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In silico CDM model sheds light on force transmission in cell from focal adhesions to nucleus

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ABSTRACT

Cell adhesion is crucial for many types of cell, conditioning differentiation, proliferation, and protein synthesis. As a mechanical process, cell adhesion involves forces exerted by the cytoskeleton and transmitted by focal adhesions to extracellular matrix. These forces constitute signals that infer specific biological responses. Therefore, analyzing mechanotransduction during cell adhesion could lead to a better understanding of the mechanobiology of adherent cells. For instance this may explain how, the shape of adherent stem cells influences their differentiation or how the stiffness of the extracellular matrix affects adhesion strength. To assess the mechanical signals involved in cell adhesion, we computed intracellular forces using the Cytoskeleton Divided Medium model in endothelial cells adherent on micropost arrays of different stiffnesses. For each cell, focal adhesion location and forces measured by micropost deflection were used as an input for the model. The cytoskeleton and the nucleoskeleton were computed as systems of multiple tensile and compressive interactions. At the end of computation, the systems respected mechanical equilibrium while exerting the exact same traction force intensities on focal adhesions as the observed cell. The results indicate that not only the level of adhesion forces, but also the shape of the cell has an influence on intracellular tension and on nucleus strain. The combination of experimental micropost technology with the present CDM model constitutes a tool able to estimate the intracellular forces.

1. Introduction

Controlling cell adhesion is a main aim in tissue engineering and biomaterials research. Indeed cell adhesion is a mechanobiological process which plays an epigenetic role influencing cell phenotype. During adhesion, transmembrane complexes such as integrins are able to connect specific proteins of the extra cellular matrix. This leads to creation and maturation of focal adhesions (FAs). Then cytoskeleton rearranges, forming stress fibers connecting FAs, and spatially organizes internal cellular organelles, such as the nucleus. FAs are able to withstand the actin-myosin contraction the cell produces to increase its stiffness and stability (Balaban et al., 2001; Del Rio et al., 2009). They are also the location for the initial mechanotransduction processes, via activation of talin, Rho-A kinase, involved in cytoskeleton contraction (Geiger et al., 2009; Wang et al., 2009). Cytoskeleton tension has been shown to play an important role in determining cell fate (McBeath et al., 2004; Bhadriraju et al., 2007; Kilian et al., 2010). Tension transmitted to the nucleoskeleton leads to nuclear deformation (Dahl et al., 2008; Nathan et al., 2011), opening of membrane ion channels and calcium entry and thereby inducing transcription of specific genes (Itano et al., 2003). Moreover, deformation of the nucleus causes repositioning of chromosomes and so affects gene transcription, as observed in cells whose nucleus is confined by both cytoskeleton and substrate microgrooved topography (McNamara et al., 2012). Understanding mechanotransduction during cell adhesion thus requires taking into account cytoskeleton tension, which appears to be a signal for mechanosensitive complexes from FAs to the nucleus.

Previous experimental works measured traction forces applied on focal adhesions (Tan et al., 2003; Fu et al., 2010; Legant et al., 2010; Rape et al., 2011). Novel techniques such as genetically encoded tension sensor microscopy or intracellular tomography were developed and give an insight in intracellular forces (Cost et al., 2015; Gayrard and Borghi, 2016; Hu et al., 2003). For

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instance, FRET-based tension sensor microscopy allows intracellular tension mapping at the molecular scale identifying regions of high and low tension. However, to date, this technology cannot quantitatively indicate the amount of force transiting through a given section of the cell. For this very reason we believe that the Cytoskeleton Divided Medium (CDM) model we proposed here can be a complementary tool to quantify forces transiting through the whole structure. Based on divided medium mechanics, the CDM model is an equilibrated system of multiple tensile and compressive interactions representing the "tensegrity like" nature of the cytoskeleton. It takes into account cell pre-stress and provides a discrete representation of the filaments of the cytoskeleton in opposition to continuum approaches of finite element modeling (Stamenović et al., 1996; Ingber 1997, 2003; Wendling et al., 2003; McGarry and Prendergast 2004; Maurin et al., 2008). Compared to classical tensegrity models, the CDM model possesses an evolving connectivity able to simulate cytoskeleton rearrangement by filament (dis)assembling (Milan et al., 2007). Stress fibers, actin cortex, microtubules, intermediate filaments and nucleoskeleton were also included in the model with specific mechanical role. The CDM model was used previously to analyze the influence of adherent shape on the mechanical state of the cell (Milan et al., 2013). This led to discriminant results yet still incomplete, because FA tractions were not measured. Indeed, a more precise computation of the intracellular mechanical state would depend on full knowledge of adhesion conditions, which must include the coordinates and forces applied on FAs. This is precisely the reason why we here combined FA traction measurement by micropost technology with a new optimization feature of the CDM model. This new feature enables the model to generate a traction force on each FA equal to what had previously been measured experimentally. With this approach, our scientific objective was to analyze the influence of substrate stiffness on the mechanical state of the cell. We considered 2 different stiffnesses of micropost arrays (Han et al., 2012). The computed cytoskeleton provided the internal distribution of intracellular tension and indicated the amount of force transmitted to the nucleus. As a result we obtain a non-trivial relationship between FA forces, intracellular tension, cell diameter and nucleus strain.

2. Materials and methods

The present CDM model is an improvement of the previous version (Milan et al., 2013) which only took cell shape into account. The CDM model has undergone extensive modifications for the sake of the present study to be cell-dependent and to act mechanically as the cell does. The new version uses FA traction measurements to simulate identical pulling forces, which the previous version did not. In the CDM model, the network of stress fibers is fully adaptable in terms of distribution, stiffness and contractility and is computed for each cell taking into account the real traction forces the cell exerts on the substrate. This is thought to better estimate the mechanical state of adherent cells.

2.1. Description of the CDM model

The CDM model represented a 15 μm diameter round cell with a 6 μm-diameter nucleus. Cell volume was divided into 12,000 spherical particles of diameters ranging between [0.4; 0.8] μm. Particle centers act as nodes of the network of interactions network within the divided medium. Nodes were classified by species: nucleus core, nucleus lamina, perinuclears, cell core, cell membrane, FAs (Fig. 1). Interactions between node species were ruled as a relationship between reaction force and gap (Milan et al., 2013). Two interaction laws, Elastic Wire and Contact, were used. Elastic Wire law acted like a virtual pre-strained elastic wire between two nodes: tension was proportional to stretching and became null when the elastic wire slackened. Contact law ruled interactions between the rigid spherical envelopes surrounding the nodes by generating necessary compression forces to prevent envelop interpenetration. The various components of the cytoskeleton and nucleoskeleton, were each modeled by specific interaction laws derived from the basic Elastic Wire and Contact laws (Supplementary Data Tables A and B). Nuclear lamina was reproduced by high-tensile LAMIN interactions between nuclear lamina nodes. Actin filaments were represented in by low-tensile interactions, termed

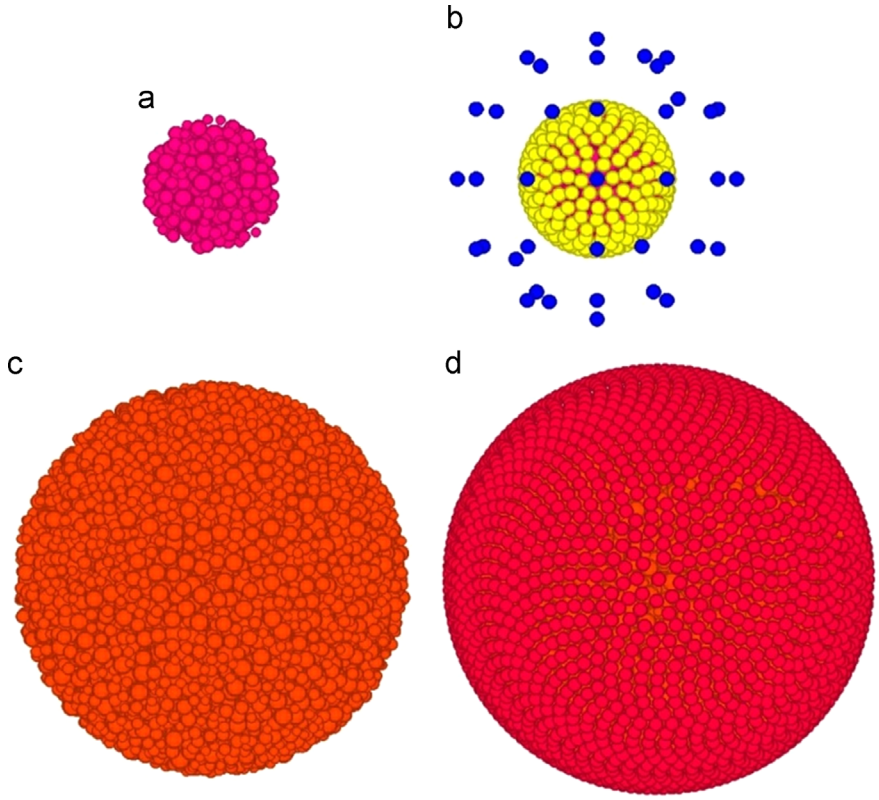

Fig. 1. Structure of the CDM model at round state (diameter=15 μm). Cell geometry is represented by a divided medium with nodes composed of different species: a) nucleus core (pink), b) nucleus membrane (yellow) and perinuclear nodes (blue) c) cell core nodes (orange) d) cell membrane (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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