Mechano-Active Cartilage Tissue Engineering using 3-Dimensional and Dynamic Environments

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Statement of Purpose: Articular cartilage is subjected to complex loading, and which play a major role in the growth, development and maintenance of the articular cartilage[1,2]. In our previous studies, mechanical stimuli enhanced the development and function of engineered cartilage tissues in elastic mechano-active poly(lactide-co-caprolactone) (PLCL) scaffolds. Also, it is known that the three-dimensional spatial organization of cells and extracellular matrix is crucial to functional cartilage formation[3]. Although hydrogels have been widely used for 3-dimensional environments, the mechanical properties of hydrogels were too weak to endure the complex loading in the body. The purpose of this study is to form substantial tissue engineered cartilage with hybrid scaffolds of fibrin gels and PLCL scaffolds in dynamic environment by mechanical stimulation.

Methods: A highly elastic scaffold was fabricated from very elastic PLCL with 85 % porosity and 300~500 μm pore size by a gel-pressing method. The scaffolds were seeded with bone marrow stromal cells (BMSCs) suspended in a solution of fibrin gels. After 24h for stabilization in the incubator, the seeded scaffolds were placed under the plunger of the bioreactor and the continuous compressive deformation was applied to them with 0.1Hz in chondrogenic medium containing 10 ng/ml TGF-β_1. And then, they were implanted into the subcutaneous dorsum of athymic mice to investigate their biocompatibility and cartilage formation. Cell-polymer constructs were characterized by WTS assay and GAGs quantitative analysis, and histological studies. For defining the gene expression for mechanical stimulation, reverse transcription-PCR was performed.

Results / Discussion: Mechano-active scaffolds having a complete rubber-like elasticity prepared by a gel-pressing method. They could be easily twisted and bended and showed almost complete (over 97%) recovery at strain applied of up to 500%. It is confirmed by the scanning electron microscope (SEM) that the cells were adhered onto the hybrid scaffolds of fibrin gels and PLCL scaffolds maintaining the round shape (Figure 1).

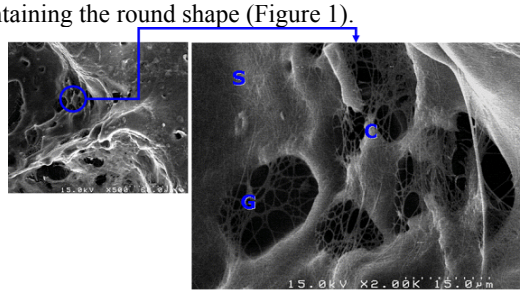

Figure 1. SEM images of cell-polymer constructs (S: scaffold, G: fibrin gels, C: cells)

The accumulation of extracellular matrix of cell-polymer constructs which was increased through mechanical stimulation showed that chondrogenic differentiation was sustained and enhanced significantly.

Histological analysis of the specimen retrieved at 8 weeks showed that stimulated implants by compression formed mature and well-developed cartilaginous tissue, as evidenced by differentiated BMSCs within lacunae. Alcian blue staining and Masson's trichrome staining indicated an abundant accumulation of sulfated GAGs and collagens respectively, which are extracellular matrices produced by differentiated BMSCs in the newly formed tissues (Figure 2).

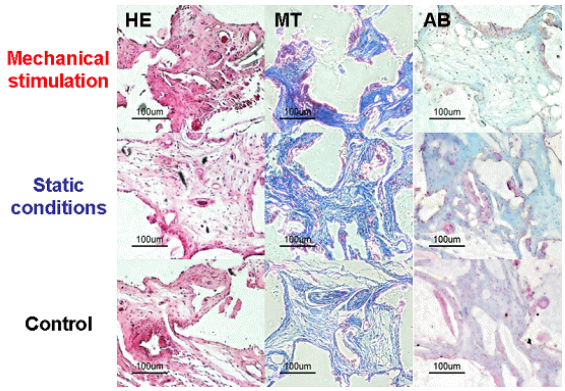

Figure 2. Histological studies of implants 8 weeks after implantation (HE: Hematoxylin and Eosin staining, MT: Masson's Trichrome staining, AB: Alcian blue staining)

Conclusions: In conclusion, the proper periodic application of dynamic compression and 3-dimensional environments of the hybrid scaffolds of fibrin gels and elastic PLCL scaffolds can encourage BMSCs to differentiate to chondrocytes, maintain their phenotypes and enhance GAGs production and consequently, improve the quality of cartilaginous tissue formed in vitro and in vivo.

Acknowledgement:
This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare (MOHW), Republic of Korea (A050082).

References:

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