In Vivo Anastomosis and Perfusion of a 3D Printed PEG Hydrogel Containing an Arteriovenous Shunt

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Statement of Purpose: Advances in 3D printing technology have promoted the development of vascularized engineered tissues. Here, we sought to develop 3D printed hydrogels which could be directly connected to host vasculature, thereby introducing blood flow and oxygenation to these constructs following surgical anastomosis. We previously developed (Sooppan, *et al*, 2015) a surgical proof-of-concept technique for implanting 3D printed silicone gels containing microchannel networks in-line in the rat femoral artery, with blood flow lasting up to three hours. **Here, we report the development of a projection stereolithography (pSLA) based technique to fabricate hemocompatible hydrogels containing a perfusable flow loop designed for direct surgical anastomosis with host vasculature.** We found that that implantation of perfusable PEG-based hydrogels can support perfusion from the femoral artery for several days *in vivo*.

Methods: Hydrogels were fabricated using 80% w/w solution of poly(ethylene glycol)diacrylate **(PEGDA)** in PBS containing a 1:1 ratio of 6:35 kDa chains (**Fig 1a**). Furthermore, gels contained 100 U/mL of heparin to help reduce clotting. Hydrogels were allowed to swell to equilibrium in a heparin/saline solution and were then adhered to a Vicryl surgical mesh which could be used to suture the gels in place within the limb (**Fig 1a)**. The hydrogels were connected to the femoral artery and vein of 14 male Wistar rats (>500 g) in an arteriovenous shunt configuration, bypassing the leg. Flow was immediately measured using laser Doppler imaging and monitored daily for up to 6 days (**Fig 1c-f**).

Results: *In vitro*, printed hydrogels had an average burst pressure of 402±12 mmHg (roughly 3x rat systolic pressure). Following *in vivo* implantation, the gels maintained secure anastomoses with host vessels at flow rates upwards of 3 mL/min. Laser Doppler imaging results showed that flow was immediately present through the engineered channels. Of 14 gels implanted, 7 lasted more than 3 days without clotting (**Fig 1b)**, with an average patency time of 3.6 ± 1.9 days.

Conclusions: Our study demonstrates rapid prototyping of a biocompatible PEG-based hydrogel containing a fluidic vessel that can be implanted *in vivo* as an arteriovenous shunt. Channels withstood arterial pressure and maintained patency for several days. This work demonstrates a crucial step towards the generation transplantable engineered organs, by demonstrating anastomosis of biocompatible hydrogels directly with host vasculature. Future work will include encapsulating cells within these gels and designing more complex vascular networks to support entrapped autologous cells.

References:

Sooppan R+. Tissue Eng Part C Methods. 2016:22(1):1-7.

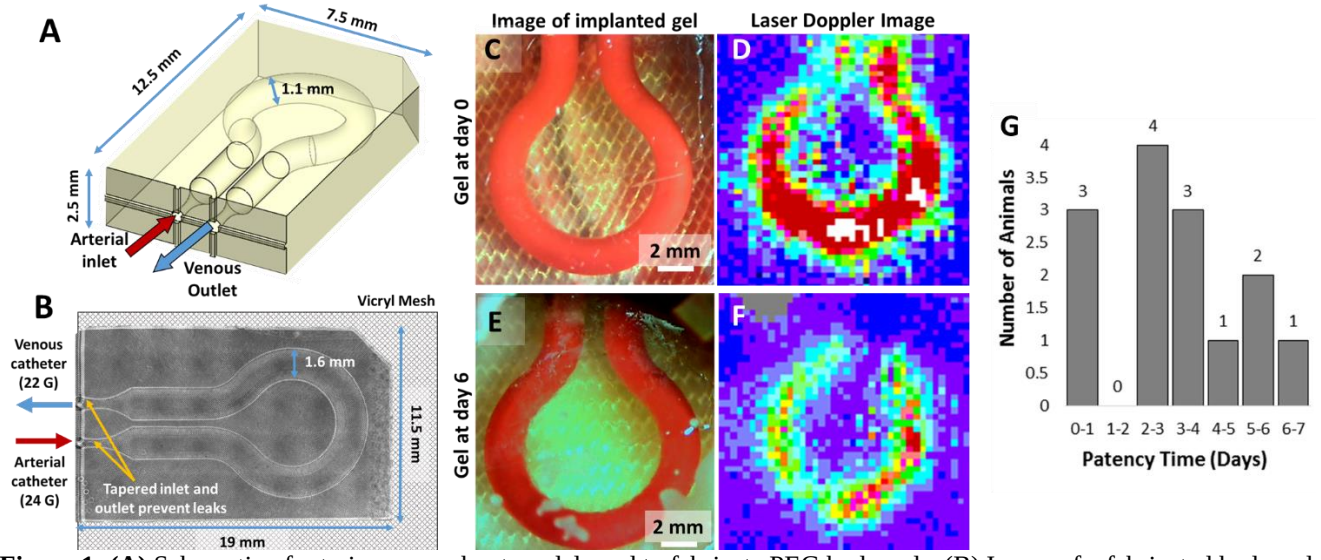

Figure 1: (A) Schematic of arteriovenous shunt model used to fabricate PEG hydrogels. (B) Image of a fabricated hydrogel containing the hairpin-shaped fluidic channel. (C-F) Image of an implanted gel and corresponding laser Doppler image showing flow rate at days 0 and 6 *in vivo*. (G) Histogram showing maximum time period for which the 14 implanted gels remained patent.