Enhanced Cartilaginous Tissue Formation with a Cell aggregate-Fibrin-Polymer scaffold 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, for preparing engineered cartilage from mesenchymal stem cells, it is known that cell density for chondrogenic differentiation of stem cells plays a significant role as well as chemical and physical stimulations[1]. In many studies, for inducing chondrogenic differentiation of cells or forming cartilage-like tissues, they used the pellet culture or micromass culture technique. By using these culture systems, they could supply the three dimensional cartilage morphology to cells, which plays important role in promoting cell-matrix interations and cell-cell interactions during chondrogenesis. Furthermore, the phenotypic features of cartilage are closely related to its three dimensional matrix of collagen and proteoglycans, which are lost in monolayer cultures[2]. In this study, we prepared an engineered cartilage using a cell aggregate-hydrogel-polymer scaffold complex capable of inducing the effective regeneration of cartilage tissue similar to natural cartilage, while retaining high mechanical strength, flexibility, and uniform morphology.

Materials and Methods: Almost homogenious sized-cell aggregates were fabricated by a hanging drop method with rabbit bone marrow stromal cells (BMSCs). The cultured BMSCs were collected by trypsin treatment and then resuspended in chondrogenic medium (DMEM, 1mM Sodium Pyruvate, 100nM Dexamethasone, 20μg/ml Proline, 37.5μg/ml ascorbic acid 2-phosphate, 1% penicillin-streptomycin, 1% Fetal Bovine Serum, 1X insulin-transferrin-selenium, 10ng/ml TGF-β_1; 3-5x10^5 cells/mL). Drops of 20 μl containing 6000–10000 BMSCs suspended in culture medium were placed on the inner side of the lid of 100 mm tissue culture dishes. Dishes were then filled with PBS to avoid loss of nutrients through evaporation and incubated for 5 days in hanging drop at 37°C in CO_2 incubator. PLCL scaffolds were fabricated with 85% porosity and 300~500 μm pore size by a gel-pressing method. We fabricated the cell aggregate-fibrin-poly(lactide-co-caprolactone)(PLCL) scaffold complex, where the cell aggregates are evenly dispersed in the fibrin gels, and the resulting fibrin gels were immobilized onto the surface of the polymer scaffold while filling up the pores. For examining the chondrogenic differentiation of seeded BMSCs and the formation of chondral extracellular matrix onto the complexes, they were cultured *in vitro* or subcutaneously implanted into nude mice for up to five weeks.

Results and Discussion: In *in vitro* experiments, cell aggregate-fibrin-PLCL scaffold complexes were fabricated and cell aggregates were tightly adhered onto the fibrin and PCL scaffolds maintaining their morphologies and chondrogenic phenotype (Figure 1).

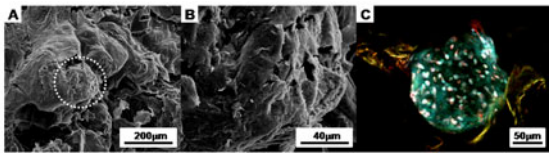

Figure 2. The images of a cell aggregate-fibrin-polymer scaffold complex (A, B) SEM images of a cell aggregate-fibrin-polymer scaffold complex, (C) a confocal microscope images of a complex at 2 day after seeding (Green: CFDA labeled cells, Blue: DAPI)

The results of *in vitro* and *in vivo* studies revealed that the accumulation of chondral extracellular matrices was increased onto the complexes seeded with cell aggregates compared to the complexes seeded with single cells. Also, we could examined that in the cell aggregate-fibrin-PLCL scaffold complexes, mature and well-developed cartilaginous tissues and lacuna structures typical to mature cartilage were evenly distributed.

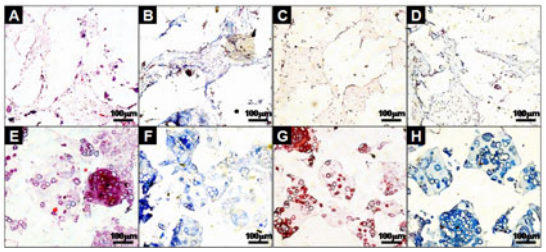

Figure 2. Histological studies of implants at 8 weeks. (H&E (A, E), Masson's Trichrome (B, F), Safranin O (C, G), Alcian Blue (D, H)) The images are of cells-fibrin-PLCL scaffold complexes (A-D) and cell aggregate-fibrin-PLCL scaffold complexes (E-H).

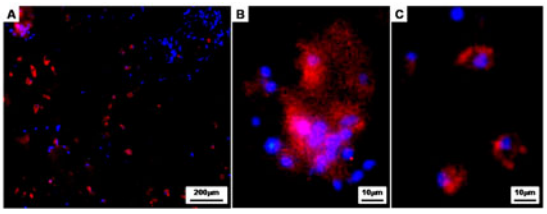

Figure 2. Immunofluorescence studies of cell aggregate-fibrin-PLCL scaffold complexes implants at 8 weeks. The sections were stained for rabbit-collagen type II (Red) and stained with DAPI.

Conclusions: The cell aggregates in the hybrid scaffolds of fibrin gels and elastic PLCL scaffolds can encourage themselves to differentiate to chondrocytes, maintain their phenotypes and enhance GAGs production and improve the quality of cartilaginous tissue formed *in vitro* and *in vivo*.

References:

1. Lynn, A. K., et. Al., J Bone Joint Surg 2004;86:1093-1099.
2. Tare, R. S., et. al., Biochem Biophys Res Commun. 2005;333(2):609-21.