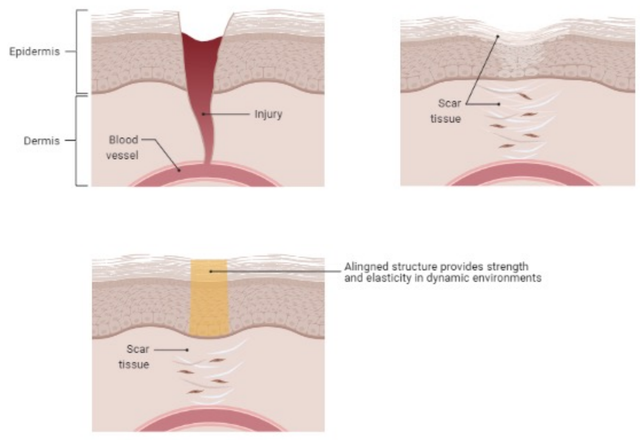
The Impact of Covalently Conjugated Alginate-Heparin Methacrylate Hydrogels on Multi-Axial Strength and HUVEC Tubular Formation

Irfan Tahir, Patrick Charron, Luis Garcia, Rachael Oldinski-Floreani*
University of Vermont, Burlington, VT, United States

Motivation: Transforming Traumatic Medical Treatment



Problem: Biomaterials which encourage wound healing in the absence of growth factors for elastic, dynamic tissues are lacking.

Elastic, dynamic tissue regeneration must recapitulate biomechanical properties

- Punctures in elastic tissue must be sealed to prevent leakage and restore normal function.
- It is imperative in the design of a wound sealant material to consider the dynamic mechanical properties of the underlying tissue and recapitulate those properties as closely as possible.
- A common downfall of wound sealants is the insufficient integration of the underlying tissue to maximize adhesion, seal the leak, and encourage wound healing.

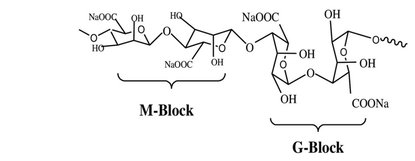
Herein, we investigated the hypothesis that a visible light crosslinked alginate-based hydrogel can support HUVEC proliferation and *in vitro* tubular network formation with the incorporation of covalently conjugated RGD and crosslinked with heparin.

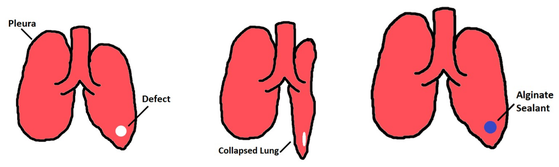
Materials and Methodology: Approaching a Functional Tissue Sealant

Alginate-Based Tissue Sealant

A natural, biocompatible tissue sealant that can be applied to the damaged pleura, preventing fluid leakage into the chest cavity.

- Sodium alginate - seaweed derivative
- Easy storage - room temperature
- Simple application process - no premixing
- Effective seal utilizing visible-light crosslinking
- Natural hydrolytic degradation

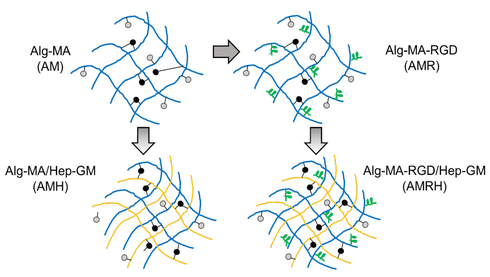




Alginate and Heparin Functionalization with RGD

Methacrylated Alginate (Alg-MA) is synthesized and functionalized with Hheparin (Hep), glycidyl methacrylate (GM) and cysteine-L-arginyl–glycyl-L-aspartic acid (cRGD).

- Material dialyzed to remove unreacted reactants and lyophilized to obtain clean, dry product.
- Alg-MA was placed in deionized (DI) water and conjugated with CRGD using carbodiimide chemistry.
- GM is added to a sodium heparin solution and stirred overnight, purified via dialysis.
- Photoinitiator system incorporated to enable visible, greenlight crosslinking *in situ.*

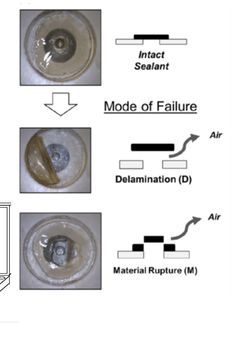


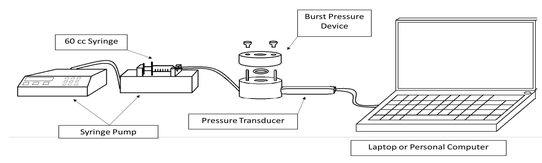
Material Group	Abbreviation
Cell Control	Cells
Alg-MA	AM
Alg-MA-RGD	AMR
Alg-MA/Hep-GM	AMH
Alg-MA-RGD/Hep-GM	AMRH
Alg-MA/VEGF	AMV
Alg-MA-RGD/VEGF	AMRV
Alg-MA/Hep-GM/VEGF	AMHV
Alg-MA-RGD/Hep-GM/VEGF	AMRHV

Physico-Mechanical Testing

Burst pressure testing is performed using a modified ASTM F2392-04 standard.

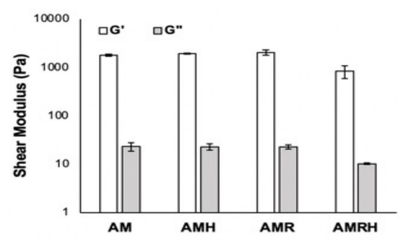
- Collagen-rich substrate used as *in vitro* test membranes.

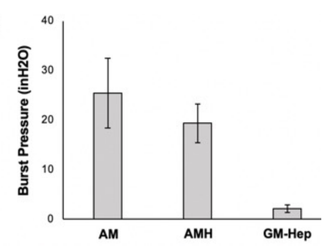




Rheology is performed to determine the shear material properties of the hydrogels during and after photo-crosslinking.

- Gelation kinetics assessed by oscillatory sweeps at 10% radial strain and 1 Hz under exposure to green light (525 nm).



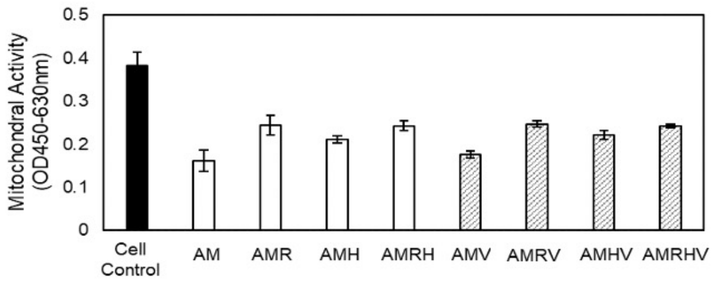


In Vitro Characterization of Alginate/Heparin Hydrogels

Hydrogel cytotoxicity

- Human umbilical vein endothelial (HUVECs) are seeded in complete media directly onto polymerized hydrogels in a 96-well tissue culture plate.
- The mitochondrial activity and cell proliferation is analyzed using a WST-8 and Pierce Protein Assay Kit.
- The ability of green light crosslinked alginate-based hydrogels to support tubular network formation are qualitatively evaluated using a phase contrast microscopy.

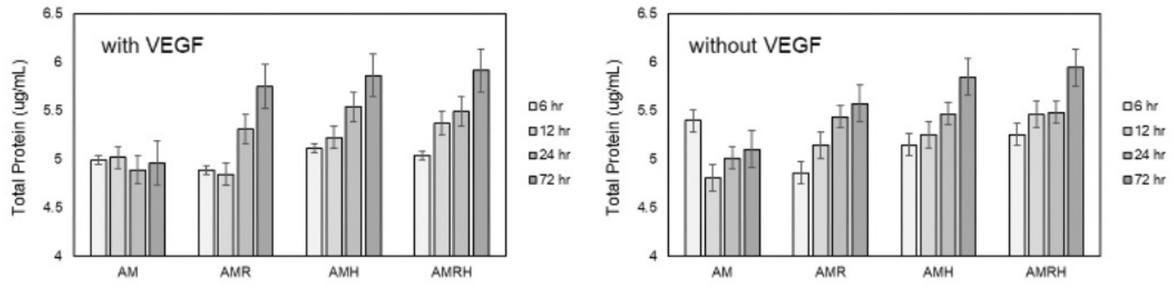
Compared to the base AM, modifications with RGD, heparin, and both RGD and heparin all resulted in increased mitochondrial activity.



Effect of VEGF: HUVEC proliferation and tubular network formation

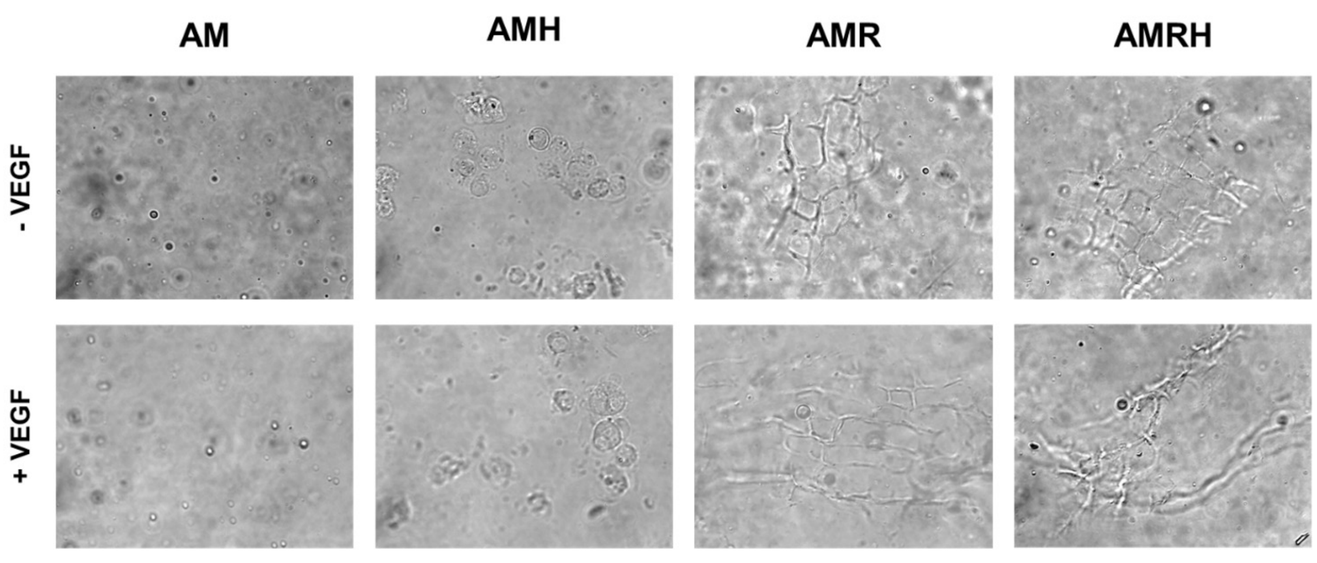
- The incorporation of growth factors, such as VEGF, into wound dressings has been investigated to promote angiogenesis and vascularization *in situ* to enhance regeneration of damaged vascularized tissues.
- It was hypothesized that an elastic and tough alginate-based hydrogel would be amendable to RGD and heparin conjugation to support cell proliferation and *in vitro* tubular network formation.
- All groups loaded with VEGF exhibited less tube formation compared to their unloaded counterpart at each time point.

While VEGF and surface-conjugated heparin have been shown to promote angiogenesis separately, together there seems to be an inhibitory effect; the VEGF-loaded hydrogels resulted in smaller networks.



Results and Conclusions

- All the hydrogel groups crosslinked under visible green light in the presence of photoinitiators
- The rheological properties and burst pressures for the modified alginate-heparin hydrogels indicate advantageous properties for use as wound dressing materials.
- HUVECs seeded gels were imaged at 6, 12, 24 and 72 hours, with 24 hours showing the greatest increase in tube formation for AMRH without VEGF.
- This experiment provides a proof of concept for using conjugated heparin and alginate copolymer hydrogels to aid in angiogenesis without the use of encapsulated growth factors.



This work was funded in part by NIH R01EB020964 (Oldinski) and the University of Vermont