Evaluation of Porous PLLA Scaffold for Chondrogenic Differentiation of Stem Cells

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Introduction

Stem cells are essential as a self-repair system in the body. They can differentiate into a variety of specialized cell types, such as chondrocytes, osteoblasts, myoblasts, and nerve cells. As an alternative to mature tissue cells, stem cells have been given much attention for their multipotency in tissue engineering and regenerative medicine. Since interactions between scaffold and cells play an important role in the tissue development *in vitro*, synthetic oligopeptides, Arg-Gly-Asp (RGD) and Arg-Glu-Asp-Val (REDV) have been immobilized onto polymeric scaffolds to improve specific cell attachment and even to stimulate cell differentiation. In this study, with poly(L-lactic acid) (PLLA) scaffold as a base material, chondrogenic differentiation of stem cells was evaluated using surface-modified PLLA scaffolds, i.e., either hydrophilic acrylic acid (AA)-grafted PLLA or RGD-immobilized one.

Experimental

Biodegradable porous PLLA scaffolds were prepared using a gas foaming method. The scaffolds were then activated by plasma, followed by subsequent grafting of AA to produce a hydrophilic polymer scaffold (PLLA-PAA). This was further processed to fix RGD peptide to make an RGD-immobilized scaffold (PLLA-PAA-RGD). Stem cells were seeded at $1x10^6$ cells per scaffold and the cell-PLLA constructs were cultured for up to 4 weeks. The chondrogenic medium contains DMEM-hg, 1% penicillin/streptomycin, 1 × insulin-transferrin-selenium (ITS+), 50 μg/ml ascorbate 2-phosphate, 40 μg/ml proline, 100 μg/ml sodium pyruvate, 100 nM dexamethasone, and 10 ng/ml transforming growth factor-β1. Using these surface-modified scaffolds, adhesion, proliferation, and chondrogenic differentiation of stem cells were evaluated through SEM, WST-1 assay, histology (H&E, Safranin O, and Masson's Trichrome staining), and RT-PCR (collagen type I, II, and X, aggrecan, and Sox 9).

Results and discussion

The surface of PLLA scaffolds turned hydrophilic (water contact angle, 45 degrees) with both plasma treatment and AA grafting. The hydrophilicity of RGD-immobilized surface was not significantly altered. When the changes in cell number of stem cells were examined by WST-1 assay, cell proliferation rate on the either PLLA-PAA or PLLA-PAA-RGD surface was obviously improved, especially with the RGD-immobilized one as compared to the control PLLA one. From histological analysis, chondrogenic differentiation was clearly identified with Safranin O staining of GAG. The staining intensity was found more enhanced in the AA- or RGD-grafted PLLA substrates. This study demonstrated that modified polymer surfaces may provide better environment for chondrogenesis of stem cells and that porous polymer scaffolds can be manipulated to promote stem cell differentiation.

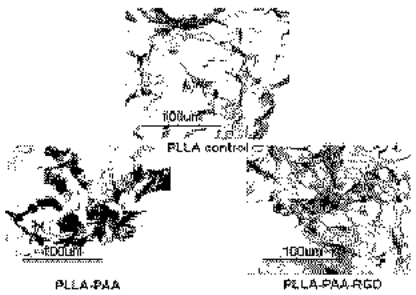

Fig. 1. Safranin O staining (x 400) of stem cell-seeded PLLA construct at 4 weeks.

References

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