Chondrogenic Differentiation of Human Adipose Tissue Derived Stromal Cells using TGF-β_1 loaded functional nanoparticle–hydrogel-PLCL complex

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Introduction: As one of treatments for the formation of functional articular cartilage, the use of tissue engineering with living cells, a biocompatible polymer and stimulations for the repair of articular cartilage was regarded unique challenges. Especially in the cartilage tissue engineering using stem cells, it is known that the chemical and mechanical stimulations are very important for the differentiation and the maintenance of the lineage[1]. Also, the three-dimensional spatial organization of cells and extracellular matrix is crucial to functional cartilage formation[2]. In our previous studies, Fibrin gel hybridization with human adipose tissue derived stromal cells (hADSCs) enhanced the development and function of engineered cartilage tissues in elastic mechano-active poly(lactide-co-caprolactone) (PLCL) scaffolds. The purpose of this study is to form substantial tissue engineered cartilage with hADSCs and TGF-β_1 loaded functional nanoparticle–hydrogel-PLCL complex with effective chemical stimulation with continuous controlled release.

Materials and 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. Functional nanoparticles were made with poly(lactide-co-glycolide) PLGA as a hydrophobic core, Pluronic F-127 as a hydrophilic surface layer, and heparin as the functional moiety by a solvent-diffusion method without chemical modification of the components and then TGF-β_1 was loaded with 67ng/μl[3]. The hADSCs suspended in a solution of fibrin gels and TGF-β_1 loaded functional nanoparticles were seeded onto PLCL scaffolds. After 2 hours for stabilization in the incubator, they were implanted into the subcutaneous dorsum of athymic mice to investigate their biocompatibility and cartilage formation. Cell-polymer constructs were characterized by GAGs quantitative analysis and histological studies. For defining the gene expression for chondrogenic differentiation, reverse transcription-PCR was performed.

Results and 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, nanoparticles and PLCL scaffolds maintaining the round shape (Figure 1). The accumulation of extracellular matrix of cell-polymer constructs which was increased through chemical stimulation with the controlled release of TGF-β_1 showed that chondrogenic differentiation was sustained and enhanced significantly. The GAG contents of implants stimulated by TGF-β_1 loaded functional nanoparticles were higher than them without nanoparticles.

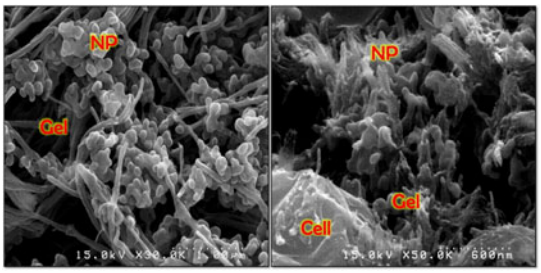

Figure 1. SEM images of cell-polymer constructs

Histological analysis of the specimen retrieved at 5 weeks showed that TGF-β_1 loaded functional nanoparticle–hydrogel-PLCL complex formed mature and well-developed cartilaginous tissue, as evidenced by differentiated hADSCs within lacunae. Alcian blue staining and immunofluorscence staining for collagen type 2 indicated an abundant accumulation of sulfated GAGs and collagens respectively, which are extracellular matrices produced by differentiated hADSCs in the newly formed tissues (Figure 2).

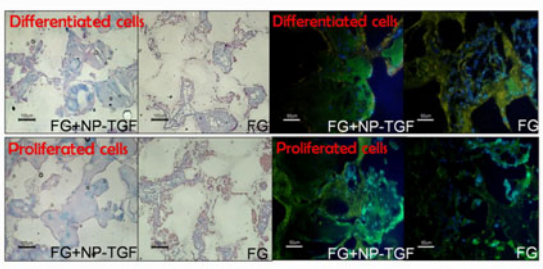

Figure 2. Histological studies of implants 5 weeks after implantation

Conclusions: The hybridization of Fibrin gel and PLCL scaffold for three-dimensional spatial organization of cells and extracellular matrix and TGF-β1 loaded functional nanoparticles for effective chemical stimulation with continuous controlled release can encourage hADSCs to differentiate to chondrocytes, maintain their phenotypes and enhance GAGs production and consequently, improve the quality of cartilaginous tissue formed.

References:

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