Novel Porous Scaffold for Releasing Bioactive Molecules

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Statement of Purpose: Application of polymeric tissue engineering scaffolds prepared by conventional techniques like salt leaching and phase separation is greatly limited by its poor biomolecule-delivery abilities.[1] Multiple cross-linking, soaking, washing, and drying procedures are often needed to incorporate biomolecules to polymeric scaffolds. To overcome this limitation, in a recent study, we developed a novel technique to fabricate degradable porous scaffolds by combining phase separation with a bovine serum albumin microbubble (henceforth MB) porogen incorporation.[2] Since MBs have been used to deliver DNA/RNA/protein and MBs can protect their content from harsh environment, such as solvent, we thus assumed that MBs would not only create porous scaffold but also deliver bioactive molecule throughout the scaffold. To test this hypothesis, insulin-like growth factor 1 (IGF-1)-loaded MBs was used to fabricate porous scaffold. We compared such IGF-1 MB scaffolds with conventional phase separated scaffolds soaked in IGF-1 to study their ability to preserve the bioactivity. We also evaluated their performance in vivo.

Methods: *Scaffold fabrication:* IGF-1 loaded MBs were synthesized by mixing the optimal concentration of IGF-1 (500 ng/ml) with BSA solution followed by sonication under nitrogen gas. Such microbubbles were incorporated in PLGA solution (7.5% w/v), stirred gently and frozen in liquid nitrogen to induce phase separation. They were then lyophilized. Phase separated scaffolds without BSA MBs served as controls. *In vitro study:* Variously treated scaffold disks were incubated in PBS and release media was collected at various time points (0, 12hrs, 1, 2, 3, 4, 5, 6 days). NIH 3T3 fibroblasts were plated on 24 well plates (10,000 cells/well) and cultured in media without serum. Release media collected at various time points was used in a cell proliferation assay. *In vivo study:* Different scaffold disks were subcutaneously implanted in Balb/c mice for a week. At the end of the study, implants and surrounding tissues were isolated and then histologically analyzed. Masson's Trichrome Blue staining was done to assess the collagen formation around the implanted scaffold.

Results: The release rates of bioactive IGF-1 were determined in vitro based on the cell proliferation rates. As expected, with simple soaking process, majority of IGF-1 was released from phase-separated scaffolds within 1 day (Figure 1) and dropped dramatically after day 2. This implies that as expected scaffolds soaked in IGF-1 had a burst release in the early stages. On the other hand, scaffold fabricated with IGF-1 loaded MBs (IGF1-MB scaffold) releases active IGF-1 at a rather consistent rate for up to 6 days based on cell proliferation assay (Figure 1). The results suggest that MBs could incorporate IGF-1 into scaffold and yet preserve its bioactivity from solvent denaturation. The bioactivity of these scaffolds was then determined in vivo using a mice subcutaneous implantation model. In agreement with in vitro observations, the phase separated control scaffolds elicited a very high inflammatory reaction as shown by the capsule thickness while had very sparse formation of collagen. The effect of IGF-1 on promoting fibroblast proliferation is not apparent in IGF-1 soaked phase separated scaffold (Figure 2A) and MB scaffolds without IGF-1 (Figure 2B) with low collagen production. However, IGF-1-MB scaffolds prompted much stronger fibroblast reactions and collagen production (Figure 2C) supporting the profound bioactivity of IGF-1 in scaffold. In addition, our results have shown that the presence of BSA in the scaffold reduce inflammatory cell accumulation adjacent to scaffold implants.

Conclusions: Building on the promising results from our recent study, here we have demonstrated that the MB scaffolds can be used to load scaffolds with active form of growth factors without additional steps of crosslinking, washing and incubation processes. To prove the concept, porous scaffolds fabricated with IGF-1-MB have been shown to increase localized collagen production while reducing inflammatory responses. In addition, pores of MB scaffolds were covered with a layer of BSA which weaken inflammatory responses and indirectly improve the biocompatibility of tissue scaffold. We believe that these novel scaffolds can be used to deliver a range of single or combination of bioactive biomolecules to substantially promote cell growth and function in degradable scaffold.

References:

1. van de Weert et al., Pharm Res 2000;17:1159-67.
2. Nair et al., Proc IEEE DEMBS 2007; 31-34..

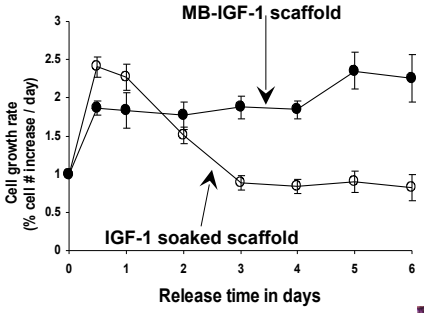

Figure 1. In vitro evaluation of IGF-1 bioactivity released from MB-IGF-1 scaffold and IGF-1 soaked scaffolds. N=3 /group

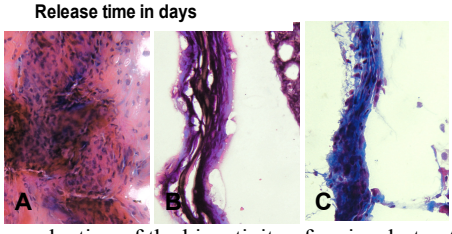

Figure 2. In vivo evaluation of the bioactivity of variously treated scaffolds. Sparse collagen formation (stained blue) in phase separated scaffolds soaked in IGF-1 (A) and MB scaffolds (B) while very high collagen formation was observed in MB-IGF-1 loaded scaffolds (C). 200X.