40 µm Diameter Pore, Precision-Templated Scaffolds Promote Infiltration of Pro-Healing Circulating Monocytes

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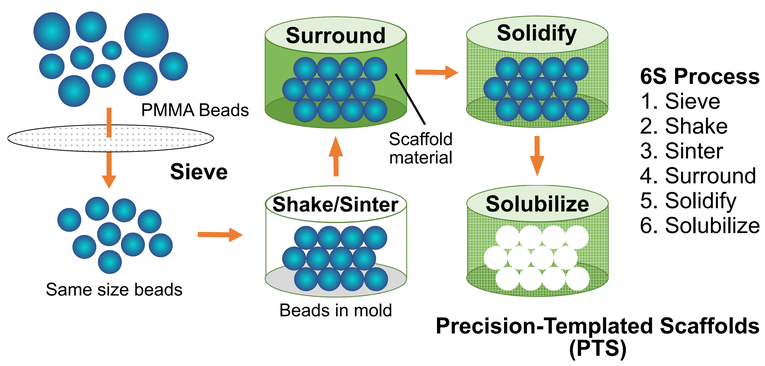
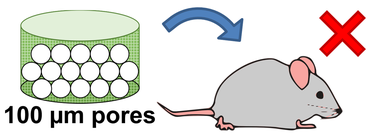
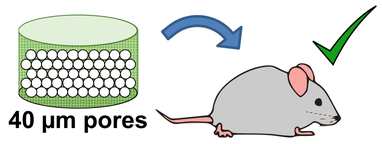
1. Introduction



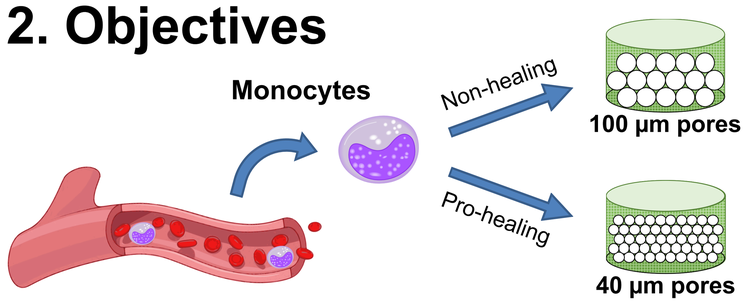


- Collagenous, avascular, fibrous tissues
- Foreign body response
- Chronic inflammation



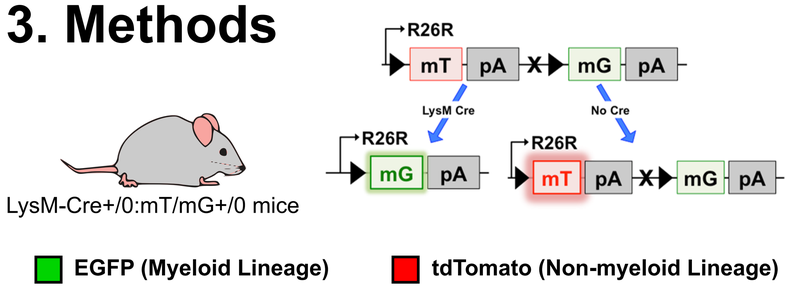
- Tissue reconstruction
- Vessel formation

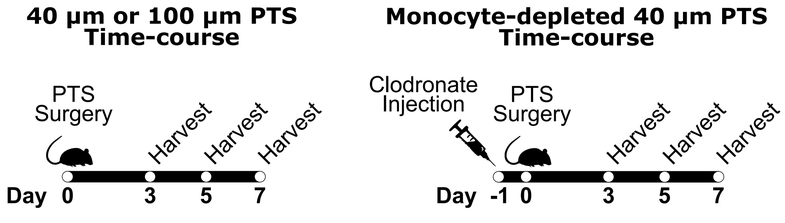
2. Objectives



Track the infiltration kinetics of circulating monocytes in the peripheral blood to subcutaneously implanted 40 µm PTS and 100 µm PTS

3. Methods





4. Results

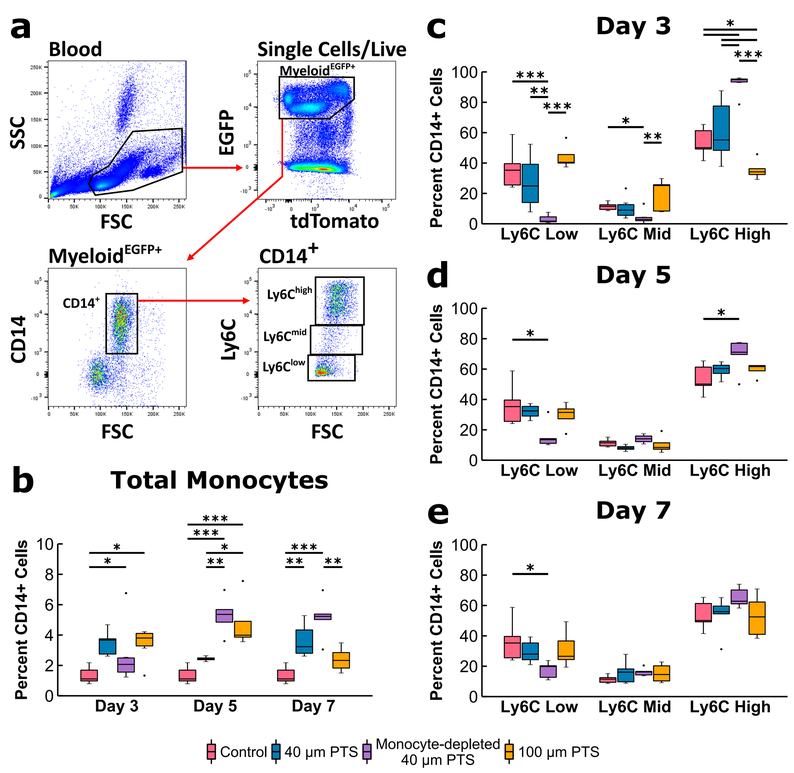


Figure 1: Flow cytometric analysis of PBMCs recovered from LysM-Cre+/0:mT/mG+/0 mice implanted subcutaneously with 40 µm PTS or 100 µm PTS, and from 40 µm PTS implanted subcutaneously in monocyte-depleted LysM-Cre+/0:mT/mG+/0 mice. (a) PBMC gating strategy. (b) Total percentage of $CD14^+$ monocytes from PBMCs. (c-e) Ly6C expression of $CD14^+$ monocytes at (c) Day 3, (d) Day 5, and (e) Day 7 post-scaffold implantation. * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$.

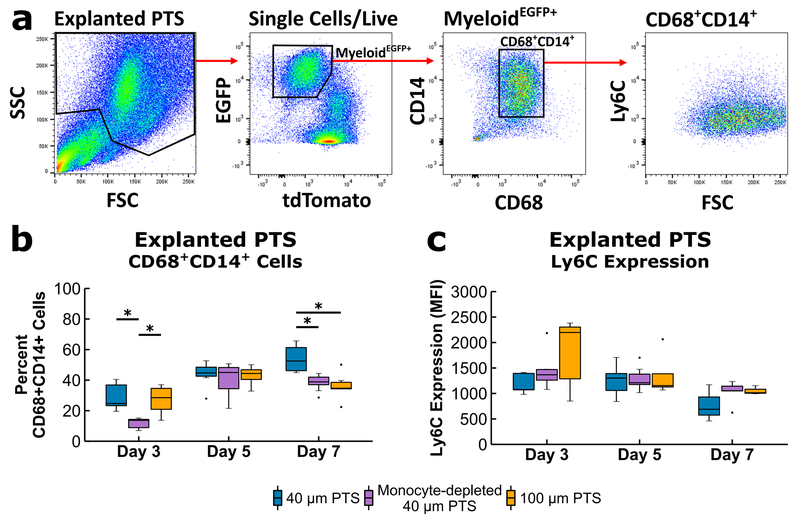


Figure 2: Flow cytometric analysis of scaffold resident cells recovered from 40 µm PTS or 100 µm PTS implanted subcutaneously in LysM-Cre+/0:mT/mG+/0 mice, and from 40 µm PTS implanted subcutaneously in monocyte-depleted LysM-Cre+/0:mT/mG+/0 mice. (a) Gating strategy for explanted scaffold-resident cells. (b) Percentage of $Myeloid^{EGFP+}CD68^+CD14^+$ cells from explanted PTS. (c) Median fluorescent intensity of Ly6C expression from explanted PTS. * denotes $p < 0.05$ and ** denotes $p < 0.01$.

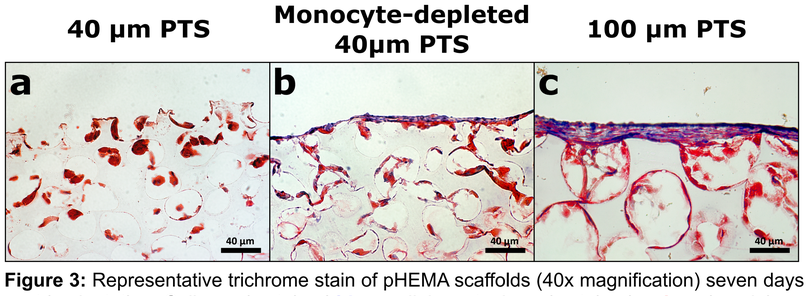


Figure 3: Representative trichrome stain of pHEMA scaffolds (40x magnification) seven days post-implantation. Collagen is stained **blue**, cellular cytoplasm is stained **red**, and nuclei are stained **black**. (a) 40 µm PTS in untreated mice, (b) 40 µm PTS in monocyte-depleted mice, and (c) 100 µm PTS in untreated mice.

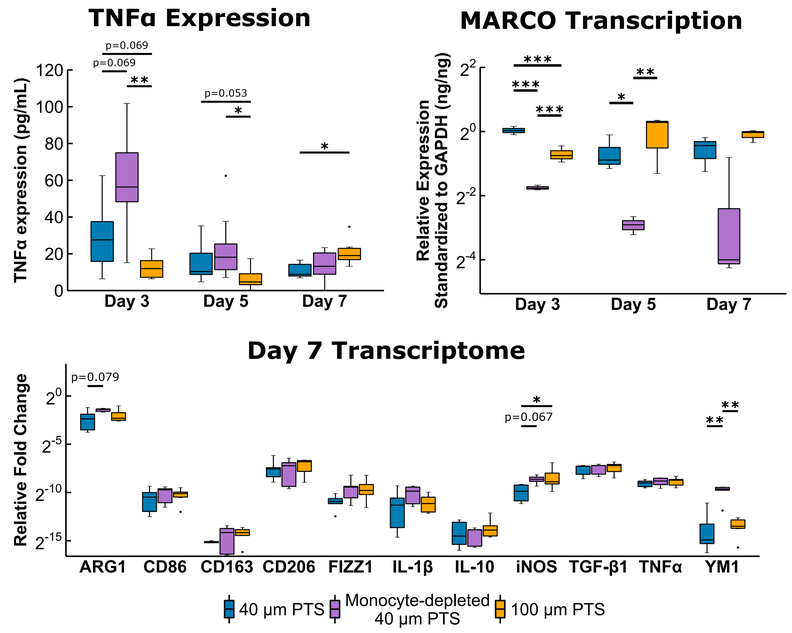


Figure 4: Cytokine secretion analysis and transcriptomics on cells resident in 40 µm and 100 µm PTS explanted from untreated LysM-Cre+/0:mT/mG+/0 mice, and cells resident in 40 µm PTS explanted from monocyte-depleted LysM-Cre+/0:mT/mG+/0 mice. (a) Conditioned medium analysis of pro-inflammatory TNFα. (b) Relative expression of MARCO transcription standardized to GAPDH. (c) Polarization transcriptome gene profiling from PTS explanted 7 days post-implantation. * denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$.

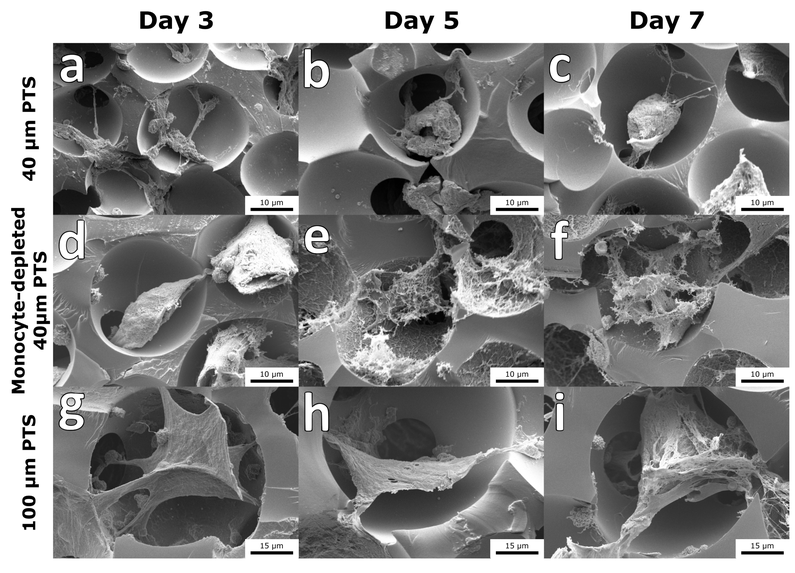


Figure 5: Scanning electron microscopy images of subcutaneously implanted scaffolds in LysM-Cre+/:mT/mG+/0 mice at Day 3, Day 5, and Day 7 post-implantation. (a-c) 40 µm PTS in untreated mice, (d-f) 40 µm PTS in monocyte-depleted mice, and (g-i) 100 µm PTS in untreated mice.

5. Conclusion

- PTS generate distinct *in vivo* circulating blood monocyte profiles dependent on pore size
- Scaffold-resident cells appear to be derived from circulating monocytes with a statistically significant increase of infiltrating monocytes within 40 µm PTS
- 40 µm PTS contain a subset of MARCO expressing monocytes that appears to be responsible for pro-healing in 40 µm PTS
- Scaffold-resident cells exhibit unique morphological changes as a function of monocyte depletion and pore size in PTS

6. Future Experiments

- Identify specific monocyte subpopulations that give rise to a regenerative, pro-healing response in the 40 µm PTS
- Understand the mechanisms that drive favorable healing outcomes in implanted PTS