Evaluation of Porcine Collagen Matrix for Cartilage Regeneration: Comparison of Two Different Membranes in Cartilage Defect Model

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Introduction: The treatment of symptomatic articular cartilage defects of the knee has evolved tremendously in the past decade. Among the several techniques such as microfracture and autologous chondrocyte implantation (ACI), subchondral bone microfracture has been widely used to recreate hyaline cartilage-like tissues from damaged articular cartilage. However, the repair is often in the form of fibrocartilage which is inferior in its properties as an articular cartilage, thus resulting in the repair tissue overgrowth [1]. Recently, in an attempt to resolve this problem, Chondro-gide, well-known as a membrane consisting of porcine collagen types I/III bilayer matrix, has been surgically approached. Many studies have confirmed an efficiency of Chondro-gide as the leading collagen matrix in the field of cartilage regenerative medicine [2].

We developed new absorbable collagen matrix, named as CARTIREGEN™ which has a porous and fibrous microstructure that is hydrophilic and allows ingrowth of chondrocytes or newly-formed cartilage tissue onto the subchondral bone.

The purpose of this study is to evaluate the effect of a new absorbable collagen matrix, CARTIREGEN™, on cartilage regeneration via comparing with the Chondro-gide.

Methods: The CARTIREGEN™ is made of pure collagen, to minimize antigenicity, which is without further cross-linking or chemical additives for excellent biocompatibility. It is a biodegradable collagen matrix derived from porcine certified by a veterinarian and is purified to prevent immunologic reactions. For osteochondral defect model, male beagles (Orient Bio Inc.) were used as experimental animal models (n = 15). Osteochondral defects were created in the medial femoral condyle with a diameter of 5 mm and depth of 2 mm using Osteochondral Autotransfer System (OATS, Arthrex, USA) recipient tool. And then, microfracture with a depth of 1.5 mm - 2.0 mm was formed. After microfracture surgery, Chondro-Gide or CARTIREGEN™ was sutured with interrupted 7-0 vicryl, and sealed with fibrin glue. At 20 weeks post-operation, regenerated cartilage tissues were harvested from euthanized beagles. The prepared samples were stained with hematoxylin and eosin (H&E), Masson’s trichrome (MT), and safranin O/fast green. To confirm the expression of two chondrogenic markers, type II collagen and aggrecans, we performed immunohistochemistry using Phycoerythrin/green fluorescent protein-conjugated secondary antibodies (Santa Cruz Biotechnology).
**Results:** First, we observed the gross morphology of regenerated articular cartilage in osteochondral defects of beagles at 20 weeks post-surgery. While tissue regeneration was rarely observed in defect control group without microfracture, the cartilage surface of the defect site was almost entirely filled with smooth, cartilage-like tissues similar to the surrounding cartilage in defect control group with microfracture, Chondro-gide, and CARTIREGEN™ group (Figure 1). Next, H&E staining showed inflammatory cells and vascularized tissue formation in defect control group without microfracture and fibrous tissue formation in the defect control group with microfracture. In Chondro-gide and CARTIREGEN™ group, however, the cartilage surface of regenerated tissues showed chondrocyte-like cells and smooth cartilage-like tissues with lacunae, thus resembling normal cartilage (figure 2A). MT staining showed that collagen contents in the Chondro-gide and CARTIREGEN™ group were higher than those in the other groups and similar to levels in normal cartilage (figure 2B). In contrast to defect control groups, Chondro-gide and CARTIREGEN™ group showed significant GAG synthesis through safranin O staining (figure 2C). As a result of immunohistochemistry, type II collagen and aggrecans was not observed in defect control group with/without microfracture. Both Chondro-gide group and CARTIREGEN™ group induced the expression of type II collagen and aggrecans on the regenerated cartilage surface. Finally, the regenerated tissues from each group were analyzed histologically, histochemically, and biochemically through O’Driscoll grading scale. As a result, defect control group without microfracture had the worst score in all groups. The average score of defect control group with microfracture was 12.3 ± 1.5 point. Each of average scores in Chondro-gide and CARTIREGEN™ group was 17.8 ± 0.9 and 18.8 ± 0.7 points respectively (figure 3).

**Discussion:** In this study, we evaluated cartilage regeneration comparing Chondro-gide with CARTIREGEN™ through gross morphology, H&E, MT, Safranin O staining, and immunohistochemistry. In gross appearance, regenerated tissue formation was observed in both Chondro-gide and CARTIREGEN™ group. But, the number of regenerated tissue which was filled with smooth and cartilage-like tissue was higher in CARTIREGEN™ group than in Chondro-Gide group. As a result of H&E and MT staining, defect control groups showed inflammatory cells and vascularized tissue formation in contrast to Chondro-gide and CARTIREGEN™ group. However, chondrocyte clustering that is hallmark of osteoarthritis was partially observed in Chondro-gide group as well as defect control groups. In Safranin O staining and immunohistochemistry, expression of aggrecans in the regenerated cartilage tissue of Chondro-gide group was weaker than in CARTIREGEN™ group although GAG synthesis and level of type II collagen was highly observed in both groups. These results indicate that both Chondro-gide and CARTIREGEN™ has significant positive effects on cartilage regeneration in a beagle model but the efficiency is higher in CARTIREGEN™ than in Chondro-gide.

**Significance:** We developed a new absorbable collagen matrix, CARTIREGEN™ which has a porous and fibrous microstructure. CARTIREGEN™ as well as Chondro-gide can be also used as a membrane for hyaline-like cartilage regeneration.
Figure 1. Gross appearance of regenerated tissue

Figure 2. Histological analysis of regenerated tissue at subchondral defect site
Figure 3. O’driscoll Histological Score

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