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Frictional Properties of Articular Cartilage-like Tissues Repaired with a Mesenchymal Stem Cell-based Tissue Engineered Construct*

Hiromichi Fujie, and Norimasa Nakamura

Abstract— **We have been developing a novel tissue engineering technique for cartilage repair using a scaffold-free tissue engineered construct (TEC) bio-synthesized from synovium-derived mesenchymal stem cells (MSCs). In the present study, the effect of TEC on the repair of chondral defect in the femoral condyle of immature and mature pigs were investigated. The permeability of TEC-treated repaired tissues was significantly higher than normal level at surface layer in immature animals, while the permeability was slightly higher than normal level at middle and deep layers in mature animals. In immature animals, the coefficient of friction of TEC-treated tissues against a glass plate was load-dependently increased, with a significantly higher value than normal level observed at a high load (280 kPa). In contrast, the coefficient of friction was load-dependently decreased in mature animals, with no significant differences from normal level observed at all loads (70, 140, and 280 kPa). It is suggested that the frictional properties of TEC-treated cartilage-like repaired tissues are recovered to normal level in mature animals, while they are unrecovered to normal level due to underdeveloped, permeable surface layer in immature animals.**

I. Introduction

The healing capacity of articular cartilage is limited [1], it is therefore required to develop effective cell-based therapies for cartilage repair. Although synthetic or animal-derived scaffolds are frequently used for cell deliveries long-term safety and efficiency of such scaffolds still remain unclear. We have been developing a new tissue engineering technique for cartilage repair using a scaffold-free tissue engineered construct (TEC) bio-synthesized from synovium-derived mesenchymal stem cells (MSCs) [2-4]. As the TEC specimen is composed of cells with their native extracellular matrix, we believe that it is free from concern regarding long term immunological effects. In the present study, the effect of TEC on the repair of partial chondral defect were investigated. The permeability and coefficient of friction of the TEC-repaired cartilage-like tissues were determined in immature and mature animal models.

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II. Method

A. Preparation of TEC and chondral defect repair

Synovium-derived cells including MSCs obtained from the synovial membrane of immature porcine knee joints at the age of 3-4 months were cultured in a monolayer in DMEM (Fig.1). Cell proliferation was performed though 4 to 7 passages. When the cell density reached to 4.0 x 10^5 cells/cm^2 (6-cm dish), 0.2 mM of ascorbic acid 2-phosphate was added to the cell culture plates, and allowed to undergo active contraction for 8 hours to develop TEC specimens [2]. Three month-old immature and 12 month-old mature 24 pigs were used as donors in the present study. They were divided into 4 groups; TEC-treated immature group (n=6), TEC-untreated immature group (n=6), TEC-treated mature group (n=6), and TEC-untreated mature group (n=6). A cylindrically shaped, partial chondral defect of 8.5 mm in diameter and 1.5 mm in depth was created in the weight-bearing area of the medial condyle of distal femur in each animal. The defects were subjected to allografting implantation of the TEC specimen in TEC-treated immature and mature groups (n=12), while the defects were subjected to no implantation in TEC-untreated immature and mature groups (n=12). Six months after surgery, cylindrically-shaped plug specimens of 4 mm in diameter and 5 mm in height were harvested from the donor sites. For comparison, plug specimens of dimensions identical to above-described were harvested from the medial condyle near donor sites to serve as immature (n=6) and mature (n=6) normal cartilage groups.

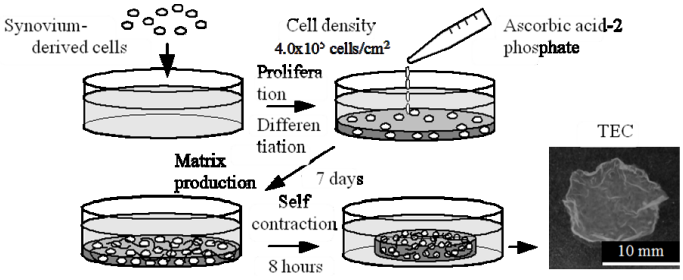

Figure1. Production of tissue engineered construct (TEC) from synovium-derived cells including MSCs.

B. Morphological observation

An atomic force microscope (AFM) (Nanoscope IIIa, Veeco Instruments, USA) was used in contact mode to scan the surface images of TEC-treated and -untreated mature and immature specimens (n=4) and control mature and immature specimens (n=2) soaked in saline solution at room

temperature. Histological observation was also performed using safranine-O for the plug specimens.

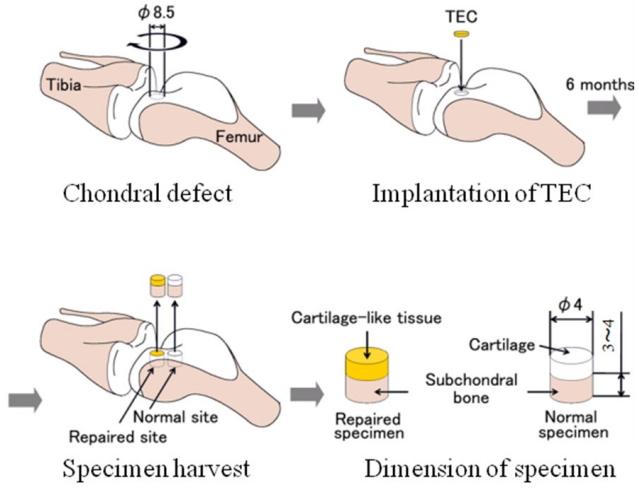

Figure 2.Allografting implantation of the TEC specimen to a chondral defect of porcine femur, and the dimension of plug-specimens harvested from donor sites.

C. Permeability tests and friction tests

Friction tests were performed for the specimens in a confined compression fashion on a flat glass plate soaked in saline solution at room temperature using a custom made micro friction tester [5]. The friction speed was 20 mm/s while the normal load was 70, 140, and 280 kPa. The coefficient of dynamic friction of the specimen was obtained immediately after frictional motion was applied. After the friction test, each plug specimen was sliced in parallel with surface to three layer specimens consisting of surface layer, middle layer, and deep layer. Thickness of the layers was between 250 and 300 μm. The permeability of the layer specimens were determined using a permeability tester under 30% of compression [6].

III. RESULTS

A. Morphological observation

Macroscopic observation indicated that the chondral defects were filled with cartilage-like tissues in TEC-treated groups, while the defects were filled with transparent tissues in TEC-untreated groups, in both the immature and mature animals (Fig.3). Histological observation indicated that the TEC-treated cartilage defects were filled with safranine-O stained tissues, while, the TEC-untreated defects had only partial coverage with safranine-O unstained tissues, in both the immature and mature animals (Fig.4). It was demonstrated that the TEC-treated cartilage was hyaline cartilage-like at intermediate-to-deep area but remained fibro cartilage-like at the surface in both the mature and immature animals. Atomic force microscopic observation indicated that TEC-treated repaired sites exhibited rough surfaces of approximately 1-2 μm in height, while the TEC-untreated sites exhibited rough surfaces of approximately 1-5 μm in height (Fig.5).

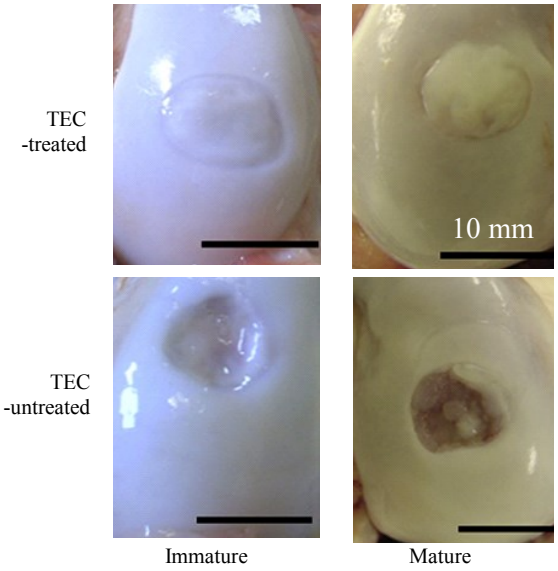

Figure 3. Macroscopic observation of TEC-treated and TEC-untreated repaired tissues of immature and mature porcine femurs.

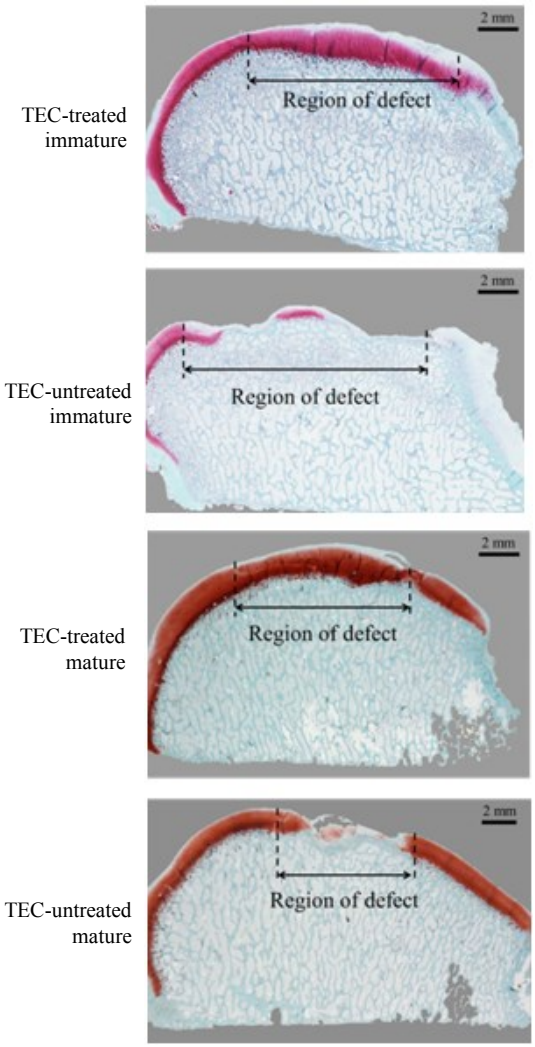

Figure 4. Histological observation of TEC-treated and TEC-untreated repaired tissues of immature and mature porcine femurs.

B. Permeability

The hydraulic permeability remained at low level in normal cartilage; between 4 and 8 x 10^{-15} m^4/Ns in immature animals, and approximately 1 x 10^{-15} m^4/Ns in mature animals. Note that, in immature animals, the hydraulic permeability was significantly increased versus normal level up to 18 x 10^{-15} m^4/Ns at surface layer. The permeability was also increased at middle and deep layers in mature animals although no significant differences was observed versus normal level.

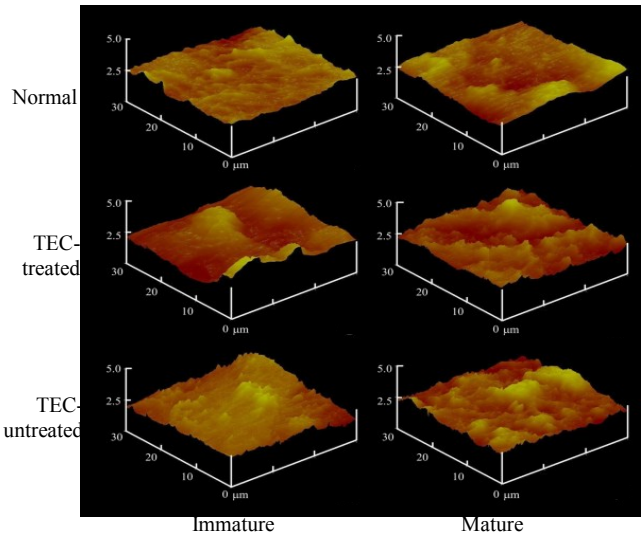

Figure 5. AFM observation of normal cartilage (upper), TEC-treated tissues (middle), and TEC-untreated tissues (lower) in chondral defects in immature (left) and mature (right) porcine femur.

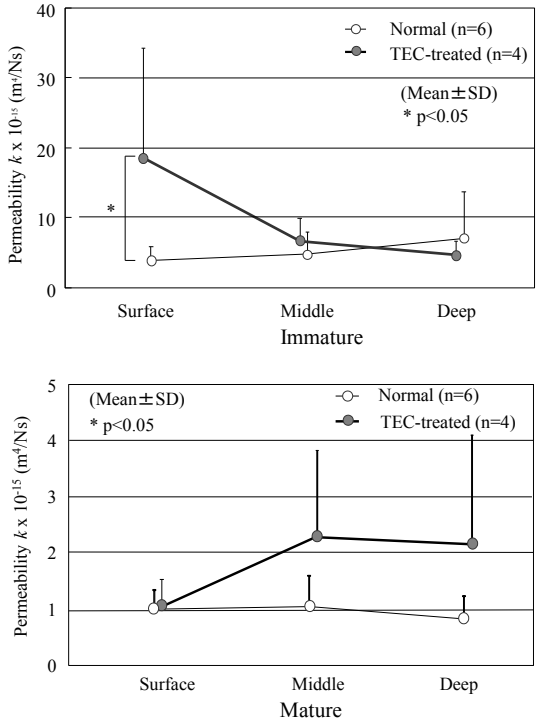

Figure 6. Permeability of the surface, middle, and deep layers of TEC-treated tissues under 30% of compression in immature (upper) and mature (lower) animals.

C. Coefficient of friction

The coefficient of friction of the TEC-treated tissues are shown in Fig.7. In normal cartilage, the coefficient of friction was load-dependently decreased in both immature and mature animals. However, the coefficient of friction of the TEC-treated tissues was load-dependently increased in immature animals. The coefficient of friction was significantly lower in TEC-treated tissues than in normal cartilage at 70 kPa, while the coefficient of friction was significantly higher in TEC-treated tissues than in normal cartilage at 280 kPa. In contrast, the coefficient of friction of TEC-treated tissues in mature animals was load-dependently decreased to approximately 0.12 at 280 kPa with no significant difference observed versus normal cartilage.

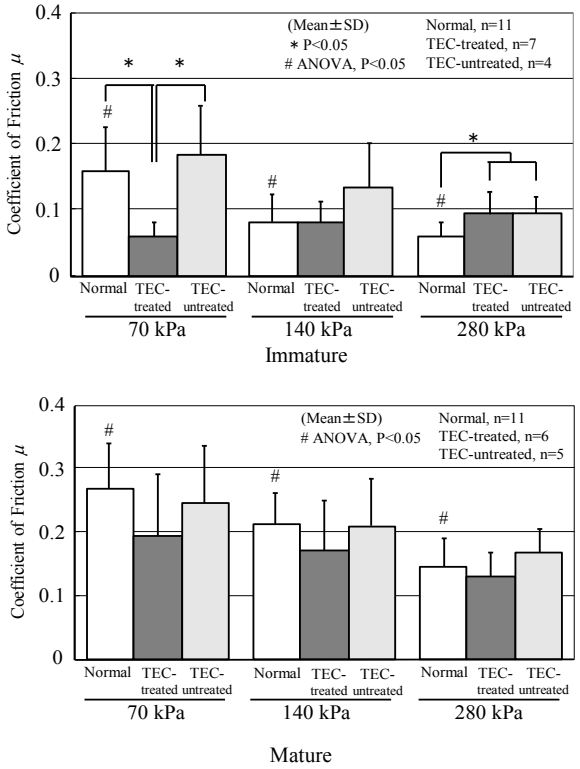

Figure 7. Coefficient of friction of TEC-treated and -untreated tissues against a glass plate at a speed of 20 mm/s as a function of applied load in immature (upper) and mature (lower) animals.

IV. Discussion and Conclusion

Partial chondral defects on the femoral condyle in pigs were repaired with synovium-derived MSCs-based TEC in the present study. As compared with tissues repaired with other methods, such as micro fracture [7], the TEC-treated repaired tissues exhibited more cartilage-like structure and properties. Morphological observations indicated that the defects were filled with hyaline cartilage-like repaired tissues, while the

TEC-untreated defects were filled with fibro cartilage-like tissues, 6 months after implantation. However, it was found that, even in TEC-treated tissues, the superficial region remained fibro cartilage-like, and the region was thicker in immature animals than in mature animals.

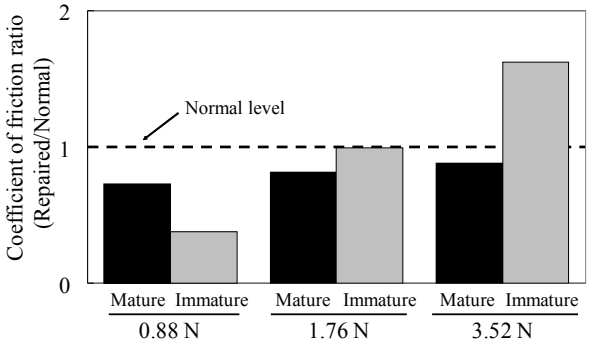

Figure 8. Ratio of coefficient of friction of TEC-treated and TEC-untreated tissues to normal level as a function of applied load.

Figure 8 indicated the ratio of frictional coefficient of the TEC-treated and TEC-untreated tissues to those of normal cartilage as a function of applied load. It is indicated that the frictional coefficient of TEC-treated tissues can be recovered closely to, or slightly better than, normal level in mature animals in all the loads. However, in immature animals, the frictional coefficient was 60% higher than normal level in TEC-treated tissues at a high load (3.52 N). This was maybe caused by the abnormality in composition found at the surface of TEC-treated immature animals. It is required to perform further studies for the improvement of cartilage repair using TEC, in particular, for the recovery of surface layer in immature subjects.

ACKNOWLEDGMENT

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