Injectable microporous scaffolds from annealed building blocks for accelerated wound healing

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Statement of Purpose: For engineered tissue regeneration, the development of interconnected microporous scaffolds that allow for extensive cell networks and collective migration without the loss of scaffold integrity is essential for effective bulk integration with the surrounding tissue. We hypothesized that an injectable microporous material would allow collective migration and bulk integration, while avoiding scaffold degradation or invasive procedures for implantation. Natural sol-gel polymers (e.g. fibrin and collagen) with a porous structure often display batch-to-batch variability, unreliable interconnectivity, and degradation rates that are difficult to optimize. Alternatively, synthetic hydrogels with microscale, interconnected pores require removal of commonly toxic porogens, limiting their severely limiting their utility in the presence of living tissue. To address the limitations of current platforms, we have created a new class of biomaterial, Microporous Annealed Particle (MAP) gels, that realizes the seamless tissue interface of injectable hydrogels and enables collective cell migration similar to previous microporous hydrogels. By combining these two abilities, we have provided an ideal biomaterial for bulk tissue integration and regeneration *in vivo*.

Methods: To produce the MAP scaffold we used an easily scalable microfluidic water-in-oil emulsion approach to portion a continuous poly(ethylene glycol) with enzymatically cleavable crosslinker pre-gel aqueous phase into nearly monodisperse scaffold building blocks (Fig. 1A). The μgel building blocks were purified simply by centrifugation into an aqueous solution of isotonic cell culture (Fig. 1B). Transitioning individual building blocks into a MAP scaffold is catalyzed by Factor XIIIa, a naturally occurring, cytocompatible enzyme responsible for stabilizing blood clots. To demonstrate regeneration, we utilized a murine skin wound healing model, addressing a tissue of interest for previous implanted porous biomaterials. Importantly, wound bed contraction, common for murine healing, was prevented using a sutured rubber splint. This approach better simulates the human healing response. We chose a chemically identical non-porous hydrogel as an injectable hydrogel control.

Results: Material properties are modifiable through a bottom-up approach by customization of the individual building blocks. We demonstrated a range of material stiffness values that span the mammalian soft tissue range (~10 to ~1000 Pa). Interconnected pore size is also modular through controlling the size of the building blocks, ranging from ~10 to ~35 μm for building blocks of 30 to 150 μm, respectively. After 5 days *in vivo*, both endothelial cells (Fig. 1C, green) and supporting pericytes (Fig. 1C, pink) were present within the MAP scaffold, while in non-porous bilateral controls only single branches of endothelial cells without supporting pericytes were present. To our knowledge, this is the first instance of early (<7 days) pericyte migration into a synthetic injectable material or implanted porous scaffold without the inclusion of exogenous growth factors. Importantly, the MAP scaffold was able to sustain the formation of complete hair follicles with adjoining sebaceous glands within the wound bed (Fig. 1D). After 5 days post-injection, lower fractions of $CD11b^+$ cells (activated leukocytes) were present both in the surrounding tissue and within the MAP scaffold relative to the non-porous controls (Fig. 1E), indicating a sustained lower level of inflammatory immune response. Ultimately this led to faster healthy tissue regeneration than with non-porous injectable controls (60% versus 100% remaining wound area after 5 days, respectively).

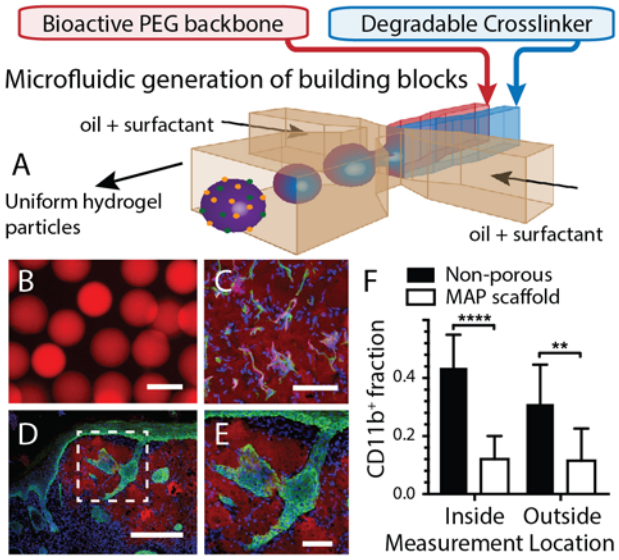

Figure 1. A) MAP building blocks are formed via segmentation of gelling aqueous solutions. B) Purified particles are a nearly monodisperse population of microspheres (scale: 100 μm). MAP scaffolds promote C) mature vascularization (scale: 50 μm; red: MAP, green: endothelial cells, pink: pericytes, blue: nuclei) and D&E) formation of hair follicles and sebaceous glands (scale: 100 μm and 50 μm; red: MAP, green: keratinocytes, blue: nuclei). E) The MAP scaffold stimulates a significantly diminished immune response inside and outside the gel compared to the non-porous control hydrogel.

Conclusions: MAP scaffolds achieve *in vivo* tissue integration and regeneration superior to chemically identical non-porous hydrogels. MAP scaffolds are injectable, display interconnected porosity, and represent a novel class of biomaterial.