Optimizing Endothelial Cell Adhesion and Invasion with Naturally-Derived, Biodegradable Hybrid Hydrogel Scaffolds

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Abstract

Background: Cellular ingrowth and neovascularization of acellular scaffolds represent the rate-limiting steps of tissue-regeneration matrix incorporation. In an effort to determine the material specifications that allow for maximal cellular invasion, we evaluated human umbilical vein endothelial cell (HUVEC) adherence and invasion into naturally-derived, biodegradable hybrid hydrogel scaffolds in an in vitro wound healing model.

Methods: Hydrogel scaffolds were fabricated to consist of the following compositions: alginate, alginate+collagen type I in 5:1 and 10:1 (w/w) ratios, alginage+chitosan in 1:1 and 2:1 ratios, and alginate+collagen+chitosan in a 10:2:5 ratio. All scaffolds were Arg-Gly-Asp (RGD)-modified. Additional alginate+collagen 10:1 scaffolds were fabricated to contain an internal biodegradable polyglactone mesh to allow for suture fixation to a recipient site. Scaffolds were seeded with $3.0x10^5$ HUVECs, maintained under standard cell culture conditions for 14 days, and fluorescently labeled with DiI-Ac-LDL. Cell density and invasion into scaffolds were evaluated using 3-dimensional confocal imaging.

Results: HUVECs were maximally confluent on alginate+collagen (5:1 and 10:1) hybrid scaffolds compared with pure alginate scaffolds (163.6±27.6 and 221±19.8 v. 5.9±0.9 cells/HPF, $p<0.001$). Cellular adhesion to alginate+chitosan 1:1 scaffolds was greater than to pure alginate scaffolds (9.8±1.4 v. 5.9±0.9, $p=0.02$) but less than collagen-containing scaffolds ($p<1.0x10^{-5}$). Cellular adhesion to alginate+chitosan 2:1 scaffolds was greater than to pure alginate scaffolds (151.6±21.4 v. 5.9±0.9, $p<1.0x10^{-7}$), unchanged from alginate+collagen 5:1 scaffolds, and less than to alginate+collagen 10:1 scaffolds ($p=0.02$). Three-dimensional imaging revealed that cells traversed the greatest distance through collagen-containing hydrogels. HUVEC adhesion alginate+collagen 10:1 hybrid scaffolds was not decreased by the presence of mesh.

Conclusions: Even at low concentrations, the addition of collagen type I to alginate matrices results in a substantial increase in HUVEC adherence and invasion. These findings provide important insights into the optimization of endothelial cell adhesion and invasion by appropriate substrate selection.

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