Comparison between Static and Rotational Culture on Chondrocyte/Silk Fibroin-based Scaffolds

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Objective: Silk fibroin-based scaffolds are natural biomaterials that have slow biodegradation and robust mechanical properties; therefore they show great promise in their application in cartilage tissue engineering. This present study was designed to test whether rotational culture could be more advantageous compared to static culture in terms of cell viability, distribution and proliferation, as well as extracellular matrix (ECM) synthesis.

Methods: Silk fibroin-based sponges prepared from 7% fibroin/water (W/V) with a 500-1180 μm pore size were used in this study. Confluent third passage rat costochondral chondrocytes were suspended at passage 4 in DMEM medium containing 10% FBS and ascorbate at $2*10^7$ cells/mL. 20 μL cell suspensions were loaded onto 3mmX6mm cylindrical sponges (0.4 million cells/sponge) by static gravity-dependent filtration through the sponge. These cell constructions were then subjected immediately to rotational culture on an orbital shaker at 15 rpm or maintained on static culture during 28 days. Cell viability was assessed using a Live/Dead® kit; the Alamar blue™ assay was used to evaluate cell proliferation; DNA content was measured to indicate cell numbers; glycosaminoglycan (GAG) production was analyzed based on DMMB-staining and normalized to DNA content; and collagen production was detected by immunofluorescence staining.

Results: At harvest, cell viability was >90% in both static and rotational culture systems. Cell distribution throughout the sponge was homogenous under rotational culture whereas more cells were found on scaffold surface and less in the center under static culture. Alamar blue assay showed time-dependent cell proliferation regardless of the culture method. After 28 days of culture, cell number increased to 1.41±0.23 million cells/sponge and 0.92±0.11 million cells/sponge for the static culture system and rotational culture system, respectively. Even though rotational culture resulted in 88.4% ± 4.9% cell loss from the scaffolds at day 1 instead of only 6.5% ± 1.4% under static culture, it induced an 18.4 fold increase in cell number compared to a 3.6 fold increase under static culture at day 28 (Fig.1A). 25.28 μg GAG/sponge were produced under static culture and 18.31 μg GAG/sponge under rotational culture. However, no significant difference between them was found after normalizing GAG to DNA content (Fig.1B). Immunofluorescence staining showed strong collagen type II expression on the cell surface under both culture conditions (Fig.2).

Conclusions: Our data indicated the good biocompatibility of this natural silk fibroin scaffold supporting cell proliferation as well as ECM production. The rotational culture system seemed to be more advantageous than static culture system in light of the even cell distribution and higher cell proliferation rate. However, the large loss of cells from the scaffolds under rotational culture suggested that it should be necessary to postpone the initiation of rotation in order to give more time for cells to attach onto scaffold under static culture. In conclusion, static loading plus rotational culture of chondrocytes on silk fibroin-based scaffold could be an effective model for *in vitro* study of cartilage tissue engineering.

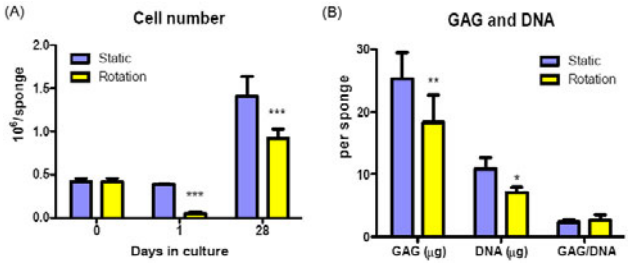

Fig.1 Cell number, GAG and DNA content in scaffold
(A) Cell number at Day0, 1 and 28;
(B) GAG and DNA content per scaffold at Day28.
*P<0.05, **P<0.01, ***p<0.001 *vs*. static; n=4

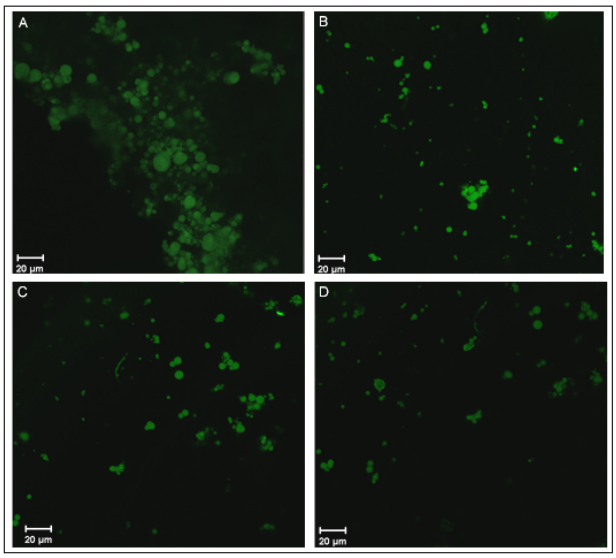

Fig.2 Immunofluorescence staining detection of collagen type II expression on cell surface
Static culture: (A) Scaffold surface, (B) Scaffold center;
Rotational culture: (C) Scaffold surface, (D) Scaffold center.

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