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Characteristics of regenerated cartilage tissue cultured under traction loading

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ABSTRACT – In this study, chondrocytes isolated from metacarpal-phalangeal joints were seeded in agarose gel. And, traction loading was applied to the surface of the gel by the roller to examine the effect of the tribological stimulation on the characteristics of the regenerated cartilage tissue elaborated in chondrocyte-agarose construct. Results indicated that the traction loading enhanced ECM biosynthesis in the surface region selectively and subsequently elaborated tissue had anisotropic structure with collagen rich surface layer covered with GAG rich superficial layer.

1. INTRODUCTION

An arthrodial cartilage covers the sliding surfaces of a diartrodial joint, and it has important mechanical functions in an articular surface. Mechanical stresses and strains exerted in articular cartilage during daily joint movements can stimulate the metabolism of chondrocytes. They play an important role to sustain the health and homeostasis of the cartilage tissue. Especially, upregulative effects of the cyclic compression and the hydrostatic pressure on the chondrocytes biosynthesis of extracellular matrix (ECM) have been studied extensively [1, 2] and utilized in the cartilage tissue engineering. However, the load which arises in a living body is not the simple in fact. Cell embedded in cartilage tissue is exposed to the dynamic and complicated stress fields. On the other hand, current regenerated cartilage does not have sufficient dynamic functionalities compared with the normal arthrodial cartilage.

In this study, the relative motion between cartilage surfaces in a joint is simulated by the rolling-sliding motion of the plastic roller on the cultured chondrocyte-agarose construct. Its effects on the formation of regenerated cartilage tissue were investigated. The chondrocyte-agarose construct, a well-established three dimensional culture system for isolated chondrocytes, has been employed as an experimental model and cultured for 2 or 3 weeks. Subsequently, effects on the ECM biosynthesis of cultured chondrocytes and characteristics of the elaborated cartilaginous tissue, such as mechanical properties and tissue structure were examined.

2. METHODLOGY

Chondrocytes were isolated from cartilage tissues harvested from metacarpal-phalangeal joints of steers using a sequential enzyme digestion method. Isolated chondrocytes were seeded in agarose gel with an initial cell density of 1 x 10^7 cells/ml and chondrocyte-agarose constructs with a diameter of 18 mm and a thickness of 2.5 mm were prepared as test specimens. The specimen was then placed in a custom-build culture dish and immersed in culture medium.

The traction loading was applied to the surface of prepared specimen by a plastic roller in the traction loading machine (Figure 1). After the specimen setup, the plastic roller with silicone rubber surface layer was brought into contact with the upper surface of specimen by a liner actuator. Then the culture dish was reciprocated horizontally while the plastic roller was oscillated. Both motions were regulated by PC-controlled AC-servo system in a coordinated manner. Consequently, the plastic roller ran over the construct specimen iteratively with a defined slip ratio and the traction load was exerted on the construct surface.

The traction loading machine was installed in a CO_2 incubator. Then, construct specimens were cultured under the traction loading for 2 or 3 weeks in this study. The slip ratio was fixed to 0 in all experiments and the traction loading was applied under pure rolling condition at 1 Hz for 12 hour a day during the culture period. To identify effects of the traction loading, a control specimen with a same initial cell density and same dimensions was also prepared and cultured simultaneously under the free swelling condition.

After the culture period, the amount of Glycosaminoglycan (GAG), and Type II collagen in the construct and culture medium were quantified. Cylindrical samples with a diameter of 5 mm harvested from the construct for quantitation assays. On the other hand, whenever a culture medium exchanges, 1 ml of culture medium was extracted and preserved in frozen storage. GAG and type II collagen contents of samples were evaluated by using dimethylmethylene blue (DMMB) assay and the Sicol collagen assay (biocolor, UK), respectively. Samples for biochemical assays were dissolved in phosphate buffered saline (PBS) based digest buffer and mixed with DMMB or Sirius red reagent in 96 well plates. Subsequently, plates were

transferred to a multi-well plate reader and the absorbance of the solutions was measured at 525 nm and 555 nm to evaluate GAG content and collagen content, respectively, by colorimetric determination.

One of the cylindrical samples was cut into thin slices with the thickness of about 1 mm and used for the immunofluorescence observation of the elaborated cartilaginous tissue. Then type II collagen and GAG were fluorescently stained with different colors to visualize the morphological characteristics of elaborated cartilaginous tissue with confocal laser scanning microscopy (CLSM).

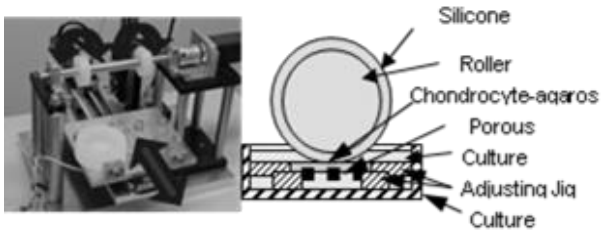


Figure 1 Traction loading culture machine.

3. RESULTS AND DISCUSSION

Constructs cultured under the traction loading had a clear sliding track on the upper surface. Therefore, samples for the analyses were collected from both inside and outside of the sliding track. Figure 2 and 3 show the result of quantification of type II collagen and GAG, respectively. These graphs show amounts of type II collagen and GAG accumulated in the construct during culture as a ratio to the control specimen. Therefore, 100 mean the same as the control specimen. The amounts of ECM in traction-loaded specimen were almost same as the control.

Figure 4 and 5 are fluorescent images of type II collagen and keratan sulfate accumulated in the traction loaded specimen and the control specimen. Keratan sulfate is a type of GAG side chain of proteoglycan. Type II collagen and keratan sulfate distributed similarly in each agarose construct probably because relatively small keratan sulfate molecules could diffuse through agarose and synthesized collagen fibers formed networks structure which trapped negatively charged keratan sulfate in the networks by electric interactions. However, clear differences in the distribution of ECM molecules could be recognized between the traction loaded specimen and the control specimen. In the control specimen, collagen and keratan sulfate were mainly accumulated around the chondrocytes and distributed evenly in agarose construct. On the other hand, collagen molecules densely accumulated near the sliding surface in the traction loaded specimen. Although keratan sulfate tended to be accumulated near the surface of agarose construct, the density of it also became higher in around the sliding surface. As a result, the traction loaded specimen had an anisotropic nature in the structure of elaborated tissue, the densely packed ECM rich articulating surface and homogeneous center and deep regions. The density of ECM molecules seems to be much higher under the sliding track and it might be responsible for the higher elastic modulus in the sliding track.

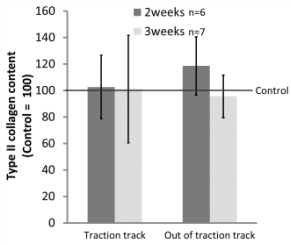


Figure 2 Amount of Type II collagen in the model.

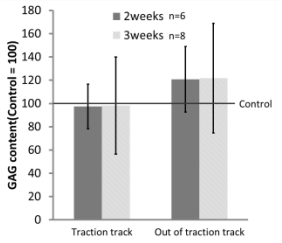
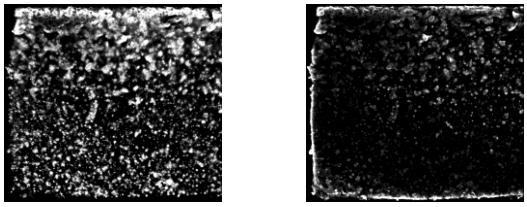
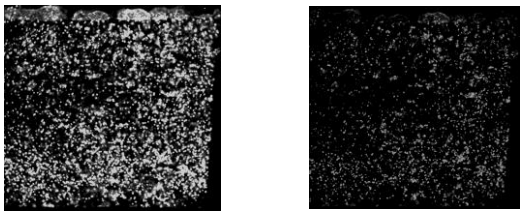


Figure 3 Amount of GAG in the model.



(a) Type II collagen (b) Keratan sulfate

Figure 4 CLSM image of the traction loaded construct.



(a) Type II collagen (b) Keratan sulfate

Figure 5 CLSM image of the control construct.

4. CONCLUSIONS

The traction loading applied to the surface of chondrocyte-agarose constructs could upregulate the biosynthesis of ECM molecules in the sliding surface region. It brought the anisotropic nature in the elaborated cartilaginous tissue and ECM rich layer was formed in the articulating surface of the construct cultured under the traction loading. Therefore, the traction loading on the surface may have a potential to make the structural anisotropy like a natural articular cartilage in regenerated cartilage tissue.

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5. REFERENCES

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