HUVEC Tubular Formation on Bio-Inspired Substrate for Promoting Angiogenesis

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Statement of Purpose: Traumatic injury to organs, either the result of acute trauma or an underlying medical condition, can be fatal if not properly treated. Tissue sealants and wound dressings are often used while treating these organ injuries.[1] The aim of this study was to determine the feasibility of heparin and arginyl-glycyl-aspartic acid (RGD) conjugated alginate hydrogels encapsulated with vascular endothelial growth factor (VEGF) as a wound dressing. VEGF is an important component in angiogenesis that binds to heparin, and RGD promotes cell adhesion.[2,3] It was hypothesized that an elastic and tough alginate-based hydrogel would be amendable to RGD and heparin conjugation to support cell proliferation and *in vitro* tubular network formation. Alginate hydrogels functionalized with methacrylic anhydride (MA), RGD, and heparin with and without VEGF were crosslinked with visible light. The material properties and burst pressure mechanics were evaluated to determine the structural integrity of the hydrogels. The promotion of angiogenesis was evaluated by directly seeding human umbilical vein endothelial cells (HUVECs) onto visible light-crosslinked hydrogels.

Methods: Methacrylated alginate (Alg-MA) was synthesized as previously described.[4,5] To form Alg-MA-RGD, Alg-MA was placed in deionized (DI) water and conjugated with cysteine-L-arginyl–glycyl-L-aspartic acid (CRGD) using carbodiimide chemistry. To form Hep-GM, glycidyl methacrylate (GM) was added to a sodium heparin solution and stirred overnight, purified via dialysis. Polymer solutions were prepared in DI water. Human VEGFA was added to the polymer solutions at a ratio of 10^6:1 (polymer:VEGF). Hydrogels without VEGF were formed as controls (see **Table 1**). Solutions were blended with photo-activators and cross-linked using visible green light LED arrays (525 nm).[4] Mechanical analysis was performed using rheometry and burst pressure testing. HUVECs were seeded in complete media directly onto polymerized hydrogels in a 96-well tissue culture plate. The mitochondrial activity and cell proliferation were analyzed using a WST-8 and Pierce Protein Assay Kit. The ability of green light crosslinked alginate-based hydrogels to support tubular network formation and the effect of heparin and RGD conjugation, and VEGF encapsulation, were qualitatively evaluated using phase contrast microscopy.

Material Group	Abbreviation
Cell Control	Cells
Alg-MA	AM
Alg-MA-RGD	AMR
Alg-MA/Hep-GM	AMH
Alg-MA-RGD/Hep-GM	AMRH
Alg-MA/VEGF	AMV
Alg-MA-RGD/VEGF	AMRV
Alg-MA/Hep-GM/VEGF	AMHV
Alg-MA-RGD/Hep-GM/VEGF	AMRHV

Table 1. List of material groups and their corresponding abbreviations for labeling.

Results: The groups without VEGF were evaluated under shear conditions and exhibited storage moduli (G′) ranging from 330-2050 Pa and loss moduli (G″) ranging from 0.4-23.4 Pa (**Figure 1**) indicating that RGD and heparin modification of the AM material is not deleterious to the properties of the pure AM hydrogel control. Burst pressure values showed that there was no significant difference between these groups (p=0.27) and both groups exceeded the physiological normal pressure of 30 cmH_2O.[6] This demonstrates a capacity for both material groups as wound sealants.

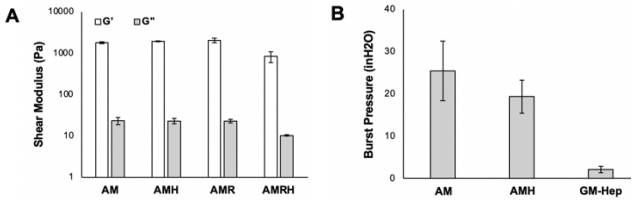

Figure 1. (A) Shear mechanical properties indicating the lack of variation in modified hydrogels and **(B)** burst pressure properties indicating the adhesive strength to collagen-based substrates.

HUVEC-seeded hydrogels were imaged at 6, 12, 24 and 72 hours. AMR and AMRH groups demonstrated successful adhesion of the cells to the substrate as evident by cells elongated morphology. Both materials exhibited angiogenic properties as microtubules were formed between cells, but a slightly greater degree of tube formation was seen on the groups containing both heparin and RGD. All groups loaded with VEGF exhibited less tube formation compared to their unloaded counterpart at each time point (**Figure 2**).

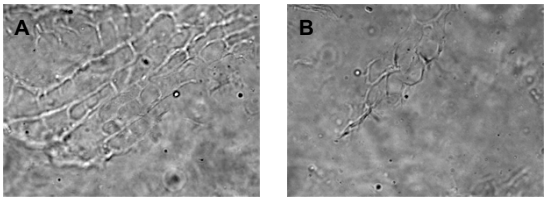

Figure 2. Phase contrast images of HUVECs after 24 hours cultured on **(A)** AMRH and **(B)** AMRHV indicating fewer tube formation in the absence of VEGF.

Conclusions: The rheological properties and burst pressures for the modified alginate-heparin hydrogels indicate promising properties for use as wound dressing materials. While VEGF and heparin have been shown to promote angiogenesis separately in other studies, together there seems to be an inhibitory effect.[7] It was found that the VEGF-loaded hydrogels resulted in smaller networks.

References: [1] Fenn,S et al., Acs App. Mater. 9(28): 23409-23419, 2017. [2] Tessler, S et al., J Biol Chem. 269(17): 12456-12461, 1994. [3] Dumbleton, J., et al. Cell Mol Bioeng. 9(2): 277-288, 2016. [4] Charron, P et al., J. Mech. Behav. Biomed. Mater. 59:314-21, 2016. [5] Fenn, S et al., J. Biomed. Mater. Res. 106.6:1229-1236, 2016. [6] Charron, P et al., *J. Mech. Behav. Biomed. Mater.,* 59:314-21, 2016. [7] Giraux, L et al., Eur J Cell Biol. 77(4):352-359, 1998.

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