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Research article

IN VITRO DEGRADATION OF ELECTROSPINNING PLLA/HA NANOFIBER SCAFFOLDS COMPARED WITH PURE PLLA

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ABSTRACT: Nano biomaterials play a basic role as scaffolds to prepare three dimensional templates and synthetic extracellular matrix environment for tissue regeneration. In this study Poly-L-lactic acid (PLLA)/hydroxyapatite (HA) nano-composite (PHnC) scaffold and PLLA scaffold were fabricated via electrospinning. The scaffolds were characterized by scanning electron microscope (SEM). In vitro degradation behaviors of three-dimensional tissue engineering porous scaffolds have been systematically investigated up to 40 days in phosphate buffer saline solution at 37 degrees C. In vitro degradation of the scaffold in phosphate buffered saline (PBS) was monitored by measuring weight loss, water uptake and pH change of the buffer during the degradation period. The results showed that, the degradation of the scaffold could be markedly influenced by HA nano-particle. It was also observed that acidification of the environment due to the PLLA degradation is suppressed by HA nanoparticles. The results obtained suggested that the PHnC scaffold could be materials of choice for bone tissue engineering.
Key words: PLLA; Hydroxyapatite; Composite scaffold; degradation

INTRODUCTION

Tissue engineering, also defined as regenerative medicine is an interdisciplinary field involving knowledge from medicine, biology, engineering and materials science fields [1].One of the basic factors is the creation of scaffold for cellular attachment, proliferation and differentiation [2]. Many of the porous composites studied to date were composed of biodegradable polymers and bioactive ceramics [3]. Poly (L-lactic acid) (PLLA) has been widely investigated in tissue engineering because of its good biocompatibility [4]. HA is a promising bone replacement material because of its stoichiometric similarity to the inorganic part of natural bone [5]. Electrospinning is a simple and effective method that has been used to produce nanofibrous scaffolds variety of materials [6]. Electrospinning is a process whereby ultra-fine fibers are formed in a high-voltage electrostatic field [7]. Scaffold degradation is one of the major considerations in the design and construction of tissue engineered scaffolds. The degradation rate of scaffolds should be matched the rate of new tissue formation, which allows a smooth transition of load transfer from scaffolds to tissue [7 and 8]. Thus, the degradation properties of a scaffold are of certain importance for biomaterial selection and design but also the long-term success of a tissue engineered construct [9]. In this study, PLLA, PLLA/HA Nano-fibers were fabricated by electrospinning and the influence of HA on the degradation of the PLLA composite scaffolds was investigated.

MATERIALS AND METHODS

Poly-L-lactic acid (PLLA) with intrinsic viscosity of 1.0 dl g^{-1} and organic solvents, chloroform and dimethyl formamide (DMF) were purchased from Sigma-Aldrich and used as received. Hydroxyapatite nanoparticle (HA) was provided by Merck-Germany. Paraformaldehyde was received from Sigma- Aldrich, Germany.

Preparation of PLLA/HA Nano-composite and PLLA scaffolds

The scaffolds were fabricated by electerospinning method [10]. Briefly, PLLA was dissolved in chloroform (9% wt/wt) and added directly to chloroform/dimethylformamide solution (10:1 v/v).for composite scaffold subsequently, the HA nanoparticles were dispersed in the solution. The weight ratio of PLLA/HA in the final solution was 10:1. The obtained suspensions were transferred into two glass syringes equipped with and infusion pump and a needle which was kept at a high DC voltage (20 kV) and the distance of electric field was fixed at 20 cm. According to the previous method [11], in order to modify the surface characteristics of the prepared scaffolds, surface plasma treatment was performed by a low frequency plasma generator of 40 kHz frequency with a cylindrical quartz reactor (Diener Electronics, Germany).

Characterization of scaffold

Scanning electron microscopy

The morphology of the scaffolds examined by scanning electron microscopy (SEM, LEO1430VP). For SEM observation, samples were coated with gold using a sputter coater.

In vitro degradation study

The scaffolds with dimensions of $1\times1cm^2$ were sterilized by immersing in ethanol (70%) overnight and exposed to UV light for 10 min. the Scaffolds of a known initial weight were placed into glass vials containing 10 ml PBS which were then incubated at 37 ^oC. The pH of the medium was recorded periodically. After any given time, samples were removed from the solution and weighed after wiping the surface with filter paper. Each sample was repeatedly rinsed with deionized water to remove any soluble inorganic salt and weighed after being completely dried at room temperature. Then, water uptake percentage and mass loss percentage of the scaffolds were calculated using the following equation:

$$\% W_U = (w_w - w_i)/w_i \times 100$$

$$\% W_L = (w_i - w_t)/w_i \times 100 \quad (1)$$

where W_U and W_L are the water uptake and mass loss percentage of sample, respectively, and w_i is the initial weight of dry sample, w_w is the weight of wet sample, w_t is the weight of dry sample at any given time.

Statistical analysis

The degree of statistical significance between control group and the composite scaffold was estimated by student's t-test, with statistical significance level of 0.05 ($P<0.05$). The data generated was calculated as the mean of the triplicate experiments and presented as mean ± standard deviation.

RESULTS AND DISCUSSION

SEM images and macrostructure of scaffold

SEM images taken from the nano-scaffolds is shown in Figure 1. The scaffolds appeared to have a porous structure with interconnected pores and thin fibers with random orientation. The surface area of composite scaffold seems rough and uneven, which can be due to the dispersion of HA nanoparticles.

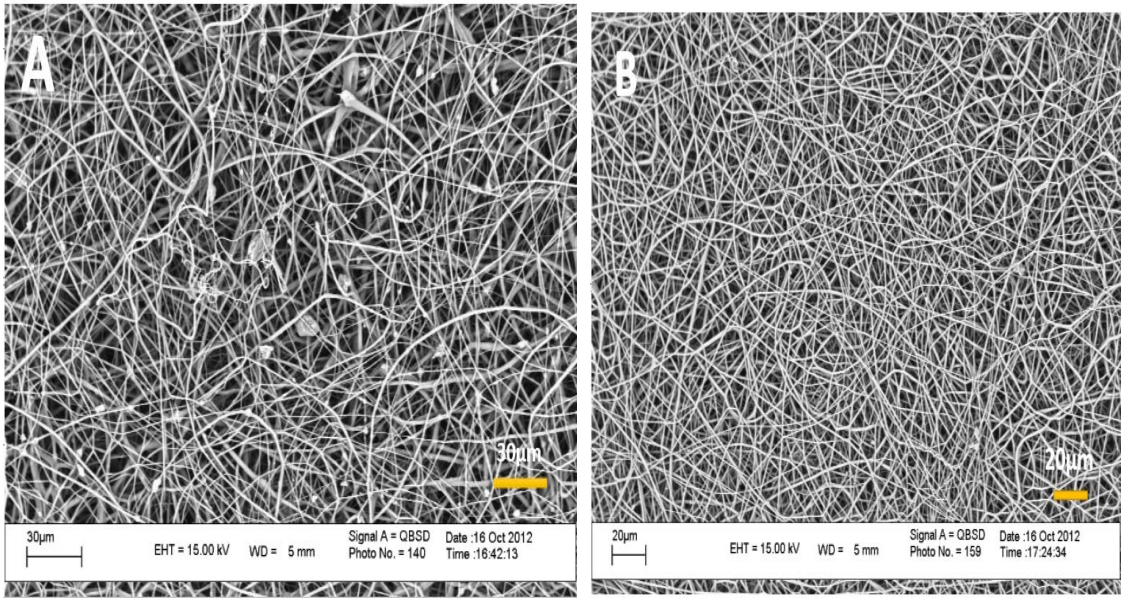
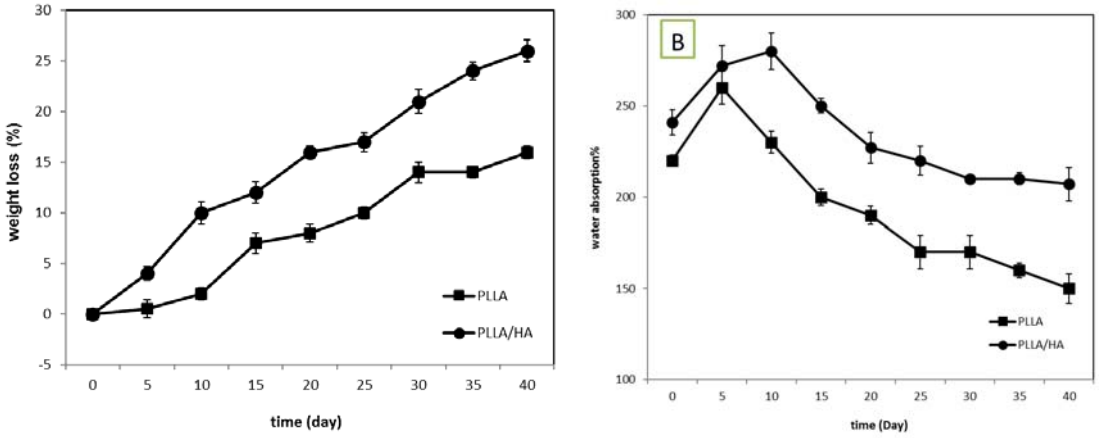


Figure 1. SEM micrograph of electrospun scaffolds : A) PLLA/HA and B)PLLA

In vitro degradation study

Various parameters including weight loss, water uptake and pH changes of PBS were examined during this experiment. The variations in are shown in Figure 2. As shown in Figure 2A, the Scaffold has lost approximately 26 % of its initial weight (equivalent to a quarter of weight of the scaffold) after 40 days. As it can be seen, weight loss in the PLLA/HA is significantly higher than that in the PLLA sample. In the present study, changes in molecular weight of scaffolds which were examined during the degradation test, over time, showed higher weight loss (figure2-A). These data was contrary to the results reported by Sui et al [12]. They reported that PLLA scaffolds showed a larger decrease in Mw in comparison to PLLA-HA scaffolds. According to Sui et al, it was the HA particles that slowed down the degradation rate of the PLLA/HA scaffolds due to their alkaline nature [12]. However, the authors did not provide details of the concentration of HA in their PLLA/HA scaffolds. It is therefore probable that the HA content of their PLLA/HA scaffolds was much higher than the HA content of the PLLA/HA scaffolds in this study (10%), and may have resulted in the difference between the results of this work and those of Sui et al. Results have shown that the increased degradation of composite scaffolds As a result of the change in hydrophobicity properties and increase the wetting properties of composite scaffolds. With increasing wettability, the scaffold is placed in direct contact with water. Some researchers have pointed out that the fiber diameter of composite scaffold is smaller than the pure scaffold. This features, increased surface to volume ratio of the composite scaffold and increased the rate of degradation [13].The change in pH over the degradation period is shown in Figure 2B. The pH drops during the degradation time up to 30 days. During the last 10 days it increases and reaches a value of 6.6. This increase is probably due to HA degradation and OH^- release from the Nano-composite scaffolds, which neutralize the acid degradation product of PLLA. The results showed that the buffer pH has decreased during biodegradation and it has increased during the last days (figure2-B). Hence, it is suggested that, the pH is increased, by introducing HA alkaline particles in buffer [14].



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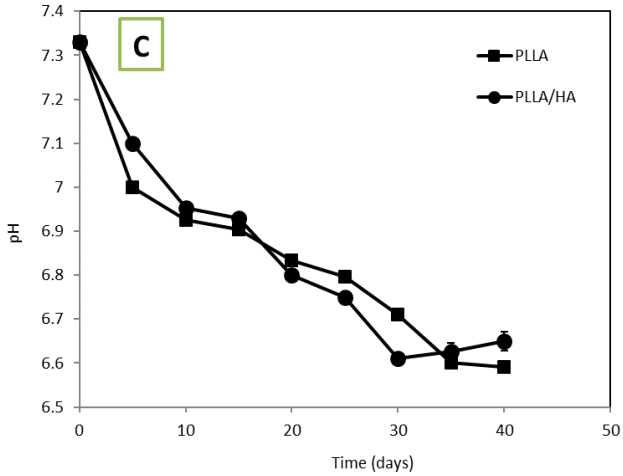

Figure 2: *In vitro* degradation study micrograph of electrospun PLLA/HA scaffold. A) Weight loss B) water uptake C) pH (Error bars indicate standard deviation)

During electrospinning of polymer/HA scaffolds, due to the tendency of HA particles to agglomerate, some of the HA particles would not have been fully embedded within the polymer fibers, as a result, some of them would have protruded from the surfaces of the fiber surfaces [15 and 16]. The water uptake over the degradation period is shown in Figure 2. According to the results, the rate of water uptake is increased during the first 10 days, and the maximum amount of it has been reached to 280% with progressive of scaffold degradation, the water uptake is reduced. This is probably due to the presence of HA particles on the surface of the scaffold. HA Induced by hydroxyl groups on the surface of the scaffold has led to increased water absorption. HA is hydrophilic, thus the presence of HA in structure of the scaffold, can significantly increase water absorption properties of scaffold (Figure 4-C). HA particles on surface of the scaffold, by increasing the hydroxyl groups on the surface of the scaffold led to an increase in its water absorption properties and hydrophilic scaffold adversely affect cell attachment, growth and migration [17 and 18].

CONCLUSIONS

PLLA and PLLA/HA composite scaffolds were successfully fabricated by electrospinning technique. The morphology showed that the scaffold had nano-fibrous network and microporous structure. According to the characteristic changes of the various properties of porous scaffolds, the degradation process is suggested that there was a decrease in the degradation rate of the composite scaffolds in PBS solution with pH 7.4 at 37°C after 40 days compared with pure PLLA scaffolds. The results suggest that the PLLA/HA scaffolds more suitable for culture of osteoblast- like cells and facilitates their application in bone regeneration. Further research can be carried out on the mechanism of enhancement and detailed biological properties.

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