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Research Article

In Vitro Biological Evaluation of Fibrous PHBV Polymer and CHA/PHBV Nanocomposite Scaffolds Developed for Tissue Engineering Applications

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Abstract In recent years, a variety of fibrous bioactive bioceramic-polymer composite scaffolds were made through electrospinning and their usefulness for bone tissue engineering was investigated. In this study, nanospheres of carbonated hydroxyapatite (CHA), which is a proven osteoconductive and biodegradable bioceramic, were synthesized using a nanoemulsion process and relatively high amounts of CHA nanospheres were successfully incorporated into electrospun poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) fibers with the aid of an ultrasonic power source. The biological evaluation of electrospun fibrous PHBV scaffolds and CHA/PHBV nanocomposite scaffolds were conducted through in vitro cell culture using the human osteoblast cell-line SaOS-2. Although both types of scaffolds supported the proliferation and spreading of SaOS-2 cells, the CHA/PHBV scaffolds caused significantly higher expression of alkaline phosphatase (ALP) activity of SaOS-2 cells than the PHBV scaffolds after 14 days of cell culture, indicating the potential of fibrous CHA/PHBV nanocomposite scaffolds for bone tissue regeneration applications.

Keywords carbonated hydroxyapatite (CHA); poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV); electrospinning; nanocomposite; scaffold; tissue engineering; SaOS-2

1 Introduction

Electrospinning has been investigated intensively in recent years for constructing fibrous tissue engineering scaffolds because the ultrafine fibers it produces mimic the nanofibrous structure of extracellular matrix (ECM) of human body tissues, which significantly enhances cell attachment and adhesion [2]. While natural, biodegradable polymers such as poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) have been electrospun into fibrous scaffolds in various tissue engineering studies [3], these scaffolds lack osteoconductivity that is desired for bone tissue engineering. A polymer scaffold can be osteoconductive if the scaffold contains a sufficient amount of bioactive bioceramics such as hydroxyapatite (HA) [5]. Therefore, the incorporation of HA particles into electrospun fibers to form fibrous HA/polymer composite scaffolds have been investigated by various research groups [1,6]. Carbonated HA (CHA) is a proven osteoconductive and biodegradable bioceramic. CHA nanoparticles, which resemble bone apatite more chemically and possess higher resorption rate than HA nanoparticles, are more suitable than pure HA for bone tissue engineering applications. They may be incorporated in tissue engineering scaffolds, thus rendering the scaffolds osteoconductive and also totally biodegradable. It is worth noting that the surface area of CHA nanospheres synthesized through a nanoemulsion process could reach 50 m^2/g [7], which is much higher than that of the commercial nano-sized HA powders. The high surface area to mass ratio of these CHA nanospheres is well suited for constructing nanocomposite scaffolds for bone tissue engineering. In the present study, both PHBV polymer and CHA/PHBV nanocomposite fibrous scaffolds were formed through electrospinning. The in vitro biological evaluation of these scaffolds was subsequently conducted through cell culture experiments.

2 Materials and methods

PHBV with 5 mol% of hydroxyvalerate (Sigma-Aldrich, USA) was dissolved in chloroform (analytical grade) to make polymer solutions. CHA nanospheres were synthesized in-house using a nanoemulsion process [7]. PHBV fibers and CHA/PHBV nanocomposite fibers with a CHA content of 15 wt.% were electrospun using the facility described previously [4]. The fabrication of CHA/PHBV fibers was conducted with the help of ultrasonification during the electrospinning process. Electrospinning was performed under the following condition: the PHBV solution concentration was 15% w/v, the solution feeding

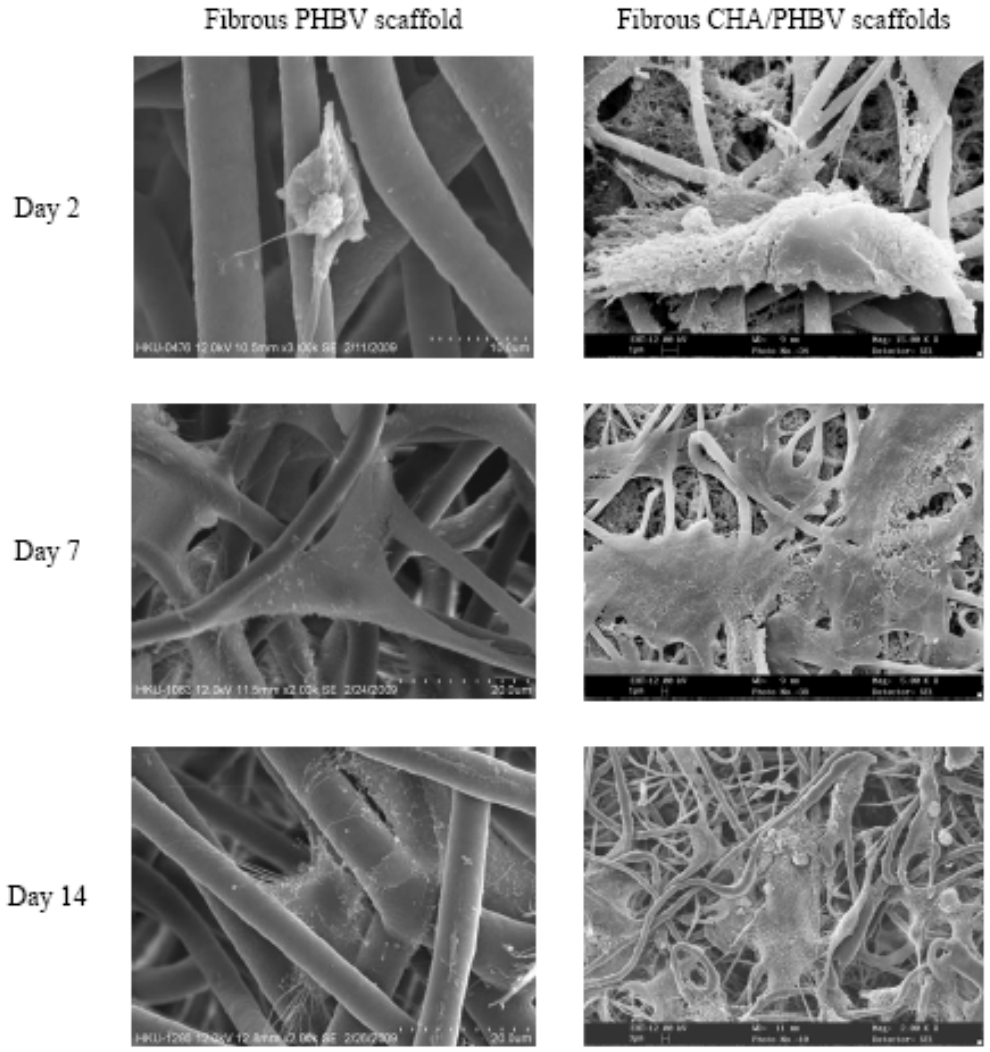

Figure 1: Morphology of SaOS-2 cells seeded on electrospun fibrous PHBV scaffolds and CHA/PHBV nanocomposite scaffolds at different time points of cell culture.

rate was 3 mL/hr, the applied voltage was 15 kV, the needle inner diameter (ID) was 0.5 mm and the working distance was 15 cm. The morphology of electrospun scaffolds was examined using SEM while the presence of CHA in the nanocomposite fibers was determined using EDX. The human osteoblast cell-line SaOS-2 was used for biological evaluation of electrospun scaffolds. SaOS-2 cells were seeded onto both types (PHBV and CHA/PHBV) of fibrous scaffolds and cultured in vitro. The morphology, proliferation and alkaline phosphatase (ALP) activity of SaOS-2 cells at different cell culture times (2, 7 and 14 days) were studied.

3 Results and discussion

The average diameter of CHA nanospheres was about 30 nm. PHBV fibers and CHA/PHBV nanocomposite fibers with fiber diameters of $5.2 \pm 0.6\,\mu$m and $5.7 \pm 0.8\,\mu$m, respectively, were successfully electrospun. While PHBV fibers exhibited a smooth fiber surface, CHA/PHBV fibers had a relatively rough surface. CHA nanospheres were found to be either encapsulated in nanocomposite fibers or attached to the fiber surface. The distribution of CHA nanospheres was homogeneous in and along fibers. The presence of Ca and P peaks in EDX spectra of CHA/PHBV fibers confirmed that CHA nanospheres were in and on composite fibers.

In in vitro experiment, SaOS-2 cells were found to adhere to both PHBV polymer fibers and CHA/PHBV nanocomposite fibers. Figure 1 shows the morphology of SaOS-2 cells seeded on electrospun fibrous PHBV scaffolds and CHA/PHBV nanocomposite scaffolds at different time points (2, 7 and 14 days). Both types of scaffolds retained their fibrous architecture throughout the cell culture period even though the scaffolds had been immersed in cell culture

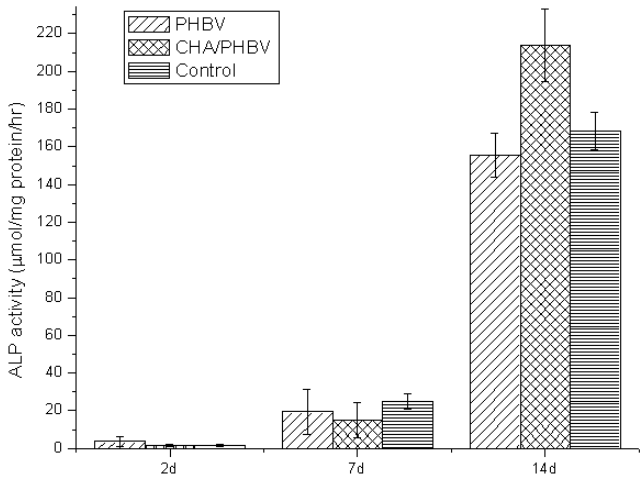

Figure 2: ALP activity of SaOS-2 cells seeded on tissue-culture polystyrene plate (the control) and two types of fibrous scaffolds (PHBV and CHA/PHBV) as a function of time during cell culture.

medium for as long as 14 days. On day 2, the cells had already attached onto the fibrous scaffolds and exhibited globular shape with diameters ranging from 5 to 10 microns. On day 7, the cells were well expanded and spread in all directions on the fibrous scaffolds. A majority of cells had diameters over 10 microns, which were larger than the average diameter of the cells after 2 days of cell culture. On day 14, the cells not only well expanded and spread but also formed numerous filopodia. With the filopodia, the cells could firmly attach to the fibers and migrate on and in scaffolds effectively by extending the filopodia. The proliferation of SaOS-2 cells on the tissue-culture polystyrene plate (TCP, i.e., the control) and the two types of fibrous scaffolds (PHBV and CHA/PHBV scaffolds) was investigated using MTT assay. The absorbance of each type of fibrous scaffolds was comparable to that of the control at every time point. The absorbance of both types of fibrous scaffolds being investigated generally increased from day 2 to day 7 but did not change significantly from day 7 to day 14, implying that SaOS-2 cells may have differentiated into mature, ECM-producing cells after the rapid proliferation period from day 2 to day 7. The ALP activities of SaOS-2 cells on TCP and both types of fibrous scaffolds were evaluated in terms of μmol of p-nitrophenol (pNP) production per milligram of protein per hour on days 2, 7 and 14 of cell culture (Figure 2). The ALP activity of SaOS-2 cells seeded on these fibrous scaffolds was comparable to that on the control on days 2 and 7. On day 14, the ALP activity expressed by the cells on the fibrous PHBV scaffold was comparable to that on the control while the ALP activity expressed by the cells on the fibrous CHA/PHBV nanocomposite scaffold was significantly higher than those on the control and the fibrous PHBV scaffold. The in vitro cell culture results showed that the ALP activity slightly increased from day 2 to day 7 and significantly increased from day 7 to day 14. These results indicated that both fibrous PHBV and CHA/PHBV scaffolds supported the expression of homogeneous phenotype of SaOS-2 throughout the cell culture period of 14 days. The high ALP activity of SaOS-2 cells seeded on the CHA/PHBV fibrous nanocomposite scaffold on day 14 suggested that the presence of CHA nanospheres in electrospun CHA/PHBV nanocomposite fibers was particularly useful for promoting the cells for the expression of ALP activity. As ALP production is one of the steps within the differentiation sequence of osteoblastic cells, significant ALP expressed by SaOS-2 cells seeded on the fibrous CHA/PHBV nanocomposite scaffolds is a clear indication that the fibrous nanocomposite scaffolds are a suitable candidate for bone tissue engineering applications.

4 Conclusions

Osteoconductive CHA nanospheres were successfully incorporated into electrospun PHBV fibers to form fibrous CHA/PHBV nanocomposite scaffolds. The enhanced ALP activity expressed by osteoblastic cells cultured on CHA/PHBV nanocomposite scaffolds indicated the promising potential of these scaffolds for regenerating bone tissue.

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