Evaluation Of Frictional Performance Of Regenerated Tissue On Fibroin Sponges Using Poly(mpc) Grafted Surface

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Disclosures:

Introduction: Cartilage repair using a fibroin sponge-based treatment is expected to be a possible new treatment for large osteochondral defects. E. Hirakara et al. applied fibroin sponge sheets seeded with cartilage cells over the osteochondral defect of rabbit knee, and reported that hyaline cartilage-like tissue with layer structure was formed within 6 weeks[1]. In addition, K. Nakagawa et al. have tried to establish a new treatment using drilling or abrasion making interface down to the bone-marrow space and covering with a fibroin sponge to regenerate cartilage tissue, which does not need culture. This technique expects the bone marrow cells to regenerate cartilage tissue with fibroin sponges in vivo.

On the other hand, Tamura N. et al. proposed a new method for evaluating the frictional performance of tissue engineered cartilage, where a poly(2-methacyloxyethyl phosphorylcholine (MPC)) grafted surface is proposed as an appropriate counter surface for cartilage friction evaluation due to the material’s ability to reduce the adhesion and reproduce natural tribological conditions[2]. In this study, frictional performance of regenerated tissue on fibroin sponges with rabbit chondrocytes or with rat MSCs was evaluated by using poly(MPC) grafted surface as a counter surface.

Methods: [Isolation and culture of chondrocytes] Chondrocytes were isolated from 4-week-old Japanese white rabbits. Articular cartilage tissue was taken from the proximal humerus, distal femur and proximal tibia. Chondrocytes were suspended at 5.2×10⁴ cells/ml with growth medium containing DMEM, 10% (v/v) FBS, 1% (v/v) antibiotic. 15 ml suspension was cultured in 75 cm² flask for 7 days. The medium was replaced at 3 days and 5 days.

[Isolation and culture of rat MSCs] Rat bone marrow stem cells (MSCs) were isolated from whole femur bone marrow aspirates of six-week-old fisher rats. A bone marrow was suspended 15 ml growth medium and cultured in 75 cm² flask for 7 days. The medium was replaced at 3 days and 5 days.

[Preparation of cell seeded fibroin sponges] Fibroin sponge scaffolds (8 mm diameter and 1 mm thickness) were carefully seeded in 24 well plates with 5×10⁵ cells suspended in 30 μl medium. Then, two types of 1.6 ml flesh medium were added to the wells. Sponges with chondrocytes were soaked in the culture medium, containing DMEM, 10% (v/v) FBS, 1% (v/v) antibiotic and 0.5 mM ascorbic acid. On the other hand, sponges with MSCs were soaked in the chondrogenic medium to differentiate them into chondrocytes.

Chondrogenic medium contains high-glucose DMEM, 50 μg/ml ascorbate-2-phosphate, 10 ng/ml TGF-β3, 10-7 M dexamethasone, 40 μg/ml proline, 100 μg/ml pyruvate, and 50 mg/ml ITS+ premix. Each medium was replaced every 2 days. We prepared fibroin sponges with chondrocytes and MSCs cultured for 7 and 14 days. Fibroin sponges with no chondrocytes and with no MSCs were defined as controls.

[Preparation of natural cartilage specimens] Natural cartilage specimens were taken from the femoral condyle of a 180-190 day-old Japanese pig. They were cut into 5 mm diameter.

[Fricntion test] The poly(MPC) grafted surfaces were prepared by atom transfer radical polymerization (ATRP) and used as a counter surface for cartilage friction evaluation. Fibroin sponges cultured for 7 and 14 days were cut into 5 mm diameter for this test. The applied pressure was 0.15 MPa and the sliding velocity was 0.8 mm/s. Friction coefficients were recorded for 1 stroke after loading periods of 600 s.

Results: Figure.1 shows frictional coefficient of regenerated tissue. In control group, there was no different between of mean values chondrocyte-seeded sponge and MSC-seeded sponge. In chondrocyte group, frictional coefficient of the sponge cultured for 7 days significantly decreased compared with that of control, and that of the sponge cultured for 14 days was inclined to further decline. On the other hand, in MSC group, coefficient of the sponge was inclined to increase with increasing culture period.

Figure.2 shows safranin-O stained sample where the staining intensity at the surface was increased with increasing culture period, but the MSC group showed little metachromasy.

Figure.3 shows collagen type I immunostaining. In chondrocyte group, there was no collagen type I, however the MSC group showed higher staining intensity at the surface with increasing culture period.

Figure.5 shows the amount of synthesized GAG. The GAG amount was significantly increased with increasing culture period. The GAG amount of chondrocyte-seeded sponge was significantly higher than that of MSC-seeded sponge.

Discussion: It is essential to investigate the tribological maturation of tissue engineered cartilage that is to be used in medical applications. The frictional performances of tissue engineered cartilage have been measured using flat counter surfaces such as
stainless steel, glass or ceramics. However, the measured friction performances were significantly inferior to those of natural cartilage, likely because of cartilage adhesion to the counter surface. In this study, a poly(MPC) grafted surface is used as an counter surface for cartilage friction evaluation, which reported to show the friction coefficients for natural cartilage to be lower than 0.01 and to increase with sliding velocities, which are equivalent to those for natural cartilage-on-cartilage friction. Lubrication capacity of fibroin sponges was improved by the existence of chondrocytes where collagen type II and GAG were produced. The MSC group showed a little synthesis of the matrix after 14 days culture, but the surface was covered by collagen type I. Kachi et al. compared frictional coefficient of regenerated cartilage on chondrocyte-seeded sponge and that on chondrocyte-aggregates-seeded sponge, and suggested that the chondrocyte aggregates inhibited formation of functional tribological structure by synthesis of collagen type I on the surface[3]. Collagen type I formed on the MSC-seeded sponge may decrease lubrication capacity of the regenerated tissue.

**Significance:** CONCLUSION: Frictional performance of regenerated tissue on fibroin sponges was evaluated using poly(MPC) grafted surface. Frictional coefficient of regenerated tissue on chondrocyte-seeded sponge decreased with culture period, whereas, that on MSC-seeded sponge increased supposedly because of collagen type I formation on the surface.

**Acknowledgments:**

**References:**
Figure 1 Friction coefficient of fibroin sponges with no cells, with chondrocyte and with MSC cultivated for 7, 14 days and natural cartilage.

Data represent mean ± SD.

(chondrocyte and natural cartilage: n=3, MSC: n=4)
(*) p<0.001, by t-test
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Figure 2. Histological photographs of fibroin sponges with chondrocyte and with MSC cultivated for 7, 14 days (Safranin-O staining). Scale bar = 200 μm.
Figure 3: Histological photographs of fibroin sponges with chondrocyte and with MSC cultivated for 7, 14 days (Collagen type I immunostaining). Scale bar = 200 μm.
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![Image](image.jpg)

Figure 4: Histological photographs of fibroin sponges with chondrocyte cultivated for 7, 14 days (Collagen type I immunostaining). Scale bar = 200 μm.
Figure 5 The amount of GAG content in fibrin sponges with chondrocyte and with MSC cultivated for 7, 14 days. Data represent mean ± SD. (n=3)
(*) p<0.001, by t-test