DEVELOPING AN ALGINATE/CHITOSAN HYBRID FIBER SCAFFOLD FOR INTERVERTEBRAL DISC ANNULUS FIBROSUS CELLS

*Shao, XX; *+Hunter, CJ
+University of Calgary, Calgary, Alberta, Canada chunter@ucalgary.ca,

INTRODUCTION

Tissue engineering approaches using biodegradable scaffolds seeded with cells are potential alternatives for the treatment of intervertebral disc disease. Alginate is an anionic polysaccharide that has been widely used as a scaffold material in cartilage reconstruction because of its biocompatibility, hydrophilicity, and relative low cost [1]. Similarly, chitosan, a cationic polymer, has been employed as an excellent biocompatible material for wound healing and tissue repair [2]. Unlike articular cartilage, the intervertebral disc has two distinct regions, the annulus fibrosus (AF) and the nucleus pulposus (NP), each containing different cell types and extracellular matrix. Therefore, the aim of the present study was to develop an alginate-based chitosan hybrid scaffold for supporting annulus fibrosus cell growth.

METHODS

Scaffold fabrication: Alginate fiber scaffolds were synthesized by a wet-spinning method in a spinner flask. Briefly, 1% sodium alginate solution was extruded into a rotating CaCl₂ bath (100 mM) through a 25G needle. A syringe pump was used to feed the alginate solution into a needle tip at a feed rate of 3 ml/min. The CaCl₂ bath was spun in a spinner flask gently so that the alginate fibers were collected around the rotating shaft of the spinner flask. After 2 minutes in the bath, the alginate loop was removed from the shaft and transferred to another static 100mM CaCl₂ bath for 1-2 hour incubation or to a 0.5% chitosan solution for 10-12 hours. This chitosan coating would produce an alginate-chitosan hybrid fiber scaffold. After the treatment, both alginate and alginate-chitosan hybrid scaffolds were rinsed in distilled water and freeze-dried for later use.

Scaffold characterization: The scaffold morphology was examined under light and scanning electron microscopy (SEM). Degradation of the scaffolds was assessed via dry mass loss after culture in the DMEM/F12 medium for various time intervals. The cyto-toxicity of the scaffold was tested using MC 3T3 fibroblasts and the MTT assay. Briefly, 3T3 cells were co-cultured with or without 4 mg alginate or alginate-chitosan scaffold pieces or latex pieces in 24-well plates. MTT tests were performed on samples each day for three days.

AF cell culture on the scaffold and gene expression: Canine AF cells were obtained from collagenase/pronase digestion of minced annulus fibrosus tissue. The cells were loaded into the scaffolds by incubating for 2 hours at 37°C, 5% CO₂. The cell/scaffold constructs were then cultured for 10 days with medium changes every two days. At the beginning of the culture, then after 4 to 5 days in culture, the cells formed into clusters or aggregates and spread along the fibers within the scaffolds (Fig. 4A, 4B). Immunohistochemical staining showed the expression of collagen type I and II in AF cells clusters (Fig. 4C, 4D). The limitation of the current study is the feasibility of alginate-based chitosan hybrid scaffold fabrication and support of annulus fibrosus cell growth. The novel scaffold had unidirectional fiber arrangement, which is similar to the anatomical structure of the AF. It is highly likely that reconstruction of a 3D shape can be made using this alginate-based loop scaffold, to fit to a degenerated AF. In comparing the scaffold properties, the alginate-chitosan scaffolds showed slower degradation (lower mass loss in physiological condition) than the alginate fibers, while other assessments were similar.

RESULTS

Scaffold morphology: Alginate loops with well arranged fine fibers were formed in the spinner flask. After cutting, chitosan coating, and lyophilization, alginate or alginate-chitosan scaffolds with unidirectionally aligned fibers were produced. The diameter of individual fibers varied between 40-100 μm (Fig. 1).

Scaffold degradation and MTT test: The alginate-chitosan scaffolds exhibited significantly lower mass loss than the alginate scaffolds at all three time points (p<0.05) (Fig. 2). Regarding the cell viability and proliferation, there was no significant difference between normal control and both scaffold groups (Algin: p=0.45; Algin-chitosan: p=0.52).

AF cell culture: AF cells attached and grew well on the fibers of the both types of scaffold. The cells retained their spherical morphology at the beginning of the culture, then after 4 to 5 days in culture, the cells formed into clusters or aggregates and spread along the fibers within the scaffolds (Fig. 4A, 4B). Immunohistochemical staining showed the expression of collagen type I and II in AF cells clusters (Fig. 4C, 4D).

Real time RT-PCR results indicated that AF cells could express functionally relevant genes on both scaffold types, including collagen I, collagen II, and aggrecan. In comparing the different scaffolds, there were no significant differences in expression level for all three genes between the alginate and alginate-chitosan scaffold groups (Col I: p=0.5; Col II: p=0.62; Aggrecan: p=0.89; data not shown).

DISCUSSION

The study demonstrated the feasibility of alginate-based chitosan hybrid scaffold fabrication and support of annulus fibrosus cell growth. The novel scaffold had unidirectional fiber arrangement, which is similar to the anatomical structure of the AF. It is highly likely that reconstruction of a 3D shape can be made using this alginate-based loop scaffold, to fit to a degenerated AF. In comparing the scaffold properties, the alginate-chitosan scaffolds showed slower degradation (lower mass loss in physiological condition) than the alginate fibers, while other assessments were similar. Both types of scaffolds could support canine AF cells growing along the fibers and specific collagen I and II protein as well as several functional genes could be detected. The limitation of the current study is the mechanical strength of the scaffold has remained unknown. Further work will focus on the biomechanical test of the scaffold fibers and related modification to achieve suitable strength for annulus fibrosus tissue engineering.

REFERENCES


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