P10

Development of Collagen Gel/Sponge Composite Scaffold For Cartilage Tissue Engineering

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Abstract

Recently, clinical application of cartilage regeneration has been investigated on the basis of tissue engineering. However, the mechanical properties of engineered cartilage tissues are relatively poor compared to biological natural cartilages. In the present study, novel hybrid scaffold was developed by composing collagen gel and collagen sponge. Human mesenchymal stem cells were cultured using the gel/sponge scaffolds in a combined culture with normal and differentiation medium up to 28days. Compression modulus increased by the normal culture for 14 days. And aggrecan tended to increase with days in culture in the differentiation culture. This study suggests that the gel/sponge composite material can be a promising candidate of scaffold for cartilage tissue engineering.

1. Introduction

Tissue engineering approach for cartilage regeneration has clinically been conducted by using chondrocytes with collagen gel scaffold[1]. However, such engineered cartilage tissue cannot be implanted into the load-bearing part of joint surface because of luck of mechanical properties such as strength. Therefore, engineering scaffolds with better mechanical properties has actively been developed in the field of cartilage tissue engineering. In previous research, we have prepared collagen gel/sponge two-phase scaffold. The study showed that a gel/sponge composite scaffold has excellent mechanical and biological properties than pure gel and sponge scaffolds. In the present study, combined culture with normal and chodrogenic medium was introduced in order to enhance cell proliferation. Human mesenchymal stem cells (hMSCs) were cultured using composite scaffold up to 28days.

2. Experimental

The type I collagen gel as the basic scaffold was produced using Cellmatrix TypeI-A. It was gelled after mixing collagen solution, 10 times concentrated MEM-α, and buffer solution. The mixing ratio was selected as 8:1:1. Collagen sponge was fabricated by the freeze-drying method using chicken cartilage collagen solution (type II

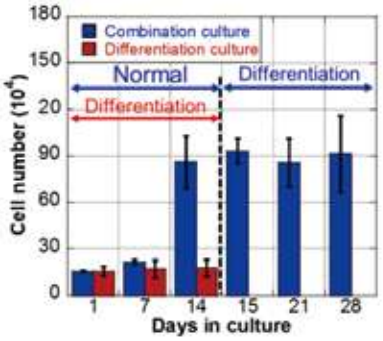


Figure 1. Variation of cell viability.

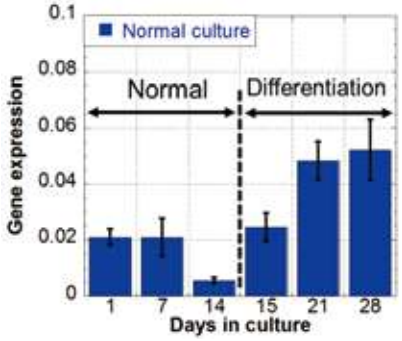


Figure 2. Variation of aggrecan.

collagen). Gel/sponge composite scaffold was produced by immersing the collagen sponge into the collagen gel. hMSCs were cultured using gel/sponge composite scaffold up to 28days. Firstly, we seeded 1.0×105 cells per one scaffold. Next, we added the normal medium(MEM-α, 10%FBS, 1% penicillin-streptomycin), and performed 14 days culture. And next, we added the differentiation medium (RPMI1640, FBS, ascorbic acid, amino acids, antibiotics), and performed 14 days culture. Compressive mechanical properties, cell viability and chondrogenic marker were measured. And also the micro-structure was observed.

3. Results And Discussion

Variation of cell viability is shown in Fig.1. The scaffold is chondrogenic differentiation cultured of 14 days from the initial condition, it is constant cell number. However, in the combined culture, cell number increased by the normal culture for 14 days and stayed at a constant value during the chondrogenic culture.

Gene expression of aggrecan which are typical proteins in cartilage tissue are shown in Fig.2. Aggrecan do not increase in the normal medium. However, they tended to increase with days in culture in the differentiation medium. Increasing chondrogenic marker implies that hMSCs were differentiated into chondrocytes.

The compressive mechanical properties of the scaffolds are shown in Fig.3. Compression modulus increased by the normal culture for 14 days. And modulus still increased during the chondrogenic differentiation culture. It is thought that the structural stability was improved by cell proliferation and extracellular matrix formation, resulting in the recovery of the compressive modulus by the normal culture for 14 days. And it is thought that if the cell number was increased by the normal culture for 14 days at the beginning, scaffold can retain a high elastic modulus after 14 days chondrogenic differentiation culture.

FE-SEM micrograph of cells on the scaffold surface and cross-section after 28 days culture are shown in Fig.4. The spherical shape of the cells implies that MSCs were differentiated into chondrocytes. The fibril structures observed in the vicinity of cells are thought to be type II collagen fibrils created by the cells.

4. Conclusion

Combined culture with normal and chodrogenic medium was introduced in order to enhance cell proliferation. Cell number effectively increased during the normal culture and still slightly increased during the chondrogenic culture with increasing aggrecan. The compressive modulus increased after the combined culture. It is thus concluded that the gel/sponge composite material developed in this study can be one of the promising candidates as a scaffold for cartilage tissue engineering.

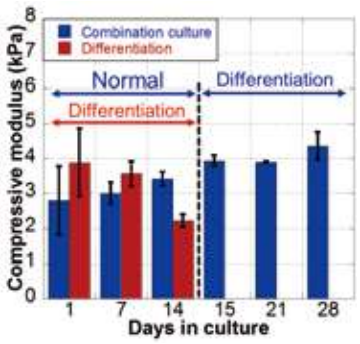
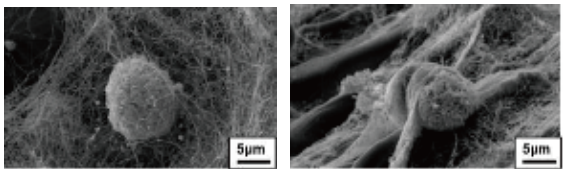


Figure 3. Compressive mechanical properties.



(a) Surface (b) Cross-section

Figure 4. FE-SEM micrographs of cells after 28 days.

Reference

[1] H.H. Lee, M.J. O'Malley, N.A. Friel, C.R. Chu.Effects of doxycycline on mesenchymal stem cell chondrogenesis and cartilage repair. Osteoarthritis and Cartilage 2013;21:385-93.

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