Fabrication of Novel Cellulosic-Based Scaffolds for Articular Cartilage Tissue Engineering by Unidirectional Freeze Drying Method

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Statement of Purpose: Osteoarthritis is a degenerative disease of articular cartilage which is second most leading cause of disability after cardiovascular diseases. It affects 15% of U.S population and costs U.S. economy around $128 billion every year. The articular cartilage (AC) has a limited capacity of self repair after damage due to its characteristic avascular structure and relatively very low cellular mitosis activity. Various method and materials had been used in order to mimic the structure and regenerate the cartilage; however, all have drawbacks, particularly in terms of long term efficacy. The AC multiphasic tissue composed mostly of solid extra cellular matrix (ECM) molecules and water. In addition to this, AC has hierarchical zones with different porosity within with unidirectional arrangement of collagen fibrils which make it difficult to replicate by tissue engineering (TE) method [1]. In order to mimic the directionality of collagen fibrils in native cartilage, we worked on a novel material known as bacterial cellulose (BC) which is secreted by *Glucanactobacter xylinus* because of its added advantages of nanofibrous structure which can mimic the native ECM, high wet tensile properties, biocompatibility, and purity [2]. Pure BC and composite scaffold of chitosan and BC were prepared by unidirectional freeze drying method as this is the most viable that has added benefit of no use of volatile solvents compared to other methods such as solvent casting/salt leaching and phase inversion [3].

Methods: *Gluconacetobacter xylinus* (ATCC 10245) was grown in a culture medium consisting of 2.5% D-Mannitol, 0.5% yeast extract, and 0.3% bacto-peptone dissolved in de-ionized water with an initial pH of 6.5. Static culture was carried out in triplicate in 500 ml flask containing 100 ml of culture medium. Cultivations were carried out at 30°C for 14 days. The BC pellicles after the cultivation period were sterilized by washing in a 1N NaOH solution at 80°C for 90 min to dissolve the remaining bacteria. The samples were rinsed with deionized water until it had an acceptable pH (~ 6). Pure BC and BC/chitosan scaffold were prepared by placing the samples inside a polypropylene tube. The polypropylene tube was then placed inside an insulating Styrofoam container such that only the bottom surface of plastic tube was exposed [4]. This Styrofoam container with the tube was then allowed to float on liquid nitrogen (-196°C) for 15 minutes (see Figure 1). During this time, the BC sheet inside the tube was unidirectionally frozen from bottom to top. The solidified BC was then lyophilized at -40°C for 2 days to remove ice crystals through the process of sublimation. The unidirectional porous morphology of BC was determined by scanning electronic microscopy (SEM). The FTIR was completed using Thermo Electron FTIR. Scans were completed between 4000- 400 cm^{-1} with 16 convolutions. Crystallinity index of the samples were found out by carrying out X-ray diffractometry. Surface area analyses were performed N_2 adsorption at 77 K by using multipoint Brunauer-Emmett-Teller theory.

Results: Figure 1 shows the SEM image of pure BC and 1wt% chitosan immersed in BC. BC with 1 wt-% chitosan (Figure 1d) was more porous than the other two samples having chitosan concentrations of 1.5 and 2 wt-%. It demonstrates an interconnected pore network in a three dimensional network with unidirectional porosity similar to pure BC scaffold. The FTIR results confirmed the presence of cellulose I_α and chitosan in composite scaffold. Table 1. shows the crystallinity index and surface area of the pure BC and composite scaffolds.

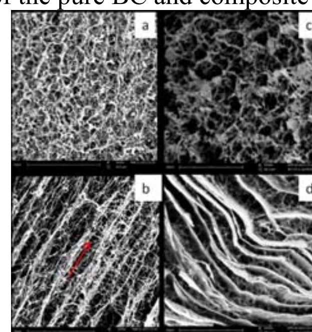

Figure 1. SEM image of a) cross section of pure BC, b) Longitudinal view showing unidirectional porosity inside pure BC, c) cross section of 1wt% chitosan with BC and d) the respective longitudinal view of sample c).

Table1. BET surface area and crystallinity index of pure BC and composite scaffolds with different weight percent of chitosan

Sample Name	Surface Area (m^2/g)	Crystallinity Index (%)
BC	201	89.45
BC-Ch-1%	29.5	83.57
BC-Ch-1.5%	22.06	83.94
BC-Ch-2%	19.85	79.46

Conclusions: Pure BC and BC-Chitosan scaffolds were prepared by a unidirectional freeze drying method. All the scaffolds show an alignment of the fibrils along the freezing direction with 3D interconnected porous structure which is beneficial for TE scaffolds. From the XRD results, the crystallinity index tends to decrease with increased chitosan concentration. In this way, the scaffold morphology such as pore size, distance between channels, and crystallinity can be controlled by carefully adjusting the chitosan concentration for cartilage tissue engineering.

References: [1] Redman SN, Oldfield SF, Archer CW. 2005, 9, 23-32. [2] Brown RM. Position Paper. [3] Xiang Z. Tissue Eng. 2006; 12:2467-2478. [4] Wu X, Liu Y, Li X, Wen P, Zhang Y, Long Y, Wang X, Guo Y, Xing, F, Gao J. 2010, 6, 1167-1177.

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