Collagen-Matrix Guided Vasculogenesis: Cell Shape and Cytomechanics Drive Vessel Morphogenesis

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Statement of Purpose: The ability to predictably and reliably guide cell fate is a critical design criterion for next generation regenerative medicine and tissue engineering strategies. Toward this end, well-established cell signaling modalities focused on cell-cell and soluble-factor signaling have been primary targets for controlling cell decision-making. However, extensive evidence now shows that the three-dimensional (3D) biophysical context of the cell microenvironment is a major determinant of 3D cell shape, cytomechanics, and therefore how cells sense and respond to various signals. Previously, we showed that in-vitro vessel morphogenesis by endothelial colony forming cells (ECFC) can be predictably modulated through specification of microstructure and physical properties of the surrounding 3D collagen-fibril matrix. We now extend this work by applying "inside-out" and "outside-in" perturbation strategies for purposes of identifying key molecular nodes of cell-matrix signaling involved in matrix-guided vasculogenesis. Furthermore, experimental results were used to inform and adapt a physical-based computational model of cytoskeletal dynamics. The coupling of such experimental and computational modeling approaches is expected to improve experimental efficiency as well as support outcome prediction, scale-up, and optimization of matrix-guided vasculogenesis for both research and medical applications.

Methods: Human umbilical cord blood ECFC were obtained from EndGenitor Technologies and cultured as described previously[1]. All type I collagen polymers, including monomers and oligomers, were derived from the dermis of market-weight pigs and characterized as we have described[2]. ECFC were entrapped within polymerized collagen matrices prepared with specific microstructure and physical properties[3]. Specific inhibitors/stimulators were added at either t=0 or t=48 hours to study modulation of early-stage vacuolization (2d) or late-stage vessel network formation (7d), respectively. Inhibitors/stimulators included lysophosphaditic acid, β1-integrin blocking antibody (MAB17781), FAK inhibitor, GM6001, TIMP2, and TIMP3. Constructs were fixed and stained with toluidine blue or FITC-conjugated UEA-1 lectin for vacuolization or vessel networks, respectively. For some experiments, constructs were stained with Alexa Fluor Phalloidin for visualization of actin cytoskeleton and/or antibodies for MT1-MMP, β1-integrin, and phosphorylated FAK.

For computational modeling, the collagen matrix was simulated as a 3D interconnected fibrous structure[4]. All physical properties of the matrix can be controlled systematically with high precision. Cells embedded in the collagen matrix are modeled as deformable viscoelastic material with acto-myosin cytoskeleton and hydrostatic pressure, surrounded by a membrane[5]. The cells are able to reversibly adhere to the collagen matrix via focal adhesions sparsely located on the membrane. Actin filaments can also bind to the focal adhesion sites. This model employs a supercomputing power to achieve large time and length scales without significantly losing the details of the collagen matrix and cells.

Results: 3D ECFC shape and actin cytoskeleton organization were found to be highly dependent upon the interplay of fibril microstructure (fibril density and interfibril branching) and stiffness of the surrounding collagen matrix. These experimental relationships were in agreement with those obtained using simulations. Furthermore, the initial cell-matrix tension-force balance appeared to be highly predictive of early-stage vacuolization and late-stage vessel network formation. In all cases, oligomer matrices outperformed monomer matrices in terms of vessel formation and persistence. Vacuolization and vessel network formation were disrupted by agents that inhibited cell-matrix adhesion or actin assembly, including FAK inhibition and β1-integrin blocking antibody. In contrast, LPA, a stimulator of actin assembly, was found to rescue, in part, the weak ECFC vasculogenesis response associated with monomer matrices and FAK inhibition. Matrix-guided vacuolization and vessel network formation were also dependent upon MMP activity. Vacuole area, lumen diameter, and early branch formation appeared to be largely driven by spatiotemporal distribution of MT1-MMP activation, which was only observed at significant levels in oligomer matrices. In contrast, MMP2 activity appeared to play a significant role in vessel elongation.

Conclusions: Collectively, this work suggests that fibril microstructure, matrix stiffness, and matrix biodegradability are critical design parameters for predictably modulating not only initial ECFC shape and cytomechanics but also downstream vessel morphogenesis processes. Previous work by others has established that a multi-molecular signaling complex involving α2β1-integrin, MT1-MMP, and cytoskeletal regulators is necessary for vacuole and lumen formation during capillary morphogenesis[6,7]. Here, we extend this work by identifying β1-integrin, MT1-MMP, and FAK as potential nodes of a force-dependent signaling complex that drives the ECFC vasculogenesis response to matrix biophysical properties. This work is expected to contribute to new and improved vascularization strategies through identification of a proposed minimum set of parameters for the design and optimization of vascular-inductive collagen-based materials.

References:

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