Polyurethane-based mechanically matched, angiogenic precision porous long-lasting elastomers (PUBMAPLE) towards *in situ* vascular engineering: a discussion on foreign body capsule assessment

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Statement of Purpose: The absence of FDA approved small diameter (≤ 4 mm inner diameter) synthetic vascular grafts and the less-than-ideal performance of FDA approved medium diameter ones suggest major opportunities for biomaterial innovations. Biomaterials that reduce foreign body capsule (FBC) and improve bio-integration may enable the recruitment of endogenous cells for the reconstruction of blood vessels *in situ*. Such an *in situ* vascular engineering approach requires pro-healing biomaterials. In this study, we developed polyurethane-based mechanically matched, angiogenic precision porous long-lasting elastomers (PUBMAPLEs) with uniform 40 μm pore size. *We hypothesized that such scaffolds will promote angiogenesis and mitigate the FBC by eliciting a vigorous and favorable immune response.* The hypothesis was tested in detail using histology and immunohistochemistry.

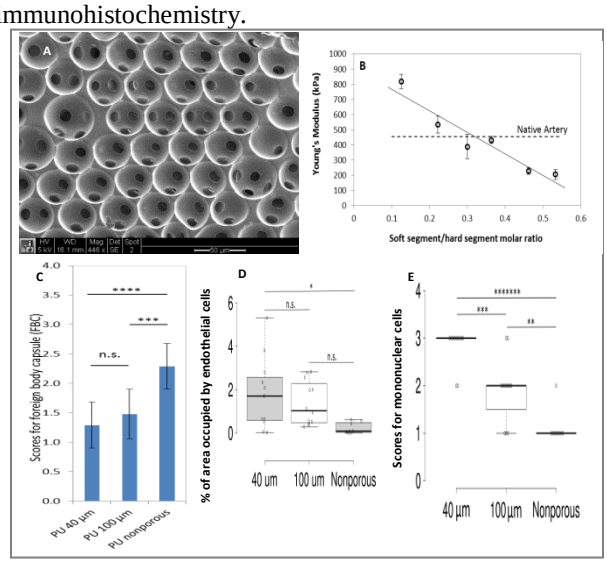

Figure 1. Structural, mechanical properties of PUBMAPLEs and biological responses to them. **A)** An SEM image of the precision porous structure; **B)** The tunable mechanical property of PUBMAPLEs; **C)** PUBMAPLEs reduce FBC; **D)** PUBMAPLEs improve angiogenesis; **E)** PUBMAPLEs recruit mononuclear cells.

Methods: We synthesized the polyurethane materials using a one-step, catalyst-free, and solvent-free reaction, and fabricated the precision-porous scaffolds using a sphere-templating method. [1] The porous structure was characterized by SEM. The Young's moduli of scaffolds were assessed by Instron testing. PUBMAPLE scaffolds with artery-matched mechanical properties (40 μm porous, 100 μm, and nonporous) were implanted subcutaneously in Balb/c mice and explants were stained with Masson's trichrome, H&E, and MECA 32 (an endothelial cell marker). Histological slides were scanned digitally, followed by quantification by a professional pathologist blinded to the study, or by software analysis of positive staining.

Results: PUBMAPLE scaffolds show an interconnected porous structure with uniform 40 μm pore size (**Fig. 1A**). The mechanical properties of PUBMAPLEs can be fine-tuned by varying the soft segment/hard segment ratio to match that of native blood vessels (**Fig. 1B**). We further assessed the severity of FBC induced by PUs with different pore sizes (40 μm porous, 100 μm, and nonporous) based on a refined, semi-quantitative scoring system that addresses the coverage, density and thickness of the FBC [1]. This assessment method is an improvement from previous methods based only on thickness [2] or density profile [3]. Both 40 μm and 100 μm porous PUs significantly reduce FBC compared to nonporous PUs (**Fig. 1C**). Only 40 μm porous PUBMAPLEs significantly increase endothelial cell abundance in the fibrous capsule (**Fig. 1D**). 40 μm porous PUBMAPLEs also significantly improve angiogenesis and cellularization inside the pores compared to 100 μm porous PU scaffolds (data not shown). These improved healing responses to PUBMAPLEs are accompanied by normal, yet elevated, levels of inflammation. For example, the density score of mononuclear cells associated with implants increases from nonporous PUs to 100 μm porous scaffolds to 40 μm porous scaffolds, with significant differences between all three groups (**Fig. 1E**). These observations support the hypothesis that the enhanced bio-integration of 40 μm porous PUBMAPLEs is driven by beneficial inflammatory processes.

Conclusion: In this study, we fabricated PUBMAPLEs with a uniform 40 μm pore size and matching mechanical property to native blood vessels. PUBMAPLEs reduce FBC and improve angiogenesis and cellularization. These pro-healing responses are likely to be driven by healthy and vigorous inflammation. PUBMAPLEs can be an ideal candidate for *in situ* vascular engineering, as well as having potential for implantable devices, tissue engineering, and drug delivery. The translational potential of these pro-healing vascular grafts is currently being investigated in a pre-clinical sheep model. Importantly, we developed a semi-quantitative scoring system that more completely describes the FBC, which contributes to our ability to accurately assess the FBC formed around biomaterials.

References:

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2. Sussman EM., et al Ann Biomed Eng. 2014;42(7):1508-1516
3. Zhang L., et al Nat. Biotechnol. 2013;31(6):553-556