Preparation of PHBV/Collagen Nanofibrous Mats and Their Tissue Compatibility Compatibilscaffolds for tissue engineering

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Introduction

In order for tissue engineering scaffolds to be useful, they must be biocompatible, support cell growth, guide and organize cells, allow tissue ingrowth, and should be able to degrade into non-toxic products as basic elements [1]. Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV) is a well-known biodegradable, biocompatible, non-toxic, and thermoplastic polyester that is produced by bacteria [2]. PHBV emerges as a new generation of PHB-based materials with properties that are adjustable via changing the hydroxyvaleric acid (HV) content [3]. Recently, much attention has been paid to the electrospinning process as a unique technique because it can produce nanofibrous scaffolds with diameters in the range between several micrometer and tens of nanometers. The electrospinning process provides a promising means for creating a tissue-engineered scaffold. Throughout the material, pore size plays a critical role in both cell adhesion and in the exchange of nutrient and metabolic waste. In biomedical applications, for example, the electrospinning technique can be used to construct wound dressings, drug delivery platform, tissue engineering scaffolds. In this study, the polymer blend solution of PHBV and collagen Type I was used to fabricate an electrospun nanofibrous scaffold. Characteristics of the nanofibrous scaffolds were investigated using ATR-FTIR spectroscopy and electron spectroscopy for chemical analysis. The behavior of the fibroblasts on the nanofibrous scaffolds was also investigated.

Experimental

Electrospinning. PHBV and collagen Type I were dissolved in HFIP at a concentration of 2 wt%. The mixed polymer solution was delivered to a metal needle connected to a high-voltage power supply (Chungpa EMT, Seoul, Korea). Upon applying a high voltage, a fluid jet was ejected from the needle. As the jet accelerated toward a grounded collector, the solvent evaporated and a charged polymer fiber was deposited on to the collector in the form of a nanofibrous web.

Surface characterization. The presence of collagen in the nanofibrous scaffolds was confirmed using a FT-IR spectrometer (Jasco-620, Tokyo, Japan). The surface chemical composition was analyzed using electron spectroscopy for chemical analysis (ESCA, ESCA LAB VIG microtech, Mt 500/1 etc, East Grin, UK), which was equipped with Mg Kα at 1253.6 eV and a 150 W power mode at the anode. A survey scan spectrum was taken and the surface elemental compositions relative to the carbon were calculated from the peak heights taking into account atomic sensitivity. Topographic images of the nanofibrous scaffold were examined using an atomic force microscope combined with an optical microscope. The tapping mode of AFM was employed to observe the nanofiber surfaces.

***In vitro* biodegradation.** The nanofibrous scaffold was cut into rectangles (20 x 20 x 0.05 mm) for an *in vitro* biodegradation test. Each specimen was placed in a test tube containing 10ml of a phosphate-buffered saline (PBS, Gibco, pH 7.0) and incubated for a requisite time at 37 ℃. After incubation, the samples were washed and lyophilized for 24 h. In order to examine the enzymatic degradation of nanofibrous scaffolds, samples were placed in a PBS containing collagenase type I or *Pseudomonas stutzeri* BM190 depolymerase. After 12 h incubation, the samples were taken out from the enzyme solution, washed with distilled water and lyophilized for 24 h. Morphological changes of the nanofibrous scaffolds were examined using a field emission scanning electron microscope.

Cell culture. In order to examine the interaction of nanofibrous scaffolds with cells, the circular nanofibrous scaffolds were fitted in a 24-well culture dish and subsequently immersed in a DMEM medium containing 10% fetal bovine serum (FBS) and 1% penicillin G-streptomycin. To the scaffolds, one ml of the NIH 3T3 cell solution was added to the scaffolds and incubated in a humidified atmosphere of 5% CO_2 at 37℃ for a requisite time. After incubation, the supernatant was removed, washed twice with a PBS, and fixed in a 2.5% glutaraldehyde aqueous solution for 20 min. The sample sheet was then dehydrated, dried in a critical point drier, and finally sputter-coated with gold. The surface morphology of the samples was then observed with a FE-SEM. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to measure relative cell viability. The proliferation of NIH 3T3 cells seeded on the nanofibrous scaffolds were determined using a colorimetric immunoassay based on the measurements of 5-bromo-2'-deoxyuridine (BrdU), that were incorporated during DNA synthesis.

Results

Morphology of electrospun nanofibrous scaffolds. Figure 1 shows the SEM images of electrospun nanofibrous scaffolds that were obtained under maximized condition. The images showed continuous fiber morphology and the fibers did not contain beads that were independent of the kind of polymer. The PHBV fiber diameters were in the range between 300 and 600 nm and it decreased after the incorporation of collagen (PHBV-Col). This is probably due to the increment of dielectric constant.

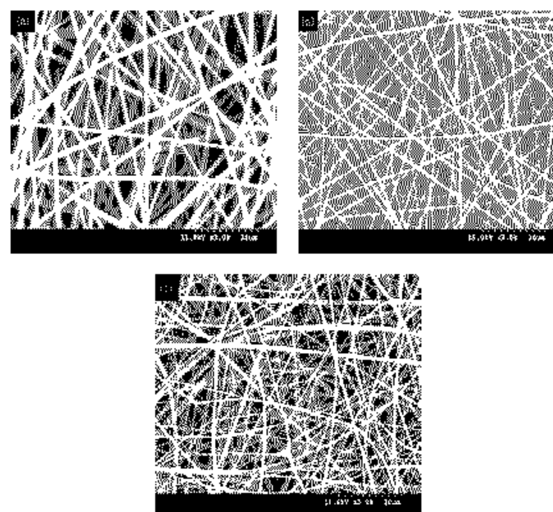

Fig. 1 SEM micrographs of nanofibrous scaffolds. (a) PHBV, (b) PHBV-Col, (c) collagen.

Characterization of nanofibrous scaffolds. The ATR-FTIR spectrum of PHBV-Col showed absorptions at 1656 and 1538 cm^{-1} based on the amide I and II bonds of collagen, respectively. In addition, strong absorption appeared at 1733 cm^{-1} based on the ester groups of PHBV. Changes in the chemical structure of nanofibrous scaffolds were investigated using ESCA. The collagen nanofiber scaffold showed three peaks corresponding to C1s (285eV), N1s (400eV) and O1s (532eV), while the PHBV nanofiber scaffold showed two peaks corresponding to C1s and O1s. The chemical composition of the nanofibrous scaffolds was calculated from the ESCA survey scan spectra. As the results, the oxygen content (36.8%) of the PHBV nanofiber surface decreased to 32.0% due to the incorporation of collagen (PHBV-Col), while the nitrogen content increased to 6.4%, indicating the presence of collagen on the surface. In order to study the surface morphology of the PHBV and PHBV-Col nanofibers, an atomic force microscope image was studied using a tapping mode. For the results, the PHBV nanofiber surface showed a relatively homogeneous color pattern while PHBV/Col showed a heterogeneous color pattern, thus showing the presence of collagen in PHBV.

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***In vitro* biodegradation.** Figure 2 illustrates the morphological changes of nanofibrous scaffold surfaces before (a, b, c) and after (d, e, f) incubation with a collagenase Type I aqueous solution. For the results, the surface morphology of the PHBV (a) and PHBV-Col (b) nanofiber scaffolds did not change after they were dipped in a PBS solution. The surface of the collagen (c) nanofiber, however, lost its original fiber morphology, probably due to swelling caused by water. On the other hand, the collagen nanofiber scaffold was partially biodegraded by the treatment of a collagenase Type I aqueous solution, as shown in Figure 2 (f). In the case of PHBV containing 30wt% collagen (PHBV-Col), the fibers preserved their original morphology within 12h after enzyme treatment (12 h). The surface morphology of the nanofibrous scaffolds before and after incubation in a PHB depolymerase aqueous solution for 12 h was studied. The surfaces of both PHBV and PHBV-Col fibers were severely eroded by the treatment of PHB depolymerase, while the collagen fibers showed very little erosion.

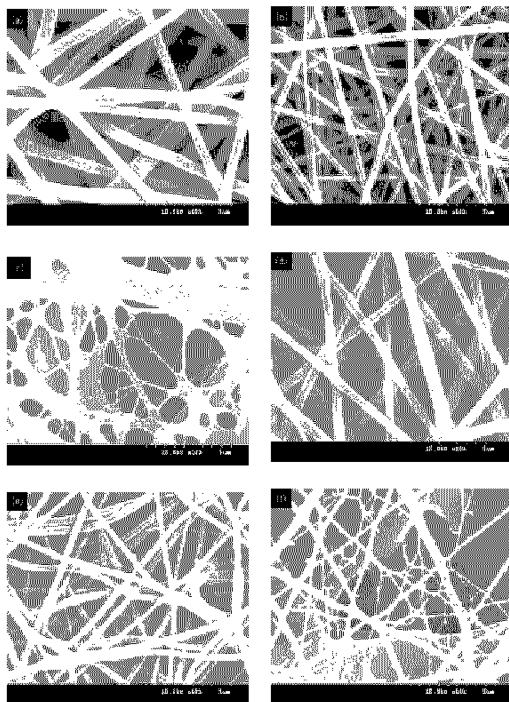
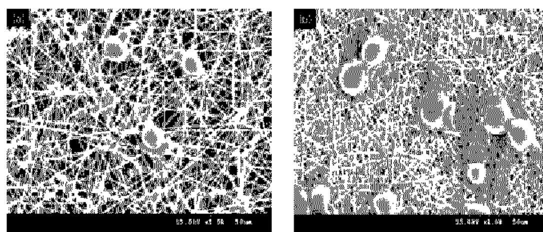


Fig. 2 SEM images of nanofibrous scaffolds incubated for 12h in PBS solution (a,b,c) and in collagenase Type I solution (d,e,f): a, d = PHBV; b, e = PHBV-Col; c,f = collagen.

Cell-scaffold interaction. Figure 3 shows the SEM images of the NIH 3T3 fibroblasts that adhered to the nanofibrous scaffolds when cultured in a Dulbecco's modified eagle medium containing 10% fetal bovine serum for 4 h. The cells well adhered to the surfaces of the PHBV, PHBV-Col and collagen nanofibrous scaffolds. In the case of the collagen nanofibrous scaffold, the fibers were swollen and connected to each other, forming a skin layer. The proliferation of cells on the nanofibrous scaffolds was examined when cultured in a DMEM with 10% serum for 20 h. Cell proliferation on the PHBV nanofibrous scaffold was significantly accelerated by the introduction of collagen Type I (PHBV-Col) ($P < 0.01$). The cell viability of fibroblasts that had been cultured for 5 days on the nanofibrous scaffolds was studied. As the results, the cell viability on the PHBV-Col was higher than that on the control PHBV ($P < 0.05$), while it was lower than that on the collagen ($P < 0.05$).



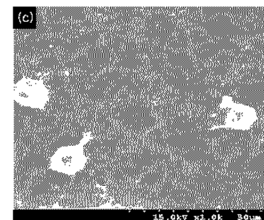


Fig. 3 SEM micrographs of NIH 3T3 cells cultured for 4h on electrospun nanofibrous scaffolds. (a) ; PHBV, (b) ; PHBV-Col, (c) ; collagen.

Discussion

Unlike a conventional fiber fabrication process, electrospinning provides a straightforward way to fabricate fibrous scaffolds with fiber diameters in the tens of nanometers. In the present study, we have studied cell behavior on nanofibrous scaffolds which was brought about by electrospinning using biodegradable PHBV and collagen Type I. Recently, a polymer blend such as poly(ε-caprolactone)/gelatin and chitosan/gelatin have been successfully electrospun. Wagner et al. [4] have fabricated elecrospun polyetherurethaneurea (PEUU)/collagen scaffolds by combining PEUU with Type I collagen at various ratios. In order to illustrate the presence of collagen in the co-electrospun scaffolds, a PHBV-Col scaffold was subjected to ATR-FTIR measurements. The resulting spectrum yielded peaks that were characteristic of collagen, as reported previously by other researchers. The enzymatic degradation of PHBV film by PHB depolymerase has been reported previously. Park et al. [5] have carried out biodegradation tests of PHBV in the form of fibrous structures or film, by using a simulated municipal solid waste aerobic composting method. They reported that the degradation of the PHBV non-woven structures was faster than the PHBV film. In this study, the surfaces of the PHBV and PHBV-Col fibers were severely eroded by the PHB depolymerase treatment. On the other hand, the PHBV-Col fibers were partially biodegraded by the treatment of collagenase solution and their cylindrical morphology was preserved.

Recently, a great deal of research has focused on the influence of scaffold microarchitecture on cell behavior. Shin et al. [6] assessed the interaction of human fibroblasts with electrospun nanofibrous scaffolds and reported that the nanofiber structures provides an environment for rapid proliferation. In our research, the PHBV-Col nanofibrous scaffold accelerated the adhesion and growth of NIH 3T3 cells as compared to the PHBV nanofibrous scaffold.

Conclusions

Both PHBV and collagen Type I were dissolved in hexafluoro-2-propanol and the polymer blend solution was electrospun to produce composite nanofibrous scaffolds. The fiber diameter could be controlled within a range of 300 ~ 600 nm. The presence of collagen in the nanofibrous scaffold was confirmed by using ESCA and ATR-FTIR. From the in vitro experiments, it was determined that the NIH 3T3 cells showed significant adherence and proliferation on the PHBV-Col nanofibrous scaffold, when compared to the PHBV control.

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References

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