INTRODUCTION

There have been many attempts to repair full-thickness articular cartilage defects using tissue-engineering techniques, focusing particularly on the regeneration of high-strength cartilage. Unfortunately, the strength is insufficient for fixation of the grafted part on large defect of joint surface. Current focus in our laboratory has been placed on the development of a novel silk fibroin sponge which, mechanically robust and can be designed to desirable mechanical specifications including ultimate tensile strength, yield point and stiffness [1, 2]. The unique performance of these fibroin sponges are now being rediscovered and reconsidered as potentially useful biomaterials for a range of applications in clinical repairs and in vitro as scaffolds for tissue engineering. Chondrocytes knocked into the fibroin sponge proliferated and possessed its normal 3-D phenotypic characteristics as we reported [1, 2]. However, according to its low-cell adhesive nature, seeding technique into the fibroin sponge is still a major problem encountered. Chondrocytes metabolism and homeostasis were known highly sensible and affected by the mechanical stimuli.

In this study, a simple magnetic stirring system was developed to improve after-seeding tissue maturity. And fibroin sponge was used as a scaffold for in vitro cartilage regeneration system. Chondrocytes were harvested from 4-week-old Japanese white rabbits and inoculated in the fibroin sponge. Cultured in 37°C incubator for 3 days, transferred to magnetic stirring system and keep culturing until another 28 days. After 3 days of cultivation, the number of chondrocytes had increased and production of extracellular matrix was observed. Well-defined cartilage-like tissue was regenerated until 28 days of cultivation. Increase of better extracellular matrix (chondroitin sulfate) was also observed by using the system.

MATERIALS AND METHODS

Silk fibroin protein solution was refined from silkworm cocoons by dissolving them in lithium bromide solution. After a water-soluble organic solvent had been added, this silk fibroin protein solution was frozen for 24 h, and then the sponge was formed by phase separation. Articular cartilage slices were taken from the proximal humerus, distal femur and proximal tibia of 4-week-old Japanese white rabbits; chondrocytes were isolated by enzymatic digestion. To inoculate the cells into the fibroin sponge, a cell suspension was filtered through the fibroin sponge, and these chondrocyte-fibroin sponge constructs were set in a CO₂ incubator at 37°C for 3 h. After 3 days static culture, the constructs were transferred to originally designed magnetic stirring system. The samples were collected on days 1, 3, 7, 14 and 28 of cultivation, and then histological observation and analysis were carried out using a digital image system (NIH image). Also, Samples were fixed in 2% paraformaldehyde in 2.5% glutaraldehyde then post fixation 2% osmium tetroxide, dehydrated in an increasing acetone series, critically point dried, coated with gold-palladium, and observed with a HITACHI S-2380N SEM to study the surface ultrastructure morphology.

RESULTS

Silk fibroin sponge construct after 14 days cultivation using the magnetic stirring system.

DISCUSSION

Until now, there are two types of scaffold mainly used for tissue engineering. The glue type, with it easily seeding and injectable abilities in clinical use, received many attentions among researchers. But the cell propagation was less seen when using this kind of scaffold. By contrast, the porous type, which provides mechanical stability to support cell adhesion and expansion require some seeding techniques.

We have reported that cartilage cells proliferate keeping its phenotypic expression on the fibroin-hydrogel sponge. Fibroin is a silk protein which has been used as biomedical suture for decades. The fibroin sponge also has a relatively high strength for fixing on the joint [1, 2]. However, uniform and high-density cartilage tissue was hardly formed of its low cell-adhesive characteristics. Our present study showed good formation of hyaline cartilage-like tissue in the pore of the fibroin sponge, and increased in the cell population the same as extracellular matrix production observed. By using the magnetic stirring system, some shear stress and/or fluid flow stimulation might be effective to form more mature cartilage-like tissue as the histological results demonstrated. Although the complexity of identifying the biophysical phenomena that occur during cartilage loading in vitro, these physical factors still need more investigations. The biochemical contents and nucleus quantification of these constructs are under progress in our laboratory.

REFERENCES


Paper No: 0907

Fig. 1: 1, 2: Macro image of fibroin-hydrogel sponge and SEM image with mean pore size 80µm. 3: Safranin-O staining of chondrocyte-fibroin sponge construct after 14 days cultivation. 4: Safranin-O staining of chondrocytes-fibroin sponge construct after 14 days cultivation using the magnetic stirring system.