

SCAFFOLD FREE GENERATION OF INTER VERTEBRAL DISC USING ROTATIONAL CULTURE SYSTEM

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INTRODUCTION

Tissue engineering methods to generate an artificial intervertebral disc has been considered a possible treatment option. Generation of a tissue using various tissue engineering methods often rely on cells and scaffolds. Recent tissue engineering studies claim that scaffold free techniques may generate better tissue formation. In articular cartilage repair, three-dimensional chondrocyte-plate, and suitable tissue-engineered cartilage was constructed without the use of scaffold. [1, 2] However, to date, there has been no report of generation of an annulus fibrosus or a nucleus pulposus tissue without the use of cell supporting scaffold. The aim of this pilot study was to investigate neither whether it was possible to form a disc tissue without the use of any scaffold nor additional growth factors using a novel technique, with a combination of static culture and rotational culture systems.

METHODS

Annulus fibrosus (AF) cells were isolated from intervertebral disc tissue of Japanese white rabbits (n=10) by enzymatic digestion and then expanded in monolayer culture. After AF cell cultures were passaged once, cells were cultured at primary static cultures for 7 days and then either cultured 2 weeks for another static culture or rotational culture. Nucleus Pulposus (NP) cells were isolated from intervertebral disc of Beagles (n= 4) by enzymatic digestion and then expanded in monolayer culture. After NP cell cultures were passaged once, cells were cultured at primary static cultures for 7 days and then cultured 1 or 2 weeks in rotational culture (Figure 1).

Primary static culture

AF and NP cells were trypsinized off the dish and applied to form a plate by using a mold with a diameter of 25 mm. Cells were suspended in DMEM/F12, FBS20% and 50 µg/ml ascorbic acid having a cell density of 2.5×10^6 cells/cm² and the cell suspension was inoculated in the mold. After 3 days, the cell suspension in the mold showed cell aggregation, an AF or NP cell plate. Then the mold was removed. The AF or NP cell plates were cultured under primary static culture condition for 7 days to form regular cylindrical shape.

Rotational culture group

After primary static culture, the plate was cultured under dynamic condition, rotational culture (70 rpm), for 2 or 4 weeks in AF cell plates and rotational culture (40 rpm), for 1 or 2 weeks in NP cell plates.

Static culture group

After primary static culture, the plate was cultured under static condition for 4 weeks in both AF and NP cell plates.

Constructed AF and NP plates were evaluated for histological analysis by Hematoxylin and Eosin staining and Safranin-O staining. Immunohistochemical staining was also performed for Type I and II collagen. In AF cell plates, biochemical analyses were also performed for proteoglycan (PG), collagen and DNA content.

RESULTS

After 2 or 4 weeks of rotational culture, AF cells formed a plate with good stability that allowed 3-dimensional handling. The rotational culture group was intensely stained with Safranin-O in comparison with static culture group. The rotational culture group was intensely stained with Safranin-O at 2 or 4 weeks (Figure 2a-c). The PG content of rotational culture group showed significant increase compared to the static culture group. The DNA content of static culture group also showed increase compared to the static culture group. The content of collagen did not show significant difference in all groups.

After 2 weeks of rotational culture, NP cells formed a plate with enough stability that allowed 3-dimensional handling with surgical pincers (Figure 2d). The rotational culture group was intensely stained with Safranin-O in comparison with static culture group (Figure 2e and f). Result of collagen II expression showed that both the rotational and static culture group were highly stained (Figure 2g and h).

DISCUSSION

AF and NP cells were both capable of forming a 3-dimensional IVD like tissue without the use of any scaffold. Both tissue generated with the use of rotational culture presented the most intense Safranin-O

and collagen II staining, suggesting that biomechanical influence of rotational culture system may become a useful method for IVD tissue engineering. Since the native AF and NP tissue possess complex matrix integrity, the relevance of scaffold free AF and NP tissue remain unclear. However, these results may be useful in development of a future technique to delay the irreversible progress of disc degeneration.

Figure 1. Methods of rotational culture and static culture

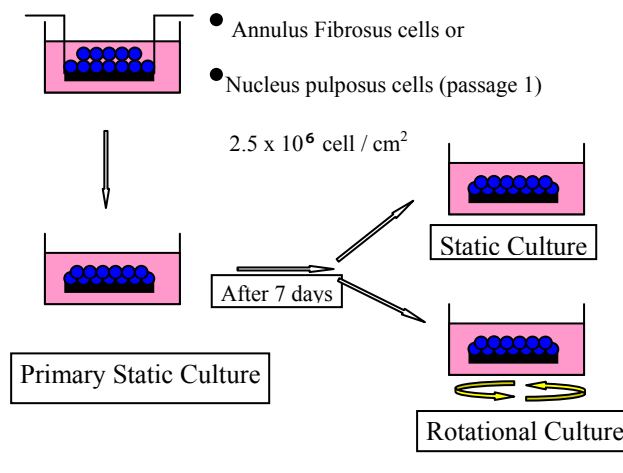
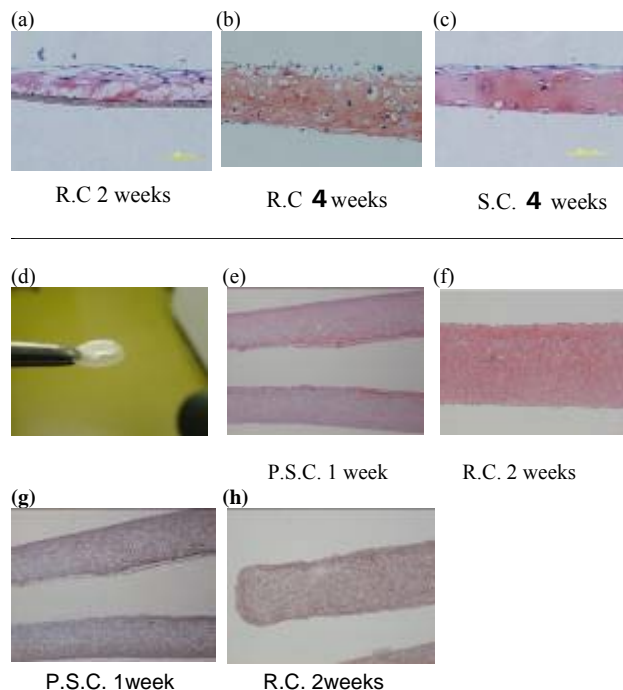


Figure 2. Safranin-O staining



REFERENCES:

- [1] Nagai T et al, Tissue Engineering 2008, in press.
 - [2] Furukawa K et al. TESHU 2004
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