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Modeling the formation of cell-matrix adhesions on a single 3D matrix fiber

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HIGHLIGHTS

- A discrete computational model in 3D is proposed for simulating the cell-matrix fiber adhesion.
- The proposed model analyzes the importance of the alignment between the matrix fiber and the cell protrusion on the size of the adhesion.
- The influence of different extracellular matrix properties on the size of the adhesion is also studied.

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ABSTRACT

Cell-matrix adhesions are crucial in different biological processes like tissue morphogenesis, cell motility, and extracellular matrix remodeling. These interactions that link cell cytoskeleton and matrix fibers are built through protein clutches, generally known as adhesion complexes. The adhesion formation process has been deeply studied in two-dimensional (2D) cases; however, the knowledge is limited for three-dimensional (3D) cases. In this work, we simulate different local extracellular matrix properties in order to unravel the fundamental mechanisms that regulate the formation of cell-matrix adhesions in 3D. We aim to study the mechanical interaction of these biological structures through a three dimensional discrete approach, reproducing the transmission pattern force between the cytoskeleton and a single extracellular matrix fiber. This numerical model provides a discrete analysis of the proteins involved including spatial distribution, interaction between them, and study of the different phenomena, such as protein clutches unbinding or protein unfolding.

1. Introduction

Cell-matrix adhesions are crucial in a wide range of biological phenomena, playing a key role in tissue formation, immune responses, cell migration or extracellular matrix (ECM) remodeling (Case et al., 2000). These interactions are performed by a large multi-protein assembly that binds both parts forming the adhesion. These adhesions are commonly known as complex adhesions when they have matured, and nascent adhesions when they begin to form, which occurs in the cell edge in like-protrusion structures such as filopodia and lamellipodia (Geiger et al., 2001). This process of cell adhesion is the mechanism that ensures structural integrity of tissue (Selhuber Unkel et al., 2010), and it is mainly regulated by mechanical processes (Discher et al., 2005; Vogel and Sheetz, 2006).

Myosin contractility and actin polymerization produce the forces responsible for the cyclic process of membrane protrusion and retrograde flow of F-actin at the leading edge (Ponti et al., 2004; Pollard and Borisy, 2003; Sabass and Schwarz, 2010). These forces are transmitted to the ECM through trans-membrane receptors of the integrin family placed on the cell membrane (Ruoslahti, 1996). These receptors serve as traction points over which the cell moves as well as sources of migration-related regulatory signals (Parsons et al., 2010; Ridley et al., 2003; Chen et al., 2012; Loeser, 2014). The integrins are bound to the actin filaments in the cytoskeleton through a clutch of proteins that include talin, α-actinin, vinculin and paxilin (Zamir and Geiger, 2001; Selhuber Unkel et al., 2010; Abbey and Bayless, 2014). On the other hand, integrins bind to protein ligands of the ECM, like fibronectin family (Ruoslahti, 1996). Finally, the formed membrane protusions must adhere to the matrix to define cell locomotion. If they do not attach, protrusions are unproductive and tend to move rearward in waves in response to the tension generated in the cell, in a process known as membrane ruffling (Borm et al., 2005). Therefore, actin retrograde flow strongly depends on cell contraction and focal adhesion size, concentration and strength (Kim and Wirtz, 2013).

Numerous studies over the past three decades have revealed a wealth of information detailing cell adhesion in two-dimensional

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surfaces. However, in *in vivo* experiments many cells are completely surrounded by ECM, which may have an influence on the size, composition and dynamics of adhesive structures. The study of cell adhesion in three-dimensional environments still remains in its infancy. This lack of knowledge together with the inherent computational cost of the corresponding simulations make these kind of 3D computational models a quite unexplored and challenging field. It is known that the way cells migrate changes between 2D and 3D environment (Harunaga and Yamada, 2011). Furthermore, in a 3D environment, cells of the same type migrate in different ways depending on the physical properties of the extracellular matrix, the degree of extracellular proteolysis and the soluble signaling factors (Petrie and Yamada, 2012; Sanz-Moreno et al., 2011; Wolf et al., 2003). Since cells use focal adhesions to sense and interact with their surroundings, it seems essential to understand adhesion behavior in order to clarify the mechanism of these migration changes.

Specific experiments in 3D environments were difficult to perform, but in the last two years this kind of studies has increased. Friedl and Wolf (2010) analyzed how the ECM architecture along with some cellular determinants (such as concentration of some specific proteins) influence the different modes of cell migration in 3D environments. Haeger et al. (2014) studied what triggers the change on the invasion mode (single or collective) of mesenchymal tumor cells, observing that the ECM mechanical properties are the determining factor. Alessandri (2013) studied how mechanical cues from the surrounding microenvironment may trigger cell invasion from a growing tumor. They used a revolutionary microfluidic technique that consists of the encapsulation and growth of cells inside permeable, elastic, hollow micro-spheres. Another interesting study is the work by Kubow and Conrad (2013), where the authors identified the different mechanisms that determine adhesion in 3D matrices, observing cells growing along the ECM fibers.

Computational modeling is a useful tool for integrating the multiple subprocesses that govern cell motility and migration. In this field, Chan and Odde (2008) investigated ECM rigidity sensing of filopodia via a stochastic model of the motor-clutch force transmission system in 2D. In their model, integrin molecules work as mechanical clutches linking F-actin to the substrate and mechanically resisting myosin-driven F-actin retrograde flow. More recently, Elosegui Artola et al. (2014) have improved this model adjusting it for two different types of integrins and adding a reinforcement mechanosensing event. This phenomenon provokes an increment on the number of adhesions when the traction forces exceed a threshold. Another interesting work is developed by Cirit et al. (2010), in which they created a model that analyzes the dynamical interplay between cell protrusion and adhesion at the cell's leading edge. Milan et al. (2013) developed a 3D model able to analyze the signals involved in cell adhesions in stem cells using the Cytoskeleton Divided Medium model (CDM). This model describes the cell like a set of particles interacting with each other, and generating a discrete force network able to mimic the discrete filament network of the cytoskeleton in the cell. The model was also implemented to simulate how a cell adheres on plain substrates by filopodia formation. With this cell model they were able to predict cytoskeleton reorganization and reinforcement during cell spreading.

In vivo, ECM consists of a myriad of fibers that are crosslinked between them, forming a complex network which serves as a scaffold for the cells to migrate. When the cell adheres to the matrix and migrates, it moves over these fibers, deforming and reorienting them. Three aspects of the ECM are crucial for cell adhesion: mechanical properties, density and grade of fiber crosslinking. In this work, we assume that the local behavior of the ECM when a filopodium adheres to it does not depend on the global properties of the matrix. We also assume that the fiber is prestressed; therefore, we focus our study on the level of fiber crosslinking. The grade and strength of fiber crosslinkings determine the difficulty of the matrix to reorganize under cell forces. Alignment of the filopodia protrusion structures with the matrix fibers is necessary for the migration. If a protrusion adheres to a fiber and they are not aligned, the protrusion tries to reorient it in order to have more surface to adhere; if this is not possible, the protrusion cannot grow further and eventually disappears.

In this work, we present a simulation model to reproduce the adhesion degree between a cell filopodium (guided by non-muscle myosins) and a collagen fiber of the ECM, depending on their relative orientations. We will detail the equations and hypotheses in which this model is based and how it is implemented in a computer algorithm.

2. Materials and methods

Myosin force-generating process in the cytoskeleton provokes actin filaments dynamics. The forces are transmitted through an adhesion complex (AC) to the extracellular matrix. These adhesion complexes consist of a clutch of proteins that include cytoskeleton proteins (paxilin, talin, vinculin and so on) and transmembrane proteins called integrins. Extracellular matrix is deformed under these forces reorienting its fibers.

Due to the high variability of the studied phenomenon, it is indispensable to assume some simplifications and hypotheses for the development of a simulation model. The computational cost and the complexity of a model that will include all the proteins involved in this phenomenon would make the problem inaccessible.

In this work, we have considered the effect of myosin proteins, only an actin filament, extracellular ligands, only a matrix fiber and adhesion complexes. Myosin exerts a force over a single actin filament causing its movement. This actin filament is oversized in order to simulate a pack of filaments of a filopodium and it moves on a plane. Force is transmitted through the ACs to the matrix fiber which posseses a 3D movement and rotation in order to try to align with the actin filopodium. The ACs bind the actin monomers with the extracellular ligands that are distributed on the matrix fiber surface. They can be found in different scenarios: bound to actin and ligands, bound only to one of them and completely free. When they are bound to actin and ligand, the adhesion grows and transmits the force to the ECM and cell. Although the ECM is a complex network of fibers, Kubow and Conrad (2013) experimentally quantified that the adhesion size mainly depends on the alignment of one single fiber.

Therefore, the simulation can be divided into three different components: Actin–Myosin contractile complex, Adhesion complexes and ECM.

The present model is a 3D extension of a previous one, developed by the authors in the 2D case (Escribano et al., 2014), with the addition of novel properties.

2.1. Actin–Myosin complex

The actin filament consists of a straight line of actin monomers, that only moves on the direction of its direction vector; therefore, only forces in this direction are considered. It is considered as a rigid solid under the assumption that the crosslinking stiffness of the matrix fibers is much lower than the union between monomers.

Myosins only exert pulling forces over the actin filament. The number of myosin heads bound to the actin filament determines the magnitude of the force. Each head is considered to produce a constant force of a fixed value. Thus, the total force is given by

$$F_m = F_c \cdot n_m,$$

where F_c is the force exerted by each myosin head and n_m is the number of myosin heads attached to the filament. The ACs bound