Design of Electrospun Poly vinyl alcohol/Chitosan Scaffold and Its Cellular Study

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ABSTRACT

Poly vinyl alcohol/Chitosan nanofibrous mat were prepared by electrospinning method with suitable pore sizes as potential matrices for soft tissue engineering. The designed scaffolds by electrospinning method evaluated by different analyses such as morphological, mechanical, and cellular analysis. Microscopic results showed diameters of poly vinyl alcohol/Chitosan nanofibers were approximately 150 nm. Mechanical investigations illustrated stress-strain curve of poly vinyl alcohol/chitosan mat indicate good flexibility with average strain and good percentage of yield stress. The cellular results reveal that addition of chitosan to poly vinyl alcohol enhances viability and proliferation of fibroblast cells, which increases the biocompatibility of the scaffold. In fact, addition of a small percentage of chitosan to the poly vinyl alcohol proved to be a promising approach for designing a scaffold.

Keywords: Chitosan, Poly vinyl alcohol; Nanofibers; Morphological and Mechanical analyses; Cell culture.

INTRODUCTION

Generally speaking, the chemical composition and structure of tissue engineering scaffolds should be optimized for supporting the reparative process in a particular tissue and attachment and proliferation of particular cells [1-5]. Fiber-based porous scaffolds, which structurally mimic the extracellular matrix (ECM), have been synthesized from numerous natural or synthetic biopolymers. These scaffolds have been specifically engineered by electrospinning platform technology, and were successfully used for nerve tissue engineering applications [6-9]. Electrospinning is a unique technology which can produce non-woven fibrous structures with fiber diameters ranging from nanometers to microns. This range of fiber sizes is difficult to achieve by other fabrication methods [10,11]. These scaffolds fabricated by these nanofibers possess an extremely high ratio of surface to volume, have adjustable porosity, and could easily be customized over a wide range of sizes, shapes, and mechanical properties, which makes them very suitable candidates for tissue engineering [12-17]. Fine-tuning of nanostructured topographical cues such as grooves, ridges, pores, and nodes is also important as they influence cell adhesion, migration, proliferation, and differentiation [5]. So far, scaffolds have been synthesized from several natural and synthetic materials using different fabrication techniques, including electrospinning. The electrospun biodegradable polymers were successfully tested for their efficacy to stimulate fibroblast regeneration, taking into account their different structural properties, such as the diameter and alignment of the nanofibers [4-23]. Of these, PVA is a non-toxic, hydrophilic, and biocompatible material which has also been used for other tissue engineering applications [24,25]. Chitosan has been widely used in this field as well [26,27]. However, most of the previous studies focus on a single polymer for fabrication of electrospun nanofibrous scaffolds. The high biocompatibility of chitosan has led several groups to use different techniques for scaffold
fabrication, such as different three-dimensional shapes including tubular conduits, using chitosan as the base material [28-30]. However, the mechanical properties of these scaffolds are still not optimal for application in human dermal tissue. It has been shown that chitosan has quite positive effects on tissue regeneration [31-33]. Hereconventional electrospinning method was employed to synthesize poly vinyl alcohol /Chitosan nanocomposites fibers and tried to modify the mechanical and biological properties of poly vinyl alcohol polymer by blending it with chitosan, and used electrospinning for fabrication of a biocompatible scaffold.

MATERIALS AND METHODS

The precursor sol for poly vinyl alcohol and chitosan was prepared from polyvinyl alcohol (PVA) (96% hydrolyzed typical average M_w 85,000–124,000 SIGMA ALDRICH) and chitosan (from shrimp shells ≥ 75% (deacetylated) SIGMA ALDRICH). Poly vinyl alcohol /chitosan solution was dissolved in acetic acid. The weight ratio of poly vinyl alcohol /chitosan was fixed at 80:20 in 2% acetic acid. The concentration of the poly vinyl alcohol /chitosan precursor was 5 wt% and mixed under magnetic stirring for 1.5 h at 75 °C. Poly vinyl alcohol /chitosan nanofibrous mat were prepared by electrospinning method. The applied voltage was 21-25 kV and the electrospinning distance was fixed at 10 cm. The characteristic morphology and diameter of Poly vinyl alcohol / chitosan nanofibrous mat were characterized by scanning electron microscopy (SEM).Tensile strength of nanofibrous mats were tested using INSTRON (5566, USA) instruments series II Automated materials testing system. The mats were cut into dumbbell shaped strips with 40 mm length and 5 mm inner width. The thicknesses of the mats were measured using the electronic digital micrometer screw gauge. The sample strips were held by pneumatic grips and the tensile force were applied at an extension rate of 2 mm/min^(-1). The tests were conducted at 20 ±2°C in 65±5% relative humidity. Triplicates were tested for each type of electrospun nanofibrous mat.

Cellular analysis

For cytotoxicity analysis, fibroblast cell suspension (L929) from mouse tails was prepared according to International Organization for Standardization 10993 standards. The control sample (TCPS; Tissue Culture Poly Styrene) were well cleaned and sterilized by the autoclave method. Individual samples were placed in Petri dishes using a sterilized pincer; 3 cc of the cell suspension was removed by pipette and poured into the control and experimental samples. Thereafter, all of the samples were placed separately in a Memmert incubator at 37°C for 24 and 48 hours. Cell proliferation was determined by the MTT assay for viable cell numbers. The MTT tetrazolium compound was reduced by living cells in a colored formazan product that was soluble in the tissue culture medium. The quantity of formazan product was directly proportional to the number of viable cells in the culture. The assays were performed by adding 1 mL of MTT solution (Sigma-Aldrich) and 9 mL of fresh medium to each well after aspirating the spent medium and incubating at 37°C for 4 hours with protection from light. Absorbance was measured at 570 nm using a Rayto micro-plate reader. Five samples were performed for each biomaterial. For microscopic study, the cultured mats with cells were washed by PBS and then fixed by gluteraldehyde (2.5%) at 4 °C for 2 h. The samples were dehydrated by alcohols and then kept with tetraoxide osmium vapors at 4 °C for 2 h. The samples were coated with gold and investigated by a scanning electron microscope (TScan, VEGA, Czech). All data are expressed as mean standard error of the mean unless noted. One-way analysis of variance with post hoc Tukey means comparison tests and unpaired Student’s t-tests were conducted with a significance level of p<0.05. A minimum of three replicate samples were used for all experiments.

RESULTS

Figure 1 shows SEM images of poly vinyl alcohol, chitosan, and poly vinyl alcohol /chitosan nanofibers prepared from the precursor. The composite systems consist of nanofiber and micro-bead structures. All images reveal that designed nanofibers have good uniformity
with regular diameter of about 100, 250 and 150 nm for poly vinyl alcohol, chitosan, and poly vinyl alcohol/chitosan respectively.

**Mechanical properties**

Figure 2a shows the stress-strain curve of poly vinyl alcohol nanofibrous mat which reveals that the sample was flexible. The percentage of strain was high however the yield stress was low. The tensile strength was 1.2 MPa and the amount of displacement was 27%. The sample was measured on the basis of the value of the modulus of elasticity. Figure 2b shows the stress-strain curve of chitosan nanofibers which reveal the low flexibility but high strain percentage, the tensile strength was 22 MPa and amount of 18% displacement. Chitosan nanofibrous mat was measured on the basis of the value of the modulus of elasticity too. Figure 2c represent the stress-strain curve of poly vinyl alcohol/chitosan mat indicate good flexibility with average strain and good percentage of yield stress, the tensile strength were 11 MPa and the amount of displacement was 21%.

![Figure 1. SEM images of poly vinyl alcohol (A), chitosan (B) and poly vinyl alcohol / chitosan nanofibers (C).](image1)

![Figure 2. Tensile stress-strain curve of nanofibrous mats. a) poly vinyl alcohol, b) chitosan, and c) poly vinyl alcohol / chitosan.](image2)
Cellular results

In this study, MTT assay was performed to evaluate the cell proliferation rate of the fibroblast cells on the poly vinyl alcohol, chitosan and poly vinyl alcohol / chitosan nanofibrous mats. As shown in Figure 3, the proliferation of cells on poly vinyl alcohol / chitosan mats was higher than on poly vinyl alcohol mats, indicating that the chitosan might have accelerated the proliferation rate of the cells. This could be attributed to the higher surface amine and the lower water contents of the swollen poly vinyl alcohol / chitosan mat. In addition, it has been shown that fiber diameters can influence cell adhesion, proliferation, and migration (Figure 4). It was shown by Bashur et al. that smaller fiber diameters favor better fibroblast cell attachment and proliferation of cells cultured on non-woven electrospun fiber meshes [36].

![Figure 3. MTT analysis of fibroblast cell culture on nanofibrous mats. Poly vinyl alcohol, chitosan, poly vinyl alcohol / chitosan nanofibrous mats, and control (TCPS).](image)

![Figure 4. SEM images of fibroblast cell adhesion on nanofibrous mats. A) poly vinyl alcohol, B) chitosan, and C) poly vinyl alcohol / chitosan.](image)

Some natural materials such as collagen, chitosan, alginate, some carbohydrates and peptides have been reported as scaffold modifiers. Chitosan has been proved and regarded as biodegradable non-cytotoxic material, which has some interesting biological activities [8,13]. The high biocompatibility of chitosan has led several groups to use different techniques for scaffold fabrication, such as different three-dimensional shapes including tubular conduits, using chitosan as the base material. However, the mechanical properties of these scaffolds are still...
not optimal for application in tissue regeneration.[31-33].
Here, we tried to modify the physicochemical and biological properties, especially mechanical property of chitosan polymer by blending it with poly vinyl alcohol, and used electrospinning for fabrication of a suitable scaffold. In our study, poly vinyl alcohol/chitosan fibers by conventional electrospinning method designed and tried to modify the mechanical and biological properties of poly vinyl alcohol polymer by blending it with chitosan. Chitosan nanofibrous mat measured on the basis of the value of the modulus of elasticity too. Results demonstrated the poly vinyl alcohol /chitosan nanofibrous mat indicate good flexibility with average strain and good percentage of yield stress. Interestingly, it was shown that the porosity of the same layers in the mats fabricated with poly vinyl alcohol and poly vinyl alcohol /chitosan blend did not differ significantly.
However, the pore morphologies were different. As mentioned above, poly vinyl alcohol solution yielded fibers with relatively large diameters, which formed pores larger than those formed by small-diameter poly vinyl alcohol /chitosan fibers. Poly vinyl alcohol /chitosan mats had a higher number of pores. It should be noted that porosity and pore morphology are important for many tissue engineering applications as they allow migration of the cells and growth of blood vessels across the scaffold and ensure effective exchange of nutrients and waste products between the cells and their microenvironment.

CONCLUSION
In this study, poly vinyl alcohol, chitosan and poly vinyl alcohol /chitosan nanofibrous mats were prepared by electrospinning method. The poly vinyl alcohol /chitosan mats have been found to exhibit better mechanical properties compared with poly vinyl alcohol or chitosan mats. Cellular investigations showed better adhesion, growth, and viability in the chitosan content nanofibers than in the poly vinyl alcohol nanofibers. These poly vinyl alcohol /chitosan mats could be used well for soft tissue engineering.

REFERENCES