Biochemical and Histological Evaluations of Articular Cartilages Preserved in Cold Storage Solution Containing Green Tea Catechin, EGCG

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Abstract: Although epigallocatechin-3-O-gallate (EGCG), a major polyphenolic constituent of green tea, has various pharmacological and biological activities including anti-carcinogenic, anti-thrombotic and anti-inflammatory effects, relatively a little is known about its beneficial effects on the non-frozen preservation of mammalian cells and tissues. In this study, articular cartilages from human knee joint were pretreated with 1 mM EGCG for 1 d and then preserved in serum-free RPMI 1640 media with 1% antibiotic-antimycotic solution at 4°C for 1, 2 and 4 wk. After cold preservation, chondrocyte viability (CCK-8 assay), biochemical and immunohistochemical composition (glycosaminoglycans and (type II) collagen), and biomechanical property (compressive elastic modulus) were assessed, respectively. Chondrocyte viability of cartilages pretreated with EGCG was significantly well-maintained for at least 2 wk with high contents of glycosaminoglycan and total collagen. These beneficial effects of EGCG pretreatment were more confirmed by histological and immunohistochemical observations showing well-preserved cartilaginous structures and delayed denaturation of the extracellular matrices in preserved specimens. The compressive elastic modulus (MPa) of cartilages pretreated with EGCG was well-maintained as much as that of fresh specimens without any increase as the progress in the preservation period. Here were also found that fluorescein isothiocyanate-conjugated EGCG were widely distributed through the matrix and clearly observed at the chondrocytes in the lacunas. Taking these results into consideration, it is suggested that EGCG may play an effective role in preserving articular cartilages, which be exploited to craft strategies for the long-term preservation of osteochondral allografts under cold storage conditions.

Key words: Epigallocatechin-3-O-gallate, cold preservation, articular cartilages, chondrocyte viability, compressive elastic modulus

1. Introduction

Articular cartilage injury is challenging to treat because of limited intrinsic healing capacity.1,2 Although the natural history of isolated cartilage lesions still is unknown,3 it generally is thought that articular cartilage injury may predispose the involved joint to accelerated degeneration.2,4 This problem is magnified by the relative frequency of cartilage injuries.5,6 The unsuitability of total joint replacements for young, active individuals has provided the stimulus to search for alternative treatments in the field of biologic resurfacing of joints. Numerous surgical treatments for chondral and osteochondral lesions have been described, but each method has certain limitations.7,8 On the other hand, tissue engineering techniques and regenerative medicine have been explored as a potential method to restore natural tissue and repair lesions.9,10 Nevertheless, no optimal method for the cryopreservation of mammalian tissue or organ as well as tissue engineered medical products and has been established.11 Also, current methods can result in a substantial loss of function and lead to damage and destruction of the cells and tissues.

The number of tissue or organ transplants has increased substantially in recent years with the advances in the surgical methods and the development of immunosuppressive agents.12,13 Ideally, tissues should be transplanted immediately.
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However, this is not always possible, and the problem of tissue preservation is very important for ensuring a successful transplantation. Therefore, it is essential to develop storage solutions that can maintain the viability and function of the tissues or organs for longer periods. Usually, a dysfunction of the transplants occurs as the result of free radicals due to ischemia, which triggers lipid peroxidation of the cell membrane when the blood flow is restarted. Particularly, ischemic osteonecrosis can produce a permanent residual deformity of the immature femoral head and epiphyseal cartilage damage. A good storage solution should prevent this peroxy lipid generation. Since this is related to cell proliferation and division, longer term tissue preservation for transplantation would become possible if cellular metabolism can be controlled.

From this point of view, our attention has been paid to (–)-epigallocatechin-3-O-gallate (EGCG), the predominant catechin from tea, since it has a wide range of pharmacological activities, including antioxidant, anticancer, anti-proliferative, anti-inflammatory and anti-thrombotic effects. Different from these biological activities of EGCG, its cytopreservative effects on mammalian cells and tissues were examined in order to design a cell- or tissue-preserving medium/solution at physiological temperature in our previous studies. The present study provided support to a scenario in which EGCG might play a key role in preserving articular cartilages by maintaining chondrocyte viability and metabolism in cartilages as well as matrix structure. Therefore, EGCG pretreatment can be exploited to craft strategies for the long-term preservation of osteochondral allografts under cold storage conditions.

2. Materials and Methods

2.1 Cartilage Specimen Collection and Cold Preservation

Human articular cartilages were obtained from knee joints of 10 outpatients (male or female, 58 - 86 years old, 15 - 20 mm in diameter and 2 - 2.5 mm in thickness) undergoing total knee arthroplastic surgery at Marunouchi Hospital, Nagano, Matsumoto-si, Japan. As shown in Fig 1, these specimens were procured under sterile conditions from the donor and placed in a storage solution[serum-free RPMI 1640 media(Sigma-Aldrich Co, St Louis, MO) with 1% antibiotic-antimycotic solution(including 10,000 units penicillin, 10 mg streptomycin and 25 mg amphotericin B per mL, Sigma-Aldrich Co)] with 1 mM EGCG(Travigo™, DSM Nutritional Products Ltd, Basel,