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Effects of Natural Cartilaginous Extracellular Matrix on Chondrogenic Potential for Cartilage Cell Transplantation

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ABSTRACT

Autologous chondrocyte transplantation (ACT) has been established to contribute cartilage regeneration over the past years; however, many obstacles need to be overcome. Recently, newer ACT technique involves cotransplantation of chondrocytes and biomaterial. Although various proposed intelligent biomaterials exist, many of them remain insufficient and controversial. In this study, we aimed to examine the effects of natural extracellular matrix (ECM) to the proliferation rate and differentiation on the chondrocytes. We first derived a natural ECM sheet from 10-μm-thick frozen sections of porcine knee cartilages. We then cultured the chondrocytes derived from a rabbit's knee on a dish precoated with the natural ECM. Then we assessed differentiation and chondrogenic potential of the cells compared with those grown in untreated culture dishes. We characterized the gene expression of chondrogenic markers, such as collagen type II, SOX-9, and aggrecan, as well as the level of ECM protein with the use of reverse-transcription polymerase chain reaction analysis. The cells cultured with the ECM sheet showed highest chondrogenic potential and differentiation. Therefore, we can induce good chondrogenesis by with the use of a natural ECM sheet on the culture dish. The readily available and easy-to-handle thin ECM sheets create an environment that promotes efficient cartilage regeneration. Our data suggest that this natural ECM scaffold improved the chondrogenic differentiation of the cells in vitro by providing a favorable microenvironment.

ARTICULAR cartilage tissue plays a crucial role in the absorbance and distribution of various mechanical loads in the joint, but it is not easy to repair a defect such as from osteoarthritis. In recent years, autologous chondrocyte transplantation (ACT) has been proven to offer cartilage regeneration. For ACT, chondrocytes, after in vitro culture, can be implanted with autologous, homologous, or heterologous implants in a number of appropriate ways. Articular cartilage is viewed as an immune-privileged tissue. Cartilage is rarely a target of autoimmune disease [1], and transplanted cartilage from unrelated donors does not elicit immune rejection responses [2,3].

An extracellular matrix (ECM) is the extracellular part of tissue that includes glycosaminoglycans (GAGs), heparan sulfate, chondroitin sulfate, keratan sulfate, hyaluronic acid, collagen, elastin, laminin, and so on. ECM usually offers structural support to the cells in addition to performing other numerous essential functions and is commonly used in cell culture systems. In tissue engineering, ECM could function as a biologic scaffold for implants and provide repair of injury after transplantation [4]. A new concept of cell culture on thin tissue sections, obtained with the use of a commercially available cryomicrotome, as a source of

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ECM, served as novel cell culture substrata that would provide in vivo tissue conditions and components [5].

In the present study, we aimed to assess the effects of natural ECM, with the use of thin tissue sections, on the proliferation rate and differentiation of chondrocytes.

MATERIALS AND METHODS

Chondrocyte Isolation

This animal experiment was approved by the Institutional Animal Care and Use Committee (KU13003) of Konkuk University. Cartilage tissues were collected from the knee joint of a 7-week-old rabbit (male New Zealand white rabbit; Samtako, Osan, Korea). The harvested tissues were then chopped and digested with 3 mg/mL type 1 collagenase (Worthington, Biochemical Co) in Dulbecco modified Eagle medium high glucose (DMEM-HG; Gibco, Walkersville, Maryland) including 1% antibiotics-antimycotics (AA; Gibco), and subsequently incubated at 65°C shaking water bath for 18 hours. The digested chondrocytes were cultured in DMEM-HG, including 1% AA, 10% fetal bovine serum (Gibco), and 1% ascorbic acid (50 μg/mL; Sigma-Aldrich) at 37°C with 5% CO_2. After cells were isolated, cell counting was conducted with the use of a Turker-Turk hematocytometer (Erma, Tokyo, Japan) with trypan blue (Gibco) staining, and the primary cells were seeded into culture plates with the seeding rates of 2×10^4 cells/cm^2.

Preparation of cartilage ECM Sheet

Articular cartilages were harvested from the knee of 6-month-old pigs acquired from a local slaughterhouse (Hwang Geum Chuk San, Seoul, Korea) immediately after they were killed. The cartilage pieces were washed with phosphate-buffered saline solution (Gibco). The cartilage pieces were cut into 10-μm sections with the use of a cryomicrotome. Each cartilage ECM sheet was spread on a glass slide and air dried. The culture dishes were precoated with

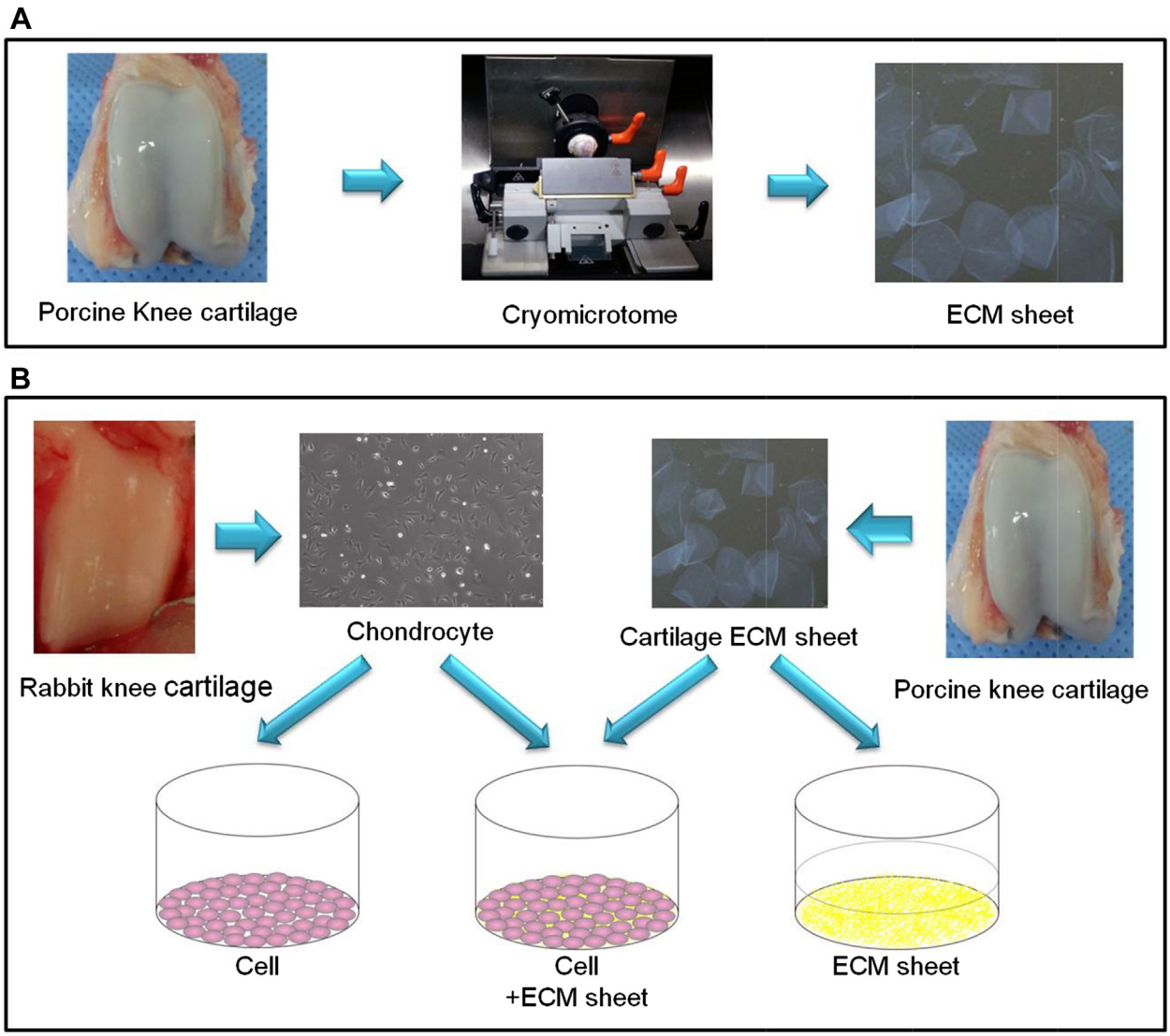

Fig 1. Schematic illustration of the process of making an extracellular matrix (ECM) sheet with the use of cartilage. **(A)** Chondrocyte seeding of the ECM sheet. **(B)** Experimental details are described in the text.