Next-generation Peptide-Functionalized Gelatin Hydrogels for Neovascularization

Corinne Curry[1], Kate Mlouk[2], Lucas Mowery[1], Hayden Paige[2], Riya Patel[1], Jeffrey Schmeckpepper[3], Ethan Lippmann[1]

[1]Department of Chemical and Biomolecular Engineering, [2]Department of Neuroscience, [3]Department of Medicine, Vanderbilt University, Nashville, TN, USA

Statement of purpose: The loss of vascular perfusion, which is associated with many diseases, can lead to ischemic limbs and improper healing. While restoration of functional vasculature could solve this problem, previous advances in the field have focused on small capillary bed formation which cannot support bulk blood flow. Arterioles, larger blood vessels surrounded with smooth muscle, are mechanically necessary for perfusion of pressurized blood flow to distal tissues. Here, a bioengineered gelatin-based hydrogel with a neural cadherin (N-Cadherin) peptide attached to the backbone is shown to promote the formation of arterioles from ex vivo tissue and after implantation in vivo. Additionally, new variations of the "GelCad" hydrogels with additional peptides have been synthesized. The newer hydrogels contain moieties that have been previously shown to enhance vascularization. These studies offer a potentially translatable solution for vascular restoration.

Methods: The modified gelatin biomaterial is synthesized by dissolving porcine gelatin in PBS and using EDC-NHS chemistry to attach the carboxylate end groups of the different peptides to the backbone of the gelatin. After lyophilizing and milling the biomaterial, the peptide attachments are confirmed using NMR. For experimental use, the biomaterials are reconstituted as a 10% solution in PBS and crosslinked by mixing with an equal volume of a 10% solution of 20 kDa 4-arm polyethylene glycol succinimidyl glutarate (PEG-SG).

Ex vivo experiments were conducted using primary C57BL/6 mouse cortical tissue embedded in three hydrogel conditions—gelatin, GelCad, and GelScram (which utilizes a scrambled version of the N-Cadherin peptide). The embedded tissue was cultured for up to a week, and immunofluorescent staining was used to image the vessel structures. Fluorescent signals from various antibodies were used to quantify the differences between the samples through ImageJ coded macros.

In vivo experiments were conducted using BALB/c mice that were subjected to femoral artery ligation. Here, after suturing of the femoral artery in two locations, hydrogels were applied on top of the exposed tissue in the subcutaneous space. After surgery, at different time points, laser doppler imaging was performed to assess perfusion in both legs and ischemic indices were used to score physical appearance. At the final endpoint, Microfil was used to cast the vessels for x-ray and microCT imaging of the vascular network within the hydrogels. Histology was also performed on the fixed tissue by a blinded pathologist to assess ischemic damage.

Results: For our initial studies, primary mouse cortical tissue was embedded in gelatin, GelCad, and GelScram hydrogels. Vessel structures were observed sprouting from the tissue in all conditions, but only in GelCad did we observe alpha-smooth muscle actin (α-SMA)+ cells lining the lectin+ vessels (Figure 1A and data not shown). These results indicated that GelCad was potentially the only condition that could yield growth of arteriole-like structures. We transitioned to the femoral artery ligation model using GelCad and GelScram hydrogels, where we observed that GelCad hydrogels could completely prevent limb necrosis and tissue degradation, whereas GelScram hydrogels could not (Figure 1B). We infer that this outcome is due to arteriole growth initiated by the GelCad hydrogels and we are currently using a Microfil technique with advanced imaging modalities to confirm this outcome (data not shown).

To build on these outcomes with the GelCad hydrogel, we have synthesized a new variant that also contains a "HepPep" peptide with the ability to bind heparin – this hydrogel is termed HepPep Cad. In initial tests, we have determined that HepPep Cad can successfully bind heparin (Figure 1C). We hope to use this hydrogel to load growth factors prior to embedding (for example, FGF2 which plays a role in collateral arteriole growth), which is expected to enhance the biological activity of the original GelCad hydrogels.

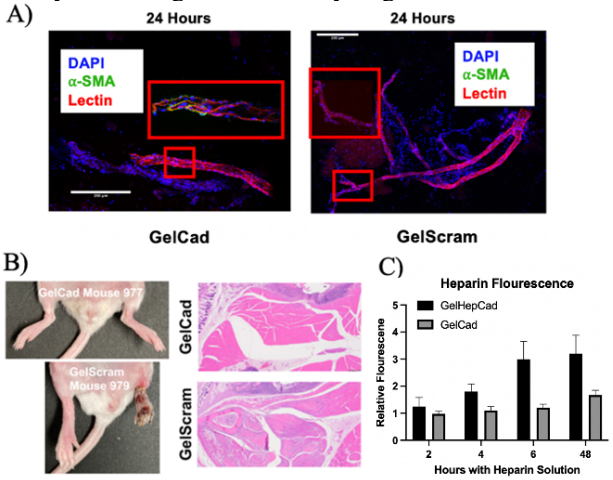

Figure 1. A) Confocal images highlighting the start of smooth muscle formation on the outside of vessels in GelCad but not GelScram hydrogels. **B)** Mice undergoing femoral artery ligation are completely healed by GelCad hydrogels, while mice receiving GelScram hydrogels exhibit extensive necrosis (N=5 biological replicates). These results were corroborated by blinded histology. **C)** After incubation with a fluorescent heparin for different times, followed by washing, GelHepCad hydrogels have higher fluorescence, indicating heparin binding.

Conclusions: Our data suggest that GelCad hydrogels are a promising technology to restore blood flow to ischemic tissues. Future studies will focus on fully characterizing the activity of the original GelCad hydrogels with comparisons to the newer variant HepPep Cad.