Modified Hyaluronic Acid Based Hydrogels to Promote Spreading and Proliferation of Endothelial Progenitor Cells

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Statement of Purpose: Cardiovascular injuries have been a major focus of current research in vascular tissue engineering.[1] Specifically, endothelialization of artificial implants has been a challenge, as these grafts do not effectively promote vascular healing without appropriate surface modifications. Circulating endothelial progenitor cells (EPCs) have been reported to speed up the healing process at the site of cardiovascular injury.[2,3] Therefore, EPCs could potentially be used as a cell source to endothelialize biomaterial surfaces. Hyaluronic acid (HA) is a major component of the extracellular matrix in cardiovascular tissues.[4] However, this material is nonadhesive, therefore limiting its use for surface endothelialization. Heparin is a well-known nonthrombogenic substrate and has the ability to interact with endothelial cells.[5] We hypothesize that combination of HA with heparin may allow to produce hydrogels which support formation of an endothelial layer. The goal of this work was to develop hyaluronic acid based hydrogel surfaces that could promote spreading and proliferation of endothelial progenitor cells.

Methods: To produce photocrosslinkable hydrogel precursors, HA, alginic acid and heparin were mathacrylated by standard chemical procedures. 1% HA methacrylate (HAMA), 1%HAMA-2% alginic acid methacrylate (AlgMA) and 1% HAMA-2% heparin mathacrylate (HepMA) hydrogels were then crosslinked following UV light exposure. EPCs, human umbilical cord vein endothelial cells (HUVECs) and macrophages were cultured in corresponding media and harvested upon reaching confluence. The recovered cells were seeded on 1% HAMA, 1%HAMA-2%AlgMA and 1%HAMA-2%HepMA hydrogels. At different time points samples were stained with Calcein AM to demonstrate cell spreading (Figure1). Furthermore, immunostaining was carried out to confirm the expression of endothelial cell markers CD31 and vWF, as well as phalloidin staining of F-actin. Quantification of experimental data was performed using ImageJ. Statistical analysis was processed by GraphPad Prism utilizing one-way ANOVA, two-way ANOVA and Bonferroni comparisons.

Results: The effect of different types of materials on EPC adhesion was found to be dependent on chemical structure. Hydrogels containing only HA did not allow for significant capture of EPCs as it is a nonadhesive biomaterial. Nonspecifically captured EPCs on that surface did not spread after 3 days of culture. To facilitate spreading and elongation of EPCs and HUVECs, 2% (w/v) heparin methacrylate (HepMA) containing HA hydrogels were synthesized. 1% HAMA-2%HepMA combination provided significantly higher cell attachment and results demonstrated formation of an endothelial monolayer on hydrogel surfaces following 3 day culture period. The highest level of EPC attachment was found to be 398±45 cells per mm^2 on 1% HAMA-2%HepMA hydrogels in 1 h and the % area covered by EPCs by day 3 was 75%±18. There were 159±57 HUVECs/mm^2 adhered on 1% HAMA-2%HepMA hydrogels in 1 h and 14%±11 of the area was covered by spread cells after 3 day culture. Macrophages, which were used as negative control for cell adhesion, exhibited significantly lower attachment on 1%HAMA-2%HepMA surfaces compared to the EPCs and HUVECs.

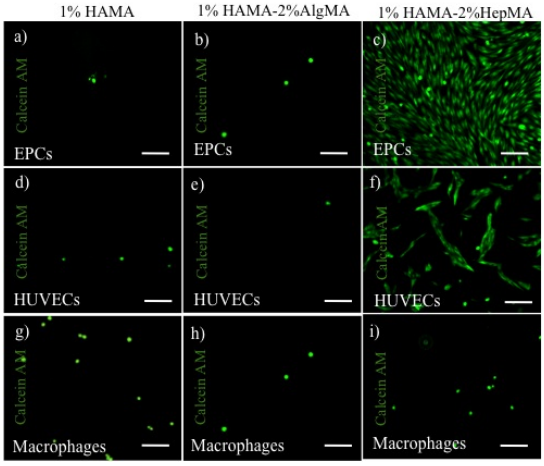

Figure 1. Cell spreading on HA-based hydrogels at day 3. Scale bars represent 100 μm.

Conclusions: Our data suggest that HA-heparin hydrogels may be a useful material to capture and promote spreading of EPCs. This strategy could potentially be useful in the treatment of cardiovascular injuries to recruit EPCs from circulating blood enhancing re-endothelialization process. Our HA-heparin based hydrogels could potentially be used to coat artificial implants and may find applications in cardiovascular tissue engineering. In future work, we will study capture of EPCs on heparin modified HA-based hydrogels under controlled flow conditions. We expect to mimic the natural environment better with shear flow and achieve an endothelial monolayer on nonthrombogenic hydrogel surfaces.

References: [1]American Heart Association, http://www.americanheart.org/, [2]Plouffe B.D. FASEB J. 2009;23:3309-3314, [3]Wu, X. Am. J.Physiol.: Heart Circ. Physiol. 2004;287:H480-H487,[4]Manasek, F.J. Circ. Res., 1976;38:331-337, [5]Barzu, T. Biochem. J. 1986;238:847-854.

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