Projection Micro-StereoLithography (PμSL) Printed PDMS Substrates to Study Flap Revascularization in an Ischemic Mouse Model

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Statement of Purpose: Revascularization is important in proper wound healing and tissue regeneration. Recently, a myocutaneous flap model was developed which provides a very effective way to examine a gradient ischemia as well as functional revascularization.[1] Upon flap creation, if a material is not placed between the flap and underlying tissue, revascularization will occur. However, if a non-porous implant is introduced, there is an inhibatory effect on revascularization and the distal flap will die. Our research has looked at the simple, inexpensive fabrication technique of projection micro-stereolithography to produce porous PDMS films.[2] Films were implanted for 10 days and perfusion analysis via laser speckle, tissue excision, histology, and immunocytochemistry were performed to examine biocompatibility, vascular tissue formation, and flap survival.

Methods: Projection micro-StereoLithography (PμSL): A modified PμSL apparatus and technique was used as described previously (Figure 1A).[2] Substrate Fabrication: A printing solution of methacryloxypropyl terminated polydimethylsiloxane (PDMS-MA) and photoinitphenylbis(2,4,6-trimethylbenzoyl)-phophine oxide (BAPO) (0.03g) was prepared as previously described.[2] Substrates were fabricated with pores (SP, n=3) and without (S, n=3) and sterilized by Sterrad®. Substrate Implantation: All animals were treated humanely following a protocol laid out by the UNM animal review committee. Previously identified myocutaneous flap mouse model was used.[1] Porous and non-porous PDMS-MA thin films of 1.5 x 3.0 cm where placed between the flap and the underlying tissue followed by suturing the wound. C57BL6 Mice were designated as S (PDMS solid) and SP (PDMS pores) and a control (n=2) with no material inserted.
Laser Speckle Perfusion Imaging: At 0, 2, 5 and 10 days each mouse underwent analysis of perfusion via a laser perfusion imager. Mice were administered anesthesia (Isoflurane®) which was maintained during imaging.
Tissue Harvest and Histology: At day 10 all mice were sacrificed by CO_2 inhalation. The entire flap was excised for examination and transected into cranial and dorsal sections to correspond to how mice were imaged during laser speckle. Once sectioned, the specimens were fixed in IHC Zinc Fixative overnight followed by paraffin embedding. Histological sections were then H&E stained and visualized optically.

Results: Substrates were prepared with thicknesses of 13.4 ± 0.1 μm (S) and 12.2 ± 0.1 μm (SP). SP films had an average pore size 271 ± 1μm with horizontal spacing of 490 ± 1 μm and 602 ± 1 μm vertical (Figure 1B). Laser speckle showed lack of revascularization in S implanted mice, with resultant distal flap necrosis. In contrast, SP implanted mice showed successful functional revascularization distally on the flap. (data not shown).

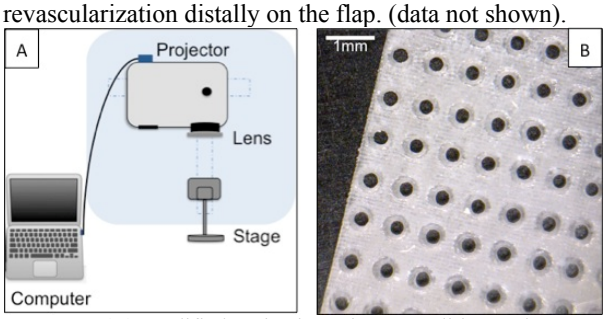

Figure 1. (A) Modified projection microstereolithography (PμSL) setup. (B) Optical image of SP implant, scale bare 1mm.

In addition, tissue harvesting and histology showed that the SP implants allowed not only for revascularization but also engraftment of the material. This can be seen by tissue growth in the pores of the H&E stained tissue sample (Figure 2A) where the growth of tissue through the pore connected the recipient bed with the flap, allowing for healthy panniculus muscle and numerous blood vessels in the muscle and dermis. In contrast, the non-porous S implantsshowed attenuated muscle due to persistent ischemia as well as significant inflammatory infiltrate in the dermis (Figure 2B).

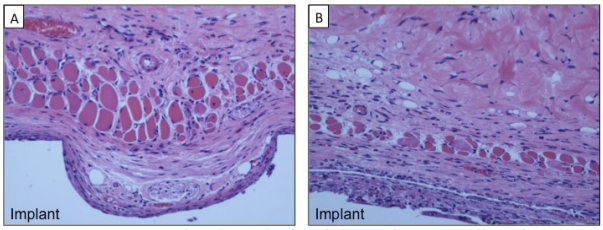

Figure 2. H&E stained sections of tissue harvested on day 10 after implant from SP (A) and S mice (B). Images were taken a 20x magnification.

Conclusions: We have fabricated porous and nonporous PDMS thin films via the simple technique of PμSL. Using a myocutaneous mouse model we have shown that the porous (SP) materials allowed for better perfusion over the solid (S) implants. The SP implants also allowed for engraftment of the material to the flap. Future implant designs will include varying the pore size to both smaller and larger as well as examining substrates with a gradient in pore size. The combination of both the mycutaneous flap mouse model and fabrication technique of PμSL presents an inexpessive model to study revascularization.

References :(1) McGuire, PG *et al*. (2010) *Journal Surg Res,* 164: e201. (2) Cicotte, KN *et al* (2012). *MRS Proceedings*, 1418: mm05.