Changes in Adhesive Force of Chondrocyte to Silk Fibroin Scaffold at the Initial State of Tissue Organization

Introduction

In vitro regeneration of cartilage tissue using scaffolds is one of the possible procedures for cartilage repair. However, chondrocytes cultured on two-dimensional substrates dedifferentiate into fibroblast-like cells and express type I collagen [1]. When using silk fibroin sponge as a three-dimensional scaffold for in vitro cartilage regeneration, we have shown that the chondrocytes proliferate and hyaline-like cartilage tissue is organized on the surface of the sponge [2, 3]. These results suggest that the chondrocytes seeded on the surface of the scaffold can proliferate while maintaining their phenotypic expression during in vitro culture period.

In this study, we focused on the adhesive performances of chondrocytes to the surface of silk fibroin substrate. Adhesive force of chondrocytes harvested from Japanese white rabbits to four types of substrates: glass, glass coated with fibronectin, fibroin film, fibroin film containing RGDs peptide, was evaluated at 3, 6, 9, 12 and 24 hours after seeding by using newly developed apparatus applying micromanipulation method, and the cell spreading area on each substrate was also measured using image processing techniques. The morphology of chondrocytes was evaluated using scanning electron microscopy.

Materials and Methods

Substrates coated on the glass plate

Three types of substrates: fibronectin (FN), fibroin films (FIB), fibroin film containing RGDs peptide (FIB-RGDm) were prepared, and the glass without coating was used as a control substrate (CON). Silk fibroin protein was made from silkworm by dissolving the cocoon in a potassium hydroxide solvent, the ultra thin glass plate (0100 Matsunami Glass Ind., Ltd.) fabricated as leaf spring (length: 45 mm, width: 1.5 mm, thickness: 30 µm) was soaked in the silk fibroin-protein solution up to the point of 15 mm from the end of the glass and dried. FIB-RGDm was made by plasmid instillation E. coli at the rate of 5 % in the silk fibroin-protein solution. In the similar manner, fibroin-protein solution was coated using ProNectin F (632-02761 Sanyo Chemical Industries, Ltd.) diluted by 10 µg/ml using PBS.

Apparatus for measuring the adhesive force of a single cell

Adhesive force of chondrocytes to the substrates was measured using ultra thin glass plate fabricated as leaf spring and micropipette aspiration equipped with a motorized stage. Figure 1 shows the schematic drawing of a principle component to measure the adhesive force of a single chondrocyte. In this study, adhesive force was calculated using the maximum bending of the glass plate observed through video microscope (DG-2 Scalar Co.) and the elastic coefficient calculated using the maximum bending of the glass plate observed after aspiration equipped with a motorized stage until the cell was detached from the surface of the substrate. All experiments were performed in Leibovitz's L-15 medium (11415-064 Gibco Invitrogen Co.), which enables cell culture without organic solvent, the ultra thin glass plate (0100 Matsunami Glass Ind., Ltd.) fabricated as leaf spring (length: 45 mm, width: 1.5 mm, thickness: 30 µm) was soaked in the silk fibroin-protein solution up to the point of 15 mm from the end of the glass and dried. FIB-RGDm was made by plasmid instillation E. coli at the rate of 5 % in the silk fibroin-protein solution. In the similar manner, fibroin-protein solution was coated using ProNectin F (632-02761 Sanyo Chemical Industries, Ltd.) diluted by 10 µg/ml using PBS.

Results and Discussion

Both adhesive force and spreading area of chondrocytes to every substrate increased with culture time (not shown in this paper). Figure 2 shows the results of changes in adhesive force per unit spreading area to every substrate with culture time. It is found that the adhesive force to the normal fibroin increased until around 6 hours after the inoculation and then decreased, while that to the substrate coated with fibroin increased gradually with cultivation time. The result suggests that the density of focal contacts rapidly increases during the period of initial cell adhesion in organizing the tissue.

Chondrocyte, which is one of the adherent cells, proliferates through the various interactions with a substrate, such as biochemistry, topography and stiffness. In addition, the morphology of chondrocyte correlates with phenotypic expression, and spherical chondrocyte is known to be able to exhibit a differentiated phenotype expressing type II collagen and produce proteoglycans [4]. Our former results showed that chondrocytes proliferated maintaining their phenotypic expression and organized a hyaline-like cartilage on the fibroin sponge [2, 3]. It might be possible that the clustering of integrins on FIB-RGDm substrate were accelerated by some mechanism. Although it must be important to analyze the expression of the adhesive protein such as integrins in our future studies, it would be obvious that the physical properties measured as one of the results through those chemical reactions are associated with the morphology of cells. It might be possible that the results of this study provide the useful information to analyze the process of tissue organization and improve the surface property of scaffold for cartilage regeneration.

References


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52nd Annual Meeting of the Orthopaedic Research Society

Paper No: 0908