FGF-2-Expanded Costal Chondrocytes Regenerate Hyaline Cartilage in Rabbit Osteochondral Defects

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Abstract: In this study, we evaluated the potential usefulness of FGF-2-expanded costal chondrocytes (CCs) for the repair of osteochondral defect in articular cartilage. Rabbit CCs were expanded approximately 107 folds up to passage 8 in the FGF-2 supplemented medium and became fully dedifferentiated. Their capacity to redifferentiate to hyaline cartilage was tested by in vitro 3D culture in the collagen scaffold and in vivo transplantation in the osteochondral defect of the rabbit knee. Those fully dedifferentiated CCs, which were seeded to collagen scaffold were successfully redifferentiated to hyaline cartilage under the chondrocyte differentiation condition for two weeks, which was featured by lacunae formation and glycosaminoglycan expression. After transplantation to the full thickness defect of the rabbit knee, both dedifferentiated CCs and redifferentiated CCs in the collagen scaffold successfully repaired the cartilage defect. The repaired tissue was confirmed to be hyaline cartilage by re-expressions of type II collagen, GAG, and aggrecan as well as histological grading scale. In conclusion, FGF-2 expanded CCs were fully dedifferentiated but retain their inherent capacity to redifferentiate to hyaline cartilage.

Key words: FGF-2, costal chondrocyte, hyaline cartilage, dedifferentiation

1. Introduction

Articular cartilage repair is very limited due to the absence of a vascular supply and the lack of stem cells in cartilage tissue.1,2 Several clinical procedures such as microdrilling and microfracture,3 osteochondral grafting4 have been developed to repair articular cartilage defects, but the success of these techniques has been limited. Recently, autologous chondrocyte implantation (ACI) has been clinically applied to repair small articular cartilage defects in young patients.3 However, ACI have also had limited success, most likely due to the difficulty in fixing chondrocytes in the defect and loss of cell viability in the transplanted area.6,7 To overcome these problems, various cell delivery systems based on 3D scaffolds have been developed. The primary function of scaffold for cartilage engineering is to deliver target cells into cartilage defect sites, support chondrocytic phenotype, and give mechanical support from the physiological load. Type I collagen scaffolds have been introduced as potential cell-carrier substances for cartilage repair and played a critical role in regulating expression of the chondrocytic phenotype and in supporting chondrogenesis both in vitro and in vivo.8,9 Tissue engineering–based therapies need high amounts of cells, but only small biopsy specimens with a low yield of cells are available, an in vitro expansion culture before a tissue engineering process is unavoidable.10 However, chondrocyte expansion is intrinsically associated with the well-known problem of cell dedifferentiation.11 Furthermore, chondrocytes have only a limited proliferative potential and the number of cell divisions they undergo in vitro decreased with age.12 The use of specific growth factor, such as FGF-2 and TGF-β, not only increases the rate of cell proliferation, but also maintains the cell ability of cells to redifferentiate upon transfer into 3D environment.13

In this study, to evaluate whether FGF-2-expanded CCs has inherent capacity to differentiate to hyaline cartilage, we made a tissue-engineered cartilage with FGF-2-expanded CCs and collagen scaffold, and then transplanted into the defect immediately after cell seeing within the scaffold or after chondrogenic culture.

2. Materials and Methods

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2.1 Isolation and Culture of Rabbit Autologous Costal Chondrocytes

All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of MCTT Research Institute. To harvest autologous CCs, twelve male New Zealand white rabbits (4 to 5 months old, Cheonan Yonam College, Korea) were anesthetized by xylazine and ketamine. The isolation of CCs was performed as reported previously. The isolation of CCs was performed as reported previously. Briefly, cartilage was minced into 1 to 2 mm³ pieces and rinsed three times in PBS. After rinsing, the minced cartilage was digested in an enzyme cocktail solution including collagenase D (2 mg/ml), hyaluronidase (1 mg/ml), and DNase (0.75 mg/ml), all from Roche Diagnostics (GmbH, Mannheim, Germany) at 37°C and 5% CO₂ overnight. The solution was then filtered through a 53-µm nylon mesh, and the isolated cells were cultured up to passage 8 in the culture medium that was composed with MSCGM (Clonetics, San Diego, CA, USA) and FGF-2 (1 ng/ml, R&D system, Minneapolis, MN, USA). To examine chondrocytic phenotype, CCs from passage 8 were observed their morphology and were stained with anti-type I collagen antibody (SouthernBiotech Associates Inc., Birmingham, AL, USA) and anti-type II collagen antibody (Chemicon International, Temecula, CA, USA).

2.2 Cell Seeding onto the Collagen Sponge and In Vitro Reconstruction of Tissue Engineered Hyaline Cartilage (TEHC)

The commercially available sponge-form type I collagen matrix (Integra®, Integra LifeSciences Corporation, Plainsboro, NJ, USA) was taken 5 mm biopsy punch and the silicon layer was removed (approximately 1 mm of thickness). The fine structure of the scaffolds was determined by scanning electron microscopy. Prior to their use in cell seeding, the scaffold was washed with PBS twice to remove isopropyl alcohol and then soaked in culture medium for 10 min. One million CCs at passage 8 were seeded within the scaffold and then the cell-scaffold construct was incubated at 37°C, 5% CO₂ for 2 h (hereafter referred to as Cell-COL). A half of the cell-scaffold constructs was cultured in chondrogenic media for 2 weeks (hereafter referred to as Ch-COL). The chondrogenic medium contained DMEM (Gibco, Grand Island, NY, USA), 1% ITS+3, 100 nM dexamethasone, 50 µg/ml ascorbic acid, 40 µg/ml proline from Sigma (St Louis, MO, USA), and 10 ng/ml TGF-β1 (Prospect technogene, East Brunswick, NJ, USA) and the medium was changed 2 times per week. Before in vivo study, Cell-COL and Ch-COL were fixed with 10% phosphate-buffered formalin. Paraffin sections (5-µm of thickness) were deparaffinized and stained with Safranin-O and fast green for morphological examination.

2.3 Efficacy Assessment of TEHC Reconstructed from the FGF-2-Expanded CC on the Repair of Rabbit Osteochondral Defect

The efficacy of the FGF-2-expanded CCs within collagen scaffold was evaluated in rabbit osteochondral defect model. After the rabbits were anesthetized, the skin in the region of the knee was shaved, washed with alcohol, and prepared with povidon-iodine solution. A defect 5 mm in diameter was created onto the patellar groove of the femoral condyle using a low-speed drill. The conical full-thickness defects extended from the surface of the articular cartilage to the cancellous bone. The depth of the defects was approximately 1.0 to 1.5 mm. The knees of animals were separated into three groups: (1) untreated control; (2) treated with Cell-COL; or (3) treated with Ch-COL. A drop of a fibrin adhesive system (Greenplast®, Greencross, Korea) was applied to fix the transplanted construct in the defect. Any cast was not applied and the rabbits were allowed to move freely after recovery from anesthesia. Intramuscular injections of cefazoline were performed twice a day for 5 days. After 6 and 12 weeks, rabbits were euthanized with an injection of potassium chloride under deep anesthesia.

2.4 Histological Evaluation of Regenerated Cartilage by TEHC

After sacrificing the animals, the gross morphology of their knees was estimated with respect to color, integrity, contour, and smoothness. The specimens in the transplanted areas were photographed, dissected, fixed with 10% buffered formalin, and decalcified using Calci-Clear Rapid (National Diagnostics, Inc., Atlanta, GA, USA). Paraffin section (6-µm of thickness) was deparaffinized and stained with Safranin-O and fast green to evaluate their histological features and GAG expression. For immunohistochemical staining, sections were permeabilized with 0.2% Triton X-100 in PBS and incubated with 0.2% hyaluronidase at 37°C for 1 hour. Antibody against type I collagen (SouthernBiotech), type II collagen (Chemicon), or aggrecan (R & D system) was applied to the specimen, and the specimen was then developed with 0.1% 3,3’-diaminobenzidine tetrahydrochloride (DAB; Vector Laboratories, Inc., Burlingame, CA, USA) in PBS for 5 minutes. Fast red dye was used for counterstaining.

The sections were evaluated blindly by two investigators using a histological grading scale following Wakitani et al. The grading scale comprised five categories with points ranging from 0 (normal cartilage) to 14 (no regenerated tissue) (Table 1).