The Effect of Using Collagen Vitrigel Containing Transforming Growth Factor Beta 1 on Articular Cartilage Repair

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Introduction: To date, we have confirmed that chondrocyte sheets are useful for the treatment of articular cartilage defects. During such treatment, we have also confirmed that humoral factors such as transforming growth factor beta 1 (TGF-β1) and prostaglandin E2 play an important role in cartilage tissue repair [1]. It was recently reported that collagen vitrigel, composed primarily of collagen type I, is useful for tissue repair. Based on these studies, we created a total-thickness defect in the articular cartilage of rabbits, and investigated the effect of treatment using collagen vitrigel containing TGF-β1 [2].

Methods: Collagen vitrigel used for this study was provided by Takezawa et al. [2] and formed into a thin round sheet of 15 mm diameter (Figure 1). The quantity of collagen in the vitrigel was 5.0 mg/cm². Eighteen Japanese white rabbits aged about 16 weeks and weighing about 3 kg were used in this study. We manufactured a total-thickness defect (diameter, 5 mm; depth, 3 mm) using a drill to the articular cartilage of the loading part of the right femor (Figure 2). We divided the 18 Japanese rabbits into three groups: nontreatment group (A), collagen vitrigel transplanted group (B) and collagen vitrigel containing TGF-β1 transplanted group (C) (n = 6 for each group). The collagen vitrigel used in group C was osmosed with TGF-β1 at a concentration of 10 ng/ml for 24 hours at 4 °C. These two types of collagen vitrigel were transplanted to cover the cartilage defect in the rabbits in groups B and C. Each rabbit was sacrificed four weeks after the operation. An incapacitance tester (Linton Instrumentation, Norfolk, England) was used to evaluate the change in pain levels in the damaged limb by assessing the weight distribution ratio (damaged limb weight distribution ratio (%) = (damaged limb load (g) / (undamaged limb load (g) + damaged limb load (g))) x 100) [3] (Figure 3). Pain levels were evaluated four times postoperatively at seven-day intervals. In addition, we performed a histological evaluation by assessing the tissue through the center of the defect after staining with safranin O for glycosaminoglycans and assessed the tissue using the International Cartilage Repair Society (ICRS) grading system.

Results: The results of the first assessment after surgery of the damaged limb weight distribution ratio (mean ± SD) were 36.3 ± 2.3% in group A, 38.9 ± 0.6% in group B and 37.2 ± 1.2% in group C. However, after four weeks. the damaged limb weight distribution ratio (mean ± SD) had improved to 42.1 ± 1.5% in group A, 44.2 ± 0.9% in group B, and 46.9 ± 1.5% in group C. At four weeks after surgery, the results for the ICRS grading of the tissue through the center of the defect were 15.5 ± 0.5 in group A, 23.3 ± 3.7 in group B, and 30.7 ± 0.8 in group C. We identified significant differences (p < 0.05) between the three
groups for both the pain and histological evaluations. There were no adverse events such as postoperative infection during this study.

Discussion: Anabolic cytokines such as TGF-β1 are known for their function in inhibiting catabolic cytokines such as IL-1, which causes cartilage defects, and they are essential in sustaining articular cartilage homeostasis [4]. Conventional collagen gel is soft and difficult to handle, as it is comprised of collagen fibers that have low density. A collagen gel of heat-denatured protein could be converted into a transparent glass-like material by evaporating both its free and bound water [5]. Takezawa et al. applied this theory and found that the manufactured gel (vitrigel) consists of high-density collagen fibers that could be made almost as dense as in vivo connective tissue by further rehydration [2]. A collagen vitrigel film was able to osmose high-polymer serum proteins of molecular weight > 100 kDa and was also able to reflect the characteristics of the materials by adding various insoluble or soluble materials [6]. It has also been reported that collagen vitrigel has been used in various studies by utilizing its ability to absorb and release cytokines. We applied these characteristics and manufactured vitrigel containing TGF-β1, which we used in our study. We could not identify any remarkable improvement in the articular cartilage defect using only the collagen vitrigel in this study. However, we were able to confirm an advantage in the treatment effect when the vitrigel was osmosed and gradually released TGF-β1. We will continue to study articular cartilage repair to find the optimal conditions, evaluate the safety profile, and assess the effects of potential treatments.

Significance: The treatment effect was enhanced when humoral factors such as TGF-β1 were gradually released to the articular cartilage defect.
Fig. 2: Total thickness defect model

Fig. 3: Incapacitance tester

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