Injectable Porous Hydrogel for Soft Tissue Regeneration

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Introduction
Injectable hydrogels are potent biomaterials for tissue engineering and regenerative medicine. Their use enables non-invasive or minimally invasive surgery, and they can be shaped to correspond to target sites. In addition, hydrogels allow for homogeneous cell distribution and high permeability. To maximize the effectiveness of hydrogels, porous structure is often required to facilitate enhanced mass transfer of metabolites, thus leading to improved cell proliferation and tissue formation in vivo [1]. Based on our hypothesis that thermo-responsive gelatin can be used for pore generation in hydrogels [2], we investigated whether tissue formation could be enhanced by the use of human fibroblasts and gelatin microbead-embedded fibrin gels.

Materials and Methods
Bovine fibrinogen and thrombin (Aldrich) were dissolved in Dulbecco’s Phosphate buffered saline (DPBS) and filtered with a syringe filter. Human dermal fibroblasts were isolated from human skin after collagenase II digestion and subsequently cultured. Cultured human dermal fibroblasts (1×10^7 cells/mL) were mixed with a fibrinogen (80 mg/mL) solution containing gelatin microbeads (25 mg/mL). This cell-gelatin containing fibrinogen solution was mixed with thrombin (40 U/mL, 2.5% w/v). For shape controlled analysis, cell containing fibrin gels with or without gelatin microbeads (diameter < 63 μm) were cast in silicone rubber molds with a diameter of 10 mm and 5 mm in height. Fibrin gels were implanted subcutaneously in the dorsum of athymic mice and then explanted at predetermined time points. To investigate microstructural changes and tissue formation, explanted fibrin samples were stained with H&E and Masson’s Trichrome staining.

Results
A porous structure was observed in the fibrin gels two weeks after implantation (Fig. 1). Under cell free fibrin gel conditions, solid fibrin gels had fewer cells in the fibrin matrix when compared to those composed of gelatin mixed porous fibrin. In porous fibrin conditions, cells resided and proliferated in the gelatin mixed fibrin both with and without cell encapsulation. Compared with solid fibrin gel condition, cells in the porous fibrin filled pores adjacent to the boundary of fibrin gels.

Discussion and Conclusions
Porous structures provide space for cell proliferation and enhance mass transfer of metabolites in tissue engineering applications. In living tissues, mass transfer of nutrients and oxygen is limited to several hundred microns from capillaries [1]. Despite the high intrinsic permeability of hydrogels, enhanced mass transfer is still required. This is clearly seen in the center region of fibrin gels in Figure 1 (a) and (b), which show that the number of cells and cell distribution are not significantly different in solid and porous fibrin. However, cells became aggregated and produced extracellular matrix within pores in the boundary region of the porous fibrin (Figure 1 d). These results indicate that gelatin based pore structures can be used in combination with injectable hydrogels to achieve enhanced tissue formation in soft tissue applications.

References

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