Application of Polyphenol (EGCG) Storage Solution to Cold Preservation of Osteochondral Tissues

Jung Yoon BAE, Dong-Wook HAN, Kazuaki MATSUMURA, Sadami TSUTSUMI, Suong-Hyu HYON.
Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507 Japan.

Introduction: Most of recent studies on green tea polyphenol, catechin and its derivatives are mainly focused on the anti-cancer effects [1]. However, less attention has been paid to the beneficial effects of epigallocatechin-3-gallate (EGCG) on the preservation of mammalian cells or tissues. Furthermore, cellular mechanism of this preservative effect of EGCG remains to be unraveled. Our earlier studies have already shown that green tea polyphenols were significantly effective in protecting mammalian cells [2,3] from reactive oxygen species-induced oxidative stress and preserving human vessels under physiological conditions [4]. In this study, the effects of EGCG-base storage solution on cold preservation of human osteochondral tissues were investigated.

Materials and Methods: To confirm the beneficial efficiency of EGCG upon cold storage of osteochondral tissues, human articular cartilages or fibrocartilages, such as hip, knee and meniscus, were preserved at 4ºC in serum-free media supplemented with 1% antibiotic-antimycotic solution according to two different protocols (Fig. 1). One protocol was preservation in RPMI 1640 media with 1 mM of EGCG (co-treatment) for 1, 2 and 4 wk. In the other protocol, cartilage specimens were preserved in fresh media for 1, 2 and 4 wk after 1 d of storage in EGCG-base media (pre-treatment). At the end of each preservation period, various biochemical evaluations including CCK-8 assay for cell viability, hydroxyproline assay for collagen quantity and dimethylmethylene blue assay for glycosaminoglycan (GAG) contents, and immunohistochemical analyses for GAG (by safranin O-fast green stains) as well as type II collagen were performed. Also, biomechanical properties including compressive modulus (MPa) were determined by dynamic visco-elastic measurements.

Results/Discussion: From the results obtained by the biochemical assays, it was demonstrated that chondrocyte viabilities of knee cartilages stored in EGCG-base solution were significantly well-maintained for at least 2 wk with high contents of collagen and GAG (Fig. 2). These preservation effects of solution with EGCG were more confirmed by immunohistochemical observations showing well-preserved cartilaginous structures in the osteochondral tissues stored in EGCG-base media. Moreover, dynamic visco-elastic compression testing revealed that compressive modulus of articular cartilages was in the same range as that of a fresh cartilage (Fig. 3).

Conclusions: Taking these results into consideration, it is suggested that EGCG addition to storage media may be a useful method for preserving human osteochondral tissues and be exploited to craft strategies for the long-term preservation of other tissues under cold storage conditions.

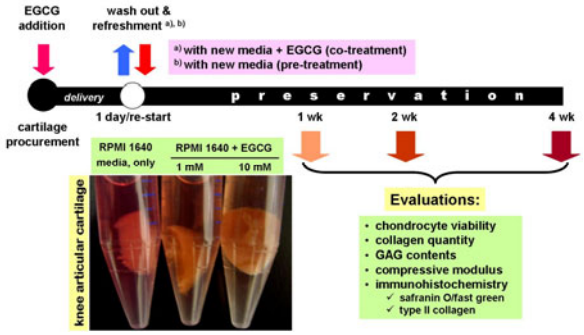

Figure 1. Experimental Scheme of Cold Storage of Human Osteochondral Tissues by EGCG-Base Solution.

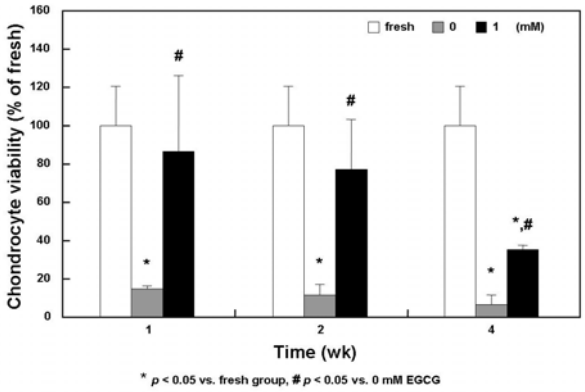

Figure 2. Chondrocyte Viability of Knee Cartilage Preserved in Storage Solution with EGCG.

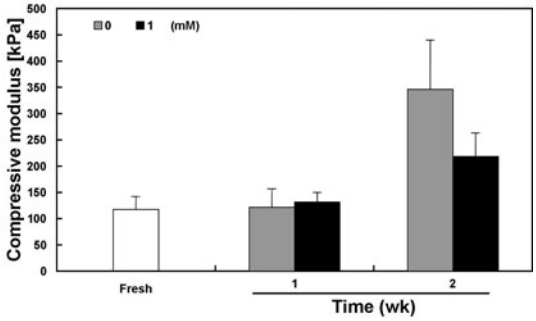

Figure 3. Compressive Modulus of Knee Cartilage Preserved in Storage Solution with EGCG.

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