Predictive Modeling of Cytoskleleton and Focal Adhesion Distribution of Spread Cells: a Discrete Paradigm Based on Attachment Optimization

Y. M. Loosli[1,2,3] and J. G. Snedeker[1,2]
[1]Orthopedic Research Laboratory, University of Zürich, Balgrist, Switzerland
[2]Institute of Biomechanics, ETH, Zürich, Switzerland
[3]RMS Foundation, Bettlach, Switzerland

Introduction: Understanding how cells interact with their neighborhood is of paramount importance for externally directing cellular behaviors (e.g. growth, migration, cycle, differentiation, etc.) and therefore useful in designing a scaffold material. The mechanical coupling between the cell cytoskeleton (CSK) and cell substrate operates via the connection of focal adhesion complexes (FAs) to actin stress fibers (SFs). FAs are aggregates of transmembrane proteins that ensure the adhesion of the cell on the substrate, whereas SFs are the contractile elements of the CSK composed mainly of actin filaments and myosin associated protein motors. The organization of these components, among others, increases cell adherence, stabilizes the cell shape and affects its mechano-stranduction properties. Therefore being able to understand the driving forces behind FA and SF distribution constitutes a powerful tool for predicting cell fate. The use of microfabricated surfaces that have been functionalized with binding ligands is a well established approach to investigate this behavior, and provides an ideal experimental benchmark for modeling purposes.
The proposed model is based on the optimization of cell adhesive pre-stress by varying SF contractibility. This rule is motivated by cellular tendency to maintain steady-state levels of adhesive strength.
Methods: To optimize cell pre-stress one can focus on the computation of the aggregate FA size: the objective function is defined as the square of the difference between the program computed total FA size and a target value (α) that corresponds to the total surface area of cell FA – which has been shown to reach an asymptotic value *in vitro*. The system variable is a vector composed of the strain (ε) existing in each SF. For this purpose the "ktrlink" function of Matlab is employed (constrained nonlinear multivariable function minimization).

$$f(\vec{\varepsilon}) = \sum FA_{size}$$

$$\min_{\varepsilon}(f(\vec{\varepsilon}) - \alpha)^2 \text{ such that } \begin{cases} FA_{size} < 10\mu m^2 \\ SF_{force} < 40nN \\ \text{SF orientation} \end{cases}$$

Eqn. 1: Objective function definition with the imposed constraints.

To compute the objective function, the linear relationship between the FA size and the SF force (generated by ε), is used (β=5.5 nNμm^{-2} [1]). The strain is assumed to be related to the force via Hooke's Law with EA=45nN [2] (E: SF cross section; A: SF elastic modulus). FA sizes are a limiting constraint (7.5-10µm) and the SF maximal force as well (20-40nN). The last constraint is imposed by the tendency of nascent FA to orient themselves toward the global SFs forces, hence favoring a unique SF with a direction close to the FA orientation. FA and SF disruption criteria are included in the algorithm (FA<0.7µm^2 and SF force<1nN). The modeled adhesive surfaces are defined similar to available experimental literature, as depicted on Fig.1.

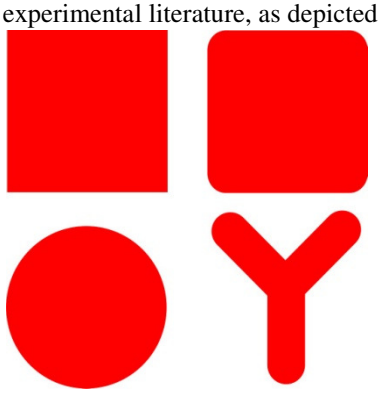

Fig. 1: Adhesive surface geometry in red. The size of each surface can be confined in a 40µm square. The filet radii are of 2.5µm.

Results: FA location and FA interconnectivity via SFs were in agreement with reported experiments. As expected we observed denser FAs at regions of high curvature (or corners) on the periphery of the cell. SFs mainly oriented toward the cell center and the longer SFs were favored.
Discussion and Conclusion: This model was driven by a set of biophysical constraints and structure-function rules that were able to replicate a realistic CSK structure of spread cells on widely varying substrate geometries. The applied phenomenological approach to modeling cell mechanisms (the rules were based on events occurring at the cellular level and not at the sub-cellular level) allows insight into how cell might reorganize themselves with downstream consequences for mechanotransduction.
A major limitation of this model is the lack of a time dependent variable, which could otherwise offer insight to cell dynamics and CSK kinetics. Another simplifying assumption is the neglected role of CSK microtubules which may influence the cell-substrate force balance.
This model can reasonably predict the FA and SF distributions with a few essential constraints that have been experimentally quantified. In the future, such a model coupled with mechanics based paradigms of cell differentiation will be useful to the development of scaffolds to optimize mechanical and material properties for the efficient regeneration of target tissues.

1. Balaban, N.Q., et al., Nature Cell Biology, 2001. **3**(5): p. 466-472.
2. Deguchi, S., T. Ohashi, and M. Sato, Journal of Biomechanics, 2006. **39**(14): p. 2603-2610.