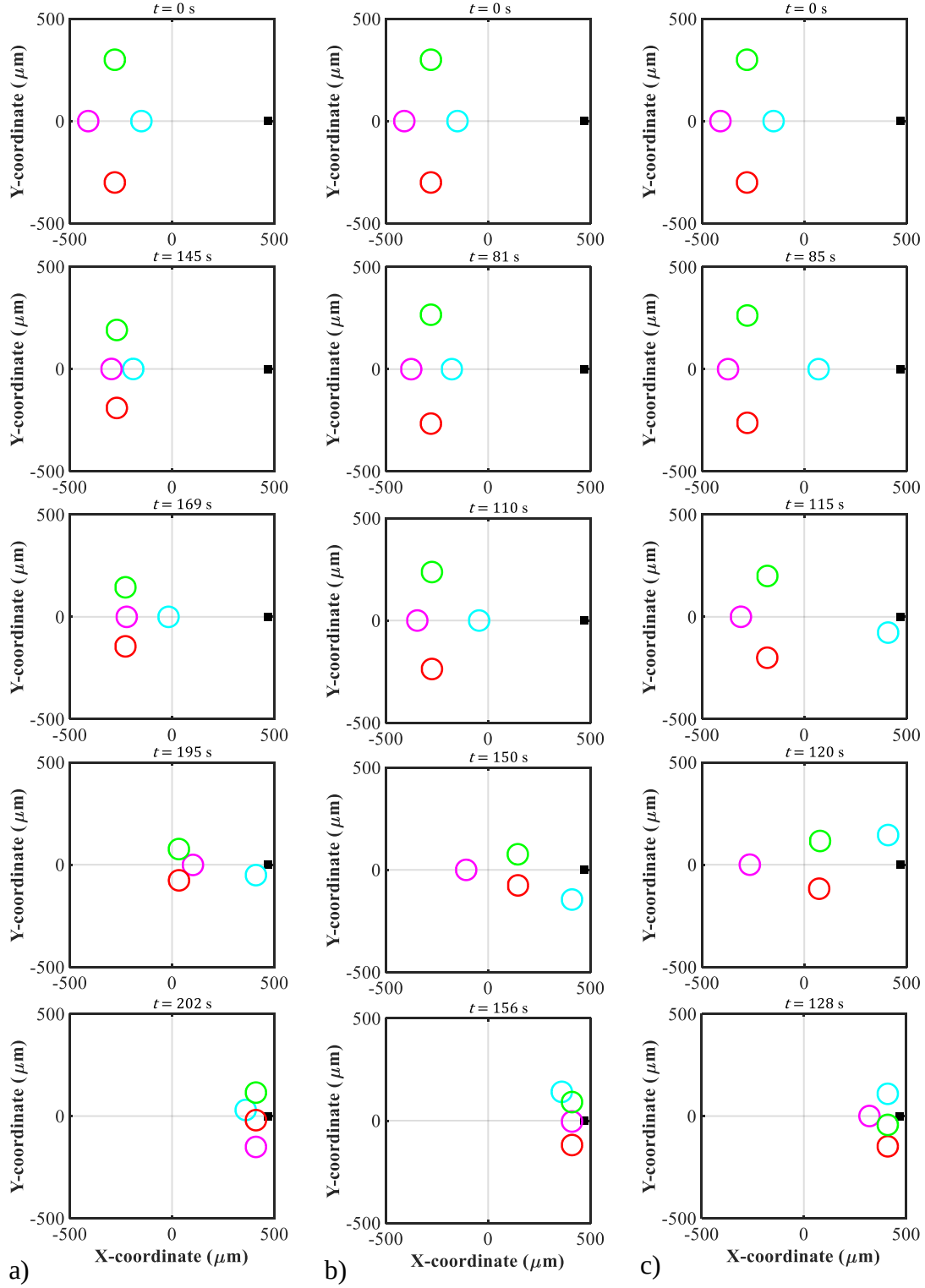


Figure 3.5. Chemo-mechanically induced collective cell migration for varying growth factor productivity. Positions of four cells (open circles) on a substrate during migration with no random motion towards a growth factor source (solid square) with a constant diffusion rate $D = 179 \mu\text{m}^2/\text{s}$ and different production rates of (a) $\alpha = 60 \text{ mol}/\mu\text{m}^2\text{s}$, (b) $\alpha = 970 \text{ mol}/\mu\text{m}^2\text{s}$, (c) $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$. The growth factor source is located on the substrate region with $X > 0$ (right). a) Initial positions of the cells at $t = 0 \text{ s}$ on the substrate region with $X < 0$ (left) in rhombus shape with a side length of $327 \mu\text{m}$, minor axis of $260 \mu\text{m}$, and major axis length of $600 \mu\text{m}$. For $\alpha = 60$

mol/ $\mu\text{m}^2\text{s}$, the cells move towards each other in the direction of the resultant strain energy density gradient at $t = 145$ s. Neighbouring cells contact each other and move towards the growth factor source (see also supplemental video V3.5A). At $t = 169$ s, the front cell moves faster towards the growth factor source; hence the chemical signal is more dominant than the mechanical cues. The front cell arrives at the source, and other cells contact each other and cluster in the direction of the growth factor source at $t = 195$ s. At $t = 202$ s, the cells reach the growth factor source and move around it along the Y-axis. The cells overlap in the absence of repulsive cellular interactions. b) Initial positions of the cells at $t = 0$ s for $\alpha = 970$ mol/ $\mu\text{m}^2\text{s}$. At $t = 81$ s, neighbouring cells move towards each other following the resultant strain energy density gradient. At $t = 110$ s, the front cell moves towards the growth factor source, and other cells continue moving towards each other in the direction of the source. The front cell arrives at the growth factor source, and the closest cells to the source move faster towards the source at $t = 150$ s (see also supplemental video V3.5B). At $t = 156$ s, the cells arrive at the growth factor source. The cells overlap in the absence of repulsive cellular interactions. c) Initial positions of the cells for $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$. At $t = 85$ s, the cells move in the direction of the chemical concentration gradient (see also supplemental video V3.5C). At $t = 115$ s, the front cell arrives at the growth factor source first. The closest cells to the source move faster towards the source at $t = 120$ s. At $t \approx 128$ s, the cells arrive at the growth factor source. (Model parameter values and sources are listed in Table 3.1 except for values provided above in this caption are estimated from Savinell *et al.* (1989)).

3.3.2.2 Chemo-mechanically induced collective cell migration for varying growth factor diffusivity

Four cells are considered on a substrate with three different growth factor diffusion rates, i.e., $D = 23, 83$, and $179 \mu\text{m}^2/\text{s}$ (Nauman *et al.* 2007), and a constant production rate of $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$. The cells are initially located in a rhombus shape with an equal side length of $327 \mu\text{m}$, a minor axis length of $260 \mu\text{m}$, and a major axis length of $600 \mu\text{m}$ (Figure 3.6a).

The first case considers the diffusion rate of the growth factor of $D = 23 \mu\text{m}^2/\text{s}$ (Figure 3.6a). The four cells primarily move towards each other. Once the cells reach each other (at $Y \approx 0$) after $t = 200$ s, they move collectively in the X-direction towards the growth factor source. Once the concentration gradient of the growth factor starts dominating the mechanical cues, the cell in front detects the concentration gradient first and moves faster than the other three cells towards the growth factor source ($t = 435$ s). The leading cell keeps moving around the source randomly in the Y-direction (see also supplemental video V3.6A). After that, two of the remaining three cells closest to the source move collectively towards the source at $t = 451$ s. All four cells reach the growth factor source at $t \approx 465$ s.

For the diffusion rate of the growth of $D = 83 \mu\text{m}^2/\text{s}$, the cells initially move towards each other (Figure 3.6b). The two neighbouring cells at $Y = 0$ reach each other first ($t = 125$ s) and start moving towards the growth factor source (at $Y = 0$) (see also supplemental video V3.6B). Once

the leading cell reaches the growth factor source ($t = 182$ s), the other three cells continue to move towards each other (at $Y \approx 0$) due to mechanical cues. The cell closest to the growth factor source moves faster than the other two cells and reaches the growth factor source site at $t = 189$ s. Once the cells reach the growth factor source, they continue to move around it randomly along the Y-direction (see also supplemental video V3.6B). Finally, all cells reach the source site and interact with each other (represented by overlap) since the repulsive force is not considered ($t = 191$ s).

The distant cell reaches the growth factor source last for $D = 23 \mu\text{m}^2/\text{s}$, whereas the two cells reach the source last for $D = 83 \mu\text{m}^2/\text{s}$, indicating the increase of mechanical effect on migration velocity with an increase in chemical diffusion rate.

For the diffusion rate of $D = 179 \mu\text{m}^2/\text{s}$, the cells initially move towards each other in the direction of the resultant strain energy density gradient, i.e., towards and along $Y = 0$, respectively (Figure 3.6c, $t = 65$ s). The cells are influenced by the release of the growth factor source before they form a collective, and the concentration gradient of the growth factor starts dominating the mechanical signals ($t = 87$ s). The cell in front reaches the growth factor source first, followed by two of the three cells closest to the source ($t = 120$ s). The cell furthest away reaches the growth factor source site last ($t = 128$ s) (see also supplemental video V3.5C).

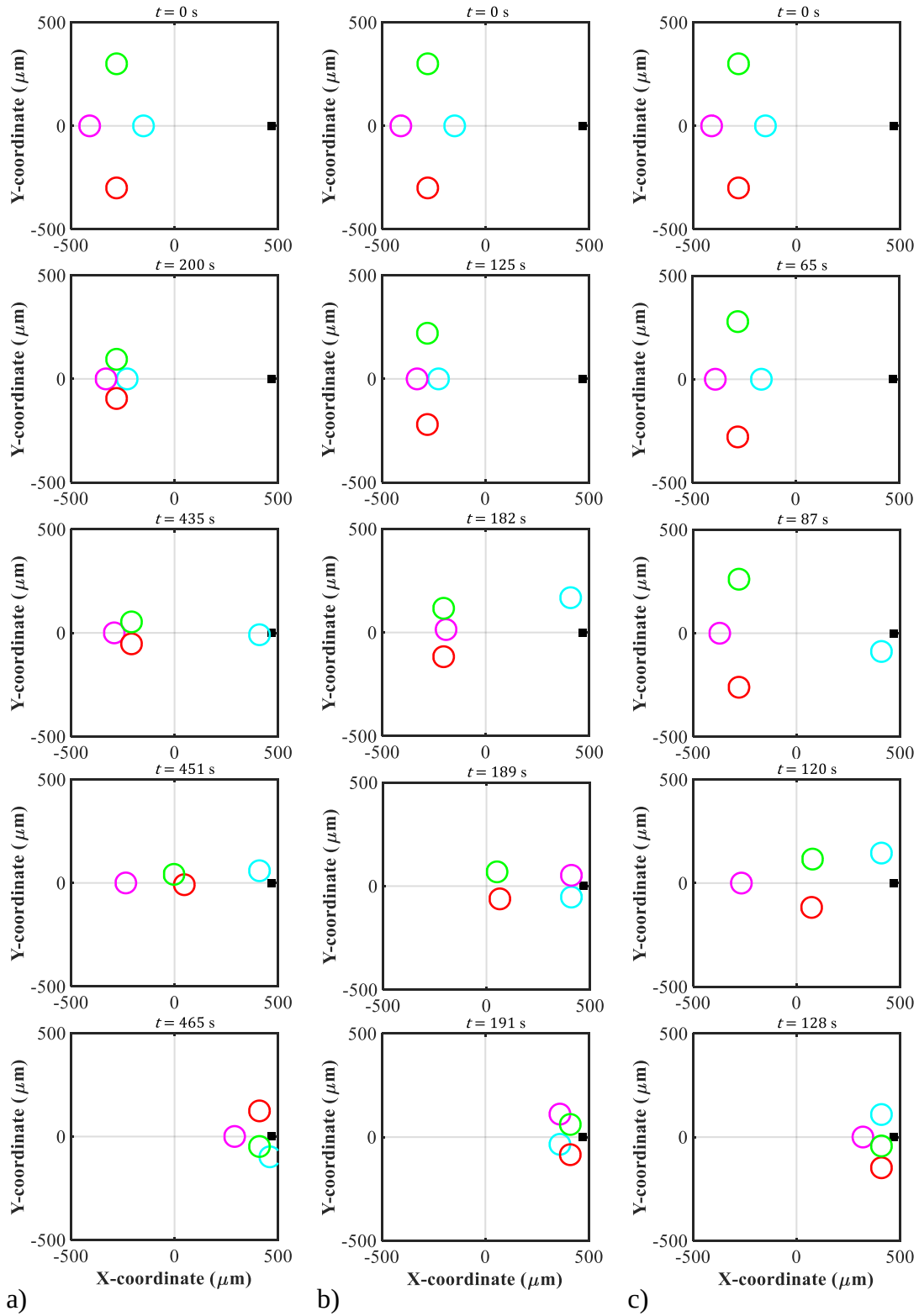


Figure 3.6. Chemo-mechanically induced collective cell migration for varying growth factor diffusivity. Positions of four cells migrating with fully deterministic motion on a substrate with three different growth factor diffusion rates of $D = 23 \mu\text{m}^2/\text{s}$ (a), $D = 83 \mu\text{m}^2/\text{s}$ (b), and $D = 179 \mu\text{m}^2/\text{s}$ (c) and a constant production rate $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$. The growth factor source is located in the region $X > 0$ (right) as a fixed point, i.e., does not move with time. a) Initial positions of cells at $t = 0 \text{ s}$. Cells are located in the region $X < 0$ (left) in a rhombus shape with an equal side

length of 327 μm , minor axis length of 260 μm , and major axis length of 600 μm . Cells migrate towards each other (i.e., towards $Y = 0$) due to the mechanical cues. Cells cluster after $t = 200$ s and keep moving around each other. Cells move into the growth factor source as a collective at first, then the front cell (i.e., closest cell to the source) moves faster towards the growth factor source and arrives at the source at $t = 435$ s (also see supplemental video V3.6A). Two cluster cells (i.e., closest to the source) move towards the growth factor source, and cells deform (represented as overlap) at $t = 451$ s. Cells keep moving around the growth factor source randomly along the Y-axis. The furthest cell arrives at the source last at $t = 465$ s. b) Initial configuration of the cells at $t = 0$ s. Cells migrate towards each other (i.e., towards $Y = 0$). At $t = 125$ s, neighbouring two cells join at $Y = 0$. At $t = 182$ s, cells keep moving towards each other in the direction of the growth factor source. The first cell closer to the growth factor source arrives at the source first, followed by the second closer cell to the source at $t = 189$ s. Once the cells arrive at the growth factor source, they keep moving around it randomly along the Y-direction (also see supplemental video V3.6B). Finally, the two cells arrive at the source last at $t = 191$ s. c) Positions of cells at $t = 0$ s. At $t = 65$ s, the cells move towards each other in the direction of resultant strain energy density at first. Once the cells detect the chemical concentration gradient, they move towards the source at $t = 87$ s (see also supplemental video V3.5C). The first cell closer to the growth factor source arrives at the source first, followed by the second closer two cells to the source at $t = 120$ s. At $t = 128$ s, the furthest cell arrives at the source last (Model parameter values and sources are listed in Table 3.1 except for values provided above in this caption are estimated from Nauman *et al.* (2007)).

3.3.3 Chemo-mechanically induced single-cell migration

3.3.3.1 Chemo-mechanically induced single-cell migration for varying growth factor productivity

Two cells 539 μm apart on the negative region ($X < 0$) of the substrate are considered with three different growth factor production rates of $\alpha = 60, 970$, and 8,000 $\text{mol}/\mu\text{m}^2\text{s}$ (Savinell *et al.* 1989) and a constant diffusion rate $D = 179 \mu\text{m}^2/\text{s}$. One cell is immobile, and the other cell is allowed to move. Both cells exert traction forces, and the stationary cell attracts the motile cell due to mechanical cues (Figure 3.7).

For the growth factor production rate of $\alpha = 60 \text{ mol}/\mu\text{m}^2\text{s}$ (Figure 3.7a), migration of the motile cell (red) is firstly directed towards the stationary cell (blue) due to the initially dominant mechanical cues ($t = 160$ s). After $t = 190$ s, the motile cell moves in the direction of the growth factor source and away from the stationary cell as the chemical concentration gradient exceeds the mechanical migratory cues (see also supplemental video V3.7A). The motile cell reaches the growth factor point source at $t = 201$ s.

For the growth factor production rates of $\alpha = 970 \text{ mol}/\mu\text{m}^2\text{s}$ (Figure 3.7b) and $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$ (Figure 3.7c), similar cell behaviour is shown as in the previous case. The single motile

cell reaches the growth factor source at $t = 147$ s for $\alpha = 970$ mol/ $\mu\text{m}^2\text{s}$ and at $t = 120$ s for $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$, indicating an increase in migration velocity with an increase in α . The cell moves at a faster velocity toward the growth factor source when α is high since the chemical concentration becomes stronger (see also supplemental videos V3.7B and V3.7C).

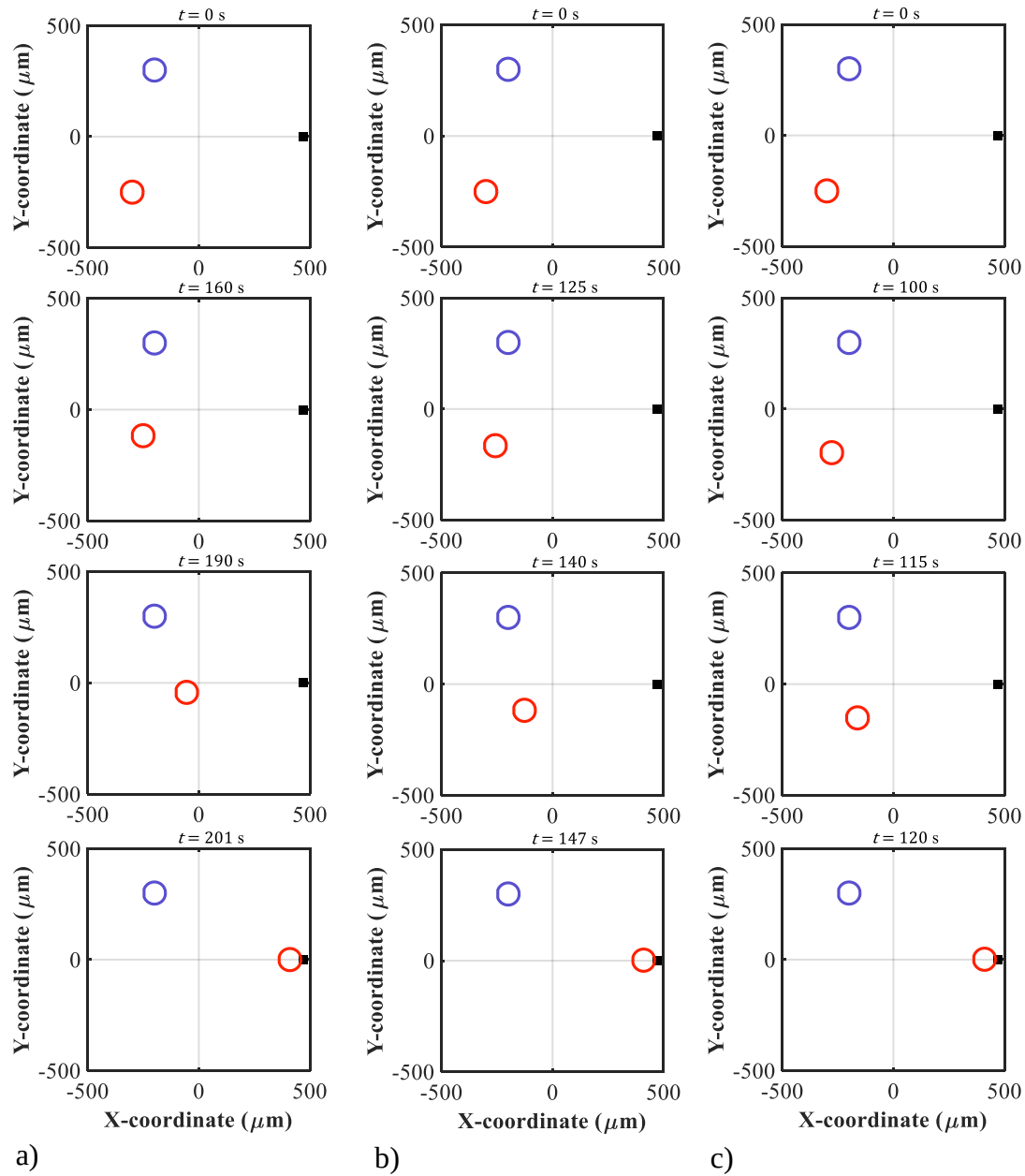


Figure 3.7. Chemo-mechanically induced single-cell migration for varying growth factor productivity. Positions of a stationary cell (blue circle) and a motile cell (red circle) migrating with fully deterministic motion on a substrate towards a growth factor source (solid square) with a constant diffusion rate $D = 179$ $\mu\text{m}^2/\text{s}$ and different production rates of (a) $\alpha = 60$ mol/ $\mu\text{m}^2\text{s}$, (b) $\alpha = 970$ mol/ $\mu\text{m}^2\text{s}$, and (c) $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$. The source is located at $(x, y) = (470, 0)$ μm and cells are initially arranged on the negative side of the X-axis (left). a) Initial position of a mobile cell at $t = 0$ s for $\alpha = 60$ mol/ $\mu\text{m}^2\text{s}$. Firstly, the motile cell moves towards the stationary cell due

to mechanical cues at $t = 160$ s. After $t = 190$ s, the cell moves towards the growth factor source. At $t = 201$ s, the cell arrives at the source and stays still around it (see also supplemental video V3.7A). b) Initial position of a motile cell at $t = 0$ s for $\alpha = 970$ mol/ $\mu\text{m}^2\text{s}$. At $t = 125$ s, the motile cell moves towards the stationary cell (see also supplemental video V3.7B). After $t = 140$ s, the motile cell moves towards the source and arrives at the growth factor source at $t = 147$ s. c) Initial position of a motile cell at $t = 0$ s for $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$. The cell migrates towards the stationary cell at $t = 100$ s. At $t = 115$ s, the cell moves towards the growth factor source (see also supplemental video V3.7C). At $t = 120$ s, the cell arrives at the source. (Model parameter values and sources are listed in Table 3.1 except for values provided above in this caption are estimated from Savinell *et al.* (1989)).

3.3.3.2 Chemo-mechanically induced single-cell migration for varying growth factor diffusivity

One motile and one stationary cell on the negative region ($X < 0$) of the substrate with a distance of $539 \mu\text{m}$ between the cells are considered with three growth factor diffusion rates of $D = 23, 83$, and $179 \mu\text{m}^2/\text{s}$ (Nauman *et al.* 2007) and a constant production rate $\alpha = 8,000$ mol/ $\mu\text{m}^2\text{s}$.

For the diffusion rate $D = 23 \mu\text{m}^2/\text{s}$ (Figure 3.8a), the migration of the motile cell is initially towards the stationary cell due to the mechanical cues. The motile cell reaches the stationary cell at $t = 297$ s and keeps moving around the stationary cell ($t = 424$ s) (see also supplemental video V3.8A). Thereafter, the cell's migration changes towards the growth factor source as the chemical signal dominates the mechanical cues. After $t = 436$ s, the motile cell reaches the growth factor source and remains stationary at the source.

For $D = 83 \mu\text{m}^2/\text{s}$ (Figure 3.8b), initial migration of the motile cell towards the stationary cell and the growth factor source due to mechanical and chemical cues is observed around $t = 175$ s. After $t = 183$ s, the motile cell starts moving towards the growth factor source (see also supplemental video V3.8B). The motile cell reaches the growth factor source at $t = 187$ s and remains still at the source.

The motile cell does not reach the stationary cell, indicating an increasing effect of the chemical cue on cell migration with an increase in the growth factor diffusion rate to $D = 83 \mu\text{m}^2/\text{s}$.

For $D = 179 \mu\text{m}^2/\text{s}$ (Figure 3.8c), the motile cell exhibits only a very small movement towards the stationary cell ($t = 100$ s), indicating that the chemical concentration gradient is more dominant than the mechanical cues. Therefore, the cell moves towards the growth factor source ($t = 115$ s) and reaches the source at $t = 120$ s (see also supplemental video V3.7C).

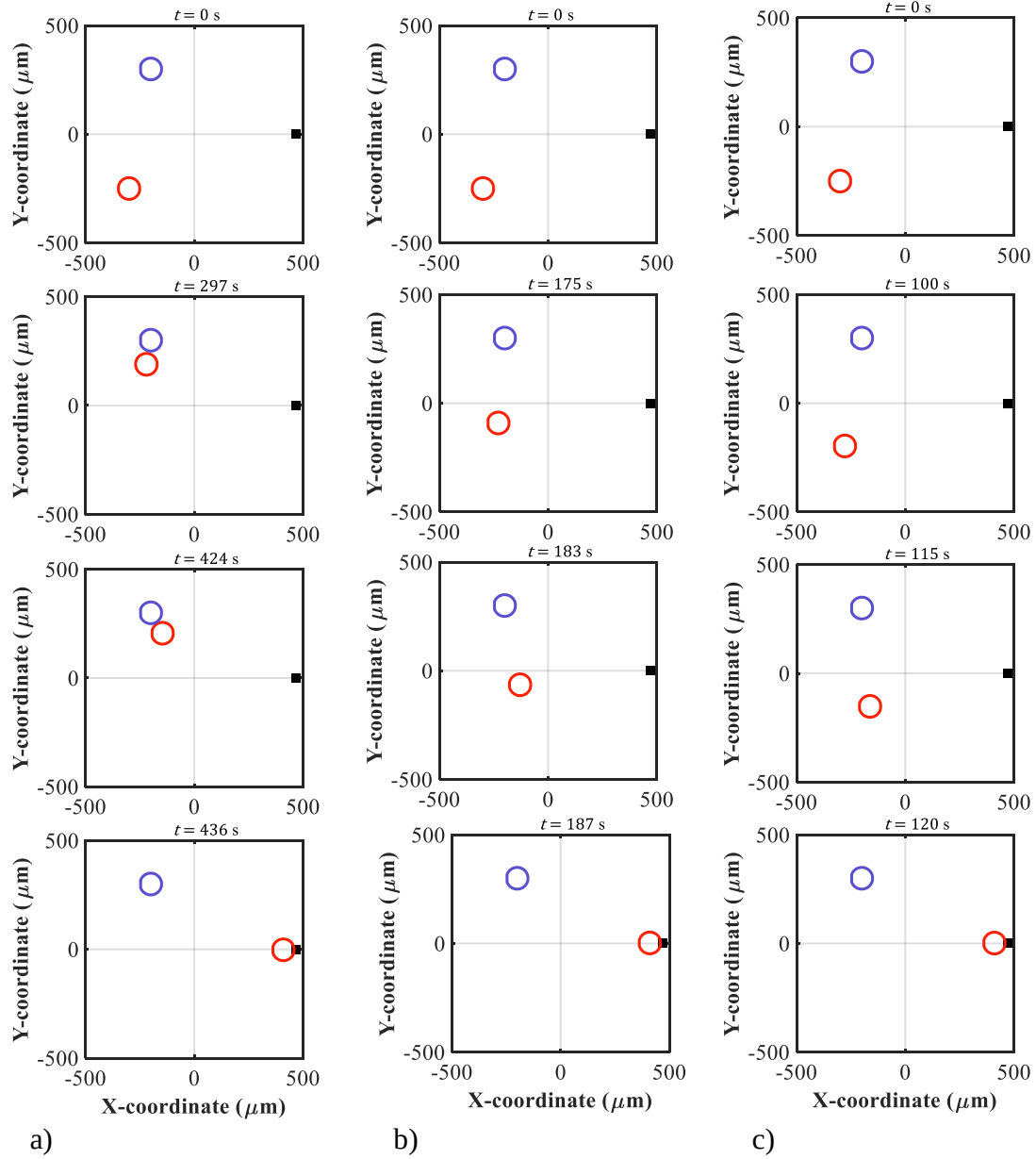


Figure 3.8. Chemo-mechanically induced single-cell migration for varying growth factor diffusivity. Positions of a stationary cell (blue circle) and a motile cell (red circle) during migration with fully deterministic motion on a substrate towards a growth factor source (solid square) with a constant production rate $\alpha = 8,000 \text{ mol}/\mu\text{m}^2\text{s}$ and different diffusion rates of (a) $D = 23 \mu\text{m}^2/\text{s}$, (b) $D = 83 \mu\text{m}^2/\text{s}$, and (c) $D = 179 \mu\text{m}^2/\text{s}$. The source is located at $(x, y) = (470, 0) \mu\text{m}$, and the initial positions of the cells at $t = 0 \text{ s}$ on the substrate region with $X < 0$ (left) are the same for three cases. a) For $D = 23 \mu\text{m}^2/\text{s}$, the motile cell migrates towards the stationary cell due to mechanical cues at $t = 297 \text{ s}$. After $t = 424 \text{ s}$, the motile cell changes migration towards the growth factor source (see also supplemental video V3.8A). At $t = 436 \text{ s}$, the motile cell arrives at the growth factor source. b) For $D = 83 \mu\text{m}^2/\text{s}$, the motile cell migrates towards the stationary cell at $t = 175 \text{ s}$. After $t = 183 \text{ s}$, the motile cell moves towards the growth factor source and arrives at the growth factor source at $t = 187 \text{ s}$ (see also supplemental video V3.8B). c) For $D = 179 \mu\text{m}^2/\text{s}$, the motile cell moves slightly towards the stationary cell at $t = 100 \text{ s}$. After $t = 115 \text{ s}$, the motile cell moves towards the growth factor source (see also supplemental video V3.7C). At $t = 120 \text{ s}$, the cell arrives

at the source. (Model parameter values and sources are listed in Table 3.1 except for values provided above in this caption are estimated from Nauman *et al.* (2007)).

3.4 Discussion

The developed model describes the mechanically induced cellular motion on a two-dimensional (2D) planar substrate with a growth factor gradient. The formalism is different from those proposed previously (Charteris and Khain 2014, Di Costanzo *et al.* 2016, Giniūnaitė *et al.* 2020, Liebchen and Löwen 2018, Tomasova *et al.* 2019) as growth factor productivity and diffusivity are considered to be parameters determining cellular migration since these two parameters are lumped into a single parameter. Therefore, the proposed model allows studying how the evolution of chemical concentration gradients guides cell migration during mechanical cellular interactions.

3.4.1 Mathematical formulation

The growth factor concentration gradient is considered to determine cellular motion in a discrete time; therefore, diffusion of the growth factor in the substrate is modelled by the transient diffusion of Green's function on a continuum. The diffusion of the growth factor is monitored using the fundamental solution of Green's function, which determines the concentrations only at the cells' positions, without the requirement of solving the entire computational field using other discretisation approaches such as the finite element approach (Hodge and Papadopoulos 2012, Vermolen 2009, Vermolen and Javierre 2010, 2012). Using the fundamental solution of Green's function requires elaborate time integration techniques to (i) estimate Green's function and (ii) track the computations of the cells' paths towards the growth factor source. However, this approach improves the numerical efficiency under qualitative precision levels by simplifying the diffusion process for semi-bounded and infinite domains compared to approaches that require solving the entire diffusion field using discretisation techniques.

Boundary conditions, such as zero flux diffusion or recurrent conditions, are essential to represent physical situations for finite bounded domains. The mathematical requirement to formulate boundary conditions is often not desirable when describing biological processes for larger diffusion fields (Prokharau *et al.* 2013). However, the fundamental solution of Green's function enables the evaluation of the growth factor concentration at any point of the substrate and at any time. Similar numerical approaches have been used to model cancer cell migration (Chen J *et al.* 2020, Vermolen *et al.* 2015, Vermolen *et al.* 2014) and wound closure (Vermolen and Gefen 2013a, b).

Several *in vitro* studies on collective cell migration in the presence of chemical sources demonstrated that cell migration is influenced by the concentration of chemical sources and the strength of chemotactic signals (McCann *et al.* 2010, Peret and Murphy 2008). Nevertheless, the role of the productivity and diffusivity of such chemical sources remains poorly understood and requires further investigation. Even though the formalism of the proposed model uses a simple approach, multiple biological processes can be easily incorporated into the developed model.

3.4.2 Migration case studies

For the migration of a single cell towards a growth factor source, the proposed model predicted a decrease in the migration time for an increase in the growth factor productivity and diffusivity (Figure 3.2a and b). This prediction agrees with experimental observations of a decreasing migration time with increasing chemical signal strength for several cell types (Peret and Murphy 2008, Smith *et al.* 2006, Smith *et al.* 2004). The decreasing migration time correlates with an increase in the average migration velocity of a single cell, i.e., from the start of growth factor release to the arrival of the cell at the growth factor source, for increasing growth factor productivity and diffusivity (Figure 3.2c and d). These findings are qualitatively similar to experimental results for increasing chemical signal strength (McCann *et al.* 2010, Oh *et al.* 2011, Smith *et al.* 2006). However, the decreasing maximum migration velocity of a single cell with increasing growth factor diffusivity (Figure 3.2d) contrasts with the decrease in migration duration. This observation may be explained by the inverse relationship between the diffusion coefficient and concentration gradient, i.e., an increase in diffusivity leads to a decrease in the concentration gradient and, hence, a weaker chemotactic cue (Peret and Murphy 2008).

For collective cell migration in the presence of a growth factor but in the absence of mechanotactic cues, the developed model predicts an increase in migration velocity with increasing growth factor productivity and diffusivity. Such migration behaviour has been reported in experimental (Schwarz J *et al.* 2016, Whitehead *et al.* 2016) and numerical studies (Charteris and Khain 2014, Mousavi *et al.* 2014a, Mousavi *et al.* 2013). Cells in a collective only start migrating once they sense the growth factor gradient. The cells closest to the growth factor source start moving towards the growth factor source first, with start time and migration velocity depending on the growth factor productivity (Figure 3.3) and diffusivity (Figure 3.4). The cell migration is affected more by a change in diffusivity than in productivity of the growth factor: The migration duration decreases approximately two-fold for a 2.2-fold increase in growth factor diffusivity (Figure 3.4b and c) compared to a 130-fold increase in growth factor productivity (Figure 3.3a and c). The growth

factor diffusivity determines the propagation velocity of the growth factor molecules in the substrate. For an increase in diffusivity, the growth factor molecules propagate faster; the growth factor gradient reaches or can be sensed by the cells earlier, and cell migration starts earlier.

The model results reveal several differences between cell migration behaviour due to combined chemical and mechanical cues and migration induced by chemical cues only. The chemo-mechanical migration of multiple cells is directed towards the growth factor source but may be distinguished into two phases relative to the growth factor release. Initially, the cells migrate towards each other due to the dominance of the mechanical cues (exerted by the cells) over the chemical cue from the growth factor source (Figure 3.5 and Figure 3.6). In this phase, the migration is predominantly mechanically induced. As more growth factor is released from the source and diffuses in the substrate, the strength of the growth factor gradient increases and the chemical cue may dominate the mechanotactic cue. At this stage, the cells start migrating towards and eventually reach the growth factor source. Similar behaviour is reported in experiments (Andrew and Insall 2007, Bosgraaf and Van Haastert 2009, Hoeller and Kay 2007, Van Haastert 2010).

Comparing the chemo-mechanical migration of one motile cell in the presence of one stationary cell (Figure 3.7 and Figure 3.8) to that of multiple motile cells (Figure 3.5 and Figure 3.6) indicates that the mechanical interactions amongst multiple motile cells delay their arrival at the growth factor source, i.e. result in slower collective cell migration compared to the single cell migration. Even though the predicted dependence of cell migration on mechanotactic cell interactions can be ascribed to the initial cell positions (Reinhart-King *et al.* 2008), these interactions do not affect the final stage of the migration, i.e., the arrival of the cells at the growth factor source. These results agree with experimental (Hale *et al.* 2010, Mittal *et al.* 2003) and numerical studies (Deshpande VS *et al.* 2006, Javierre *et al.* 2009a, Mousavi *et al.* 2013, Tomasova *et al.* 2019).

As observed for the chemically induced cell migration without mechanical cues, chemo-mechanical cell migration is also more affected by the variation of the growth factor diffusivity than the growth factor productivity. The migration duration decreases approximately 1.5-fold for a 2.2-fold increase in growth factor diffusivity (Figure 3.6b and c) compared to a 130-fold increase in growth factor productivity (Figure 3.5a and c).

3.4.3 Limitations

Although the results generated with the proposed model are in qualitative agreement with previously reported experimental data for cell migration in complex environments, the model has some limitations.

The model approach does not consider the maximum migration rate towards the growth factor source. The signal strength that attracts the cells increases over time since the growth factor source is immobilised and non-soluble. In contrast, experimental studies reported that cells have limited velocity to move towards the source (Bosgraaf and Van Haastert 2009, Sixt and Lämmermann 2011, Whitehead *et al.* 2016).

The model uses a simple approach to describe the cell motion once the cells reach the growth factor source. The signal strength that attracts the cell becomes very large since the growth factor source is immobilised and non-soluble. In addition, the distance between the cells and the growth factor source, described in Eq. (3.6), becomes very small.

The model formalism considers the productivity and diffusivity of the growth factor to be constant over time during the migration process. In contrast, *in vitro* studies reported that these parameters depend on the composition of the growth factor (Peret and Murphy 2008, Tomasova *et al.* 2019) and the substrate (Cai *et al.* 2010, Smith *et al.* 2006). Therefore, considering the productivity and diffusivity of the growth factor as linear and isotropic are simplifications. Implementing anisotropic growth factor productivity and diffusivity may need a different numerical approach or an additional numerical scheme in the current approach in future studies.

The model includes some assumptions to simplify the approach, such as a circular cell shape, whereas the cell shape changes during migration (Marzban *et al.* 2019). Also, the number of cells for the chemo-mechanical simulations is limited to four, and cell deformation has been incorporated as partial overlapping. Moreover, stochastic processes, such as randomness of cell motion and cell proliferation, death, and growth, have not been considered in the current model. However, the model performs well as it captures the essential features, such as the mechanotactic cell-cell interactions and chemically induced cell migration.

Even though the chemical interactions between cells have not been considered, an extension of the model will not change the findings. However, the complexity of the model will increase as more parameters need to be determined from experiments.

3.4.4 Recommendations

The model results indicate that, with some extensions, it is possible to include an alternative shape of the chemical source from the square, corresponding to different phenotypes. Extending the proposed model to include many chemoattractant sources that emit several signals with different concentration rates is also possible. These chemoattractant sources can be considered mobilised or immobilised, and the concentration gradient can be soluble or non-soluble. This could be done by extending the numerical solution of the diffusion equation by using convolution reach with the Green function, where a reaction term derives from the chemical signal that is secreted by the external sources such as bacteria or cells like it has been done, for instance, in Vermolen and Gefen (2013a, 2013b). The acquired pattern from these equations can be applied to quantify the sensitivity of the cells concerning the secreted chemical signal.

3.5 Conclusions

A mathematical model for cell migration has been developed based on two stimulus types, i.e., chemical and mechanical. For a weak chemical concentration gradient, the cells migrate toward one another due to the inter-cellular mechanical cues, whereas cells migrate toward the chemical source for a strong chemical concentration gradient that dominates the mechanical signals.

This model can provide qualitatively useful information to predict collective cell migration *in vivo* or *in vitro* due to the flexible approach to consider the effects of several cues on cell migration simultaneously.

4 Modelling of chemo-mechanically induced migration of cells in 3D engineered matrices

This chapter presents a mathematical cellular chemo-mechanical model to predict the migration of cells in a collective in 3D microenvironments. The new model extends the models for cell migration on 2D elastic substrates described in Chapters 2 and 3.

4.1 Introduction

ECM is a 3D non-cellular material that exists in all organs and tissues and provides structural support for cells within tissues (Frantz *et al.* 2010). The ECM structure is essential for regulating cellular behaviour, including transforming chemical and mechanical signals, cell migration, differentiation, proliferation, and death (Lu *et al.* 2012). Cells have multifaceted responses to complex cues from the surrounding ECM, such as chemical factors, adhesion gradient, and mechanical responses.

Cells interact mechanically with the ECM and neighbouring cells by deforming the surrounding ECM region. These deformations direct neighbouring cells to the dominant signals (Hadjipanayi *et al.* 2009, Oakes PW 2018). Mechanical forces generated by cells through focal adhesions during migration depend on cell type and physical properties of the cellular environment (Bangasser *et al.* 2017, C ndor *et al.* 2017, Doyle and Yamada 2016, Style *et al.* 2014). Cells identify differences in the ECM composition, topography, and physical changes such as pore size (Caswell and Zech 2018, Charras and Sahai 2014, Yamada and Sixt 2019), elasticity or plasticity (Boekhorst *et al.* 2016, Buxboim *et al.* 2010a, Chaudhuri *et al.* 2020, Talkenberger *et al.* 2017), and stiffness (Ehrbar *et al.* 2011, Lam *et al.* 2014, Matte *et al.* 2019). Cells recognise differences in the ECM stiffness and preferentially migrate towards stiffer ECM regions (Boekhorst *et al.* 2016, Talkenberger *et al.* 2017) based on the strength of mechanical signals they detect.

Chemical gradients in the microenvironment trigger cell receptors and induce cell migration up or down the signalling stimulus gradient. Cells migrate favourably to obtain nutrients or to escape toxins. Cells can produce chemical cues that attract or repel other cells. The chemical stimulus that is produced by the cells or of extracellular origin correlates with cell migration and gene expression. Cells sense the chemical stimulus gradient while they migrate using their surface receptors.

Cellular locomotion in 3D matrices depends on several factors, such as ECM environment, cell type (individual or collective migration), and tissue functional requirements. The ECM environment includes, for example, molecular composition (collagen, fibronectin), and component concentration (constant or varied).

Collective cell migration in the 3D ECM has been described for different physiological and biophysical processes such as embryogenesis (Knecht and Bronner-Fraser 2002, Kurosaka and Kashina 2008, Martin and Parkhurst 2004), angiogenesis (Li YB *et al.* 2023, Zgheib and Liechty 2016), osteogenesis (Chen X *et al.* 2013, Etich *et al.* 2023), morphogenesis (Loganathan *et al.* 2016, Nerger *et al.* 2017), immune and inflammatory responses (Bogle and Dunbar 2010, Hettler *et al.* 2013, Luster *et al.* 2005), wound healing (Arwert *et al.* 2012, Brugues *et al.* 2014, Lan *et al.* 2010, Shaw and Martin 2009), cancerous tumour invasion (Franz *et al.* 2002, Geraldo *et al.* 2013, Jayatilaka *et al.* 2017, Koch *et al.* 2012, Lauand *et al.* 2014, Simon *et al.* 2014, Stock *et al.* 2014, Tien Anh *et al.* 2013).

Several experimental studies and mathematical models considered the mechanical communication due to traction forces and the chemical interaction of collective cells in the 3D ECM (Chen J *et al.* 2018b, 2020, Fang *et al.* 2022, Li T *et al.* 2016, Mao *et al.* 2016, Merino-Casallo *et al.* 2018, Mousavi *et al.* 2014a, Ng *et al.* 2016, Whitehead *et al.* 2016). These investigations aimed to record every cell's locomotion to predict collective cell migration in 3D. There is a lack of models that deal with the migration process involving multi-signalling cues in the extracellular matrix.

The current study presents a mathematical model predicting the migration of individual cells in a colony due to mechanotactic cues based on mechanical cell-matrix and cell-cell interactions and a growth factor gradient in 3D extracellular environments.

4.2 Methods

4.2.1 Mechanically induced cell migration

A set of cells, i.e., cells in a collective, is considered embedded in and deforming an elastic ECM. The deformation gradient induced by a normal cell force in an elastic ECM has been considered in several experimental studies (Ben-David and Weihs 2021, Nerger *et al.* 2017, Polacheck and Chen 2016). The same mechanism described in Chapter 2 is used to simulate the traction forces applied by cells to the ECM. The deformation gradients by pulling forces of collective cells result in strain energies and strain energy densities (i.e., strain energy per unit volume) in the ECM. Cells identify the strain energy density gradient induced in the 3D matrix by neighbouring cells. The strain energy density function (Figure 4.1) is approximated by using the finite element methods in Abaqus (Dassault Systèmes, Providence, RI, USA) for this study (Abdallahman, unpublished data). For simplicity, cells are assumed to be the same type with a spherical shape.

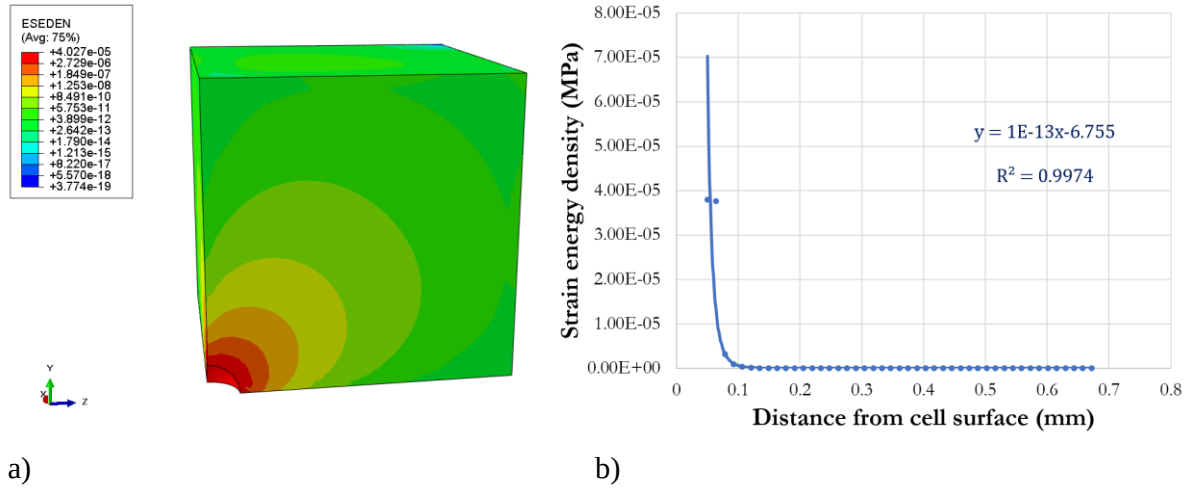


Figure 4.1. Strain energy density in an elastic ECM around a single cell due to distributed pulling force obtained using the finite element method. a) Strain energy density gradient by a spherical cell in the ECM. b) Strain energy density versus distance from the surface of a spherical cell with radius $R = 0.05$ mm. The strain energy density normal to the cell-ECM interface decays exponentially from the cell surface (Abdallahman, unpublished data for this study).

The strain energy density, Ψ_i^0 , around a cell i , with spatial center position $\mathbf{C}_i(t) = (X_i(t), Y_i(t), Z_i(t))$ at a particular time t , is:

$$\Psi_i^0 = \frac{1}{2} E_m \varepsilon^2, \quad (4.1)$$

where E_m is the elastic modulus of the ECM and ε is a strain of the ECM at the cell surface. The matrix strain, ε , is obtained from Hooke's law (Atanackovic and Guran 2000):

$$\varepsilon = \frac{1}{E_m} \frac{\mathcal{F}}{\mathcal{A}} = \frac{1}{E_m} \frac{\mathcal{F}}{4\pi R^2}, \quad (4.2)$$

where $\mathcal{F}$ is the distributed traction force in the ECM, $\mathcal{A}$ is the cell surface area, and R is the cell's radius. By substituting ε into Eq. (4.1), the strain energy density can be written as:

$$\Psi_i^0 = \frac{\mathcal{F}^2}{2(4\pi R^2)^2 E_m}. \quad (4.3)$$

The strain energy densities resulting from the ECM deformation around other cells j that cell i senses in the domain are:

$$\Psi(\mathbf{C}_i) = \Psi_j^0 \left(\frac{N_{ij}}{R} \right)^{-\gamma} \quad \text{for all } i, j = \{1, 2, \dots, n\}, \quad (4.4)$$

where Ψ_j^0 is the strain energy density from other cells j , and N_{ij} is the surface distance between cell i and other cells j , i.e.,

$$N_{ij} = \|\mathbf{C}_j - \mathbf{C}_i\| - 2R,$$

with $\|\mathbf{C}_j - \mathbf{C}_i\|$ as the distance between the centres of cell i and other cells j . γ is the mechanical signal attenuation ratio and its magnitude (Merkel *et al.* 2007):

$$\gamma = \frac{E_m}{E_c},$$

with E_c as the elastic modulus of the cell.

The sum of all strain energy densities that each cell i senses is:

$$\Psi(\mathbf{C}_i) = \Psi_i^0 + \sum_{\substack{j=1 \\ j \neq i}}^n \Psi_j^0 \left(\frac{N_{ij}}{R} \right)^{-\gamma}, \quad \text{for all } i, j = \{1, 2, \dots, n\}. \quad (4.5)$$

The first term in Eq. (4.5) describes the strain energy density around cell i due to the ECM deformation induced by the cell's pulling force. The second term in Eq. (4.5) represents the strain energy densities resulting from the ECM deformation induced by the pulling forces of neighbourhood cells j .

The migration direction of cell i is towards the resultant direction of the strain energy density:

$$\mathbf{v}_i = \sum_{\substack{j=1 \\ j \neq i}}^n \Psi_j(\mathbf{C}_i) \mathbf{u}_{ij}(t) \quad \text{for all } i, j = \{1, 2, \dots, n\}, \quad (4.6)$$

where $\Psi_j(\mathbf{C}_i)$ is the strain energy density from other cells j that cell i senses, and $\mathbf{u}_{ij}$ is the unit vector connecting centres of two cells, i.e.,

$$\mathbf{u}_{ij}(t) = \frac{\mathbf{C}_j - \mathbf{C}_i}{\|\mathbf{C}_j - \mathbf{C}_i\|} \quad \text{for all } i, j = \{1, 2, \dots, n\}.$$

The normalised migration unit vector is:

$$\hat{\mathbf{v}}_i = \frac{\mathbf{v}_i}{\|\mathbf{v}_i\|} \quad \text{for all } i, j = \{1, 2, \dots, n\}. \quad (4.7)$$

The magnitude of collective cells' movement towards each other during a time frame Δt is proportional to the strength of the ECM deformation:

$$\mathbf{C}_i(t + \Delta t) - \mathbf{C}_i(t) = \frac{\Delta t \Psi(\mathbf{C}_i(t)) \hat{\mathbf{v}}_i}{2 \int_0^R \beta(\Lambda) d\Lambda}, \quad (4.8)$$

where $\beta(\Lambda)$ is the adhesion friction coefficient of the cell-ECM, and Λ denotes the position of a point in the cell that varies in the interval occupied by the cell as time evolves, i.e., $\Lambda = [-R, R]$.

4.2.2 Cell-ECM interaction

Every single cell in a collective is assumed to be a homogenous rigid component. The friction coefficient of the cell-ECM adhesion, $\beta(\Lambda)$, is considered to depend linearly on the concentration of cell receptors binding to the ECM ligands. Therefore, the friction coefficient $\beta(\Lambda)$ of the cell-ECM interaction is:

$$\beta(\Lambda) = \beta_0 n_b(\Lambda), \quad (4.9)$$

where β_0 is the adhesion strength of an integrin attached to the ECM ligand, and n_b is the concentration of bound integrin receptors to the ECM ligands.

The interaction between each cell and the microenvironment is outlined in Chapter 2. The most important steps are mentioned here in brief for completeness. Suppose that the concentration of free integrin receptors is n_f and the concentration of the ECM ligands is n_s . Assume that the cell

receptors attachment to the ECM, the cell receptors detachment from the ECM, and the diffusion of free integrin receptors change instantaneously compared to the migration time scale, then:

$$n_b(\Lambda) \xrightleftharpoons[K_r \phi_r(\Lambda)]{K_f} n_s(\Lambda) + n_f(\Lambda), \quad (4.10)$$

where K_f is the bond formation rate and it is assumed that is constant, and $K_r \phi_r(\Lambda)$ is the dissociation rate that varies along with the position in the cell.

Suppose that the diffusion of free integrin receptors is n_{fi} and the density of the ECM ligands is n_0 . Assume that n_{fi} is constant since the diffusion of free integrin receptors n_{fi} is considered an instantaneous mechanism. Also, assume that the density of ligands n_0 is constant and saturated. As a result, the ligand depletion is negligible:

$$\begin{aligned} n_f(\Lambda) &= 2\pi R \times n_{fi}, \\ n_s(\Lambda) &= 2\pi R \times n_0. \end{aligned} \quad (4.11)$$

The concentration of integrin receptors binding to the ECM ligands can be written as:

$$n_b(\Lambda) = \frac{K_f}{K_r \phi_r(\Lambda)} n_f(\Lambda) n_s(\Lambda). \quad (4.12)$$

Suppose that the dynamic of cell adhesion is:

$$\phi_{r0} = Y_r + (1 - Y_r) \frac{\Lambda}{2R}, \quad (4.13)$$

where $Y_r \geq 1$ denotes the increment of dissociation rate from the front to the rear of the cell.

Assume that the total number of integrin receptors, N , remains constant over time, then the density of free integrin receptors n_{fi} can be obtained from:

$$\begin{aligned} N &= \int_{-R}^R (n_b(\Lambda) + n_f(\Lambda)) d\Lambda, \\ &= 2 \int_0^R (n_b(\Lambda) + n_f(\Lambda)) d\Lambda, \end{aligned} \quad (4.14)$$

$$= 2n_{fi} \left[2\pi R^2 + \frac{K_f}{K_r} n_0 4\pi^2 R^2 \int_0^R \frac{d\Lambda}{\beta_r(\Lambda)} \right].$$

The total number of integrin receptors N is determined from Gracheva and Othmer (2004) and Zaman *et al.* (2005). The integrin adhesion strength β_0 is obtained from Ananthakrishnan and Ehrlicher (2007) and Schwarz US and Soiné (2015). With parameter $B = 4\pi^2\beta_0 n_0/K_r$ representing the cell-ECM interaction, the density of free integrin receptors n_{fi} is:

$$n_{fi} = \frac{N}{2 \left[2\pi R^2 + \frac{K_f B R^2}{\beta_0} \int_0^R \frac{d\Lambda}{\beta_r(\Lambda)} \right]},$$

and the adhesion friction coefficient of the cell-ECM interaction $\beta(\Lambda)$ is:

$$\beta(\Lambda) = 4\pi^2\beta_0 \frac{n_0}{K_r} \frac{R^2 K_f n_{fi}}{\beta_r(\Lambda)} = \frac{B K_f R^2 n_{fi}}{\beta_r(\Lambda)}. \quad (4.15)$$

4.2.3 Chemically induced cell migration

Diffusion of a chemoattractant source, i.e., a growth factor source, over time that attracts cells in a collective, is studied. Diffusion of the growth factor concentration at cell positions in the ECM is:

$$\frac{\partial G}{\partial t}(t, \mathbf{H}) - \mathcal{D}\Delta G(t, \mathbf{H}) = \Gamma(t)\delta(\mathbf{H} - \mathbf{C}_i), \quad (4.16)$$

$$G(t, \mathbf{H}) = 0, \quad t = 0,$$

where $G(t, \mathbf{H})$ is the concentration of the growth factor in the ECM, $\mathbf{H}(x, y, z)$ represents the growth factor position, $\mathcal{D}$ is the diffusion coefficient of the growth factor, $\Gamma(t)$ is the production rate of the growth factor diffuses in the ECM over time, $\Delta = (\partial^2/\partial x^2 + \partial^2/\partial y^2 + \partial^2/\partial z^2)$ is the Laplace operator, and $\delta(\mathbf{H} - \mathbf{C}_i)$ is the Dirac Delta function, which is zero in all points except in the origin, and it satisfies $\int \delta d\Omega = 1$, if the entire origin is contained in Ω .

Initially, the cells are located in a region where the growth factor concentration is null. The growth factor is considered as an immobile point source and the diffusion of the growth factor is instantaneous with time. Therefore, the boundary conditions of the growth factor diffusion in the ECM are:

$$\frac{\partial G}{\partial t}(t, \mathbf{H}(x, y, z)) = 0, \quad \text{where } x = \text{const}, \quad \text{and}, \quad (4.17)$$

$$\text{const} \leq x \leq \infty, \quad -\infty \leq y \leq \infty, \quad -\infty \leq z \leq \infty.$$

The concentration gradient of the growth factor is obtained by solving Eq. (4.16) by using the fundamental solution (Green's Function) and Duhamel principle (Evans 1998):

$$G(t, \mathbf{H}) = \int_0^t \frac{\Gamma(s)}{[4\pi\mathcal{D}(t-s)]^{3/2}} \exp\left(-\frac{\|\mathbf{H} - \mathbf{C}_i(s)\|^2}{4\mathcal{D}(t-s)}\right) ds, \quad (4.18)$$

Cells migrate from low-concentration to high-concentration regions caused by the concentration gradient away from the growth factor source. The concentration gradient of the growth factor that triggers the motion of the cells is:

$$\nabla G(t, \mathbf{C}_i) = \int_0^t \frac{\Gamma(s)(\mathbf{H} - \mathbf{C}_i(s))}{16(\pi)^{3/2}[\mathcal{D}(t-s)]^{5/2}} \exp\left(-\frac{\|\mathbf{H} - \mathbf{C}_i(s)\|^2}{4\mathcal{D}(t-s)}\right) ds. \quad (4.19)$$

The solution of Eq. (4.19) is obtained using the Trapezoidal rule with the parameters listed in Table 4.1. The integration time is divided into a discrete set of points, i.e., $t_h = h\Delta t$, where Δt is the time step, and h denotes the time step size for the numerical integration.

4.2.4 Chemo-mechanically induced cell migration

The growth factor source is considered to be on one side of the ECM, and cells are on the opposite side of the source in the ECM (Figure 4.2).

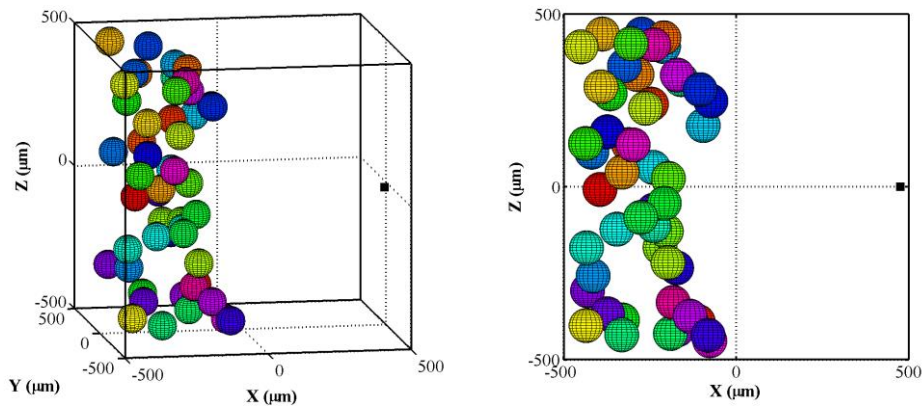


Figure 4.2. Initial cell positions (spheres) and a growth factor (solid square) in the ECM. The growth factor diffuses and attracts the cells in the ECM. The growth factor is considered an immobile point source located at $\mathbf{H}(x, y, z) = (475, -110, 0)$.

To assess the migration of collective cells in the presence of the growth factor gradient and the mechanical stimulus, i.e., the chemo-mechanically induced migration of cells, Eq. (4.8) is combined with Eq. (4.19):

$$\mathbf{C}_i(t + \Delta t) - \mathbf{C}_i(t) = \frac{\Delta t \Psi(\mathbf{C}_i(t)) \hat{\mathbf{v}}_i}{2 \int_0^R \beta(\Lambda) d\Lambda} + \xi \nabla G(t, \mathbf{C}_i(t)) \Delta t, \quad (4.20)$$

where ξ denotes the signalling rate parameter of the cell's sensitivity to the growth factor concentration gradient that each cell experiences as it migrates.

Cell migration is partially a random process. The cellular random motion is incorporated into the model by the standard Brownian vector $\Delta \mathbf{W}(t) = (x(t), y(t), z(t))$ with three independent samples from the normal distribution with expected value zero and variance Δt , i.e., $\Delta \mathbf{W} \sim \mathcal{N}(0, \Delta t)$. Thus, the migration of collective cells with partial stochastic motion is:

$$\mathbf{C}_i(t + \Delta t) = \mathbf{C}_i(t) + \frac{\Delta t \Psi(\mathbf{C}_i(t)) \hat{\mathbf{v}}_i}{2 \int_0^R \beta(\Lambda) d\Lambda} + \xi \nabla G(t, \mathbf{C}_i(t)) \Delta t + \ell \Delta \mathbf{W}(t), \quad (4.21)$$

where ℓ stands for possible changes due to uncertainties such as cell composition, tissue composition, and access to necessary chemicals. $\ell \Delta \mathbf{W}$ is commonly referred to as a random walk. The definition of random walk can be found, for instance, in Bressloff (2014) and Karatzas and Shreve (2012).

4.2.5 Cell migration simulations

The computational domain of the 3D ECM is assumed as a cube with the dimensions of $1,000 \mu\text{m} \times 1,000 \mu\text{m} \times 1,000 \mu\text{m}$. The cells are initially distributed randomly in the ECM, and the initial cell location differs for each simulation run. The cells are considered spheres with a radius of $R = 50 \mu\text{m}$. Hypothetic values and their sources, if applicable, have been used for input parameters introduced in the proposed model and reported in Table 4.1 unless stated otherwise. Each simulation runs with a 1 s time step.

Table 4.1. Parameters and values used in the model.

Parameter	Description	Value and unit	Reference
$\mathcal{F}$	Cell force	1 nN	(Sarangi <i>et al.</i> 2017, Steinwachs <i>et al.</i> 2016).
R	Cell radius	50 μm	(Balzer <i>et al.</i> 2012).

E_c	Elastic modulus of a cell	0.74019 kPa	(Ding <i>et al.</i> 2017).
E_m	Elastic modulus of ECM	5 kPa	(Oria <i>et al.</i> 2017, Wang T <i>et al.</i> 2017).
N	Total number of cell receptors	10^5	(Gracheva and Othmer 2004, Zaman <i>et al.</i> 2005).
β_0	An integrin adhesion strength	$50 \times 10^{-12} \text{Ns}/\mu\text{m}^3$	(Ananthakrishnan and Ehrlicher 2007, Schwarz US and Soiné 2015).
B	Cell-ECM interaction parameter	$7.0708 \times 10^{-5} \text{Ns}/\mu\text{m}^3$	(Ananthakrishnan and Ehrlicher 2007, Sakamoto <i>et al.</i> 2011).
K_f	Bounded integrins formation rate	10^{-9}	(Sakamoto <i>et al.</i> 2011).
γ_r	Integrins dissociation rate	10^5	(Sakamoto <i>et al.</i> 2011, Schwarz US and Soiné 2015).
Γ	Production rate of the growth factor source	$8,000 \text{ mol}/\mu\text{m}^3\text{s}$	(Savinell <i>et al.</i> 1989).
$\mathcal{D}$	Diffusion rate of the growth factor source	$103 \mu\text{m}^2/\text{s}$	(Di Costanzo <i>et al.</i> 2016, Ma <i>et al.</i> 2010, Nauman <i>et al.</i> 2007).
ξ	Growth factor signalling rate	10 s^{-1}	(Vermolen <i>et al.</i> 2015).
h	Size of time step for the numerical integration	0.01 s	(Chen J <i>et al.</i> 2018a).
ℓ	Possible changes in cell motion due to ECM uncertainties	0.1538 – 1.6667	
Δt	Time step	1 s	

Since cells can impinge on each other during migration, the resulting cell deformation is considered in the model and presented by an overlap of the cells. Repulsive forces corresponding to the strain energy density due to cell-cell contact are considered to prevent 100% cell overlap by a negative attraction value in Eq. (4.7). However, the cells are permitted to overlap up to 100% during chemical concentration gradient due to the absence of inter-cellular forces.

The migration of cells in a collective due to mechanotactic cues and the effect of ECM stiffness (E_m) are investigated first. Thereafter, the migration of cells in the presence of a growth factor

concentration gradient is demonstrated. Finally, the migration of cells as a response to the cellular mechanotactic cues, ECM stiffness, and the growth factor concentration gradient are studied.

The proposed model has been implemented in MATLAB (The MathWorks, Inc., Natick, MA, USA) with the computational algorithm illustrated in Figure 4.3.

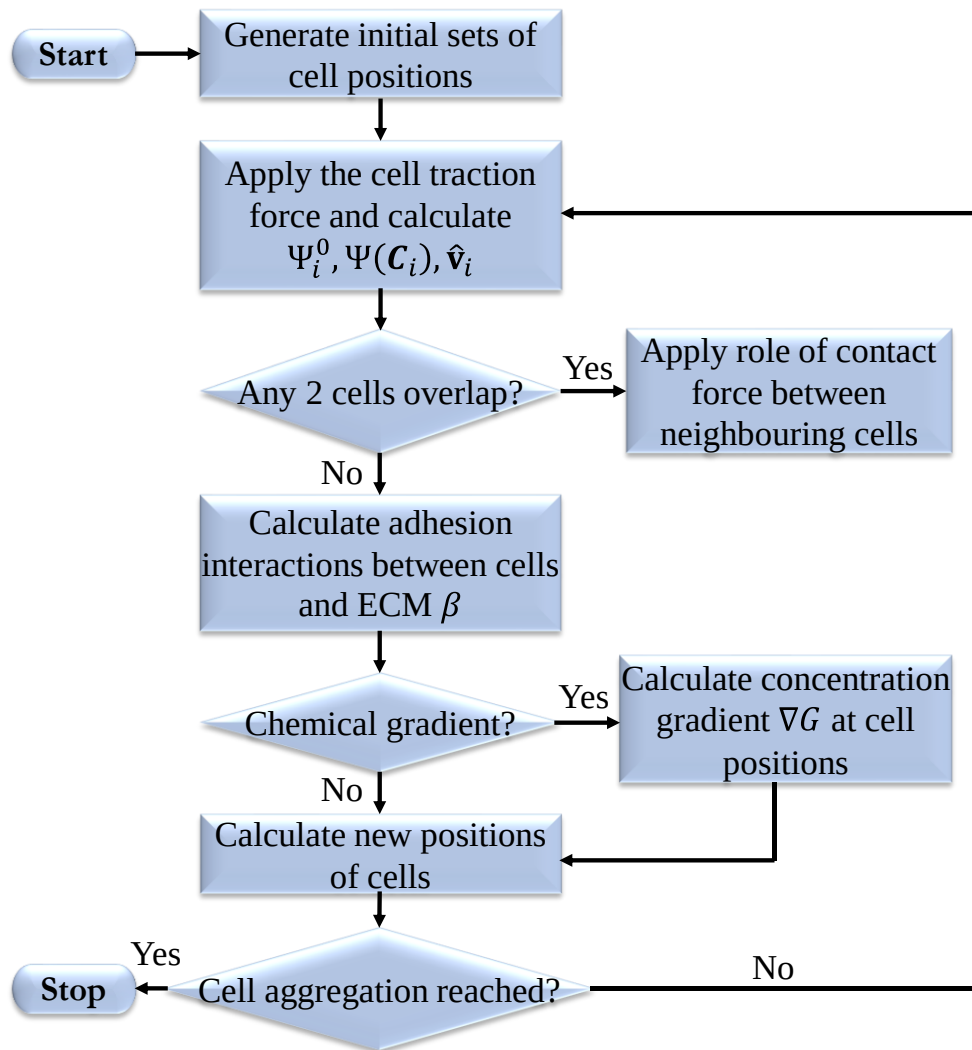


Figure 4.3. Schematic illustration of computation implantation for collective cell migration with mechanistic cellular interactions and chemical gradient. Cells apply traction forces and deform the ECM resulting in strain energy density around cell i , Ψ_i^0 , total strain energy densities cell i sense, $\Psi(C_i)$, and migration unit vector. Then, adhesion applied by cell surfaces to the ECM β is computed. After that, the chemical concentration gradient ∇G at the cell positions is calculated. This implementation is repeated for new cell positions until cells form a cluster or aggregate.

4.3 Results

4.3.1 Mechanically induced migration of collective cells

4.3.1.1 Cell migration for spatially uniform ECM stiffness

Migration of collective cells with Brownian motion (random movement) due to mechanical cues in a 3D ECM with a constant elastic modulus of $E_m = 5$ kPa is investigated. Initially, the cells are randomly located in the ECM with a dimension of $1,000 \mu\text{m} \times 1,000 \mu\text{m} \times 1,000 \mu\text{m}$.

For twenty-five cells with stochastic perturbed motion (Figure 4.4a), the cells primarily move towards each other in the dominant direction of the resultant strain energy density in the ECM (Figure 4.4b, $t = 330$ s). The cell-cell interaction in the ECM depends mainly on the initial cell locations. The random perturbed motion adds variability to the direction and magnitude of the migratory displacement at each time step. All cells move into the centre of the ECM and form a cluster after $t = 660$ s (Figure 4.4c). Cells in contact deform (displayed by overlap) in the absence of repulsive forces. The stochastic perturbations do not change the final cluster configuration (also see supplement videos V4.4-3D and V4.4-2D).

For 50 cells (Figure 4.5b) in the same ECM as in the previous case, the individual cells initially move towards and interact with neighbouring cells. The cells start forming small clusters ($t = 600$ s). The cells contact each other and deform (shown as overlap) as the repulsive inter-cellular forces are not considered in the model. In the final arrangement (Figure 4.5c, $t = 1,200$ s), the cells remain distributed in the ECM and form several clusters in the ECM, i.e., the cell clusters do not move further, and cells tightly adhere to neighbouring cells in each cluster (see also supplementary videos V4.5-3D and V4.5-2D).

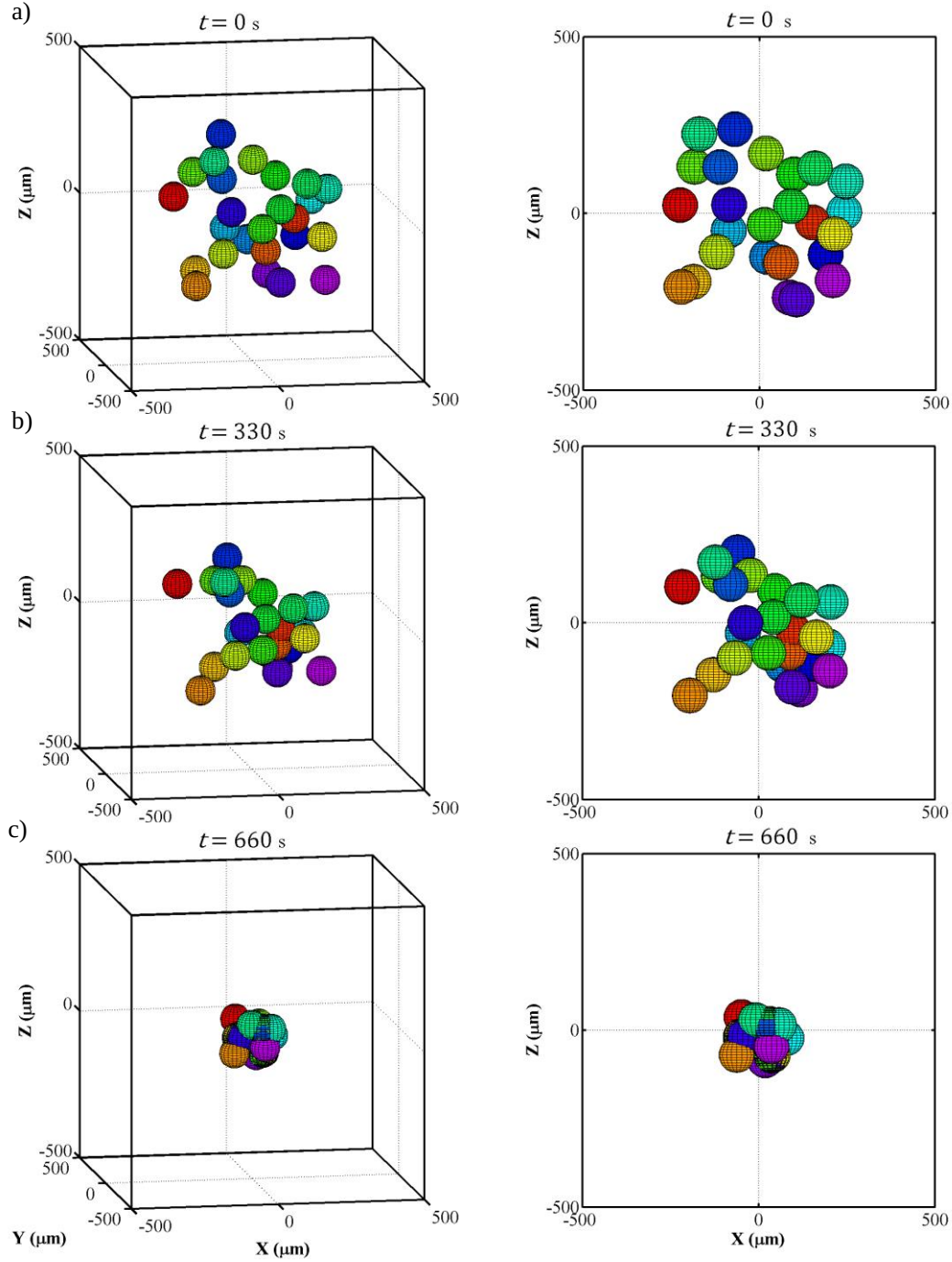


Figure 4.4. Migration of 25 cells in the 3D ECM with constant stiffness ($E_m = 5$ kPa) with random motion as a function of time. A) Initial positions of the cells are distributed randomly in the ECM at $t = 0$ s. b) Neighbouring cells move towards each other directed by the resultant strain energy density at $t = 330$ s. c) At $t = 660$ s, all cells in the ECM reach each other and form a cluster at the origin of the ECM at (0, 0, 0). Cells deform (represented by overlap) as the repulsive forces are not considered in the model (see also supplemental video V4.4-3D and V4.4-2D). (Model parameter values and sources are listed in Table 4.1).

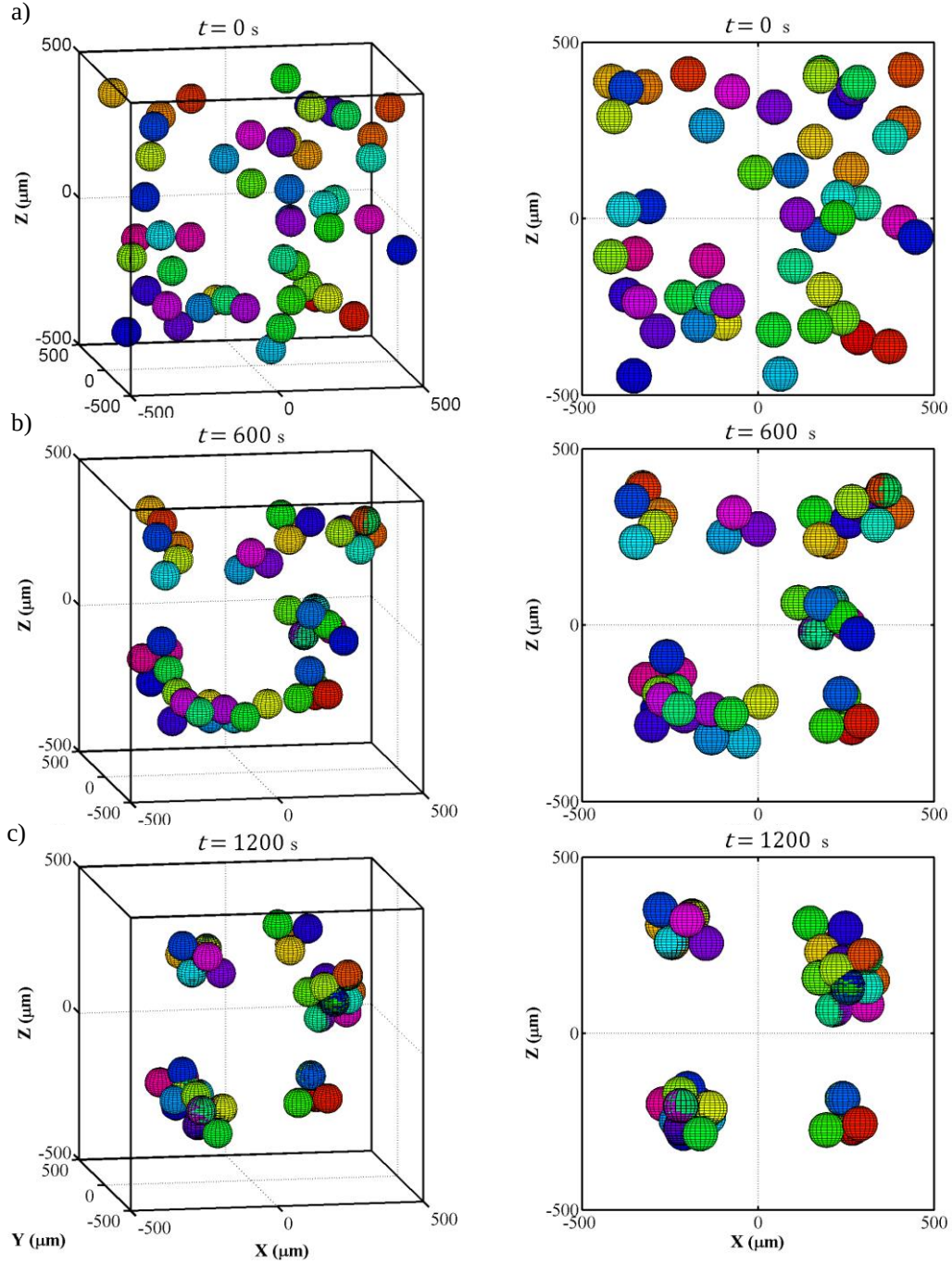


Figure 4.5. Migration of 50 cells with stochastic perturbed motion in the 3D ECM with constant stiffness of $E_m = 5$ kPa as a function of time. A) Initial random positions of the cells in the ECM at $t = 0$ s. b) Cells migrate towards neighbouring cells following the resultant strain energy density and start forming clusters at $t = 600$ s. The cells deform (displayed by overlap) once they are in contact in the absence of the inter-cellular forces. C) At $t = 1,200$ s, the cells form distributed clusters in the ECM and do not migrate further (see also supplemental video V4.5-3D and V4.5-2D). (Model parameter values and sources are listed in Table 4.1).

4.3.1.2 Cell migration for spatially varying ECM stiffness

The effect of ECM regions with different stiffness on the migration of 50 cells located randomly in the ECM ($t = 0$ s) is studied. The ECM is divided along the Y-Z plane into two regions at $X = 0 \mu\text{m}$, with a stiff region of $E_m = 35$ kPa (Buxboim *et al.* 2010a, Oria *et al.* 2017) for $X < 0$ and a soft region of $E_m = 5$ kPa for $X \geq 0$ (Figure 4.6a).

With randomly perturbed motion, the cells initially move towards each other in both regions due to the mechanotactic responses (Figure 4.6b, $t = 270$ s). Cells close at or near the stiffness change at the ECM region interface ($X = 0 \mu\text{m}$) move from the soft region to the stiff region, but not vice versa. However, the cells at the boundary between stiff and soft ECM regions (Figure 4.6c, $t = 540$ s) move along the ECM interface at $X = 0 \mu\text{m}$ towards $Y \approx 0$. The single cells in the soft ECM region form two-, three-, and four-cell clusters and move collectively towards the cells in the stiff ECM region. Similarly, the cells in the stiff region form clusters and move collectively towards the cells in the soft region. Cells in the soft region move with larger velocities than cells in the stiff region. All cells form a single cluster and aggregate slightly more towards the stiff ECM region in the ECM centre (Figure 4.6d, $t = 810$ s) (see also supplement videos V4.6-3D and V4.6-2D).

The migratory displacement of two cells towards each other with fully deterministic motion in the 3D ECM is considered for different values of the elastic modulus (stiffness) of the ECM. The initial distance between the two cells of $1,370 \mu\text{m}$ is the same for each case. The migrated distance of the two cells towards each other at any time, i.e., the migration velocity, increases with decreasing the stiffness of the ECM (Figure 4.7). The two cells impinge on each other due to the absence of inter-cellular forces, and therefore, the final distance between the two cells is smaller than the cell diameter ($2R = 100 \mu\text{m}$).

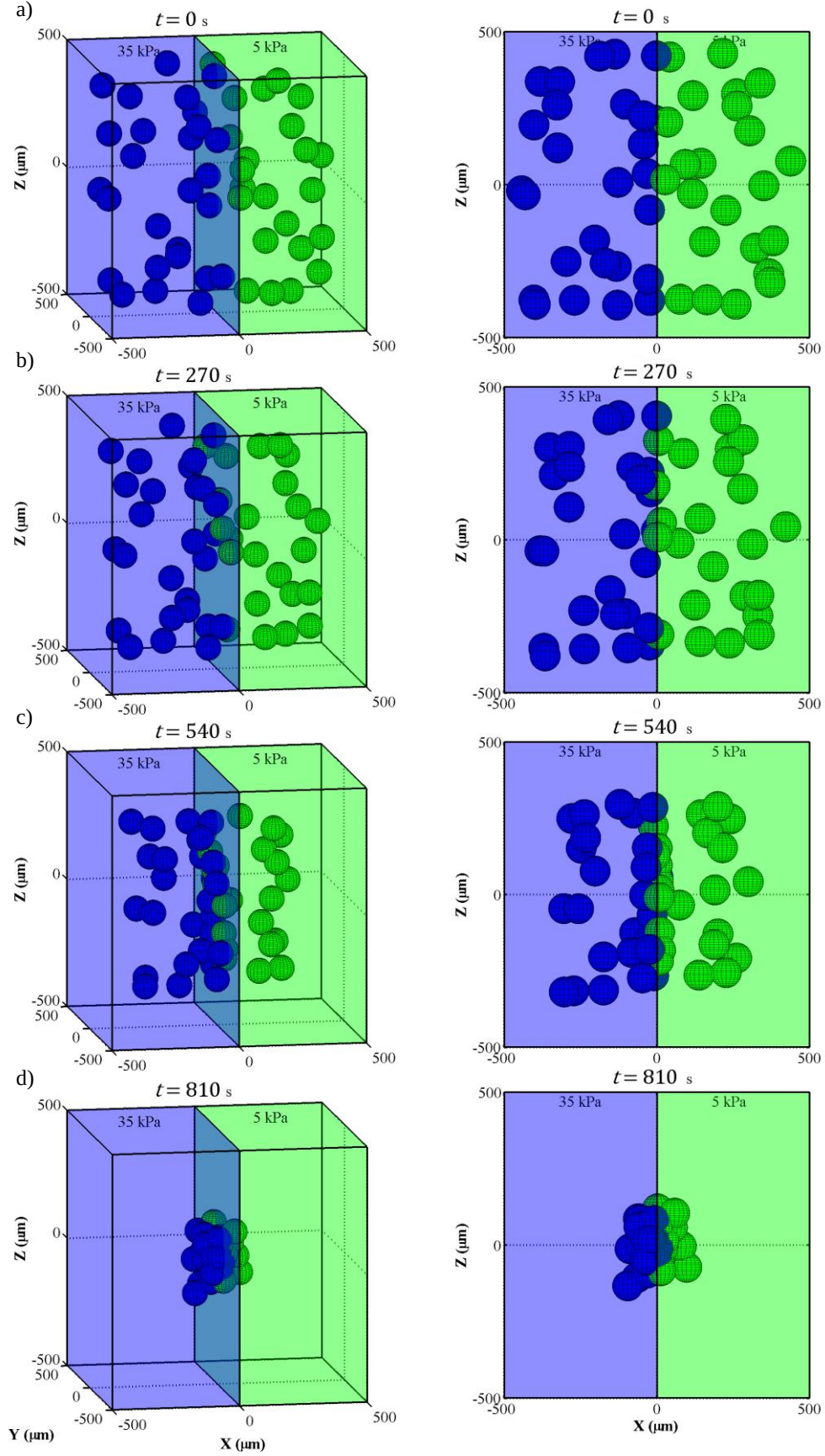


Figure 4.6. Positions of 50 cells migrating with random motion in the 3D ECM with two different elastic moduli E_m (stiffness) as a function of time. E_m changes along $X = 0 \mu\text{m}$ from $E_m = 35 \text{ kPa}$

in the region with $X < 0$ (blue, stiff region) to $E_m = 5$ kPa in the region with $X \geq 0$ (green, soft region). A) Initial positions of cells are located randomly in the ECM at $t = 0$ s. b) Cells in both stiff and soft ECM regions migrate towards each other due to the mechanical cues at $t = 270$ s. c) The cells in the soft region migrate farther and have larger velocities than those in the stiff region at $t = 540$ s. Cells in contact deform since repulsive cellular forces are not considered. D) The cells from both regions form a cluster at $t = 810$ s. Cells in the soft ECM region migrate more into the stiff ECM region, whereas cells in the stiff region migrate less in the soft ECM region (see also supplemental videos V4.6-3D and V4.6-2D). (Model parameter values and sources are listed in Table 4.1 except for $E_m = 35$ kPa provided above in this caption is estimated from Buxboim *et al.* (2010a) and (Oria *et al.* 2017)).

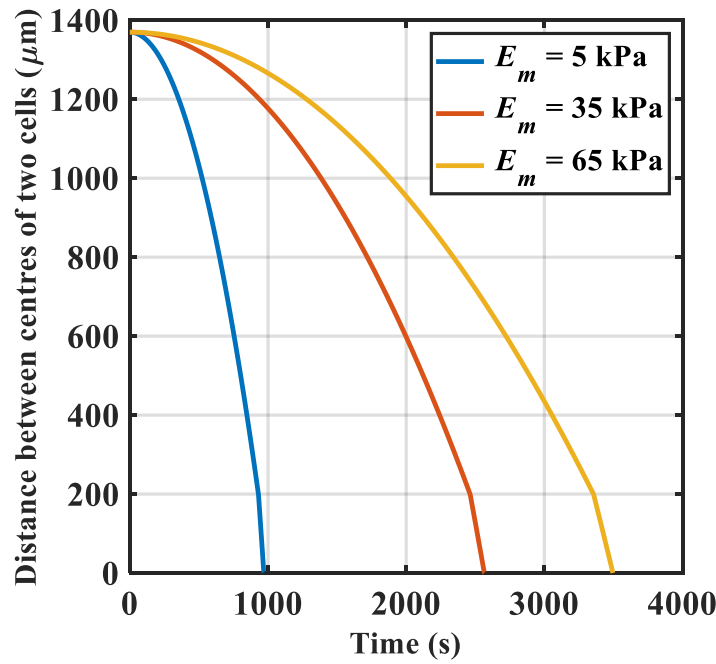


Figure 4.7. Distance between centres of two cells approaching each other versus time in the absence of random motion in the ECM for three different elastic moduli E_m (stiffness). Cell velocity decreases with increasing ECM elasticity E_m . (Model parameter values and sources are listed in Table 4.1 except for E_m values provided above in the Figure label are estimated from Buxboim *et al.* (2010a) and Oria *et al.* (2017)).

Migration of 50 cells due to mechanical interactions in the ECM with three regions of different elastic moduli of $E_m = 5$, 35, and 65 kPa is investigated. The cells are located randomly in the ECM initially (Figure 4.8a, $t = 0$ s). The ECM of $1,000 \mu\text{m} \times 1,000 \mu\text{m} \times 1,000 \mu\text{m}$ is divided into three regions with step-wise changes of the elastic modulus at $X = -170$ and $170 \mu\text{m}$, with of $E_m = 65$ kPa (Oria *et al.* 2017) for $X < -170 \mu\text{m}$ (left), $E_m = 35$ kPa (Buxboim *et al.* 2010a, Oria *et al.* 2017) for $-170 \mu\text{m} \leq X < 170 \mu\text{m}$ (middle), and 5 kPa for $X \geq 170 \mu\text{m}$ (right). For easier reference, the notations E_{m-65} , E_{m-35} , and E_{m-5} are introduced.

The cells primarily move towards and connect with neighbouring cells. However, cells in the E_{m-65} region near the E_{m-65}/E_{m-35} interface move into the softer E_{m-35} region, and cells in the E_{m-35} region move towards the E_{m-35}/E_{m-5} interface. Cells in the E_{m-5} region move towards the stiffer E_{m-35} region and form clusters at E_{m-35}/E_{m-5} interface with cells in the E_{m-35} region. Similarly, cells in the E_{m-35} region move towards the stiffer E_{m-65} region and form clusters at E_{m-35}/E_{m-65} interface with cells in the E_{m-65} region. This process is faster in the E_{m-5} region than in E_{m-35} region (Figure 4.8b, $t = 840$ s). The cells in the E_{m-65} and E_{m-35} regions remain distributed longer than the cells in the E_{m-5} region. The cell clusters at the E_{m-35}/E_{m-5} and E_{m-65}/E_{m-35} interfaces move into and merge with cells in the stiffer region, i.e., E_{m-35} and E_{m-65} , respectively, again faster in the E_{m-35} than the E_{m-65} region (Figure 4.8c, $t = 1,700$ s). The overall movement tendency of the cells is towards the stiffest E_{m-65} ECM region. The cells in contact deform (shown as overlap) as inter-cellular forces are not considered in the model. In the final configuration, the cells form a single cluster at the E_{m-65}/E_{m-35} interface, with a larger part of the cluster located in the E_{m-65} region (Figure 4.8d, $t = 2,500$ s) (see also supplemental videos V4.8-3D and V4.8-2D). Whereas the cluster formation of 50 cells is completed after $t \approx 810$ s in an ECM with two stiffness regions cluster (Figure 4.7), this process takes $t \approx 2,500$ s in an ECM with three stiffness regions in sequential order, indicating a decrease in migration velocity with an increasing number of stiffness regions in the ECM.

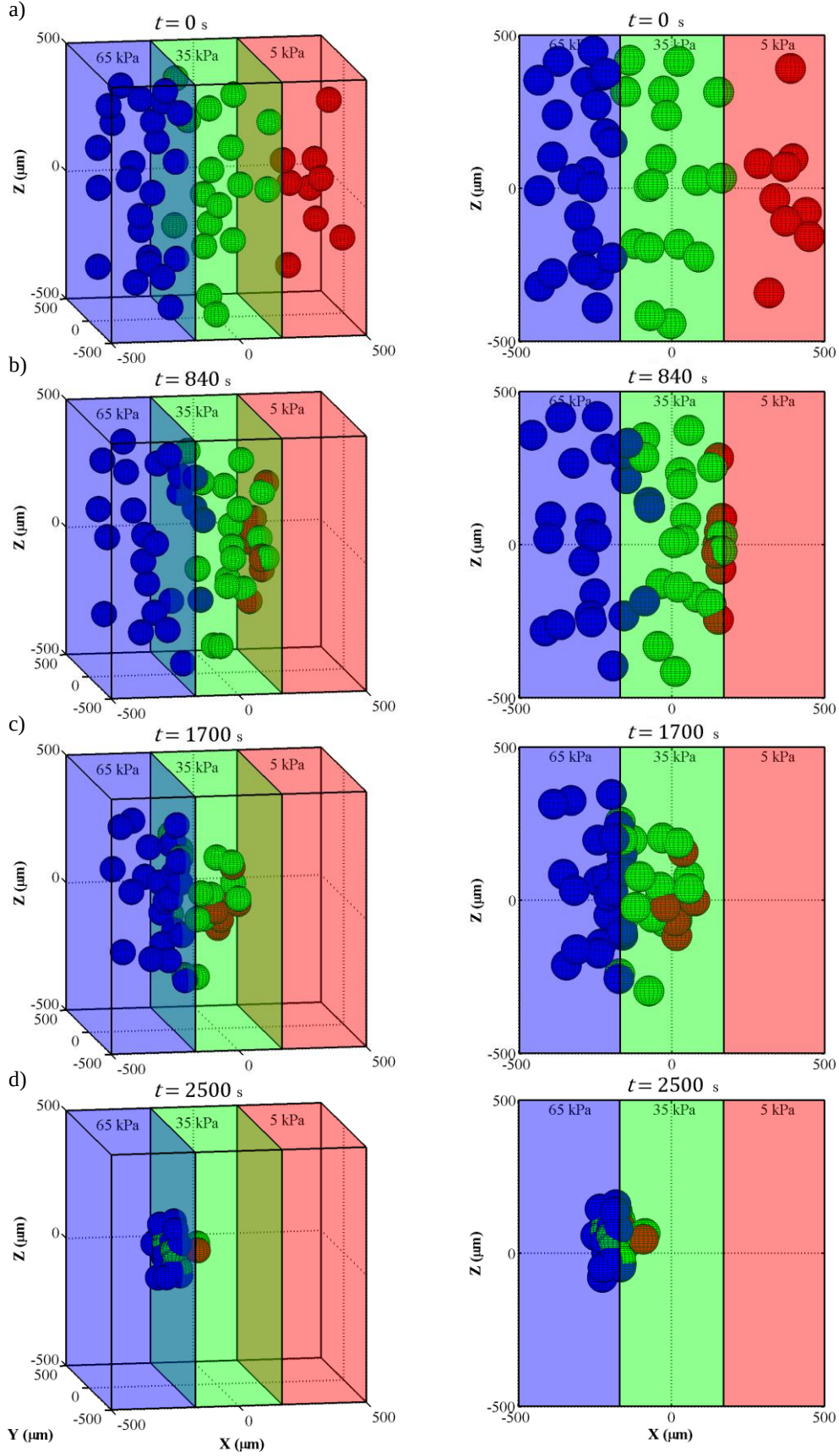


Figure 4.8. Positions of 50 migrating collective cells with random perturbed motion in 3D ECM with varying elastic modulus E_m (stiffness) as a function of time. The ECM region boundaries are

in the Y-Z plane, with $E_m = 65$ kPa for $X < -170$ μm (left blue), $E_m = 35$ kPa for -170 $\mu\text{m} \leq X < 170$ μm (middle green), and $E_m = 5$ kPa for $X \geq 170$ μm (right red). a) Initial configuration of 50 cells located randomly in the ECM at $t = 0$ s. b) At $t = 840$ s, the cells preferentially migrate towards neighbouring cells due to mechanical interactions. Cells in the left region, but close to the middle region interface at $X = -170$ μm , migrate towards and into the middle ECM region. Similarly, cells in the middle region, but close to the right ECM region interface at $X = 170$ μm , migrate towards and into the ECM right region. After that, cells in the right ECM region migrate towards cells in the middle region and form clusters at $X = 170$ μm . Cells in the right region migrate faster than the middle and left regions. Cells in contact deform (represented by overlap) due to the absence of inter-cellular forces. c) After $t = 1,700$ s, cells in the right region reach the cells in the middle region and continue to migrate collectively towards the cells in the left ECM region (i.e., towards $X = -170$ μm). The cells in the middle region migrate faster than in the left region. d) At $t = 2,500$ s, the cells from the left and middle regions reach each other and form a single cluster. Cells in the middle ECM region migrate more towards and into the left ECM region, whereas cells in the left region migrate less towards the middle region (see also supplemental videos V4.8-3D and V4.8-2D). (Model parameter values and sources are listed in Table 4.1 except for E_m values provided above in this caption are estimated from Buxboim *et al.* (2010a) and Oria *et al.* (2017)).

Fifty cells are considered in an ECM with a non-sequential step-wise change of the elastic modulus E_m along $X = -170$ and 170 μm , with $E_{m-65} = 65$ kPa for $X < -170$ μm (left), $E_{m-5} = 5$ kPa for -170 $\mu\text{m} \leq X < 170$ μm (middle), and $E_{m-35} = 35$ kPa for $X \geq 170$ μm (right). Initially, cells are located randomly in the three regions (Figure 4.9a, $t = 0$ s).

The collective cell migration generally starts with the movement of single cells preferentially towards neighbouring cells. However, cells in the E_{m-5} region near stiffness interfaces move towards the E_{m-65} and E_{m-35} region, respectively (Figure 4.9b, $t = 350$ s). After that, the cells in the E_{m-35} region form a cluster at the E_{m-5}/E_{m-35} interface and move collectively towards and into the E_{m-5} region. The cells in E_{m-65} region cluster at the E_{m-5}/E_{m-65} interface and also move as a cluster towards and into the E_{m-5} region. This process occurs faster in the E_{m-35} than in the E_{m-65} region (Figure 4.9c, $t = 750$ s). The cells in contact deform (represented by overlap) in the absence of inter-cellular forces. Finally, the cells aggregate at the E_{m-65}/E_{m-5} interface and cluster slightly more in the E_{m-5} ECM region (Figure 4.9d, $t = 970$ s) (see also supplemental videos V4.9-3D and V4.9-2D).

The cells cluster for the three sequential changes in ECM stiffness after $t \approx 2,500$ s (Figure 4.8), whereas this takes $t \approx 970$ s for the three non-sequential changes in ECM stiffness, indicating an increase in migration velocity with an increase in the ECM stiffness gradient.

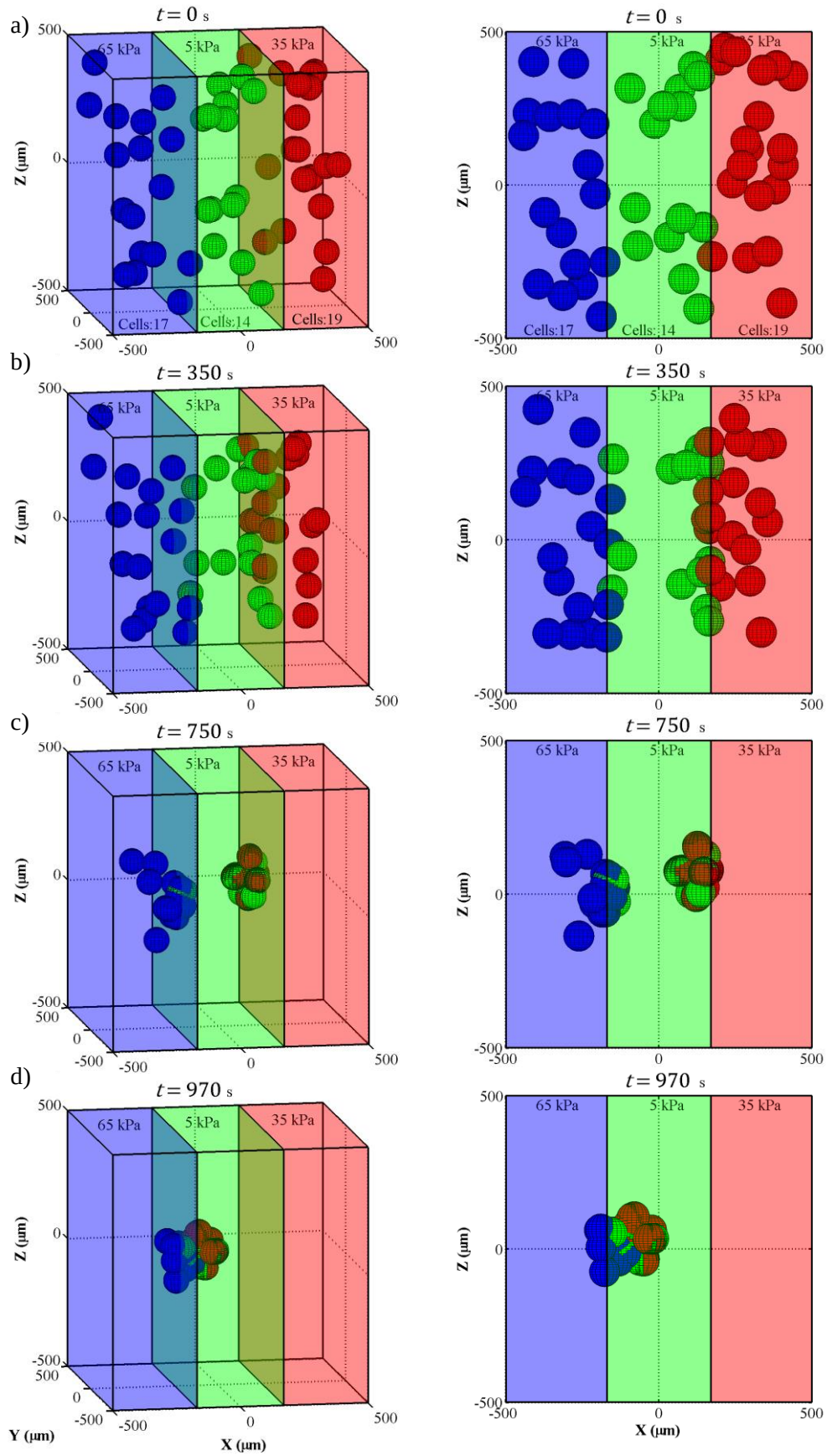


Figure 4.9. Positions of 50 migrating cells with stochastic motion in 3D ECM with varying elastic moduli E_m (stiffness) as a function of time. Region boundaries are in the Y-Z plane, with $E_m = 65$

kPa for $X < -170 \mu\text{m}$ (left blue), $E_m = 5 \text{ kPa}$ for $-170 \mu\text{m} \leq X < 170 \mu\text{m}$ (middle green), and $E_m = 35 \text{ kPa}$ for $X \geq 170 \mu\text{m}$ (right red). a) Initial cell locations are distributed randomly in the ECM at $t = 0 \text{ s}$. b) At $t = 350 \text{ s}$, the cells preferentially migrate towards neighbouring cells due to mechanical cues. Cells in the middle region, but near the stiffness changed with $X = -170 \mu\text{m}$, migrate towards and into the left ECM region. Similarly, cells in the middle region, but near the stiffness changed with $X = 170 \mu\text{m}$, migrate towards and into the ECM right region. Cells deform (shown as overlap) once they are in contact in the absence of repulsive forces. c) The cells form clusters at $X = -170$ and $170 \mu\text{m}$ at $t = 750 \text{ s}$. d) The cells form a cluster at the ECM region interface with $X = -170 \mu\text{m}$ at $t = 970 \text{ s}$ (see also supplemental videos V4.9-3D and V4.9-2D). (Model parameter values and sources are listed in Table 4.1 except for E_m values provided above in this caption are estimated from Buxboim *et al.* (2010a) and Oria *et al.* (2017)).

For an ECM with three regions of non-sequential stiffness changes with 50 initially randomly located cells presented in Figure 4.9, different final configurations of the cell cluster are observed (Figure 4.10). The first case considers 13, 18, and 19 cells in the E_{m-65} , E_{m-5} , and E_{m-35} regions, respectively. The cells cluster at the E_{m-5}/E_{m-35} interface slightly towards the E_{m-5} ECM region after $t = 960 \text{ s}$ (Figure 4.10a) (also see supplemental videos V4.10a-3D and V4.10a-2D). In the second case with 16, 21, and 13 cells located in the E_{m-65} , E_{m-5} , and E_{m-35} ECM regions, respectively, the cells cluster in the centre of the E_{m-5} region after $t = 950 \text{ s}$ (Figure 4.10) (also see supplemental videos V4.10b-3D and V4.10b-2D).

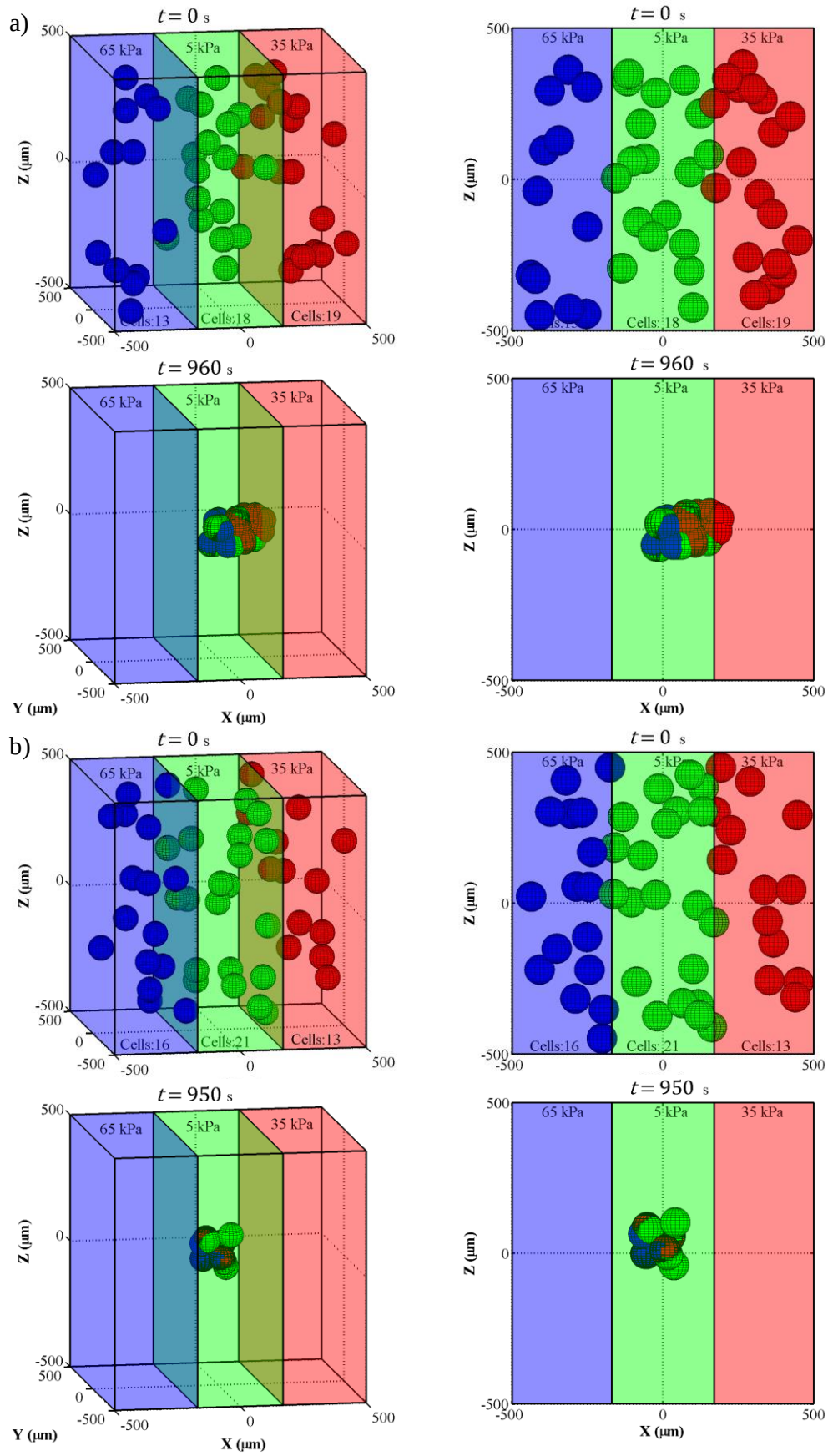


Figure 4.10. Initial and final configuration of 50 migrating cells with random perturbed motion in 3D ECM with varying elastic modulus E_m (stiffness) as a function of time. The ECM region

boundaries are in the Y-Z plane, with $E_m = 65$ kPa for $X < -170$ μm (left blue), $E_m = 5$ kPa for $-170 \mu\text{m} \leq X < 170 \mu\text{m}$ (middle green), and $E_m = 35$ kPa for $X \geq 170 \mu\text{m}$ (right red). The initial arrangement of the cells is located randomly in the ECM for the two cases at $t = 0$ s. a) The cells reach each other and aggregate at the ECM region interface with $X = -170 \mu\text{m}$ at $t = 960$ s slightly towards the middle region (see also supplemental videos V4.10a-3D and V4.10a-2D). b) In the final arrangement of cells, the cells aggregate into the ECM centre (middle region) at $t = 950$ s (see also supplemental videos V4.10b-3D and V4.10b-2D). (Model parameter values and sources are listed in Table 4.1 except for E_m values provided above in this caption are estimated from Buxboim *et al.* (2010a) and Oria *et al.* (2017)).

4.3.2 Chemically induced migration of collective cells

Collective cell migration due to a growth factor signal in the ECM with constant E_m is studied using 50 cells located randomly in a half-region $X < 0$ of the ECM with the dimension of $1,000 \mu\text{m} \times 1,000 \mu\text{m} \times 1,000 \mu\text{m}$. The growth factor concentration at the initial cell positions is zero (Figure 4.11a, $t = 0$ s). The growth factor is located at $(475, -110, 0)$ with a production $\Gamma = 8,000$ mol/ $\mu\text{m}^3\text{s}$ and a diffusion rate $\mathcal{D} = 103 \mu\text{m}^2/\text{s}$. The growth factor source is assumed to be stationary, and diffusion of the growth factor is considered to be instantaneous with time. Therefore, the release of the growth factor starts at $t = 1$ s. Fully deterministic motion (without stochastic motion) of the cells is considered.

The cells closest to the growth factor source start moving towards the source after the chemical release is started, and one cell reaches the growth factor source first at $t = 490$ s (Figure 4.11b). The cells with initial positions furthest from the growth factor source do not move as they do not sense the chemical concentration gradient (see also supplemental videos V4.11-3D and V4.11-2D). Once cells reach the growth factor source, they randomly move around the source along the Y-Z plane (Figure 4.11c, $t = 700$ s). The cells contact each other and deform (shown as overlap) in the absence of inter-cellular forces. All cells reach the growth factor source after $t = 997$ s (Figure 4.11d).

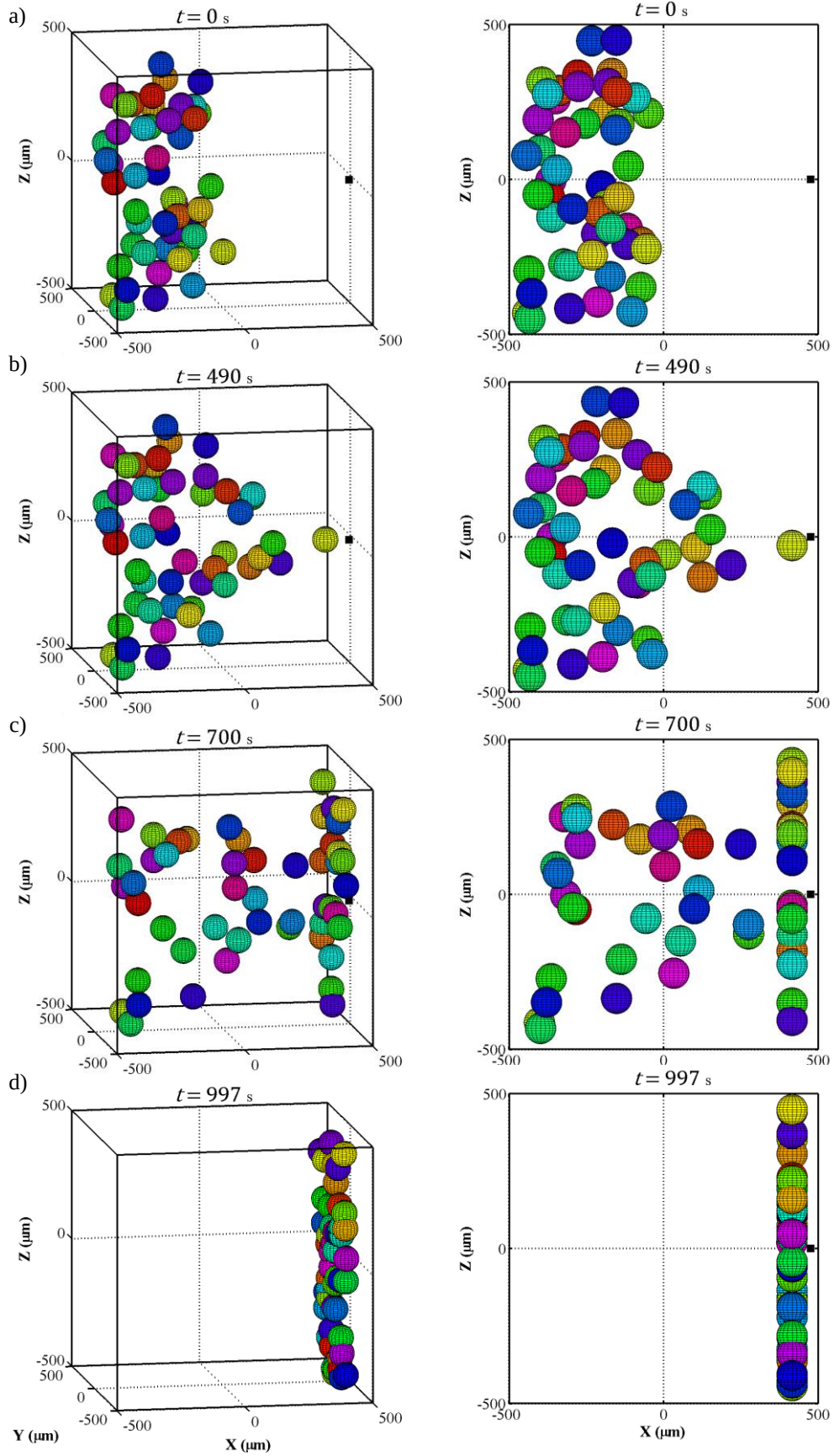


Figure 4.11. Chemically induced collective cell migration. Positions of 50 cells (spheres) migrating with fully deterministic towards a growth factor source (solid square) with a production rate $\Gamma =$

8,000 mol/ μm^3 s and a diffusion rate $\mathcal{D} = 103 \mu\text{m}^2/\text{s}$ in the ECM with a constant stiffness $E_m = 5 \text{ kPa}$. a) Initial configuration of the cells distributed randomly in the ECM region with $X < 0$ (left) at $t = 0 \text{ s}$, and the growth factor source is located in the ECM region with $X > 0$ (right). b) The closest cells to the growth factor source start migrating towards the source, and one cell reaches the source first at $t = 490 \text{ s}$. The cells furthest from the source do not migrate towards the growth factor source as they do not feel the chemical concentration gradient (also see supplemental videos V4.11-3D and V4.11-2D). c) The cells keep moving randomly around the growth factor source along the Y-Z plane after they reach the source at $t = 700 \text{ s}$. d) All cells reach the growth factor source at $t = 997 \text{ s}$. (Model parameter values and sources are listed in Table 4.1).

4.3.3 Chemo-mechanically induced migration of collective cell

4.3.3.1 Cell migration for spatially uniform ECM stiffness

The migration of 50 cells, located randomly in a half region $X < 0$ of the ECM with the dimension of $1,000 \mu\text{m} \times 1,000 \mu\text{m} \times 1,000 \mu\text{m}$ and a constant E_m , due to mechanical cues and a growth factor signal is investigated. The growth factor is assumed to be stationary and is located at $(475, -110, 0)$ with a production rate $\Gamma = 8,000 \text{ mol}/\mu\text{m}^3\text{s}$ and a diffusion rate $\mathcal{D} = 103 \mu\text{m}^2/\text{s}$. The growth factor concentration gradient at the initial cell positions is zero (Figure 4.12a, $t = 0 \text{ s}$). Diffusion of the growth factor is considered to be instantaneous with time, and release of the growth factor starts at $t = 1 \text{ s}$. Stochastic random motion is considered.

The cells primarily move towards neighbouring cells due to the mechanical interactions in the direction of resultant strain energy density in the ECM at $t = 340 \text{ s}$ and start to form clusters (Figure 4.12b). The cells deform (displayed by overlap) once they are in contact since repulsive inter-cellular forces are not considered. After that, the cells nearest the growth factor source start moving collectively towards the source. The first cell reaches the growth factor source at $t = 511 \text{ s}$ (Figure 4.12c). All cells reach the growth factor source in the final configuration at $t = 1,377 \text{ s}$ (Figure 4.12d). The cells continue moving around the source randomly along the Y-Z plane (also see supplemental videos V4.12-3D and 4.12-2D).

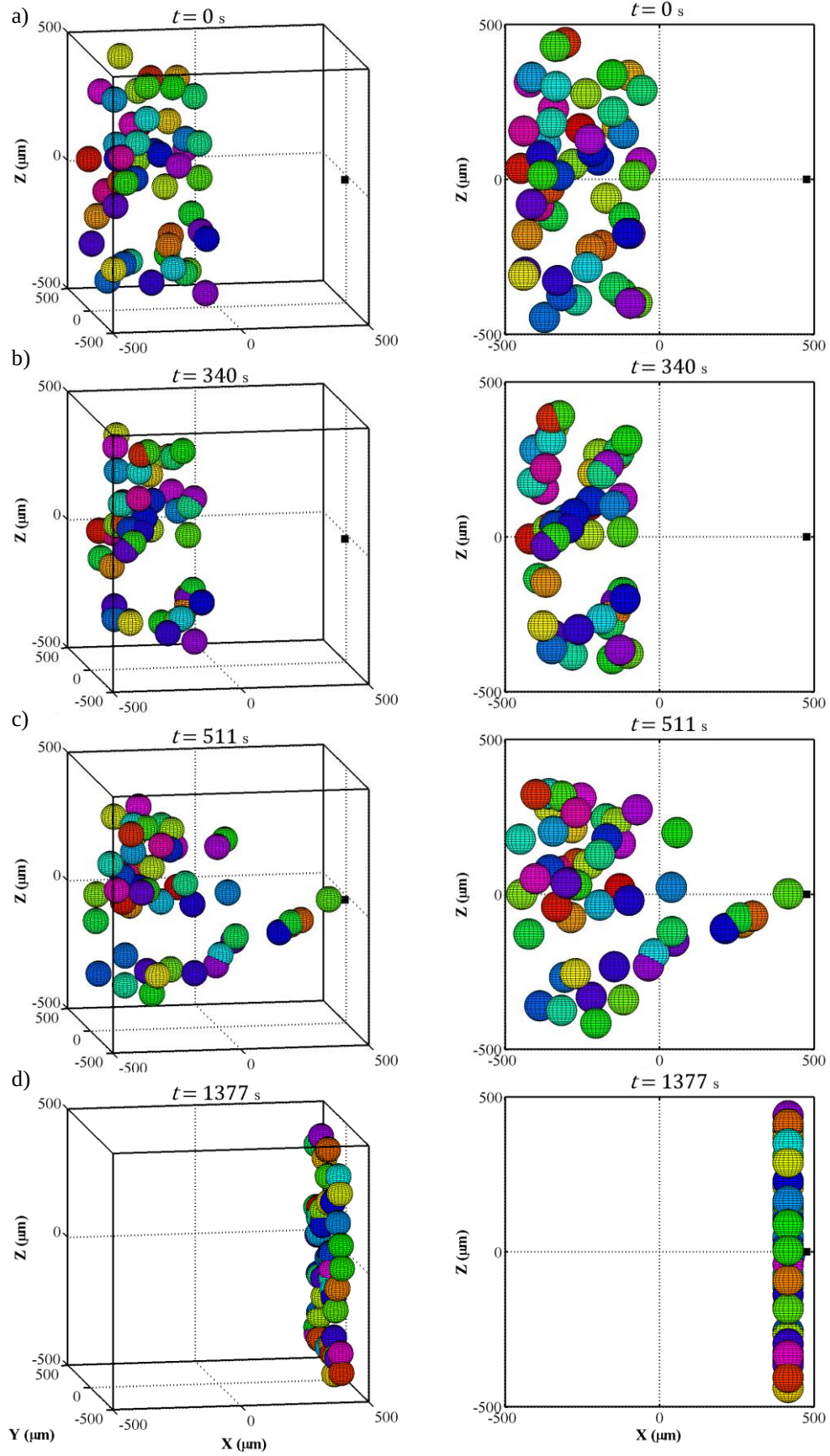


Figure 4.12. Chemo-mechanically induced collective cell migration. Positions of 50 cells (spheres) migrating with stochastic motion towards a growth factor source (solid square) with a production

rate $\Gamma = 8,000 \text{ mol}/\mu\text{m}^3\text{s}$ and a diffusion rate $\mathcal{D} = 103 \mu\text{m}^2/\text{s}$ in the ECM with a constant stiffness $E_m = 5 \text{ kPa}$. a) Initial arrangement of the cells distributed randomly in the ECM region with $X < 0$ (left) at $t = 0 \text{ s}$, and the growth factor source is located in the ECM region with $X > 0$ (right). b). The cells migrate towards each other due to the mechanical interactions at $t = 340 \text{ s}$. Once the cells are in contact, they deform (represented by an overlap) as the repulsive forces are not considered in the model. c) The cells migrate collectively towards the growth factor source. The first closest cell to the growth factor source reaches the source at $t = 511 \text{ s}$. The cells reach the growth factor source and keep moving around the source along the Y-Z plane randomly (also see supplemental videos V4.12-3D and V4.12-2D). d) All cells reach the growth factor source at $t = 1,377 \text{ s}$. (Model parameter values and sources are listed in Table 4.1).

4.3.3.2 Cell migration for spatially varying ECM stiffness

Migration with stochastic motion of collective cells due to mechanical interactions in the ECM with three regions of different elastic modulus $E_m = 5, 35$, and 65 kPa and a growth factor source with a production rate $\Gamma = 8,000 \text{ mol}/\mu\text{m}^3\text{s}$ and a diffusion rate $\mathcal{D} = 103 \mu\text{m}^2/\text{s}$ is studied. 50 cells are arranged randomly in the region $X < 0$ of the ECM with the dimension of $1,000 \mu\text{m} \times 1,000 \mu\text{m} \times 1,000 \mu\text{m}$. The ECM is divided into three different regions with a sequential step-wise change of the elastic modulus: $E_m = 65 \text{ kPa}$ ((Oria *et al.* 2017), E_{m-65}) for $-500 \mu\text{m} \leq X < -250 \mu\text{m}$, $E_m = 35 \text{ kPa}$ ((Buxboim *et al.* 2010a, Oria *et al.* 2017), E_{m-35}) for $-250 \mu\text{m} \leq X < 0 \mu\text{m}$, and $E_m = 5 \text{ kPa}$ (E_{m-5}) for $0 \mu\text{m} \leq X \leq 500 \mu\text{m}$) (Figure 4.13a, $t = 0 \text{ s}$).

The cells closest to the growth factor source, in the ECM region with E_{m-35} , start moving towards the growth factor source and into region E_{m-5} . Cells in the ECM region with E_{m-65} move towards cells in the ECM region with E_{m-35} (Figure 4.13b). After that, the cells initially farthest from the growth factor source in E_{m-65} and E_{m-35} regions, respectively, interact mechanically with neighbouring cells and move collectively towards the growth factor source (Figure 4.13b, at $t = 1,600 \text{ s}$). At $t = 2,070 \text{ s}$ (Figure 4.13c), the cells initially located closest to the source, in the E_{m-35} region, reach the growth factor source. The cells farthest from the growth factor source form clusters and move as clusters towards the growth factor source. At $t = 3,300 \text{ s}$ (Figure 4.13d), the cell initially in the E_{m-65} region closest to the growth factor sources (i.e., the E_{m-65}/E_{m-35} interface) reaches the growth factor source. All cells reach the growth factor source at $t = 6,468 \text{ s}$ (Figure 4.13e). Once the cells reach the source, they continue to move around it randomly along the Y-Z plane (also see supplemental videos V4.13-3D and V4.13-2D).

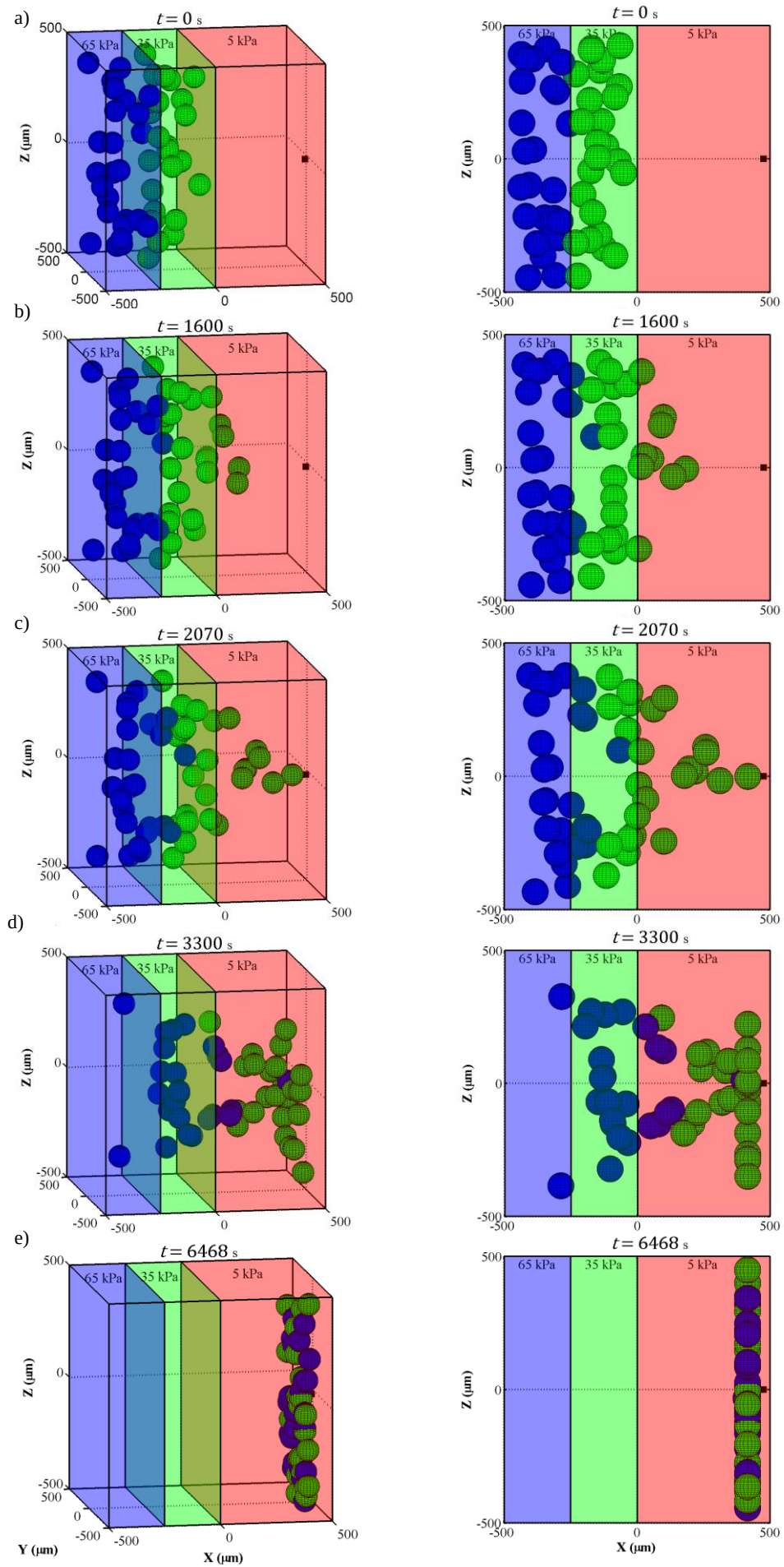


Figure 4.13. Chemo-mechanically induced collective cell migration. Positions of 50 cells (spheres) migrating with random perturbed motion towards a growth factor source (solid square) with a production rate $\Gamma = 8,000 \text{ mol}/\mu\text{m}^3\text{s}$ and a diffusion rate $D = 103 \mu\text{m}^2/\text{s}$ in the ECM with varying stiffness E_m . E_m changes along $X = -250 \mu\text{m}$ and $0 \mu\text{m}$ with $E_m = 65 \text{ kPa}$ for $X < -250 \mu\text{m}$ (left blue), $E_m = 35 \text{ kPa}$ for $-250 \mu\text{m} \leq X < 0 \mu\text{m}$ (middle green), and $E_m = 5 \text{ kPa}$ for $X \geq 0 \mu\text{m}$ (right red). a) Initial positions of the cells distributed randomly in the ECM region with $X < 0$ at $t = 0$ s, and the growth factor source is located in the ECM region with $X > 0$. b). The closest cells to the growth factor source in the middle region with $E_m = 35 \mu\text{m}$ start migrating towards the source. The cells in the left region with $E_m = 65 \mu\text{m}$ migrate towards the cells in the middle region with $E_m = 35 \mu\text{m}$. The cells in the left and middle regions interact mechanically with neighbouring cells and migrate collectively towards the growth factor source at $t = 1,600$ s. c) The closest cells in the middle ECM region reach the source at $t = 2,070$ s. The cells farthest from the growth factor form clusters and migrate towards the source. d) The first cell is initially located in the left region with $E_m = 65 \mu\text{m}$ reaching the growth factor source at $t = 3,300$ s. e) All cells reach the growth factor source at $t = 6,468$ s. Once the cells reach the growth factor, they keep migrating around it randomly along the Y-Z plane (also see supplemental videos V4.13-3D and V4.13-2D). (Model parameter values and sources are listed in Table 4.1 except for E_m values provided above in this caption are estimated from Buxboim *et al.* (2010a) and Oria *et al.* (2017)).

4.4 Discussion

The proposed model describes the migration of cells due to mechanical cues and a chemical signal in a three-dimensional (3D) ECM. The presented approach is different from previous studies (Camley and Rappel 2017, Fang *et al.* 2022, Giniūnaitė *et al.* 2020, Heck *et al.* 2015, Kim M-C *et al.* 2018, Mousavi *et al.* 2014b, Vittadello *et al.* 2018) in the sense that it considers the mechanical properties of the 3D microenvironment, cell-ECM adhesion, and cellular interaction with growth factor source. The initial goal of the developed model is to analyse the evolution of growth factor concentration gradient on cellular motion in ECM with spatially varying stiffness.

4.4.1 Mechanically induced cell migration in ECM with uniform stiffness

The developed model indicates the dependence of (i) the mechanical cell-cell interactions in the 3D ECM and (ii) the final stage of the collective cell migration on the initial cell positions. The 25 cells form one single group at the centre of the ECM as the final migration configuration (Figure 4.4), whereas the 50 cells form five discrete groups distributed in the ECM domain (Figure 4.5), the latter of which has been observed experimentally (Gong and Mills 2018) and computationally (Gonçalves and Garcia-Aznar 2021). The 25 and 50 cells were initially seeded in the computational ECM domain at different volumetric densities, i.e., 274 and 50 cells/ mm^3 , respectively. Hence, the prediction of a single versus multiple final clusters may be explained by (i) decreasing strength of

intercellular mechanical signals with increasing distance between the cells and (ii) weaker mechanical signals, i.e., deformation gradient in the ECM, amongst neighbouring groups than amongst neighbouring cells in each group (George M *et al.* 2017).

The model indicates that the migration duration is affected by the number of cells in a collective. The migration velocity decreases approximately two-fold for a two-fold increase in cell number (Figure 4.5 vs Figure 4.4). It is proposed that an increase in the number of mechanical interactions between cells in a collective increases the time required for the cells to migrate to their final destination and form final clusters in the ECM. This result is in qualitative agreement with numerical studies reporting that the increased number of cell interactions decreases the overall migration velocity (Ahmed *et al.* 2023, Mousavi *et al.* 2014a).

Even though the model results in different final cell cluster configurations for different simulations (Figure 4.4 and Figure 4.5), the overall migration direction with stochastic movement is the same for each simulation run. It is worth noting that cell aggregation in the centre of the initially seeded cell cloud (coinciding with the centre of the computational ECM domain) (Figure 4.4) agrees with previous computational studies on cell migration in environments with uniform stiffness (Chen J *et al.* 2018b, Mousavi *et al.* 2014b).

4.4.2 Mechanically induced cell migration in ECM with spatially varying stiffness

For collective cell migration in an ECM with two different stiffness regions, cells in the same ECM region initially migrate towards each other at a speed depending on the respective ECM stiffness before the cells in the soft ECM region migrate towards the stiff ECM region (Figure 4.6). It is argued that the mechano-sensing of the ECM regions with different stiffness is weaker than the mechanical signals received from other cells in the same ECM region, which has also been reported in previous numerical studies (Borau C. *et al.* 2011, Dudaie *et al.* 2015).

The cells in a sub-group in each ECM region aggregate in the ECM centre across the stiffness change, however, favouring the stiffer ECM region. Cells originating in the soft ECM region move partially into the stiff ECM region. In contrast, cells originating in the stiff ECM region do not cross the stiffness interface (Figure 4.6). This behaviour is in qualitative agreement with other numerical studies reporting that cells do not move from a stiff to a soft region (Borau C. *et al.* 2011, Dokukina and Gracheva 2010, Kim M-C *et al.* 2018).

For collective cell migration in an ECM with three regions of sequentially changing stiffness, the model predicts initial cell movement towards neighbouring cells, i.e., in the direction of the dominant mechanical cues from other cells compared to the mechanical cue from the ECM stiffness. Some cells detach from their groups and move from high ECM stiffness regions to low ECM stiffness regions (Figure 4.8). This is observed for cells near the stiffness changes and can be ascribed to a weaker cell-cell interaction than the cue based on the ECM stiffness changes. The overall migration is directed towards the stiffest ECM region. This behaviour is based on a stronger mechanotactic cue from the higher ECM stiffness than the ECM deformation caused by two neighbouring cells (Borau C. *et al.* 2011, Dudaie *et al.* 2015). Cell clusters in ECM regions with lower stiffness are attracted to the ECM regions with higher stiffness.

Collective cell migration in the three sequentially decreasing ECM stiffness regions leads to a substantially longer migration period of $t = 2,500$ s (Figure 4.8) to form a cluster at the higher ECM stiffness interface compared to the migration in the two ECM stiffness regions of $t = 810$ s (Figure 4.6). The migration duration increases approximately 3.1-fold for the three consecutively decreasing ECM regions (Figure 4.8) compared to the two ECM regions (Figure 4.6) for the same number of cells.

The migration of cells in the ECM with two non-sequential stiffness changes (stiff, soft, and intermediate) is directed towards the soft ECM region but may be distinguished into two steps relative to the mechanical signals from the surrounding ECM environment. Initially, cells in the soft middle region near the two stiffness interfaces move towards the stiffer and intermediate ECM regions (Figure 4.9) due to the dominance of the mechano-sensing cue from the ECM stiffness changes over the mechanical cues from neighbouring cells in the soft region. Cells start forming clusters at the stiff/soft and soft/intermediate ECM interfaces. At this stage, mechanical cues from the cells become dominant over the mechanotactic cues from the ECM stiffness changes, and the cell cluster at the soft/intermediate stiffness interface migrates towards and joins the cluster at the stiff/soft stiffness interface and completes the migration. However, the model predicted slight variations for the last migration stage with regard to the location of the final cell cluster (Figure 4.10). The cell clusters originating at the stiffness interfaces move into and join in the soft ECM region or at the soft/intermediate interface. A similar promotion of cellular interaction forces from stiff to soft ECM regions is observed experimentally by Isomursu *et al.* (2022).

The model results reveal several differences between cell migration behaviour in an ECM with two non-sequential stiffness changes and two sequential stiffness changes. Compared to migration in the ECM with two sequential stiffness changes (Figure 4.8), the non-sequential change in ECM

stiffness (Figure 4.9 and Figure 4.10) may accelerate the cell's arrival at their final destination, i.e., the overall migration velocity decreases due to the longer duration required to form the final cluster compared to the two sequential changes in ECM stiffness. The mechanical contact force among clusters may be greater than the detected effective mechanical cues from ECM stiffness regions. The migration velocity in the ECM with three sequential stiffness regions is approximately 2.5-fold lower (Figure 4.8) than in the ECM with three non-sequential stiffness regions (Figure 4.9).

4.4.3 Chemically and chemo-mechanically induced cell migration in ECM with uniform stiffness

For collective cell migration in the presence of a growth factor but without mechanical signals in an ECM with uniform stiffness, the proposed model predicts that cells only start migrating once they sense the chemical concentration gradient. The cells closest to the growth factor source start moving towards the growth factor source first, followed by cells with increasing distance to the growth factor source (Figure 4.11) as the strength of the growth factor gradient increases in the ECM over time (Whitehead *et al.* 2016).

The model results reveal several differences between cell migration in the presence of a growth factor only and migration induced by chemical and mechanical cues for a uniform ECM stiffness. The chemo-mechanically induced migration initially is towards neighbouring cells due to the dominance of the mechanical cues, i.e., ECM deformation gradient between two neighbouring cells, over the chemical signal from the growth factor source (Figure 4.12). With increasing growth factor release in the ECM over time, the chemical signal dominates the mechanical cues as the strength of the growth factor concentration gradient increases. Overall, cell migration is directed towards the growth factor source. Such migration behaviour has been reported in experimental studies (Tomasova *et al.* 2019, Whitehead *et al.* 2016).

The migration velocity towards the growth factor source for uniform ECM stiffness is approximately 1.5-fold slower for chemo-mechanical (Figure 4.12) than chemically induced cell migration (Figure 4.11). The mechanical interactions amongst the cells delay their arrival at the growth factor source, which is also reported by Ahmed *et al.* (2023).

4.4.4 Chemo-mechanically induced cell migration in ECM with spatially varying stiffness

The proposed model predicts that the collective cell migration in the presence of a growth factor and mechanical cues in an ECM with sequential stiffness change is directed towards the growth factor source located in the softest ECM region. This behaviour suggests that cells move from the stiff to the soft ECM region due to the dominance of the chemical signal from the growth factor source over the mechanical cues from neighbouring cells and based on the ECM stiffness changes (Figure 4.13).

Comparing the chemo-mechanically induced cell migration for uniform (Figure 4.12) and sequentially changing ECM stiffness (Figure 4.13) indicates that, in the latter case, the cell interactions in each of the different ECM stiffness regions increase the time to reach the final configuration at the growth factor source, i.e., decrease the overall migration velocity. The migration velocity towards the growth factor source for the chemo-mechanical cell migration is approximately 4.7-fold faster for uniform ECM stiffness (Figure 4.12) than two sequential ECM stiffness changes (Figure 4.13).

4.4.5 Limitations

The results generated with the developed model are in reasonable agreement with previously reported experimental data and computational studies for cell migration over complex environments. As the model for chemo-mechanically induced cell migration in a 3D ECM presented in this chapter uses algorithms for mechanically and chemically induced cell migration on planar substrates developed in the previous studies (described in Chapters 2 and 3), the limitations for these algorithms similarly apply here.

In addition, the model approach considers the ECM around the cells to be a smooth continuum, free of small-scale heterogeneities in the ECM, and the elasticity and porosity of the ECM are lumped into a single parameter. However, this will not change the current findings.

The model includes some simplifying assumptions, such as a spherical cell shape, whereas the cell needs to elongate during migration (Lees *et al.* 2015, Marzban *et al.* 2019). Also, the elasticity of the cells is considered constant during migration. However, these simplifications do not affect the conclusions of this work.

Even though the properties of the matrix governing cell-ECM interactions such as the molecular composition of the matrix (for example fibronectin, collagen, elastin), the concentration of matrix components (for example different densities of fibronectin or proteoglycans), matrix remodelling by forces or proteases, pore sizes in the ECM, and the ECM crosslinking have not been considered

in the current model. The complexity required to generate accurate simulations of *in vivo* or *ex vivo* studies will increase as more parameters need to be determined. However, the chemo-mechanical changes in the ECM are sufficient to observe a transition in the migration direction.

4.4.6 Recommendations

The same recommendations as in Chapters 2 and 3 apply to the current model. However, there are some additional recommendations for the chemo-mechanically induced cell migration in a 3D ECM.

It is possible to consider different and varying cell elasticity during the migration with some extensions in the presented model with adjustment to the numerical solution of the strain energy density function or by using different equations in future work.

The current model considers the ECM as linear elastic. This is not suitable for describing cell migration in all ECM types. The formalism is generic in this sense since nonlinear elasticity and variable plasticity can be incorporated into future work using different equations. However, essential features of the migration behaviour of collective cells based on two stimulus types, i.e., mechanical and chemical, in 3D microenvironments are captured.

The model results indicate that the effect of production and diffusion rates of the growth factor can be studied with spatially varying ECM stiffness. Extending the numerical approach for the chemical equation formalism, described in Eq. (4.16), will allow us to define non-linearity and anisotropic of the production and diffusion rates of the growth factor.

4.5 Conclusion

A computational model has been developed for collective cell migration induced by two cues, i.e., strain energy density gradients in the 3D ECM and a growth factor source. Cell migration is determined by the mechanical interactions between cells in the ECM, the mechanical properties of the ECM, the initial configuration of cell locations in the ECM, and a growth factor source. The ECM stiffness and chemical concentration gradient regulate the rate and directionality of the migration. The model provides qualitative results demonstrating collective cell migration in complex 3D environments with several cues.

5 Overall conclusions and recommendations

This research aimed to develop a mathematical model for chemo-mechanically induced collective cell migration in engineered extracellular matrices and demonstrate the predictive capabilities for the migration of individual cells in a collective based on the mechanical properties of the matrices and a growth factor gradient in extracellular matrices. The objectives of the research are repeated below for convenience to achieve the above-mentioned aim:

1. To develop a mathematical cellular mechano-sensing model on planar elastic substrates and demonstrate the predictive capabilities for the migration of cells in a collective on planar elastic substrates.
2. To extend the mathematical model for the mechanically induced cell migration on the planar elastic substrates to capture the effect of a growth factor gradient on the migration of individual cells in a collective.
3. To develop a mathematical cellular mechano-sensing model based on mechanical interactions between a cell and its surroundings in three-dimensional (3D) engineered matrices and illustrate the predictive capabilities for the migration of cells in a collective in the extracellular matrix (ECM).
4. To establish a mathematical model to describe and predict the effect of a growth factor gradient on collective cell migration in the 3D microenvironments.

The aim and objectives were all achieved, as described in detail in the preceding chapters. The current chapter summarises the main findings of this research and highlights the novel aspects of the research. The major recommendations to extend this research for future work are also summarised.

5.1 Mechanically induced migration of cells on planar elastic substrates (objective 1)

A mathematical model is developed in Chapter 2 for cellular mechano-sensing on planar elastic substrates. The prediction for the migration of individual cells in a colony on planar substrates is described. The model considers the mechanical cell-cell interactions mediated through the substrate due to perpendicular traction forces. The model formalism considers the adhesion friction coefficient of the cell-substrate interaction as a viscous force. Cell locomotion, division, and death are considered semi-random processes.

The deformation of an elastic substrate by a single cell, representing a mechanotactic cue for neighbour cells, decreases with increasing substrate elasticity and decreasing substrate thickness. It is observed that the mechanical interactions between cells in colonies (i) delay their arrival at the final location and (ii) increase the magnitude of the strain energy density and local velocity compared to the migration of two cells. The developed model predicts that early cell interactions and migration depend on the initial cell positions, but the final configuration of the cell migration does not.

The substrate elasticity and thickness regulate the rate, orientation, and directionality of the migration. The migration velocity increases for decreasing substrate elasticity and thickness. The migration of cells in a collective on a substrate with regions of different stiffness elasticity and thickness, respectively, is directed towards the stiffer substrate regions after the mechanotactic cue from the substrate stiffness overcomes the mechanical signal based on the substrate deformation gradient induced by neighbouring cells in the same substrate region. Additionally, it is observed that the overall migration velocity is slower on substrates with non-uniform than uniform elasticity and thickness.

Novel aspects: Previous computational models focused on capturing individual cell migration on the substrate governed by the mechanical communication of cells through substrate deformation due to traction forces. Other models additionally incorporate cell-cell interactions in cell aggregates to predict collective cell migration. The model presented in the current work improves upon the previous cellular mechano-sensing models as it considers the mechanical cell-cell interactions through substrate deformation and adhesion friction of the cell-substrate interaction. The model demonstrates capabilities to predict how changes in substrate elasticity and thickness guide the migration of individual cells in a colony.

5.2 Chemo-mechanically induced migration of cells on a planar substrate (objective 2)

Diffusion of a growth factor gradient on a planar substrate is combined with the mechanical model (described in Chapter 3) to simulate the migration of cells in collectives induced by two concurrent cues, i.e., of chemical and mechanical origin. The model considers the mechanical interactions between cells and interactions of cells with a growth factor source. The diffusion of the growth factor on the substrate is defined using the fundamental solution of Green's function. Stochastic processes are not considered in this model.

The production rate and diffusion rate of the growth factor regulate the rate and directionality of the migration. The time required for a single cell to migrate to the growth factor source decreases with increasing the growth factor production and diffusion rates. However, the maximum velocity of the cell towards the growth factor source increases with increasing production rate and decreasing diffusion rate of the growth factor.

The migration velocity for collective cells in the presence of a growth factor but in the absence of mechanotactic cues increases with increasing the growth factor production rate and diffusion rate. The cell migration is affected more by variation in the diffusion rate than the production rate of the growth factor.

The migration behaviour of collective cells due to combined chemical and mechanical cues is directed towards the growth factor source once the chemical cue dominates the mechanotactic cues, i.e., substrate deformation gradient of neighbouring cells. The developed model predicts slower collective cell migration to the growth factor source than single cell migration.

Novel aspects: This is the first study to investigate the effects of the growth factor production rate and diffusion rate on cell migration combined with mechanical cell-cell interactions. The growth factor production rate and diffusion rate are lumped into a single parameter in the experimental and computational studies, while the two parameters have different effects on the migration behaviour.

5.3 Chemo-mechanically induced migration of cells in 3D engineered matrices (objectives 3 and 4)

The models developed in Chapters 2 and 3 have been extended to predict the collective cell migration governed by the mechanical properties of extracellular matrices and a growth factor source in the 3D microenvironments (Chapter 4). The model considers the migration of cells induced by two stimulus types, i.e., chemical and mechanical.

The cellular mechano-sensing model is based on the strain energy density gradients in the ECM originating from cellular traction forces. The model formalism considers the adhesion friction coefficient of the cell-ECM interaction as a viscous force. Cell locomotion is considered a semi-random process.

The developed model predicts the dependence of (i) the mechanical cell-cell interactions in the 3D ECM and (ii) the final stage of the collective cell migration on the initial cell positions. The migration velocity decreases with increasing cell number due to increased mechanical cell-cell interactions.

The model predicts that the rate, orientation, and directionality of the cell migration depend on the ECM stiffness (i.e., the elastic modulus of the ECM). Migration of cells due to mechanical cellular interactions in the ECM with two and three regions of sequential and non-sequential differences in the elastic modulus is studied. The overall migration in an ECM with two and three sequentially different stiffness regions is directed towards the stiffest ECM region once the mechanical cue from the ECM stiffness dominates the mechanical cues from other cells in the same region. However, the overall migration in the ECM with three non-sequential stiffness regions (i.e., stiff, soft, and intermediate) is directed towards the soft ECM region. The developed model predicts an increase in the migration velocity for decreasing ECM stiffness. The collective cell migration is faster in an ECM with two stiffness regions than three sequentially different stiffness regions. Also, the migration for three non-sequential ECM stiffness regions is faster than for three sequential stiffness regions.

The chemical model is monitored using the fundamental solution of Green's function. In addition to the ECM stiffness, the growth factor regulates the rate, orientation, and directionality of the cell migration. The migration velocity towards the growth factor source for a uniform ECM stiffness is observed to be slower for the chemo-mechanical than chemically induced cell migration. The mechanical interactions amongst the cells delay their arrival at the growth factor source. The

chemo-mechanically induced cell migration is studied with three sequential ECM stiffness regions. The overall migration is directed towards the growth factor source located in the softest ECM region. The model predicts a decrease in the overall migration velocity for the chemo-mechanically induced cell migration with the sequentially ECM stiffness regions compared to the uniform stiffness.

Novel aspects: This is the first mathematical model to investigate the effects of the mechanical properties of the cellular microenvironment, cell-ECM adhesion, and growth factor gradients on the migration of collective cells in a 3D cellular environment. This model allows for predicting the migration behaviour of individual cells in a colony under different conditions and environmental properties, which is an additional novelty.

5.4 Recommendations

The models can also be extended to include different cell types that exert different traction forces and exhibit different cell shapes. Further, different cell elasticity can be considered, and the cell elasticity can be varied during the migration through adjustments to the solutions of the strain energy density functions or using different equations. Cell-cell junctions and repulsive forces between cells resulting from cells impinging on each other have not been considered in the current models and offer another avenue for extension.

The developed models consider the substrate (in Chapters 2 and 3) and the 3D ECM (in Chapter 4) to be linearly elastic. Linear elasticity has limited suitability for describing cell migration in physiological cellular environments. The formalism employed is generic and allows for incorporating nonlinear elasticity and variable plasticity using different equations.

The models consider the adhesion friction interaction between cells and the environment, i.e., cell-substrate and cell-ECM interactions, as a viscous force in a one-dimension. The formalism is generic and allows for incorporating the other adhesion interactions using different equations in future work depending on the cell phenotypes.

Extending the mechano-sensing models to include gradual stiffness changes, i.e., gradients, in the cellular environment, i.e., 2D substrate or 3D ECM, offers capabilities to represent *in vitro* and *in vivo* conditions more closely.

Extensions of the chemical part of the models may include alternative shapes of the chemical source and multiple chemical sources that emit signals with different concentration rates. The

chemical sources can be immobile (as in the current work) or mobile and the concentration gradient can be soluble or non-soluble. Such extensions can be implemented by adjusting the numerical solution of the diffusion equation by using convolution reach with the Green function. A reaction term derives from the chemical signal secreted by external sources such as bacteria. Extending the numerical approach for the chemical equations formalism also allows for defining non-linearity and anisotropy of the production and diffusion rates of the growth factor.

A further extension to the chemo-mechanically induced cell migration model is the variation of production and diffusion rates of the growth factor with spatial ECM stiffness gradients.

The simulation results, in particular for Chapter 4, need to be compared to experimental data to assess the validity of the current models in future research. Extensions and potential adjustments of the current models might be required to validate the model's predictions.

Such extensions will expand the model to predict different processes involving chemo-mechanically induced cell migration, for example:

1. Evoked wound closure with the necessary chemotactic ability.
2. Infection of wounds with bacteria that trigger the release of lactates (chemicals), which suppress cell migration.
3. Response of immune cells to viral infections by allowing various phenotypes to find their viral prey.
4. Initiation of tumours through the spread of cells and the response of the immune system to cancer growth.
5. Utilisation of drugs and other modalities of treatment to inhibit disease development.
6. Muscular growth (hypertrophy) as a result of exercises to increase muscle mass.

Publication plan for this PhD thesis

One manuscript reporting on the study described in Chapter 2 has been published as:

- Ahmed RK, Abdalrahman T, Davies NH, Vermolen F, Franz T. Mathematical model of mechano-sensing and mechanically induced collective motility of cells on planar elastic substrates. *Biomech Model Mechanobiol*, 2023, 22(3), 809-24. <https://www.ncbi.nlm.nih.gov/pubmed/36814004/>.

Two manuscripts reporting on the studies described in Chapters 3 and 4 are planned:

- Ahmed RK, Abdalrahman T, Davies NH, Vermolen F, Franz T. Mathematical model of chemical-sensing and chemo-mechanically induced collective migration of cells on planar substrates. Target journal: *Biomechanics and Modeling in Mechanobiology*.
- Ahmed RK, Abdalrahman T, Davies NH, Vermolen F, Franz T. Mathematical model of chemo-mechanically induced collective motility of cells in three-dimension extracellular matrices. Target journal: *PLOS Computational Biology*.

Data availability

The video files of the cell migration simulations presented in the results sections of Chapters 2, 3 and 4 are available on the University of Cape Town's institutional data repository (ZivaHub) as *Ahmed R, Abdalrahman T, Davies NH, Vermolen F, Franz T (2024). Video files for PhD thesis by Ribam Ahmed titled Mathematical modelling of growth factor-induced cell migration in 3D engineered matrices. University of Cape Town, Media, <https://doi.org/10.25375/uct.24588114> from 1 January 2025 or upon request.*

The custom MATLAB code files of the three mathematical models supporting this thesis can be accessed on the University of Cape Town's institutional data repository (ZivaHub) as *Ahmed R,*

Abdalrahman T, Davies NH, Vermolen F, Franz T (2024). Software code for PhD thesis by Riham Ahmed titled Mathematical modelling of growth factor-induced cell migration in 3D engineered matrices. University of Cape Town, <https://doi.org/10.25375/uct.24586425> from 1 January 2025 or upon request.

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