TGF-β-grafted PLLA Scaffold for Chondrogenic Differentiation of Adipose-derived Stem Cells

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Statement of Purpose

Adipose-derived stem cells (ASCs) are capable of differentiating into connective tissue-forming cell lineages.[1] Known as an alternative cell source to chondrocytes, many studies have utilized them for *in vitro* cartilage formation under an appropriate 3D environment, along with the use of growth factors, i.e., transforming growth factor (TGF), bone morphogenetic proteins (BMP), and insulin-like growth factor (IGF-I). Growth factors would stimulate cartilaginous matrix prodution. In particular, TGF-β is a multifunctional protein that promotes the proliferation of ASCs and enhances the chondrogenic differentiation *in vitro*. Therefore, a combination of a growth factor and a porous scaffold might be a plausible tactics in improving cartilage-forming efficacy of ASC.[2] In the present work, we hypothesized that this novel TGF-β1-grafted PLLA scaffolds may be a suitable platform for the induction of chondrogenic differentiation of ASC.

Materials and Methods

Dual pore PLLA scaffolds were prepared using a solvent casting and gas foaming method. The scaffold surface was then activated using argon plasma treatment and *in situ* grafting of acrylic acid (AA). The resultant carboxyl groups were readily activated in a mild aqueous solution of EDC/NHS/MES buffer (pH 5.6), followed by the grafting of heparin. The heparin-grafted PLLA was then soaked in PBS solution of TGF-β1 for 2 h at room temperature. The release of TGF-β1 was monitored for predetermined tines at 37˚C. Surface properties of the modified PLLA substrates were analyzed by ATR-FTIR, ESCA, and contact angle measurement. The amount of immobilized heparin was determined using toluidine blue and TGF-β1 was assayed using ELISA kit (Quantikine® TGF-β1 ELISA, R&D Systems). ASCs were then seeded onto either TGF-β1-grafted PLLA or PLLA control scaffold and cultured in a serum-free chondrogenic medium, DMEM supplemented with ascorbic acid, ITS^+, dexamethasone, and TGF-β1 for 2 and 4 weeks, respectively. The experimental groups of PLLA-PAA-Hep-TGF-$\beta1^-$ and PLLA-PAA-Hep-TGF-$\beta1^+$ were cultured without and with TGF-β1 in chondrogenic medium, respectively. Cell proliferation was assessed by WST-1 assay. Chondrogenesis of MSCs in the TGF-β1-grafed PLLA was examined from histology and gene expression.

Results and Discussion

As the surface of PLLA scaffolds turned hydrophilic, the hydrophilicity of the TGF-β1-grafted surface was similar to the modified PLLA. In the ESCA data, PLLA film showed two separate peaks corresponding to carbon and oxygen peaks. However, TGF-β1-grafed PLLA film surfaces were enriched with nitrogen atoms. From histological analysis, after 4 weeks of culture on the TGF-β1-grafted surface, H&E staining showed that cellularity was obviously improved, especially with the TGF-β1-grafted one as compared to the PLLA control. Chondrogenic differentiation was clearly identified with Safranin O staining of GAG (Fig. 1). The staining intensity was found more enhanced in the TGF-β1-grafted PLLA constructs at 4 weeks. This study demonstrated that the modified polymer surfaces may provide more favorable environment for chondrogenesis of stem cells and that TGF-β1-grafted polymer scaffolds can continuously release the growth factor into culture medium, stimulating chondrogenic differentiation of ASC.

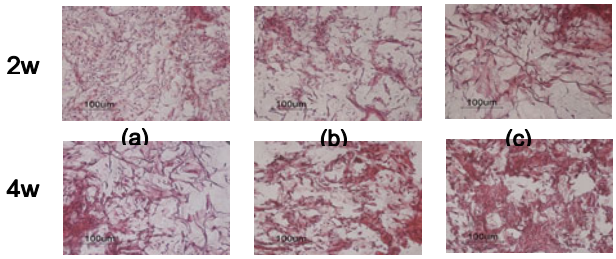

Fig. 1. Safranin O staining of ASC-cultured PLLA constructs at 2 and 4 weeks (x 200): (a) PLLA, (b) PLLA-PAA-Hep-TGF-$\beta1^-$, and (c) PLLA-PAA-Hep-TGF-$\beta1^+$.

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References

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