Int. J. Oral Maxillofac. Surg. 2016; xxx: xxx–xxx
http://dx.doi.org/10.1016/j.ijom.2016.03.002, available online at http://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Research Paper
Tissue Engineering

Production of three-dimensional tissue-engineered cartilage through mutual fusion of chondrocyte pellets

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Abstract. In this study, the mutual fusion of chondrocyte pellets was promoted in order to produce large-sized tissue-engineered cartilage with a three-dimensional (3D) shape. Five pellets of human auricular chondrocytes were first prepared, which were then incubated in an agarose mold. After 3 weeks of culture in matrix production-promoting medium under 5.78 g/cm^2 compression, the tissue-engineered cartilage showed a sufficient mechanical strength. To confirm the usefulness of these methods, a transplantation experiment was performed using beagles. Tissue-engineered cartilage prepared with 50 pellets of beagle chondrocytes was transplanted subcutaneously into the cell-donor dog for 2 months. The tissue-engineered cartilage of the beagles maintained a rod-like shape, even after harvest. Histology showed fair cartilage regeneration. Furthermore, 20 pellets were made and placed on a beta-tricalcium phosphate prism, and this was then incubated within the agarose mold for 3 weeks. The construct was transplanted into a bone/cartilage defect in the cell-donor beagle. After 2 months, bone and cartilage regeneration was identified on micro-computed tomography and magnetic resonance imaging. This approach involving the fusion of small pellets into a large structure enabled the production of 3D tissue-engineered cartilage that was close to physiological cartilage tissue in property, without conventional polyper scaffolds.

Key words: tissue engineering; cartilage; chondrocyte; culture; pellet.

Accepted for publication 2 March 2016

Cartilage is present not only in the limbs and vertebrae, but also in the face. It is present in the nose, ears, eyelids, and mandibular condyles, and maintains the facial morphology and movement. Facial cartilage may be lost due to congenital malformations such as cleft lip nasal deformities, or through trauma or extensive resection of cancer. This may result in functional losses in the face, markedly impairing quality of life. Furthermore, arthritis of the mandibular cartilage also leads to a deterioration of activities of daily life due to severe pain and trismus. Therefore, the treatment of impaired cartilage in the

0901-5027/000001+09

face is an important issue in the field of oral and maxillofacial surgery. Although autologous cartilage transplantation has been performed for these diseases involving the facial cartilage, there are related problems; for example, a sufficient volume of tissue cannot always be obtained and the procedure is highly invasive at the donor site. Therefore, cartilage tissue engineering is being investigated.

As regenerative medicine for facial cartilage, chondrocytes collected from auricular cartilage and cultured have been injected to fill spaces generated after the removal of silicon used for cosmetic rhinoplasty.[1] Since auricular chondrocytes show a high rate of division and can be cultured relatively easily,[2] they represent a favorable cell source for cartilage regenerative medicine. However, significant hypoplasia or severe deformation in congenital malformations and arthritis cannot be treated by this method. Thus, the present investigators developed a method to produce regenerative cartilage of a specific shape and hardness by incorporating porous poly-L-lactic acid (PLLA). This has been applied to cleft lip nasal deformity patients.[3]

Since PLLA is a biodegradable polymer, the transplant using porous PLLA is eventually organized into the host tissue.[4,5] However, the absorption process may be similar to that of foreign body reactions.[6,7] Moreover, the volume reduced by absorption may lead to a risk of graft deformation in the future. Thus, it is necessary to develop a method that creates mechanical strength and a three-dimensional (3D) morphology without using a scaffold of biodegradable polymers, such as porous PLLA. This may be achieved by producing a cartilage matrix of cultured auricular chondrocytes *in vitro* and subsequently reconstructing the cartilage tissue. However, chondrocytes cultured in a monolayer condition lose their original cartilage matrix-producing ability and dedifferentiate.[8] In order to re-start cartilage matrix production, cultured chondrocytes should form small aggregates (pellets), because the mesenchymal stem cells (MSC) – the progenitors of chondrocytes – undergo a mesenchymal condensation before differentiating into cartilage.[9] However, cultured chondrocytes alone may not be able to form pellets if their cell-to-cell adhesion force is too weak and insufficient.

Thus, an initial attempt was made by the present investigators to apply atelocollagen, a type of medical hydrogel.[10,11] It was found that cartilage matrix accumulation was promoted by incubation with a matrix production-promoting medium. However, it was known empirically that only pellets of approximately 1 mm in size could be prepared using this method, due to the limitations of substance exchange.[11] To overcome this, it was next attempted to mass-produce 1-mm pellets. An agarose mold was developed in which many pellets could be cultured simultaneously while maintaining a sufficient substance exchange (Fig. 1).[12] Compressive force was applied to these pellets (Fig. 1) for the purpose of bringing them into close contact, thereby inducing interactions among the matrices produced and adhesion between the matrices and cells, fusing many pellets.

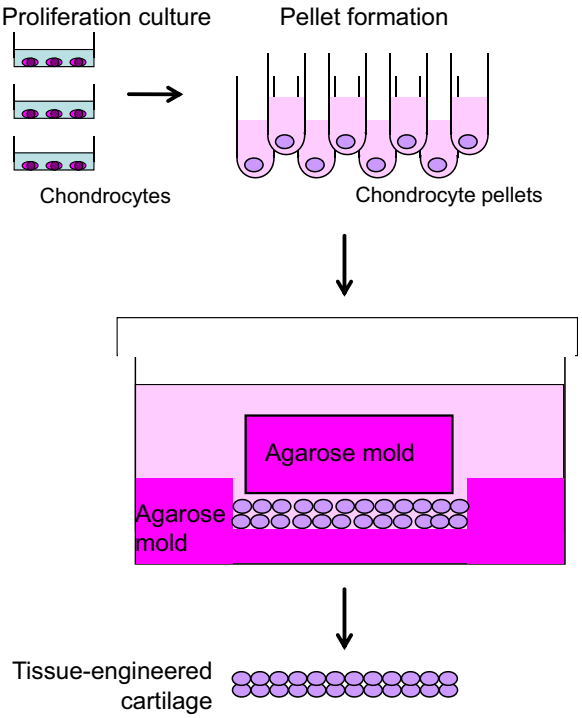

Fig. 1. Production of the tissue-engineered cartilage. Many chondrocyte pellets were prepared. These pellets were compressed in an agarose mold in order to produce the tissue-engineered cartilage.

The aim of the present study was to establish a method to produce large-sized tissue-engineered cartilage with a 3D shape, without using any polymer scaffold, by promoting the mutual fusion of chondrocyte pellets in the agarose mold. The conditions for preparing and fusing the chondrocyte pellets were investigated. In addition, subcutaneous or articular transplantation of the tissue-engineered cartilage was performed in beagles to verify its utility.

Materials and methods

Chondrocyte culture

Human auricular chondrocytes were isolated from microtia patients undergoing surgery to the ear. After obtaining informed consent, the perichondrium was detached from about 2–3 g of excised residual auricular cartilage, and the cartilage was cut into 1-mm^3 pieces using a surgical knife. These pieces were incubated in 0.15% collagenase solution (Worthington Biochemical Corporation, Lakewood, NJ, USA) at 37 °C for 24 h in a thermostat with shaking.[13] The lysate was filtered through a cell strainer with a pore size of 100 μm. After removing the residue, the filtrate was centrifuged at 500 × *g* for 5 min, and the human auricular chondrocytes were isolated. The isolated chondrocytes were seeded in a collagen-coated plastic tissue culture dish (Iwaki, Scitech Division, Asahi Techno Glass Co. Ltd, Chiba, Japan) at a density of 2500 cells/cm^2 and were cultured in chondrocyte growth medium (Dulbecco's modified Eagle medium nutrient mixture F-12 (DMEM/F12) containing 5% human serum, 100 ng/ml fibroblast growth factor 2 (FGF-2), and 5 μg/ml insulin)[14,15] in an incubator at 37 °C/5% CO_2. The medium was changed twice a week. Before reaching confluence, the cells were treated with trypsin–ethylenediaminetetraacetic acid (EDTA) solution and passaged. These chondrocytes were used in the experiments after two passages.

Canine auricular and articular chondrocytes were isolated from beagle dogs

Please cite this article in press as: Hoshi K, et al. Production of three-dimensional tissue-engineered cartilage through mutual fusion of chondrocyte pellets, *Int J Oral Maxillofac Surg* (2016), http://dx.doi.org/10.1016/j.ijom.2016.03.002