KU LEUVEN

FACULTEIT INGENIEURSWETENSCHAPPEN

Where shall I go? The mechanosensing adventures of a computational single cell

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Thesis submitted for the degree of
Master of Science in
Biomedical Engineering

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Academic year 2018 – 2019

Acknowledgements

These last five months have been short but intense. We could think that after finishing the Bachelor's degree and passing all the master's courses, to write a master's thesis would be a piece of cake; but we would be wrong. And the difficulty does actually not lie in the complexity of carrying through with a thesis itself, but with all the stress that our subconscious, consciously or not, put on our shoulders. Indeed, to complete a master's thesis is a good task to test ourselves. That is why that, even if I wanted, I would not be able to measure all the things that I have learnt both this last semester as well as last year, nor all the happiness that this area of science has brought me -and this is for real, I am not kidding in here! For this reason, I would like to thank some people who have helped me to get here.

In first place, I want to thank my parents for giving me the option of getting such a good education. Even in the toughest times, they supported me to not give up and go ahead. This can be spread out to my sister, who is always ready to pass on her happiness, and the rest of the family in general, who always believed in me.

In second place, I would like to thank my supervisor Diego A. Vargas. I could not have expected a more attentive, encouraging and patient mentor to guide me through this master's thesis. I also thank my promoter, professor Hans Van Oosterwyck, and the rest of the group of Mechanobiology and Tissue Engineering group at KU Leuven. Their enthusiasm for keeping learning new things and improving day by day is contagious. Next, I want to thank the KU Leuven for hosting me as an Erasmus student and thus giving me the opportunity to do this work. Finally, special thanks to the Flemish Supercomputer Centre (VSC) for giving support to my simulations.

In third place, my sincere gratitude goes to the University of Zaragoza and the Master's degree in Biomedical Engineering. I do not lie when I say that thanks to this master's studies I have found my true vocation in life. Applied mathematics becomes much interesting when they are together with biomedicine and you feel like you can contribute your bit to the world with something you actually enjoy. In this regard, I would like to thank specially Mª Ángeles Pérez Ansón, coordinator of this master's degree as well as a very good mentor. Also, I want to thank both José Manuel García Aznar and Mª José Gómez Benito, professors of the Mechanobiology course and hence the first ones introducing me to this topic.

And last but not least, I am very grateful to my boyfriend, Jurgen Kersschot, who is the reason for exchanging Spain's sunny skies for Belgium's grey ones -so that must be love- and whose support has also guided me along this path like a lighthouse in the night.

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Abstract

Cell adhesion and migration play an essential role in processes within the human body such as embryogenesis, tissue regeneration or cancer. Thus, to fully understand the behaviour of the mechanisms that regulate them would be a big step in biomedicine. In recent years, computational models have been postulated as firm candidates in terms of cellular research, since they constitute a very powerful tool complementary to traditional *in vitro* research: thanks to them, we are able to analyze what is happening within the cell even at subcellular level. In this work, we used a computational model to approach such cellular processes by studying the mechanical stimuli that govern the interaction of a cell with its environment. In particular, our interest resided in analyzing how the cell exerts traction through its actomyosin stress fibers by sensing the substrate stiffness, which is known as mechanosensing; as a consequence, the cell is deformed and this allows cell migration. To mimic some biological functions regarding cell-matrix adhesions, Bell's model and fiber maturation were implemented in the computational model. From the results we obtained after running some simulations, it is shown that there are many factors that influence cell traction. For instance, the total amount of focal adhesions at a certain time determines the number of fibers exerting force at the same time, which is translated as a higher force. Also, if those focal adhesions are able to live longer, there are more fibers coexisting. Substrate stiffness also plays an important role: as stiffness increases, stress fibers mature further and thus exert higher forces on the substrate; in addition, it also determines the size of the contact interface between the cell and the substrate. All in all, computational methods give quantitative and qualitative data with a lot of detail; hence, further research in this line is indeed a big step forward.

Preface

«That brain of mine is something more than merely mortal, as time will show.»
Ada Lovelace (and she was right!)

According to an East Asian belief, a red thread of fate connects two people who are destined lovers, regardless of place, time or circumstances. I want to believe that, in a similar way, biology and mathematics have been always connected since the beginning of time: waiting patiently for someone brave enough to take the risk of studying them together, so they can meet one more time and receive each other like an old good friend.

Leonardo of Pisa (c. 1170 - c. 1250), better known as Fibonacci, was one of these brave people. Starting with the growth of rabbits poulation through different generations, he proposed a sequence of numbers who became the Fibonacci numbers. Together with the golden ratio, this Italian mathematician tried to understand the surrounding nature with mathematical tools. As far as we know, this could be the first attempt of meeting between our two lovers in this little story.

Some centuries later, Daniel Bernoulli (1700 - 1782), Swiss mathematician and member of the distinguished Bernoulli family, gave them another encounter when his study about the smallpox mortality and its relationship with the efficacy of vaccination was published in 1766.

Following this attempt, mathematics and biology got finally together forever and ever during the first years of the 20th century. William Hamer and Ronald Ross with their studies about epidemic behaviour, the epidemic model of Kermack & McKendrick... a new era had started. But let us go back in time for a moment.

When some years earlier Ada Lovelace (1815 - 1852), English mathematician, published in 1843 the so-considered first algorithm in history, she could not know that she was opening the door to a whole new world of possibilities. Well, we know that they say: love is an open door. It is indeed for this biology-mathematics relationship. I wonder what would have happened if she, one of our main representatives of women in science, had lived longer. Or if she could have worked with some other scientists.

Considering these hypotheses, I would like to imagine the scenario in which Ada Lovelace met Theodor Schwann and Matthias Jakob Schleiden, founders of the cell theory, since they three were coetaneous. With the ideas of the former -Ada thought that the analytical engine designed by her mentor, Charles Babbage, was meant to do more than simple calculations- and the knowledge about the cell of the latters, would they have ever imagined their legacy? A computarized study of biological processes!

As a mathematician, I was also attracted by this whole biology-mathematics relationship. That is why, if I had a time-turner, I would like to invite all of these scientists from the past to share a cup of tea with us in the current time. I wonder if they would be amazed, satisfied or proud as a parent is of their children's success. I wonder what they would say. I wonder about the honour of meeting them, since they inspired new generations of scientists to get what we want to get, to become what we want to become: truthful mathematicians, engineers, physicists, physicians... scientists all in all, in search of understanding life and the whole world.

Laura Lafuente Gracia

Chapter 1

Introduction

Along this master's thesis we are going to study an episode of the mechanosensing adventures of a computational single cell. This leads us then to some questions, starting with *what is mechanosensing?*, going through with *why do we want to study it as a computational problem?* and ending with *why is it interesting and how can it help the world?*, among others.

As the preface has introduced, the relationship between mathematics and biology started long ago because there was a good reason for it. As a student, I was delighted about this topic and wanted to study it further. Having said that, how are computational models and the study of cells specifically related?

In this first chapter we will go through these issues, trying to answer some main questions and setting a goal for this master's thesis. Some biological concepts are also presented, so that we start with a solid background.

1.1 Motivation

A cell is the smallest unit of life in a living organism, as well as our protagonist in this work. Besides, a cell is close to be a living organism itself: they are born and they can die, they feed, they breath, they reproduce... and, to do these things, sometimes it is advantageous to migrate to other territories and explore their surroundings. That is the start of our journey: cell migration.

Cell migration, together with cell adhesion, is a complex process that plays an essential role in embryogenesis, wound healing, tissue regeneration, angiogenesis, metastasis and tumor invasion (see figure 1.1). Regarding the last ones, we know that they are involved in cancer: when the cells invade the surrounding tissue (that is indeed *tumor invasion*), the tumor is said to be malignant and then is considered a cancer [1]. This cell invasion includes a period of migration in which the cancerous cell leaves the tumour before entering blood or lymphatic vessels. To detect this invasiveness is a key element since secondary tumors,

the so-called metastases, can be generated at other areas of the body.

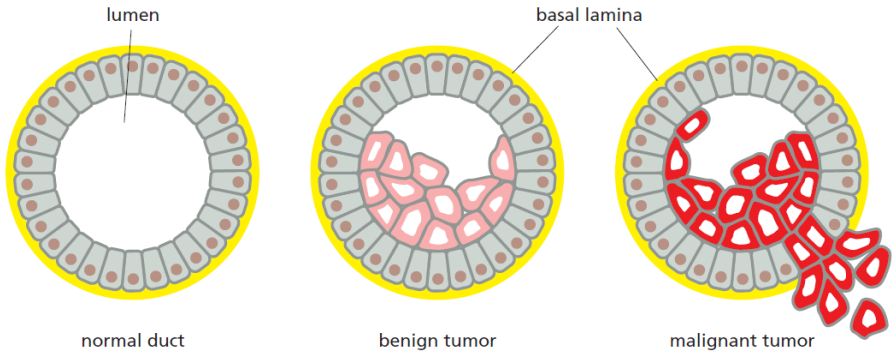

FIGURE 1.1: Benign tumor vs. malignant tumor. Cell migration occurs in the latter one. From [1], Fig.20-3.

In fact, the word «cancer» is an important matter in medical research. Nevertheless, it is estimated that about one in five of us will die of cancer [1]. Therefore, to develop a computational model that helps to understand the behaviour of cell migration would be indeed something very interesting to do. But what are the main aspects that guide this cell migration? Is it directed by any kind of mechanism or stimulus? Of course it is! In fact, the interaction of a cell with its environment is governed by electrical, chemical and mechanical phenomena. In this work, our interest lies in this last one.

Therefore the motivation is clear: understanding cell migration would help to understand better some events that take place in the body, which play a fundamental role when studying diseases that are still waiting for a cure, such as cancer.

I truly believe it is worth doing such a study, so let us do it! Let us try to contribute our bit with this work.

1.2 Cell mechanics

General literature: [1] (ch. 16, 19 & 20), [2] (ch. 8 & 10), [3]

As we have said, our protagonist -the cell- is a very small unit of life: its size is measured using the micrometre scale (μm). This means that, in general words, it is going to be hard to see what happens at a (sub-)cellular level. Therefore, when we want to study the cell and its migration (and the processes involved), the μ-size can lead us to a problem: that by experimental methods it is hard to measure important parameters like e.g. force.

There exist, of course, some different techniques, like the atomic force microscopy (AFM), the laser optical tweezer (LOT) or the parallel microplates [4], [5], among others,

that work with no problem and are widely used to measure and study cells *in vitro*. However, these techniques sometimes may not be good enough, mainly because they require a lot of time (the cells first need to be cultivated, then they will be measured in different time steps) and also they are hard to apply in *in vivo* like conditions. Besides, if we want to get a wide range of nice results, we need to reproduce the experiment with the same conditions and that is not always possible or easy. Not to forget that the experiment will be performed with one specific type of cell. So if we want to study another one, the whole process must be repeated all over again.

Until now. Because now we also have computational methods, which are a powerful technique that comes to solve some of these problems or worries. Meaning that our experiments go from *in vitro* to *in silico*, opening the door to a full new world of possibilities (well, this sounds familiar -thanks, Ada Lovelace!). But this is not going to be easy either. Luckily, the cell has been studied for long time, so there exist a lot of models and approaches to mimic or analyze its behaviour.

Now then, before modelling a cell, we need to know about the basis of cellular mechanics. Because, once more, the phenomena that will encourage our cell to migrate will be mechanical: mechanosensing!

1.2.1 Cytoskeleton and viscoelastic behaviour

The cell is a viscoelastic material, meaning that its behaviour depends on time, and this is mainly determined by the cytoskeleton [6]. It also presents a pre-stressed network structure [7] that will be our starting point in the model.

The cytoskeleton (CSK) is one of the main constituents for cell mechanics, together with the plasma membrane, the nucleus and the cytoplasm. The CSK is the skeleton of the cell and provides a cell with mechanical support: it changes the cell shape, coordinates the cell movements, exerts mechanical forces and senses the extracellular matrix (ECM). This is indeed the most important part for us, because we want to tell about this kind of adventures: the mechanosening ones!

The CSK consists of a biopolymer network of three major components that are linked by plectin: filamentous actin (F-actin), intermediate filaments and microtubules, being the F-actin the main constituent of the CSK. Therefore we will focus on the study of these F-actin microfilaments, because they will help us to analyze how the cell senses the extracellular environment (that is, the ECM) through the CSK.

1.2.2 Cell adhesions: how the cell senses the ECM through the CSK

As we were saying, one of the functions of the CSK of the cell is to sense the ECM. This allows the cell to have interactions with the environment, by forming cell-matrix adhesions.

What is a cell-matrix adhesion? A cell-matrix adhesion is a protein complex that

makes the interaction between the cell and the ECM possible. It is generated when an actin filament (intracellular CSK attachment) is connected to integrin (transmembrane adhesion protein) via talin (intracellular anchor protein), as shown in figure 1.2. At the same time, integrin is bound to an ECM protein, e.g. fibronectin.

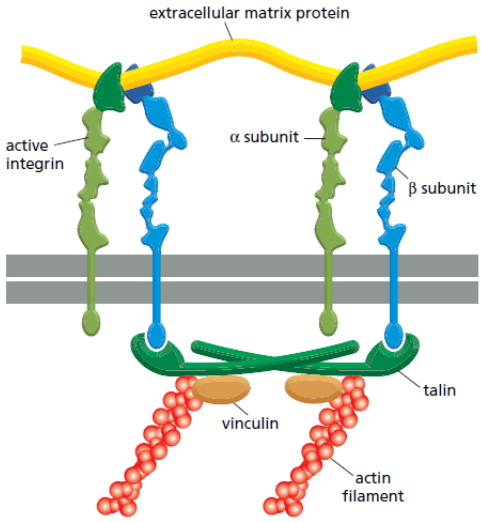

FIGURE 1.2: Cell-matrix adhesion. Note that integrins consist on two subunits, α and β. From [1], Fig.19-45.

The cell-matrix adhesions formed by integrins may be small and weak or large and durable [1]. When the actin filaments have enough time to create strong attachments, they become the so-called focal adhesions (FA) and then the cell is able to attach to the substrate. Thereby, integrins attach the cell CSK to the ECM and sense whether the cell-matrix adhesion has taken place, by transmiting stresses.

In relation to these stresses, it is reported that the higher the applied force is, the more integrin binds to talin; consequently, the focal adhesions acquire an elongated shape [8], as we will see now.

Are there different types of cell-matrix adhesions? Yes. As we just said, the cell-matrix adhesions can be transient or durable; what is translated into different types of adhesions. Accordingly, we find four types: nascent, mature, fibrillar and rear adhesions [9], which are shown in figure 1.3. Our interest lies in the mature adhesions, the ones that are in a growing state.

Indeed, the mature adhesions correspond to the previously mentioned FA. They evolve slowly over time and are linked to actomyosin stress fibers, that is, the actin-myosin complex that forms within the cytoskeleton. Actomyosin fibers are contractile due to the myosin protein, which acts like a sub-cellular motor pulling on actin filaments; as a

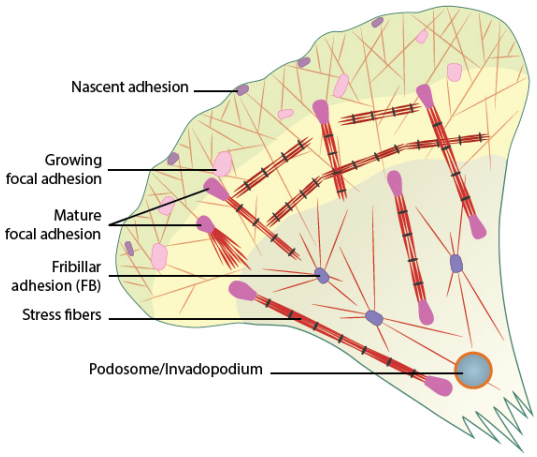

FIGURE 1.3: Cell-matrix adhesion complexes. From [10], *What are cell-matrix adhesions?*

consequence, cell motility and force generation are enabled [10].

Hence, this means that in the cell-matrix adhesions: (1) the FA transmit mechanical forces bidirectionally inside-outside the cell and vice versa, and (2) both FA proteins and actin filaments work as mechanosensors [11].

Therefore, we conclude that the cell senses the ECM through the CSK, that is, there is mechanosensing. In addition, mechanical signals are converted into chemical signals, thus intracellular pathways are activated and then there is also mechanotransduction.

1.2.3 How does the cell migrate?

Cell polarization. When mechanosening occurs, the cell becomes polarized, meaning that it chooses a direction of migration by placing the CSK at the front and the nucleus at the rear. This becomes the beginning of cell migration.

We define two methods of cell migration (figure 1.4): random migration, where the cell follows a Brownian-like motion, or directed migration, where the cell responses according to different stimuli: chemical stimuli (chemotaxis), stiffness gradient (durotaxis), gradient of adhesion sites (haptotaxis), etc.

In our computational model, cell polarization is predefined such that migration direction is determined. Once this is set, cell migration is guided by mechanosensing of the substrate stiffness: the stiffness of the ECM will be a parameter that will be changed in order to study different situations.

Migration cycle. Once the cell is polarized, the migration cycle starts. In short, what happens is that there is cell contraction, then the cell pushes and consequently the back part

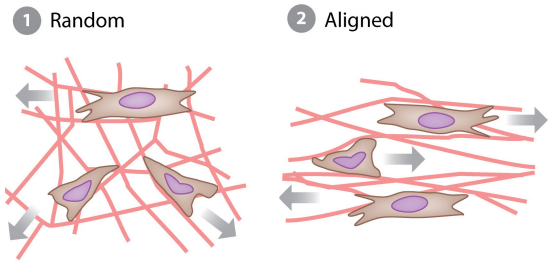

FIGURE 1.4: Random vs. directed migration. Adapted from [12], Fig. 3.

is detached. A very nice schematic shows this in figure 1.5. But in a slightly more extended way, we find four steps in the migration cycle:

1. **Protusion:** cell polarization and protusion of the lamellipodia due to actin polymerization in the front.

2. **Attachment:** adhesion to the substrate via integrins, the cell adhesions maturate and FA are created, which causes lamellipodia formation.

3. **ECM remodelling:** contraction of the cytoplasm due to traction force, with the consequent detachment of rear adhesions.

4. **Release:** rear retraction and cell displacement toward the direction of cell migration.

What are lamellipodia and filopodia? When we talk about the migration cycle, we can distinguish two areas of the cell: the leading edge moving forward and the trailing edge following the main bulk of the cell. In relation with this, lamellipodia and filopodia are extensions at the leading edge. The difference between them resides in that whereas lamellipodia are flat, wide extensions containing actin networks, filopodia are finger-like and contain cross-linked actin bundles [2]. As a result, the leading edge will be thinner and stiffer. In our computational model, both lamellipodia and filopodia can be observed.

What regulates the migration mode? [12] Finally regarding cell migration, some regulators of migration mode are reported in the literature, as shown in figure 1.6.

First of all, cells can move either **individually or collectively**. Individual migration is mediated by CSK activity, however it is very limited since there are no interactions with any surrounding cells. On the other hand, collective migration uses cohesive groups of cells who coordinate their CSK activity via their cell-cell junctions. As a result, the movement is more effective when the cells move in groups. In this work, a single cell is considered, thus we will have individual migration.

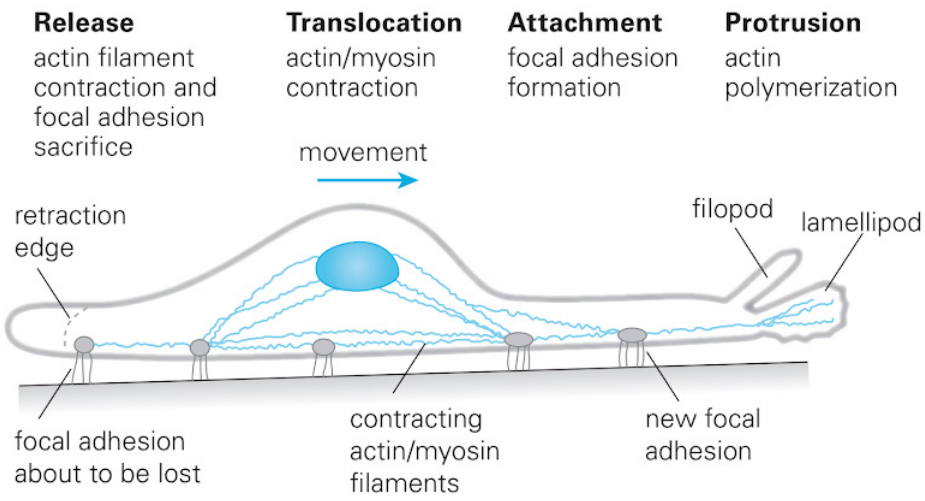

FIGURE 1.5: Major steps in cell migration, where the cell migrates toward the right. From [2], Fig. 10-15.

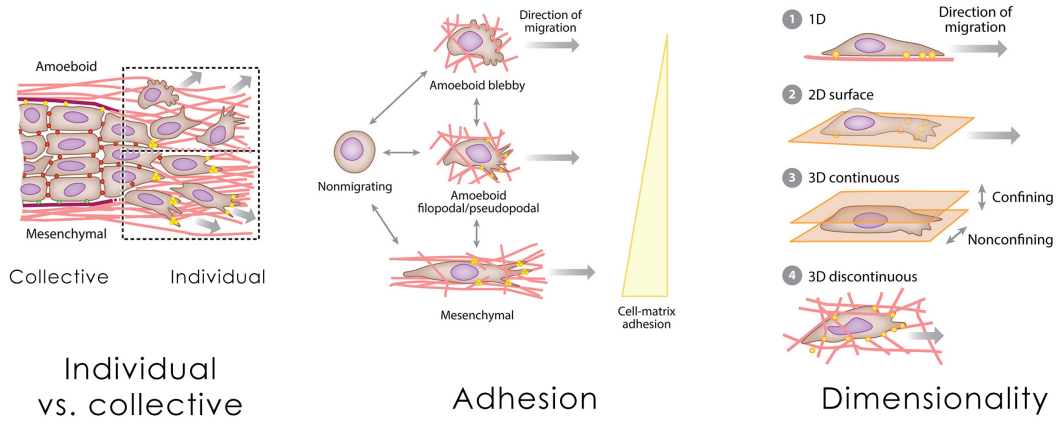

FIGURE 1.6: Regulators in cell migration. Adapted from [12], Fig. 1-3-7.

In second place, **cell adhesion** is defined by the CSK organization and vice versa, since the CSK defines how cells move, as stated above. When we have defined the migration cycle in the previous page, we were talking about mesenchymal movement of the cell. Nonetheless, single-cell migration can also follow an amoeboid movement. The difference between both modes resides in the strength of the cell-matrix adhesions: strong, mature adhesions (FA) lead to mesenchymal movement, whereas weak, nonadhesive interactions results in amoeboid-like movement. *Mesenchymal migration* generates an elongated cell shape, due to cell polarization with protusions at the leading edge (e.g.

lamellipodia), and traction force. Besides, it is followed not only by position change but also by tissue remodelling, deposition of ECM, maintenance and repair during migration. On the other hand, *amoeboid movement* tends to bring on a roundish cell shape whose leading edge is highly dynamic since it only reaches to generate pseudopodia, i.e. temporary filopodia. It is followed by a limited ability to remodel tissue while migrating, but also by a capacity of readily crossing membrane barriers.

Lastly, there are also some **tissue determinants** like the dimensions, topography, space and organization of the tissue or substrate in which cells are placed. In response to this, migrating cells generate deformation of the cell body. Besides, «cells moving with moderate to high adhesion force and contractility pull and deform ECM meshworks, including fibrillar fibrin and collagen» [12], meaning that the substrate in which cells are placed can also be deformed by them. In the computational model we are going to use, a flat surface is presented as a substrate; therefore, migration will take place across a 2D surface. This means that, as stated in [12], «because of infinite lateral space, cells can spread, can form a broad leading lamellipod gliding along the substrate and can freely change direction». However, our computational single-cell will be predefined to migrate only in one direction in order to restrict variability in the simulations. The cell will be placed at one end of a rectangular substrate and move towards the other end.

1.2.4 Computational model vs. traditional *in vitro* experiments

Now that we have refreshed some cell mechanics knowledge, we can go back to our main issue: modelling a cell. The idea is to prove how powerful the computational methods are and why they can really help us in research. Note that is not a war against *in vitro* experimentation, but a perfect complement to get better results.

When modelling a cell *in silico*, some general considerations can be taken. Firstly, the viscoelastic behaviour of the cell is easy to reproduce by using a spring-damper system. Also, the cell presents a pre-stressed network structure. In terms of our model, this means that, before studying anything else, we must first find this state: the so-called equilibrium state. For us this is the first step out of two when modelling the cell behaviour, it corresponds to the cell spreading part and was already solved by Odenthal et al. [6]. So we will focus on the second step: what happens after the cell has spread.

Secondly, regarding to our initial worries, time is not a problem anymore with a computational model: now time is only a number that can be set or changed depending on how long we want to carry out the experiment. Besides, with a computational model we have easy reproducibility while maintaining stochasticity. As we will see in next chapter, both the creation and lifetime of FA are given by probabilities in our model. Therefore, we can generate a new experiment only by sending a new job of the model. The conditions of the experiment will stay the same as long as we keep the same values of the parameters, but the outcome can differ due to the effects of stochastic processes in the simulation. In case we want to change these values, we will get another set of conditions -which will be great to analyze how the experiment changes when changing some characteristics! This

means that we can also redefine the type of cell we want to simulate: by changing e.g. its Young's modulus or Poisson's ratio, we could study another type of cell. Keep in mind that in this work a generic, non-specific cell will be used.

Thirdly, with a computational model we are able to *see* what is happening. By saving variable values at each simulated time step, such as position and force of particles making up the simulated cell, we can both visualize cellular deformation and obtain quantitative values for every point in the cell at any point in the simulation. This means that we will be able to study both qualitative and quantitative the behaviour of our single-cell, whereas with an *in vitro* experiment it would be hardly possible. Together with the previous stated point, we could see, for instance, when there is a transition from amoeboid movement to mesenchymal migration.

All of these reasons should be new motivation for our work, as well as should make us realize of this powerful technique that computational methods constitute. As I said before, I truly believe that this kind of study is completely worth -now even more!

1.3 Research goal and outline of the thesis

Finally, to summarize after this first introductory chapter, what do we want to get with this work and how are we going to do it?

1.3.1 Goal: what do we want to do?

The main goal of this master's thesis is to gain insight into the effects of mechanosensing in migration while accounting for the effects of mechanical properties of the cell, which leads to cell deformation, and the substrate. This has been extensively studied *in vitro* [15], also by the Mechanobiology and Tissue Engineering group at KU Leuven [16]. Now, we want to reproduce this experiment but *in silico*, i.e. with a computational model.

1.3.2 Outline: how are we going to do it?

First of all, as we have already presented, some biological concepts were necessary to understand the underlying nature of the problem. As scientists, it is fundamental that we know the reason why we are doing such a study and the behaviour of the element to study.

Furthermore, the starting point of this work lies in the work of Diego A. Vargas, i.e. my supervisor. From the model presented in [6] by Odenthal et al., he developed a model of the migrating cell with two fronts, which mimic the leading edge and the trailing edge during cell migration.

The basic idea of the computational model, as well as the model formulation, is found in chapter 2. Multiple simulations are carried out to explore scenarios corresponding to different conditions and their outcomes, along with their analysis, are presented in chapter 3. Finally, some conclusions and future prospects are shown in chapter 4.

Chapter 2

Description of the model

Since the mechanosensing adventures we want to narrate are the ones that a computational cell lives, we have to introduce now the computational model that we are going to use. Therefore, in this second chapter the basic ideas to understand how the model works and which mathematical principles it uses are given.

2.1 Basic idea: spring model and Hooke's law

According to the Oxford Dictionary [14], a *spring* is «an elastic device (...) that can be pressed or pulled but returns to its former shape when released, used chiefly to exert constant tension or absorb movement». In other words, this means that a spring is an elastic body and, as a consequence, it can recover its original shape after being deformed by a force.

For small deformations, linear elasticity can be considered and thus described by Hooke's law:

$$F = k \cdot x \tag{2.1}$$

where $k \equiv$ spring constant, i.e. stiffness of the spring and $x \equiv$ length or displacement of the spring from its initial position.

Sometimes Hooke's law is also written as

$$F = -k \cdot x \tag{2.2}$$

if we want to talk about the restoring force, which is the opposite to the force that caused deformation.

This linear elasticity can be also described as a linear relation between stress σ (force applied per unit area) and strain ε (measure of the deformation), whose definition is as follows:

$$\sigma = \frac{F}{A} \quad \text{and} \quad \varepsilon = \frac{\Delta L}{L} \tag{2.3}$$

Now, by the definition of the modulus of elasticity E or Young's modulus,

$$E = \frac{stress}{strain} = \frac{\sigma}{\varepsilon} \tag{2.4}$$

linear elasticity is defined. By using the definitions in (2.3), this expression is indeed equivalent to the one given by Hooke's law in (2.1):

$$E = \frac{\sigma}{\varepsilon} \overset{\sigma}{=} \frac{F}{A} \cdot \frac{1}{\varepsilon} \overset{\varepsilon}{=} \frac{F}{A} \cdot \frac{L}{\Delta L} \implies F = E \cdot A \cdot \frac{\Delta L}{L} = \frac{A \cdot E}{L} \cdot \Delta L = k \cdot x \tag{2.5}$$

Where the corresponding units are:

$$E = \left[\frac{N}{m^2}\right] \qquad k = \left[\frac{N}{m}\right] \qquad x = [m]$$

With these concepts in mind, let us go back to our main issue here: a single-cell placed on a substrate. That is what we want to model.

According to the regulators of migration explained in section 1.2.3, both the cell body and the substrate can suffer deformability during cell migration. Since the cell is able to migrate thanks to the focal adhesions (FA) that are attached to the substrate (ECM), let us take this particular interaction to simplify our problem: the interface FA-ECM. Both of them will suffer small deformations during cell migration, therefore they can be modelled as molecular springs (with linear elasticity) connected in a series configuration. Either if we want to reproduce an *in vitro* experiment, where the ECM would be inside a petri dish or culture flask, or an *in vivo* experiment, where the ECM would be the body itself, the substrate will be bound to something immovable. In this way, the spring representing the ECM will be fixed at one of its ends and connected to the spring representing the FA by its other end. This situation is represented in figure 2.1.

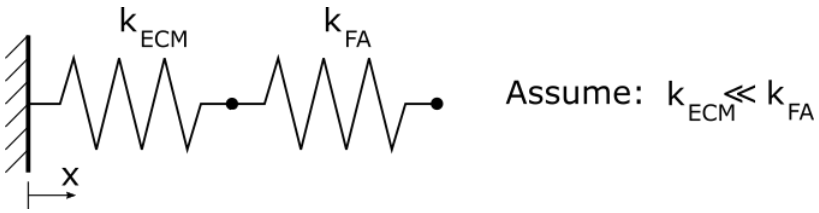

FIGURE 2.1: Spring system representing the basic idea of the interaction FA - substrate during cell migration. $k_{FA} >> k_{ECM}$ is assumed.

Thus, both cell adhesions and substrate are modelled as Hookean springs. Since we consider mesenchymal movement of the cell, the adhesions represent strong, mature FA; resulting in a cell moving with moderate to high adhesion force which can deform the

ECM. Therefore, we take the next assumption:

$$k_{FA} >> k_{ECM} \tag{2.6}$$

With this assumption, when a force is applied and pulls the spring system, the FA spring will suffer a small displacement ΔL, whereas the ECM spring will suffer a larger ΔL. When different substrate stiffnesses are considered, the softer the substrate is, the larger the displacement ΔL will be, since it will be easier to deform; as depicted in figure 2.2.

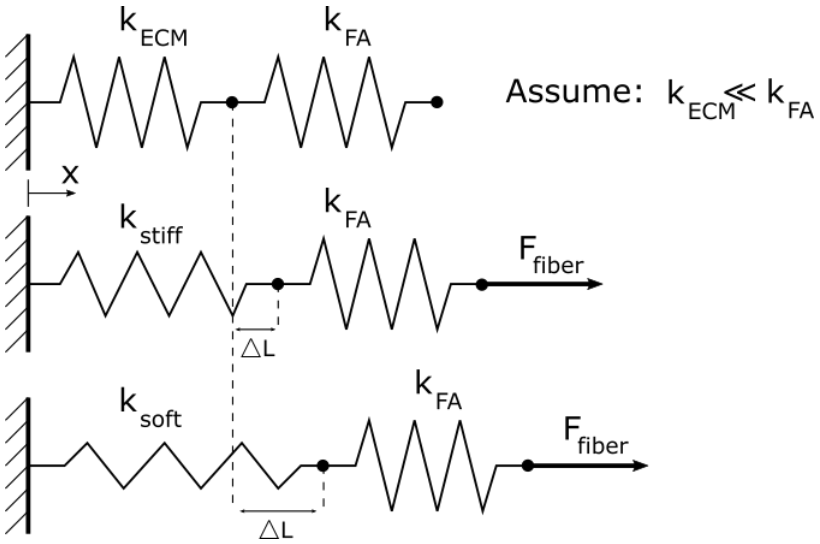

FIGURE 2.2: Spring system representing deformable ECM and FA connecting a node of the cell boundary with a substrate plane. Image shows the effect of deformation by application of cytoskeletal force (F_{fiber}) via a stress fiber. Different deformations are expected from the same force for different stiffness values of the substrate (kECM).

To finish, note that the force is exerted by the actomyosin stress fibers, which, as explained in 1.2.2, enable cell motility and force generation when pulling on.

2.2 Computational model

Recapitulating, our aim is to reproduce cellular tractions through a computational model to study cell deformation and how the cell exerts traction over the substrate through the actomyosin fibers and, with it, analyze the effect of substrate stiffness (sensed through mechanosensing) on cell migration.

In other words, we want to model different interactions within the cellular environment. Thus, a discrete mathematical model can be used, e.g. an agent-based model. This kind of method is very helpful in cellular mechanics, where we have μ-size components to study,

large data sets and the reproducibility of the experiments is an important factor. The main characteristic of a discrete computational model is its ability to describe elements in the subcellullar scale (e.g. proteins), giving a quantitative analysis and showing their evolution along time. On the other hand, its main disadvantage is its high computational cost, which will make our simulations longer than desired.

Agent-based models can be divided in categories, depending on whether a lattice is considered or not. Our interest lies in a deformable cell model (DCM), which uses a lattice-free method. With this type of model, «mechanical stresses in the cell can be computed to subcellular level, cell shape is explicitly given and complex deformations can be straightforward represented» [17]. In addition, «due to solving an equation of motion, the time scale is well defined» [17]. Deformable cell models can also incorporate models for specific adhesion, e.g. focal adhesions at lamellipodia, and non-specific adhesion, e.g. proteins generating cell adhesions along the whole cell.

All together, it sounds like something very big and complex. But for a better understanding of such a model, some general considerations can be taken. In this way, we can deconstruct the model in three pieces or levels, as shown in figure 2.3, that will be next studied separately:

1. Cellular level: cortex of the cell *(in 2.2.1)*
2. Substrate: ECM where the cell is placed *(in 2.2.2)*
3. Subcellular level: focal adhesions and actomyosin stress fibers, i.e. the responsible parties for cell migration, which we want to study *(in 2.2.3)*

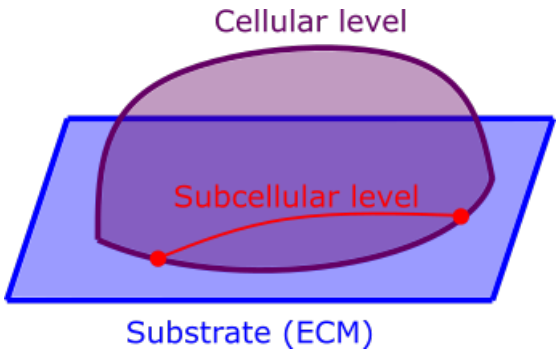

FIGURE 2.3: Deconstruction of the computational model in three levels: cellular (cortex), substrate (ECM) and subcellular (FA and fibers).

Thanks to this computational model, we will be able to assess with a lot of accuracy e.g. the lifetime of FA, their maximum force or rupture, as well as cell traction or speed.

Finally, before going into more detail, let us remark that after building our computational model and getting the corresponding results with the simulations, the model

must be validated. Since our model uses as starting point the one described by Odenthal et al., the validation of the cortex model can be found in [6].

2.2.1 Cellular level: cortex

At the cellular level, the deformable cell model presented by [6] is used. This model is intended to describe the behaviour of red blood cells (RBCs); however, in our assumptions a non-specific cell is considered. Since the cell has a viscoelastic behaviour, the cell cortex «in DCM is discretized by a number of nodes which are connected by viscoelastic elements» [17]. In addition to this, the model describes the mechanical interactions taking place at the cell boundary in contact with the substrate (ECM in our case). A lattice-free, particle-based method is used to calculate interaction forces within such contact interface, as well as the resulting movement based on the equation of motion. Therefore, the key novelty presented by this model is that there is a special emphasis on the interaction between the cell and its environment, not only on the general mechanical behaviour of the cell. To keep the local curvature of the cell mesh in contact with its environment, the surface of the cortex of the cell is triangulated and then the contact mechanics are calculated by applying Maugis-Dugdale theory [6].

The cortex nodes interact through viscoelastic potentials. Each viscoelastic potential can be simplified by a simple spring-damper system, which is shown in figure 2.4, and the equation of motion for node i is:

$$\sum \vec{F}_i = m_{node} \cdot \vec{a}_i \tag{2.7}$$

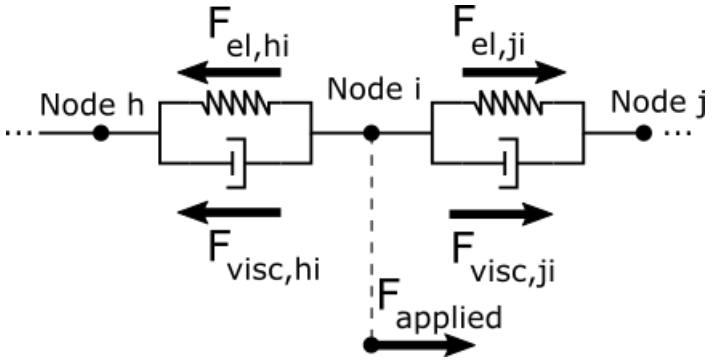

FIGURE 2.4: Schematic representing the internodal spring-damper systems, which can be used to explain the equation of motion for viscoelastic behaviour.

Now, the environment in which cells live is determined by a low Reynolds number, then cell motion is dominated by viscous forces and inertial forces are not taken into account. Therefore, the equation of motion is now:

$$\sum \vec{F}_i = m_{node} \cdot \vec{a}_i = 0 \tag{2.8}$$

Since the expression for the damper is $F_{visc} = c \cdot v$ and the one for the spring is given by Hooke's law,

$$\sum \vec{F}_i = 0 \implies F_{applied} - (F_{el,hi} + F_{visc,hi}) + (F_{el,ji} + F_{visc,ji}) = 0 \tag{2.9}$$

$$\implies F_{applied} = k \cdot (x_{hi} - x_{ji}) + c \cdot (v_{hi} - v_{ji}) \tag{2.10}$$

Where $F_{applied}$ represents all the external forces applied on the node, including the force sensed by the cell through the focal adhesions and applied through the stress fibers.

Putting all the nodal equations together and taking into account conservation of cell surface and volume, due to the limited compressibility of the cell cortex, we get the global matrix equation:

$$\underline{F} = \underline{\Gamma} \cdot \underline{v} \tag{2.11}$$

At each time-step the position of the nodes is updated by solving this equation by a forward Euler integration scheme. Further details can be found in [6].

2.2.2 Substrate

In our model, the stiffness of the substrate (kECM) can vary. The interaction of the substrate with the cell can be simply explained by the two-springs system stated in section 2.1, which is better illustrated in figure 2.5.

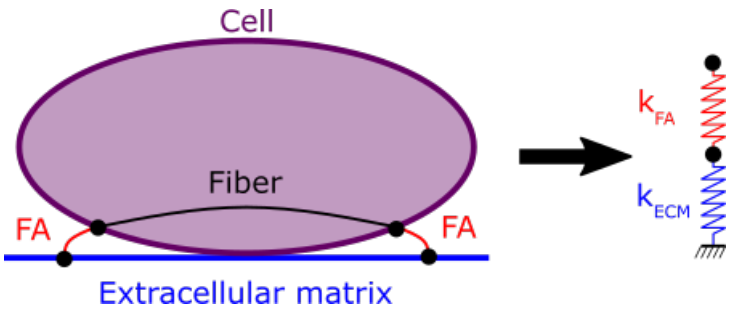

FIGURE 2.5: Schematic overview of the interaction cell-substrate, where both the focal adhesions of the cell and the extracellular matrix are considered Hookean springs. Thus, the interface FA-ECM is a spring system in series configuration.

Following the triangulation used in the cortex model, the surface of the substrate will be also triangulated. Therefore, we can have the desired interaction between the cell and its environment. This can be seen in figure 2.6.

Note that, according to figure 2.6, the substrate surface has two well diferentiated parts: an inner rectangle in which the cell is placed (in red) and an outer edge surrounding it (in blue). The former is the interesting one for the model and corresponds to the ECM. That

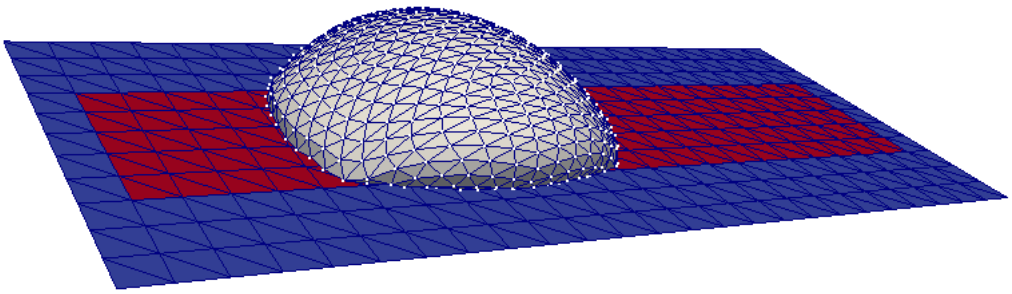

FIGURE 2.6: Visualization of the computational model, where the triangulated surface of both the cell (in white) and the substrate (in blue and red) can be seen.

is, if our model was an *in vitro* experiment, the inner rectangle would be e.g. a collagen gel and the outer edge a petri dish. Therefore, when the substrate stiffness is modified, it only varies in this inner rectangle. Also, the cell can only create adhesions in this inner part, since there will be no interactions between the cell and the substrate in the outer edge.

Finally, the cell is located at one of the ends of the substrate at the beginning of the simulation and the model sets a greater probability to migrate towards the opposite side. This aims to get some stability during the migration of the cell when running the simulation, which will help us for a better analysis of the results.

2.2.3 Subcellular level

At the subcellular level we find the focal adhesions and stress fibers, hence this is where most of the effort was spent in implementation to be able to model cell migration.

How are adhesions created? Adhesions cannot be generated all over the cell-substrate interface, but only at the cellular fronts in areas behind the edges corresponding to a cellular lamella, i.e. leading edge and trailing edge. That is, we use a two-fronts cell. Now, how are these adhesions created by the model? The process is described and illustrated (figure 2.7) below.

1. The center of mass of the cell nodes in contact with the substrate is obtained
2. The vectors $-\vec{e_x} = (-1,0,0)$ and $\vec{e_x} = (+1,0,0)$ are defined with their origin in the center of mass, pointing to the trailing and leading edge of the cell respectively
3. An angle $\pm\alpha/2$ is taken with respect to each vector, thereby defining an area on each front

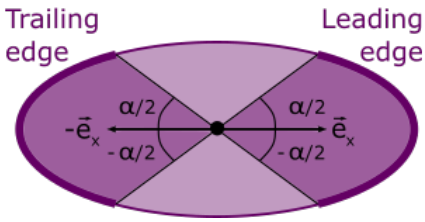

FIGURE 2.7: Schematic showing the process followed by the model in order to create cell-matrix adhesions

4. Nodes within the fronts can form an adhesion with a point on the substrate plane based on a probability and if they are within a cut-off distance. The point on the substrate is determined by projecting the cellular node on the surface. This point must be within the area of the substrate plane with the ECM (figure 2.6).

Consequently, there will be only focal adhesions at the two fronts of the cell. Thus, the two-fronts single cell is well defined.

How are the fibers created? After focal adhesions (FA) are randomly generated in each cell front, stress fibers are created. These fibers will connect two FA, each one from a different edge, meaning that there will be $FA/2$ stress fibers.

How do adhesions disappear? Since focal adhesions are mature cell-matrix adhesions, they will have a natural lifetime. But they can also break if they are subjected to too much force by the stress fibers, detaching the cell from the substrate. This gives some parameters to consider.

The lifetime of focal adhesions is a parameter that we want to study. In the literature, some authors [18, 19] have established that mature adhesions of fibroblasts live more than 1500 seconds [18] in a collagen culture and between 20 and 40 minutes [19] when fibrinogen is used. Inspired by this, we will take a range of different lifetime values. Since our interest resides in a stochastic model, we will take the probability of the focal adhesions to disappear at every second of the simulation, instead of simply taking a constant value which would give us a fixed lifetime. Such probabilities will be obtained by using the survival function of the exponential distribution. Besides, focal adhesions will become stronger as they maturate and this will be implemented in our computational model by using Bell's model [20]. The mathematical nature of these concepts is later explained in 2.3.1.

Regarding the stress fibers, fiber maturation due to mechanosensing stimuli can be taken into consideration. The main idea is that when a fiber reaches equilibrium, it is not able to deform the substrate anymore; hence, it is reinforced, being able to exert more force again, pulling on the focal adhesions. This rigidity sensing has been described by some authors [21, 22]. What it is about and how we are going to implement it, following Hooke's law, is explained in 2.3.2. To be sure that focal adhesions do not break due to the force exerted by a single stress fiber, a rupture force parameter, i.e. a force threshold above the maximum force exerted by this single stress fiber, is defined for the focal adhesions. In this way, we make sure that focal adhesions disappear due to their lifetime and not because of the fiber force.

With these considerations, the subcellular level in the computational model will look like figure 2.8.

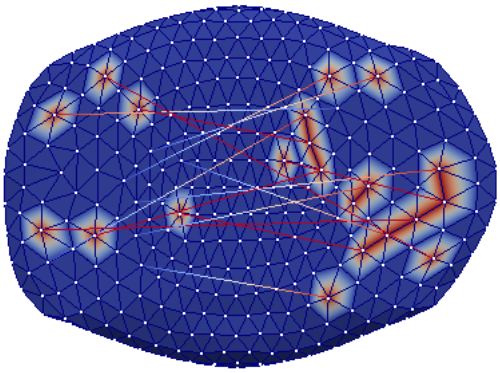

FIGURE 2.8: Visualization of the computational model at the subcellular level seen from below at a certain timestep. Red coloured areas correspond to focal adhesions, which may form adhesion complexes, whereas straight lines represent the stress fibers.

2.3 Mathematical model

Now that we have seen the different parts in which the model can be deconstructed, let us study with further detail the mathematical models that are implemented to study the lifetime of the focal adhesions and the maturation or reinforcement of the stress fibers, since they are known biological mechanisms of mechanosensing.

2.3.1 Bell's model

In 1978, Bell's model [20] introduced specific, reversible binding of the adhesion molecules by focusing on the dynamics of one adhesion, either cell-cell or cell-matrix type. Besides, it was the first model for cell adhesion taking into account spatial limitations, like e.g. membrane diffusion, by modelling the interactions between adhesion molecules according to the enzyme kinetics models, e.g. Michaelis-menten kinetics equation for enzymatic reactions. In this way, two rates of formation (k_{on}) and dissolution (k_{off}) of adhesion complexes were proposed, under the assumption that the encounter complex is transient and thus it only exists in small concentration. With these statements, Bell provided an equation which gave «a theoretical description of the temporal evolution of adhesion between cells with a homogeneous distribution and a constant number of adhesion molecules», as well as «an equilibrium concentration of cell-cell adhesions depending on binding dynamics» [4]. Note that this was postulated for cell-cell adhesions, but it works as well for cell-matrix interactions.

In addition, and here comes the most interesting part for our model, Bell stated that to separate cell-cell or cell-matrix adhesions, a force is needed. This means that the dissociation of a focal adhesion would be not only determined by its lifetime, but also by an external force to which it would be subjected. However, due to this force, focal

adhesions would be able to maturate according to the force they sense through mechanosensing and, thereby, their lifetime might vary. And that is exactly what we are going to implement in our model.

The idea is to model how focal adhesions mature with force: since more force is applied in order to dissociate the cell-matrix adhesion, the focal adhesion maturates due the mechanosensing of this force and thus it is reinforced, making the rupture event less likely, i.e. decreasing the probability of fiber rupture. This drop in probability is applied as an exponential factor.

How do we apply it? From the kinetic theory of the strength of solids, Bell applied to his equation for adhesion dynamics the following function for the lifetime of a bond depending on the force applied on it:

$$\tau(f) = \tau_0 exp[-(\gamma f - E_0)/kT] \tag{2.12}$$

Where $f \equiv$ force applied on the bond, $\tau_0 \equiv$ natural lifetime of the bond and E_0, kT, γ are enzymatic reaction-related parameters non-relevant for our computational model.

Since we do not use the same dynamics, because we model an adhesion complex rather than simply an integrin-ligand interaction, our parameters are likely different. Besides, we are considering the probability of a focal adhesion to disappear after a given lifetime instead of a simple lifetime value. Therefore, inspired by function 2.12, we take the following adapted expression to describe the probability P of a focal adhesion (FA) to live a determined lifetime depending on the force applied on such FA (i.e., the force of the cell-matrix adhesion):

$$P(F) = P_0 exp(-zF/F_{threshold}) \tag{2.13}$$

such that:

$P_0 =$ natural probability of FA lifetime
$z =$ drop in rupture probability
$F =$ force of the cell-matrix adhesion
$F_{threshold} =$ maximum force that FA can withstand before rupture

Note that P_0 is calculated according to the survival function of the exponential distribution, as shown below, and both z and $F_{threshold}$ are set in the simulation.

How do we calculate the natural lifetime of focal adhesions? As said before, we will not use simple lifetime values, but probabilities of focal adhesions to live during a determined time and disappear afterwards. For this purpose, we will use the survival

function $S(t)$ given by the exponential distribution of probability. This function estimates the probability to survive beyond a specified time. In our case, we have rather the opposite event, $1-S(t) =: Y \equiv$ the probability of a focal adhesion to dissociate after a certain time.

By definition,

$$\begin{aligned} S(t) = 1 - F(t) \quad &\Longrightarrow \quad Y = 1 - S(t) = 1 - (1 - F(t)) = F(t) \\ Y \sim Exp\left(\lambda = \tfrac{1}{E[X]}\right) \quad &\Longleftrightarrow \quad f(t) = \lambda \mathrm{e}^{-\lambda \mathrm{t}} \; \forall t \geq 0 \\ &\Longleftrightarrow \quad F(t) = 1 - \mathrm{e}^{-\lambda \mathrm{t}}, \; F(t) \in [0,1] \\ \text{For t = 1 second:} \quad &\quad F(1) = 1 - \mathrm{e}^{-\lambda} \end{aligned} \tag{2.14}$$

Where $f(t)$ and $F(t)$ are respectively the density and the distribution function of the exponential distribution and $E[X]$ is the average expected lifetime of adhesions we want to study. Also, note that we take $t = 1$ because the probability is taken at every second of the simulation.

Therefore, to calculate the corresponding probability for every lifetime value, we will only need to evaluate the following expression:

$$F(lifetime) \equiv F(t = 1) = 1 - \mathrm{e}^{\frac{-1}{lifetime}} \equiv P_0 \tag{2.15}$$

2.3.2 Mechanosensing: fiber maturation

The external force experienced by the focal adhesions and thus taken into account by Bell's model is the force that fibers exert, as shown in figure 2.2. However, this force is not going to be a constant, as we are about to see.

The cell senses the rigidity, i.e. stiffness, of the substrate by applying forces to it through the cell-matrix adhesions. Some authors even conjectured that «integrins provide a rigidity sensing mechanism through their binding and unbinding rates» [21], meaning that this sensing could be indeed related to the formation and dissolution of adhesion complexes. In any case, due to this mechanosensing of forces, stress fiber maturation occurs.

What is fiber maturation? Biologically, as stress fibers contract due to the action of the myosin motors, once no further contraction occurs, a pause is observed. During this pause, recruitment of proteins in the focal adhesion follows and recruitment of more myosin molecules in the fibers is hypothesized. After this, a stress fiber can exert higher forces and the substrate is further deformed [22]. We aim to recreate this step growth in force exerted by stress fibers.

How is fiber maturation implemented? When a stress fiber exerts force, eventually equilibrium is reached between the fiber force and the pull-back force of the spring system which represents the cell-matrix adhesions. When this happens, the stress fiber is reinforced

and then increases its pulling force. This reinforcement of the fiber is implemented in our model as a multiplicative factor n, as shown in figure 2.9.

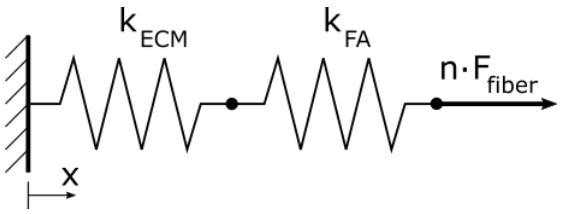

FIGURE 2.9: Spring system where fiber maturation is considered. As a result, the stress fiber is reinforced by using a multiplicative factor.

As we have already seen in section 2.1, a soft substrate is easier to deform than a stiff substrate when the same force is applied, according to Hooke's law. As a consequence, the spring representing the substrate suffers a larger displacement in the former case, which means that the time to reach equilibrium will be longer than for the latter case. For this reason, also the spring representing the focal adhesion will become fully stretched (i.e. reaching equilibrium) later in soft substrates (since our assumption is $kECM << kFA$), meaning that its length may not vary so easily. Following this reasoning, a stress fiber is earlier reinforced in stiff substrates; therefore, it can exert again more force in shorter time when compared with soft substrates. This situation is shown in figure 2.10.

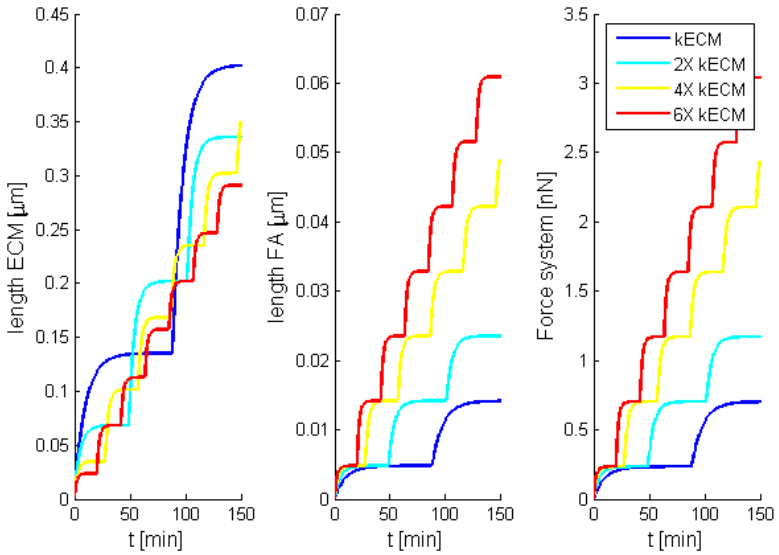

FIGURE 2.10: Substrate stiffness determines maturation rate. In time higher stiffness results in a higher exerted force but similar substrate deformation, as observed in experiments [22]. Figure courtesy of Diego A. Vargas (2018)

Thereby, it is concluded that substrate rigidity regulates mechanosensing, which makes this phenomenon a very interesting one to consider when modelling our computational model. In addition, «what determines rigidity sensing is the number of steps taken before reaching a (...) force level, which activates adhesion reinforcement» [22]. This is important, because the higher the multiplicative factor n is, the more the fiber is reinforced and, consequently, the higher the force pulling on the focal adhesions is, being able to cause focal adhesions to break, causing unbinding of the cell-matrix adhesions.

In our model, fiber reinforcement is allowed to happen five times and focal adhesion rupture happens when a force threshold is exceeded, defined as it follows:

$$F_{threshold} \gtrapprox 5 \cdot F_{fiber} \tag{2.16}$$

Where F_{fiber} is the force before maturation starts. Which means that when a stress fiber is completely reinforced, the force it can exert will be close to the force that a focal adhesion can hold. But when the fiber force surpasses the rupture threshold, the focal adhesion will not be able to manage it and it will break.

Finally, note that in the model the fiber is only reinforced when no change in the length of the spring representing the focal adhesion is detected.

Chapter 3

Results and discussion

With the computational model described in chapter 2, a total of 240 simulations have been run. There was a wide range of possibilities to study different combinations of parameters, but, at the end, we have mainly focused on studying the influence of the substrate stiffness *(kECM)* -since we want to study cell mechanosensing- and the lifetime of focal adhesions *(FA lifetime)*. For this purpose, the chosen values have been the following:

- kECM = {2.5e-4, 5.0e-4, 1.0e-3, 2.5e-3, 5.0e-3, 1.0e-2} N/m
- FA lifetime = {18, 31.5, 45, 58.5, 72} minutes

With what we get thirty different combinations. Each kECM-FA lifetime combination has been simulated eight times with a duration of 48 hours. Note that this would be equivalent to a total of 240 *in vitro* experiments. However, only one computational model and a few changes of parameters are necessary... Well, computational models are a powerful technique indeed!

3.1 Post-processing

The results obtained with the simulations are divided into two groups: qualitative and quantitative. The qualitative results have been obtained and analyzed by using Paraview, where we have been able to study how the computational cell advanced along the substrate. This has helped us to observe how the cells corresponding to the different conditions differ from each other after 48 hours. For obvious reasons, the generated videos cannot be shown here, but they will be shown during the final presentation of this work. On the other hand, a post-processing of the data has been performed in MATLAB in order to get the quantitative results of our simulations. These are the results that are presented below in this chapter.

To analyze numerically the results of the simulation, we have selected the most relevant output parameters. Each output is interpreted and depicted as five average charts

with standard deviation of the mean (confidence interval at 5%), in which the last 8 hours of the simulation have been taken as a representative sample.

Why do we consider the last 8 hours? We have taken the last 2, 8, 16 and 24 hours of one of the sets of simulations, specifically that of *FA lifetime = 18 minutes*. By visual inspection of the results, we see that between 8, 16 and 24 hours there is not too much variation. The case of the last 2 hours does vary a little more, because there is variability that would average out for longer periods of time, so we discard it as a representative sample of the entire data set. Since the last 8/16/24 hours show practically similar results, it is accepted that the last 8 hours are a representative sample for the last 16 and 24 hours. In this way, we also avoid that fluctuations along time can cover other results, e.g. those that happen due to rupture events.

Now, is it also a representative sample for the entire population of the data? At the beginning the data are transient, because all simulations are initialized in the same state, hence it takes some time before the difference between conditions becomes evident and a certain stability is reached. For this reason, the first steps of time are discarded, thus obtaining a biased data set. For this biased set, the last 8 hours we are taking are representative; and, after all, it is what interests us since it is where we have the stability in cellular migration of the model. Of course, if we wanted to compute a proper mathematical sensitivy analysis to see if the sample is representative or not according to the statistical significance, we could use e.g. an statistical hypothesis testing for the means. In any case, the visual inspection of the data confirms that the last 8 hours are a representative sample.

Let us now move on to the study of the selected outputs.

3.2 Quantitative results

Number of FA

This first parameter will determine in a strong way the behaviour of all other outputs, since it is the number of focal adhesions (FA) and, as a consequence, an approximation of the number of stress fibers that we can find in the computational cell. The results are shown in figure 3.1.

From the Y-axis, coexistence of FA according to lifetime is deduced: there are more coexisting adhesions when their lifetime is longer, which shows how a longer lifetime also means more time to reach equilibrium in ECM displacement leading to stress fiber maturation and higher force which in turn, due to Bell's model, stabilizes the adhesions. This is quite obvious, because new FA adhesions can be generated while the «old» ones are still alive due to their longer lifetime. However, there seems to be an exception at the highest stiffness values for the two longest lifetimes: there are less FA than for the previous cases. This can be attributed to the fiber rupture that happens in these conditions, as we will see later.

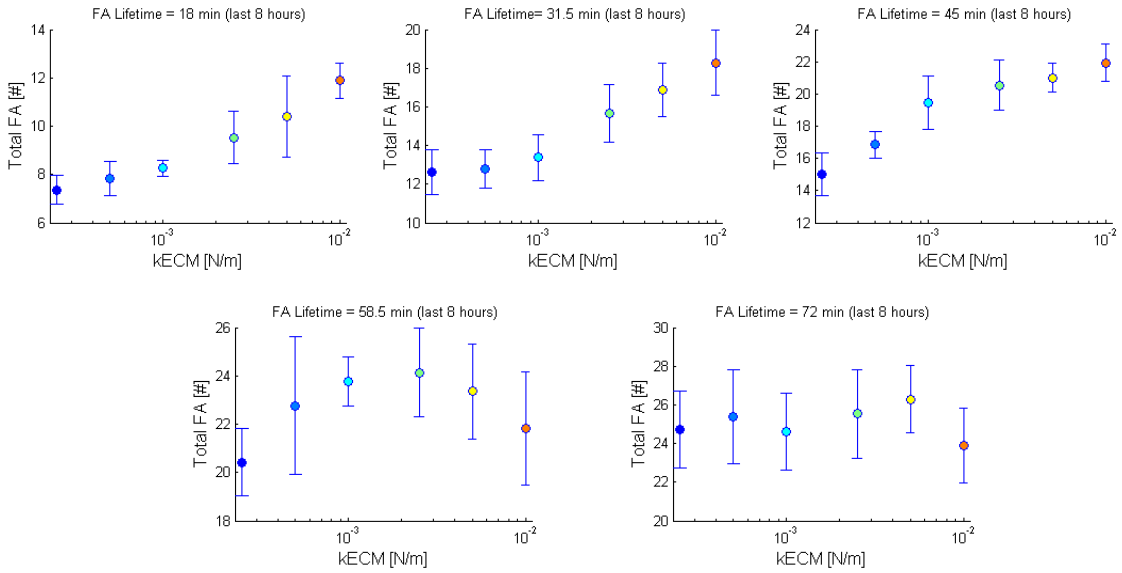

FIGURE 3.1: Number of FA depicted for five different lifetimes of the FA and six different values of kECM.

Average FA life

Average FA refers to the average lifetime (given in minutes) of all coexisting focal adhesions at each time step of the simulation. The results are shown in figure 3.2.

It is interesting to show the effect of mechanosensing according to substrate stiffness, as we wanted to study, and the results concurred with Bell's model, explained in subsection 2.3.1. This also tells us that the results are as expected from having implemented Bell's model: in soft substrates, the FA lifetime is quite similar to the initial value given, indicating that the force applied by the stress fibers is insufficient to stabilize the FA. But as stiffness increases, the probability of FA to disappear decreases exponentially and, as a consequence, their average lifetime quickly rises.

However, FA show a similar average lifetime in stiffer substrates. Actually, we can see that FA live shorter in the substrate with the highest stiffness, in comparison with the previous cases. Again, this may be attributed to fiber rupture due to fiber maturation: since fiber reinforcement becomes more frequent (see figure 3.3), more and more fibers break (see figure 3.6), which results in a shorter average lifetime.

In any case, it is clear that, due to Bell's model, the probability of FA dissociation drops and, consequently, adhesions live longer than their expected natural lifetime. Besides, since a fiber is deleted in the model as soon as both FA that it connects are disassembled, then also the lifetime of the stress fibers are defined indirectly by the FA lifetime.

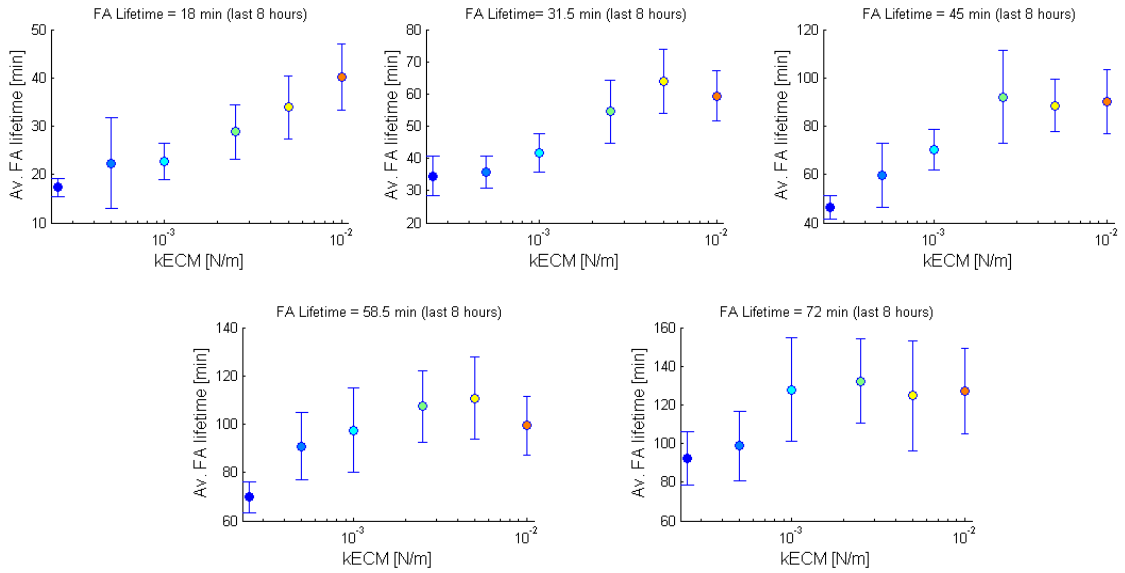

FIGURE 3.2: Average FA life depicted for five different lifetimes of the FA and six different values of kECM.

Fiber maturation: multiplicative factor

The multiplicative factor $n \cdot F_{fiber}$ shows how many times, until the maximum of five, the fiber has been reinforced; meaning that the more it is reinforced, the more force is exerted. In another way, we could interpret this output as a description of how forces evolve in our spring system. The results are shown in figure 3.3.

As explained for the fiber maturation model in subsection 2.3.2, a stress fiber is earlier reinforced in stiff substrates. Therefore, greater multiplicative factors can be achieved in stiffer substrates; as the X-axis shows. This is due to the mechanosensing of the substrate rigidity by the cell, as we were expecting.

On the other hand, if we take a look at the Y-axis we see that also greater multiplicative factors are reached when the FA lifetime is longer, meaning that their reinforcement will be greater. Due to the fact that FA live during more time, they also have more time to be reinforced, because it will take longer for the FA to disappear. Thus, stress fibers keep pulling on the kECM-FA spring system until equilibrium is reached and fiber maturation, i.e. reinforcement, takes place.

Besides these general trends, no difference in fiber maturation on low stiffness substrates is observed in the cases of short FA lifetimes (18, 31.5 *min*). This indicates that we find a minimum lifetime of FA required for mechanosensing to play a role in cellular tractions. This is confirmed by figure 3.5. Similarly, we see that for the highest FA lifetime (72 *min*), at high stiffness values ($2.5x10^{-3}$ to $1x10^{-2}$ N/m) there starts to be also no difference in

maturation. This suggests that, for these stiffness values, 72 *min* is enough time for all fibers to fully maturate, i.e. n=5. This would explain the saturation in cellular traction in the corresponding condition (see figure 3.7, lifetime FA = 72 *min*).

As a conclusion, more force will be exerted by the more mature fibers; which concurs with the average force and the total cell traction, as we will see below.

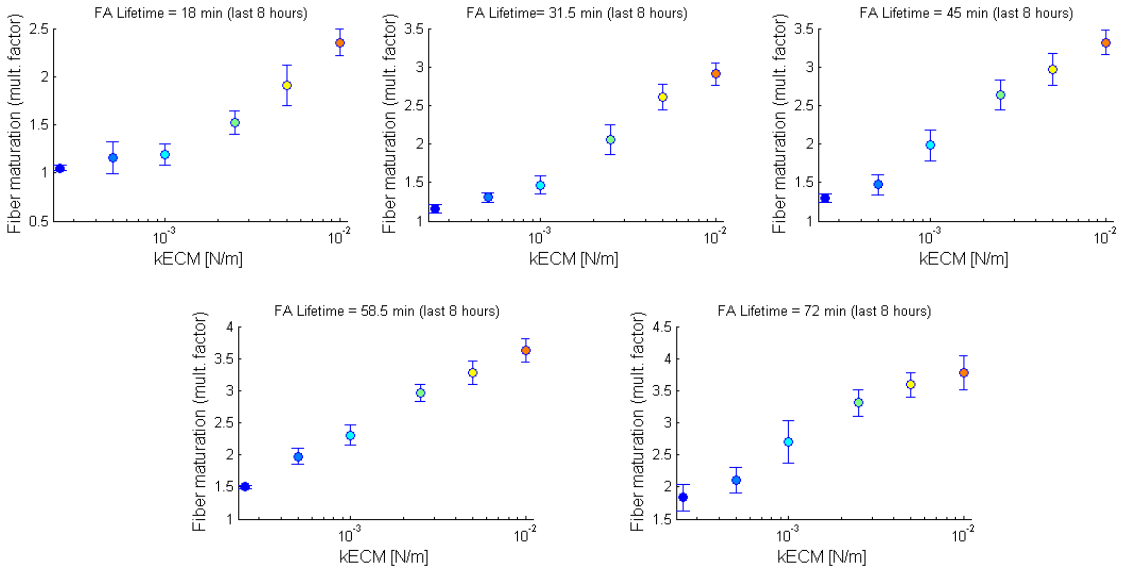

FIGURE 3.3: Fiber maturation depicted for five different lifetimes of the FA and six different values of kECM.

Contact area of the cell

The contact area of the cell-matrix interface is given in $[\mu m^2]$ units and represents how much the cell is spread during cell migration. The results are shown in figure 3.4.

In a way, it also represents how much the substrate can be deformed: a soft substrate can be easily deformed, because, thinking on the spring model representing the system, the stress fibers can pull on the ECM spring for some long time. Therefore, we should expect less contact area with increasing stiffness, since it becomes harder to pull on. If we take a look at the results, this is true for most of all the situations, except one: for lifetime = 18 *min* it first increases a bit before going down as expected. Why this happens still remains as a mystery, but here comes an hypothesis: for the three first substrate stiffnesses (kECM), there are so few FA (and, consequently, even less stress fibers: FA/2) that the cell is not attached to the substrate and thus it freely expands, which is translated into a larger contact area. This leads us to wonder if what is then happening here is an amoeboid-like movement, as a contrast to our assumption of mesenchymal migration. Note that the lack of adhesions

is due to the short time that FA live: when the existing ones disappear, no new FA have been generated yet.

Thereby, contact area seems to be also affected by the lifetime and not only by the substrate stiffness: when the FA can live longer, we see that the area is smaller. At low stiffnesses, the fibers do not really do much, since the substrate can be pulled on without the need of fiber reinforcement; so the focal adhesions are the only ones preventing the protusions to stretch the cell. As the stiffness is higher, the fibers maturate more so they exert more force (by fiber maturation), which is mechanosensed by the FA and consequently, by Bell's model, they live longer. If the FA live longer, the stress fibers can contract the cell during longer time. As a results, the cell has its contact area reduced.

All in all, we can conclude that the effect of FA lifetime and substrate stiffness values determines whether contraction or protusion forces dominate, where the former situation occurs due to fiber maturation and the latter due to lack of this.

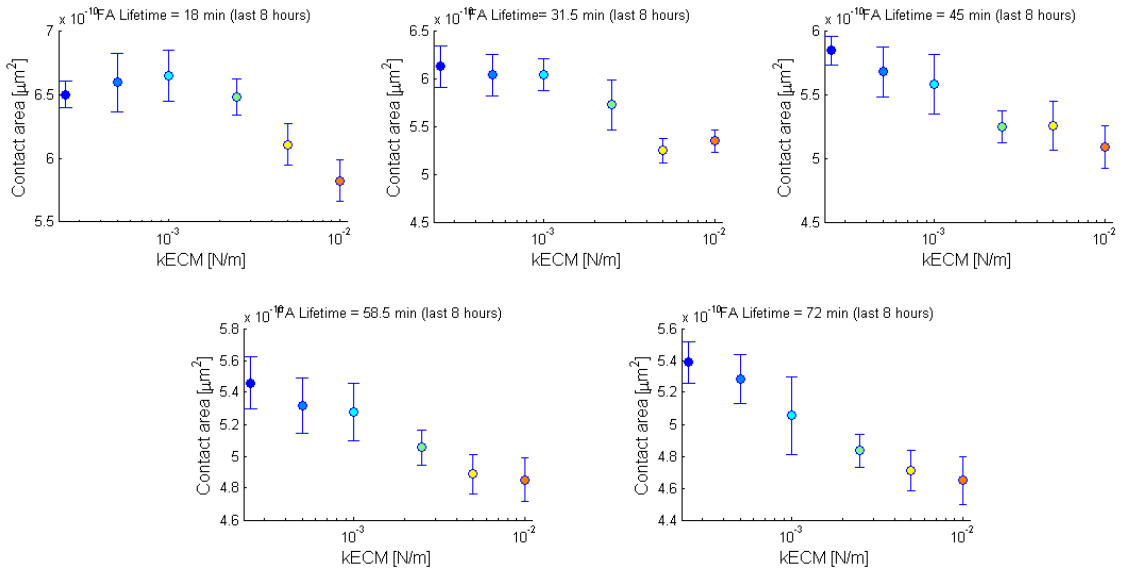

FIGURE 3.4: Contact area of the cell depicted for five different lifetimes of the FA and six different values of kECM.

Average force

The average force output is given in [nN] units and it represents the average force in all focal adhesions. This means that it is the force exerted by the stress fibers and sensed by the cell through the cell-matrix adhesions, but, since we take the average force, the results we get can be interpreted as the force sensed in one FA. The results are shown in figure 3.5.

First, we can see that the average force is higher in stiff substrates than in soft substrates. In addition, regardless of FA lifetime, it follows the same trend in its dependence on substrate stiffness and shows almost the same values. Nevertheless, if we take a closer look to the values, we do find differences. As the FA lifetime increases, the values of the average force increase as well. This slight rise is indeed due to fiber maturation: once again, for stiff substrates the fibers suffer an earlier reinforcement. In average, this reinforcement is also present, which results in a higher average force exerted per fiber and sensed by one single FA.

Besides, we should not forget that we imposed a force threshold above which fiber rupture happens. This gives us a clue about why the maximum force is similar in all cases of FA lifetime with the stiffest substrate. The stress fibers are being reinforced each time after system equilibrium has been reached, but only until they achieve a total of five reinforcements (due to the configuration of our model) or the FA cannot hold the fiber force any longer, after which the FA break. Therefore, there is a condition of maximum force that is imposed in the system. With this condition, it is obvious that we cannot expect higher values for the average force.

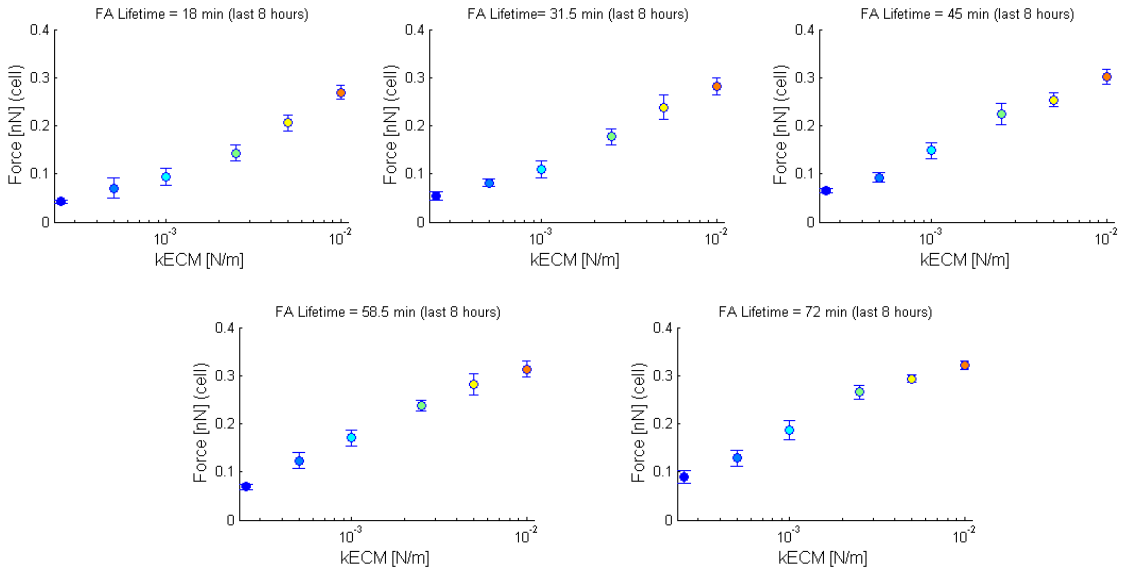

FIGURE 3.5: Force in all FA of the cell depicted for five different lifetimes of the FA and six different values of kECM.

FA rupture

Fiber rupture is a cumulative output which tells how many FA have been broken at the end of every simulation. Note that only rupture is considered, therefore it is related to fiber maturation: when the force threshold is exceeded after fiber reinforcement, then the corresponding FA break. Remind that, by our definition of force threshold in expression

2.16, a single fiber cannot cause rupture, since the maximum force it can exert at its strongest is below such threshold. However, the effect of multiple fibers combined can exceed this threshold, thus causing FA rupture. The results are shown in figure 3.6. No dissociation, which is natural unbinding of the cell-matrix adhesion, is considered here: only rupture is taken.

This rupture event is of course explained by the fiber maturation model, which has been already widely discussed. In any case, we can appreciate how fiber rupture rises due to both increase of substrate rigidity and longer FA lifetime, as we were expecting, since the number of FA will be bigger.

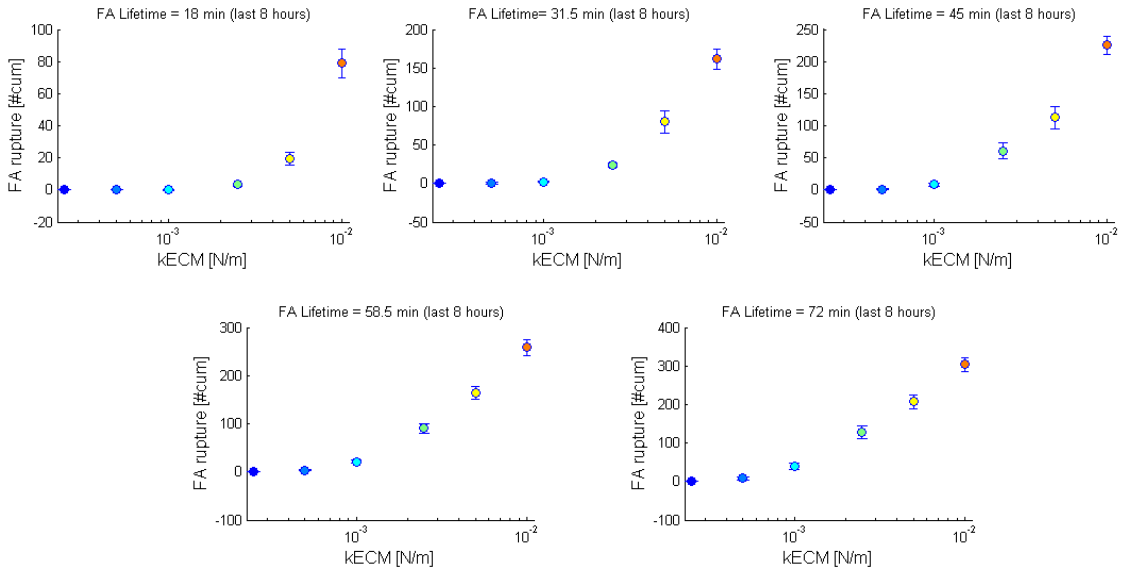

FIGURE 3.6: FA rupture depicted for five different lifetimes of the FA and six different values of kECM.

Cell traction over substrate

The cell traction is the total magnitude of the traction force over the substrate (ECM) given in [kPa] units. As traction is force over unit of area (contact area here), this output is directly related to some previous results that we have already discussed. Its results are shown in figures 3.7 and 3.8.

Let us start first with the force. In one of the previous parts, we have talked about the average force sensed by one FA. This average force was not really influenced by the lifetime of the FA. However, the number of the FA increases when they live longer, because there are more FA at the same time, which means that the total force exerted over the substrate increases as well with the FA lifetime and the substrate stiffness.

Regarding to the contact area, we have seen that it decreases with higher stiffness and with increasing lifetime. All together, the cell traction will increase with stiffness and FA lifetime as can be seen in the figures: as substrate stiffness and FA lifetime take greater values, the exerted force increases while the contact area decreases, as a result we get higher cell traction.

By considering only the average values shown in figure 3.7, we can summarize our data in a very intuitive surface plot as in figure 3.8. There we can nicely see how cell traction evolves. All in all, these results match what we expected to see with these simulations.

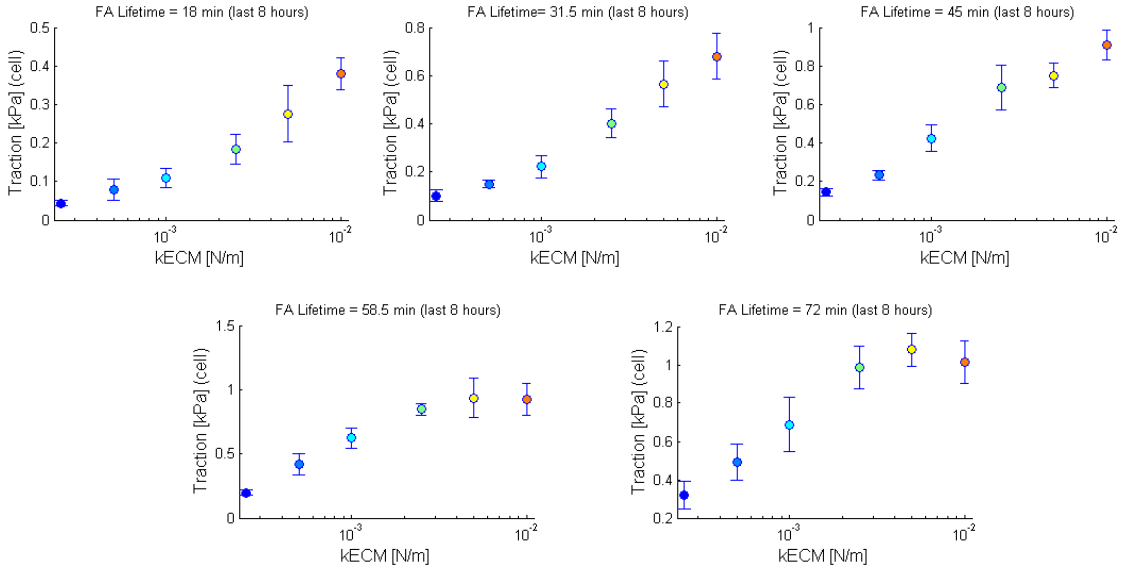

FIGURE 3.7: Cell traction over substrate depicted for five different lifetimes of the FA and six different values of kECM.

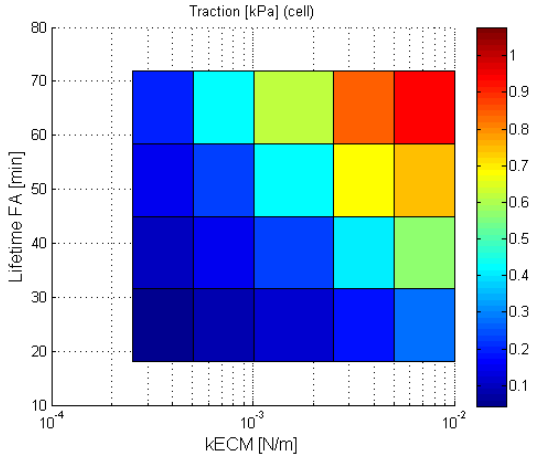

FIGURE 3.8: Cell traction depicted as a surface where all the different kECM-lifetime combinations of our analysis are shown. An increase of cell traction from bottom-left to up-right can be seen, i.e. traction increases both in the direction of increasing FA lifetime and increasing substrate stiffness.

Cell displacement

The displacement of the cell along the substrate is given in [μm] units and it is calculated from the center of mass of the cell. The results are shown in figures 3.9 and 3.10.

If we take a look at figure 3.9, our first impression is that it is a bit hard to describe. Apparently, there seems to be higher displacement with higher substrate stiffness, but this only happens for the conditions corresponding to the three stiffest substrates. For the lower stiffnesses, it rather happens in the opposite way: smaller displacement as subtrate stiffness increases. In both cases some exceptions can be seen. In addition, if we relate these results with the number of FA, we find that in general more FA imply less displacement.

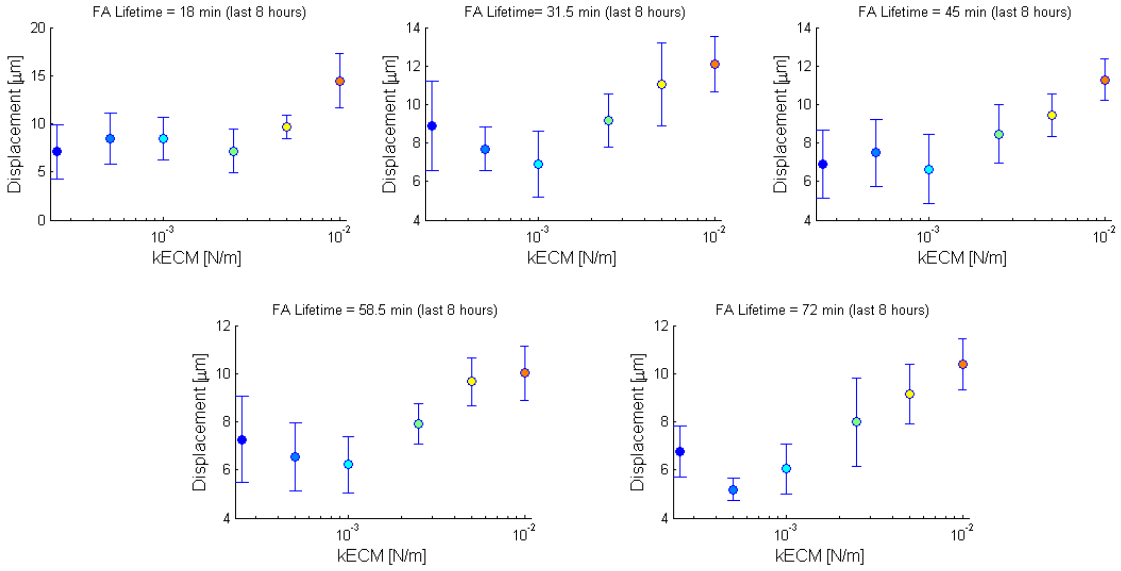

FIGURE 3.9: Displacement of the cell depicted for five different lifetimes of the FA and six different values of kECM.

For a better understanding of this behaviour, we can take a look at figure 3.10, which summarizes the same results. Here, the surface plot can be divided in three bands: bottom-left area, diagonal blue band and up-right area. In the bottom-left area we find soft substrates and less FA with shorter lifetime. However, the displacement here is higher than in the diagonal band. On the other hand, in the up-right area there are stiff substrates and more FA which live longer, and the displacement in this area is the highest. This leaves the blue band as the area with intermediate stiffnesses and where smaller displacements happen. Therefore, we conjecture a possible explanation: the blue band could be a valley that acts like a transition state from one migration mode (amoeboid-like movement) to the other one (mesenchymal movement). As was explained in subsection 1.2.3, mesenchymal migration is characterized by a greater amount of FA and higher traction, which concurs with the results found in the up-right area, leading to a higher directional movement. In

comparison, a lower amount of FA are found in the bottom-left area, which would correspond to an amoeboid-like movement. In this regard, we can consider figure 3.10 a sort of phase diagram that shows two areas of higher displament where two different migration modes are separated by a third region of no migration.

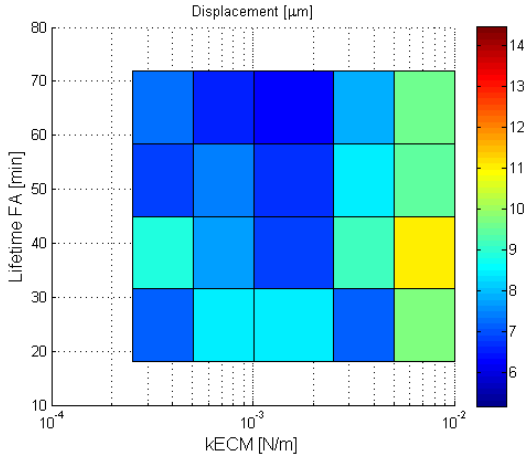

FIGURE 3.10: Displacement of the cell depicted as a surface where all the different kECM-lifetime combinations of our analysis are shown. A diagonal blue band can be seen from the up-left corner to the bottom-right one, dividing the surface into three areas.

Speed of cell migration

The speed of cell migration, given in $[\mu m/h]$ units, is also calculated from the center of mass of the cell. It represents the speed per hour by substracting the position of the cell from its position an hour before, which is done in a continuous way at every step of the simulation after the first hour. The results are shown in figure 3.11.

What we first see is how large the standard deviations are. This is understandable, since we have already seen large standard deviation bars in the displacement, shown in figure 3.9, as speed is the derivative of the displacement and, consequently, disparity between the different experiments (i.e. simulations) is enlarged. This is of course reasonable due to the stochastic character of the simulations, because it is quite unlikely that a cell is placed at the same position and at the same time for different experiments.

Secondly, it seems that migration speed is higher when the FA lifetime is shorter. If we relate these results with the ones of the displacement, in the previous section, this further supports our hypothesis on the different migration modes: for a longer FA lifetime we have more FA, meaning that the cell is more attached to the substrate and migration happens according to a mesenchymal, slower behaviour. On the other hand, when there are less adhesions due to a shorter FA lifetime, amoeboid-like movement dominate the migration mode, giving a faster cell.

Finally, regarding to substrate stiffness, no clear conclusions can be made.

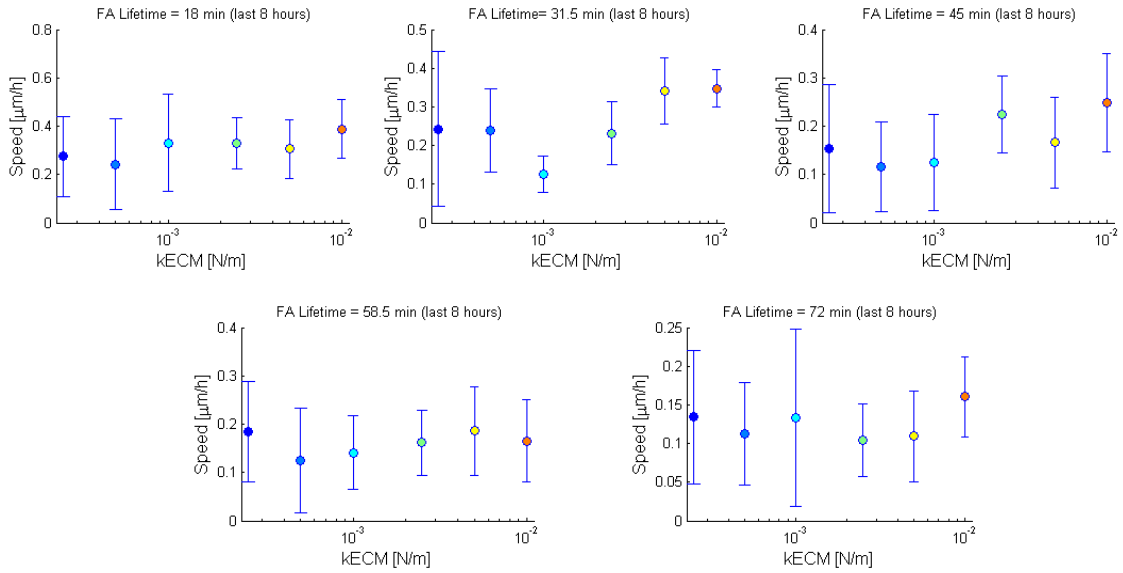

FIGURE 3.11: Speed of cell migration depicted for five different lifetimes of the FA and six different values of kECM.

Chapter 4

Conclusion and future prospects

This thesis has investigated the potential of the computational models to simulate the behaviour of a single cell to sense substrate stiffness and migrate accordingly. This has been possible thanks to the presented model, which mimics real experiments made *in vitro* and reproduces cell deformation and the force exerted through the fibers. The obtained results are a tangible proof of how powerful the computational models are, since they provide us the chance to perform diverse experiments under the same conditions and study in detail, both qualitatively and quantitatively, what happens even at the subcellular level. For this reason, computational models could be the key to further research in the field of biomedicine, allowing us to understand how the cell responds to different stimuli, which is essential to comprehend how some diseases or processes within the body work.

To conclude, let us summarize some general thoughts from the results of this master's thesis and motivate some future prospects that could be carried out.

4.1 Conclusions

In first place, we can conclude from the results that the cell migrates according to biological principles: focal adhesions are formed and dissociated, creating cell-matrix adhesions with which the cell senses the substrate and moves along it. One remarkable fact is that, after 48 hours of simulation, the cell does not seem to move too far, even with few adhesions. In other words, the cell of our simulation is slow. To improve this we want to increase the number of fibers to exert larger forces and the number of adhesions, and in both ways speed the process of cell front retraction. We anticipate this to increase cell speed to match experimental data, meaning better results for the migration speed and cell displacement. The reason why we focus on these parameters here is because of the interesting conjecture that they have aroused: the possible transition state from amoeboid-like movement to mesenchymal migration. If this was indeed the case, it would be very interesting to have more data (e.g. after running the simulation during more time) to prove or reject our hypothesis. From the literature, it is known that the ability to bind the ECM is a determinant of the migration mode [23, 24], which our results could corroborate in terms of the substrate stiffness. Our model additionally could quantify the degree of adhesion at which this change in migration mode occurs.

In second place, regarding to the models considered, both Bell's model and fiber maturation seem to be able to mimic the natural behaviour of the cell: the former one in relation to focal adhesions maturation, dissociation and rupture (as seen e.g. when average FA lifetime was discussed) depending on the substrate rigidity, whereas the latter one reproduces how fibers are reinforced or stalled after certain time when the whole system of the cell-matrix adhesion has reached equilibrium (as seen e.g. when we discussed fiber maturation and FA rupture), which also happens due to the mechanosensing of the substrate stiffness. Although the literature has provided evidence that the phenomena that both models represent are actually happening in the cell (in terms of mechanical stimuli), we wonder how our computational cell would response if only one of these two models was implemented: would it shed light on the necessity to use both models in our simulation? Or perhaps would it inspire us to use a different approach to mimic this behaviour of the cell?

In third place, the goal at the beginning of this work has been fulfilled: we have been able to study how the cell exerts traction over the substrate through its fibers. Besides, the results were as we expected, as it has been discussed in the corresponding section.

Finally, it remains to be said that, due to the limited time of this master's thesis, the suggestions here presented in order to improve our results stay as future prospects.

4.2 Future prospects

As intuited in the previous section, there is still a lot of work to do in relation with this topic and, particularly, with this computational model.

First, both Bell's and fiber maturation models have been implemented in our model. As stated, it would be interesting to analyze what happens, how the cell responds in a different way, when only one of them is considered -or even if none of them are taken. In this way, we could think of another three possible situations to have a full study in regarding these implementations.

Secondly, some other parameters could be modified. For instance, with respect to the substrate, here we have mainly varied its stiffness, but some other good ideas come to mind, e.g. substrate width and shape. Different hypotheses can be launched in relation to both. Regarding the width, how would the migration speed change? Our hypothesis states that a narrower substrate would imply lower velocity, i.e. slower cell migration, and that, since less focal adhesions would be created (due to less space would be available), the cell would exert less traction. Regarding the shape, we have seen that the cell adapts itself to the substrate shape, but so far we have only tried a rectangular shape. How would this affect cell migration if we had e.g. a T-shape?

All in all, further research is necessary to develop a realistic computational model that can completely reproduce the behaviour of cell migration in different environments, in aim to fully understand some body processes or diseases, as e.g. cancer.

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