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Biomimetic mineralization of electrospun poly(lactic-co-glycolic acid)/multi-walled carbon nanotubes composite scaffolds in vitro

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ABSTRACT

Biomimetic mineralization is an effective method to improve the biocompatibility and bone inductivity of certain materials. In this study, composite scaffolds composed of poly(lactic-co-glycolic acid) (PLGA) and multi-walled carbon nanotubes (MWNTs) were prepared by electrospinning. Subsequently, the scaffolds were immersed in a simulated body fluid (1.5×SBF) at 37 °C for 7, 14 and 21 days for biomimetic mineralization. Scanning electron microscopy, Raman spectroscopy, and X-ray diffraction were used for characterization. It was found that the electrospun scaffolds had extremely resemblant structural morphology to the natural extracellular matrix. After mineralization, apatite crystals were deposited on the PLGA/MWNTs composite scaffolds. The mineralized PLGA/MWNTs composites may be potentially useful in tissue engineering applications, particularly as scaffolds for bone tissue regeneration.

1. Introduction

In tissue engineering, an ideal scaffold should mimic the fibrous structure of the extracellular matrix (ECM). At present, electrospinning is an attractive method of preparing fibers from both natural and synthetic polymers. The scaffold produced by electrospinning has appropriate porosity, the very high aspect ratio and huge specific surface area, which features a structural similarity to the natural extracellular matrix [1,2]. Moreover, the electrospun scaffold can promote cells attachment, proliferation and differentiation [3].

Multi-walled carbon nanotubes (MWNTs) have gained widespread attention due to their unique structure and remarkable mechanical, thermal and electrical properties [4–7]. Moreover, they also have good biocompatibility [8]. Therefore, they may serve as a candidate for biomedical applications. Poly(lactic-co-glycolic acid) (PLGA), a copolymer composed of poly(lactic acid) (PLA) and poly (glycolic acid) (PGA), has good biocompatibility, biodegradability and mechanical properties. It was approved by U.S. Food and Drug Administration (FDA) for clinical research and so far has been one of the most commonly used biodegradable polymers in tissue engineering [9]. However, the relatively poor hydrophilicity and bioactivity make PLGA poorly interact with osteoblasts cells [10,11]. In contrast, hydroxyapatite (HA), which is a major inorganic component of natural bone, has good hydrophilicity, excellent bioactivity and osteoconductive properties [12]. Therefore, hydroxyapatite (HA) coatings have been widely used to improve the biocompatibility and bioactivity of the hydrophobic materials [13], such as polycaprolactone (PCL), poly(lactic acid) (PLA) and PLGA. Among various HA coating methods considered, biomimetic mineralization has attracted much attention over the last twenty years. Biomimetic mineralization is defined as a complex process that involves the controlled nucleation and growth of HA on the surfaces of various inorganic and organic substrates after immersion in a simulated body fluid (SBF) which is a metastable calcium phosphate solution supersaturated with respect to apatite and has a similar composition to that of human blood plasma [14–17]. Compared with other HA coating methods, the advantage of biomimetic mineralization is that it simulates the natural bone formation process without using special equipments and strict conditions. The SBF soaking method is a relatively easy and fast way to mineralize certain materials.

In the present study, we are aiming at biomimetic mineralizing the electrospun PLGA/MWNTs composite scaffolds using the SBF soaking method, as a foundation for further study of the mineralized scaffolds for tissue engineering application.

2. Experimental

2.1. Materials

PLGA (Mw = 100,000 g/mol) with a lactide/glycolide ratio of 75:25 was purchased from Chengdu Zhuoxin Co. Ltd. (Chengdu, China). Carboxyl MWNTs (diameter: 8–15 nm, length: 0.5–2 μm, –COOH content: 2.56 wt.%, purity>95 wt.%) were purchased from Chengdu Organic Chemistry Co. Ltd. (Chengdu, China). Dimethyl formamide (DMF), trichloromethane (TCM) and other chemical reagents (A.R.) were purchased from Chengdu Kelong Co. Ltd. (Chengdu, China).

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2.2. Electrospinning of PLGA/MWNTs composite scaffolds

PLGA/MWNTs composite scaffolds were prepared using an electrospinning technique. Briefly, PLGA was dissolved in a mixture of TCM and DMF (volume ratio 7:3) at the concentration of 25% w/v. And then the MWNTs (1.5% w/v) were added to the PLGA solution. The mixture was then ultrasonicated for 1 h at room temperature to disperse the MWNTs. The electrospinning apparatus utilized in this study consisted of a 10 ml syringe, a blunt-end steel needle (inner diameter = 0.27 mm), a syringe pump (KD Scientific, Model 100), a collector (ground) electrode (aluminum foil), and a high voltage supply (MDG, Dongwen High Voltage Inc. China) which was able to generate positive direct-current voltages ranging from 0 to 45.0 kV. The PLGA/MWNTs solution was loaded into the syringe, to which the needle was attached. The needle was pointed downward toward the ground electrode and a constant positive voltage (20 kV) was applied to the needle. The distance between the needle tip and the ground electrode was 13 cm and the syringe pump, which was used to feed the polymer solution, was set at a flow rate of 0.2 ml/min. A fluid jet was formed from the needle and fibers were sprayed onto the cover slips (22 mm × 22 mm), which were put onto the aluminum foil to collect the fibers. All experiments were carried out in air and the ambient condition was 25 °C and 60% humidity.

2.3. Structural and morphological characterization of the composite scaffolds

The surface morphology of the electrospun scaffolds were observed with scanning electron microscopy (SEM, JSM-5900LV, JEOL, Japan) at an accelerating voltage of 20 kV. The average diameter of the electrospun fibers was determined by the measurement of 50 single fibers from the SEM photographs using SigmaScan Pro 2.0 software (Systat Software Inc.). Raman spectrum of the sample was measured with a LABRAM-HR (JY.FRANCE) Raman spectrometer.

2.4. Biomimetic mineralization of the composite scaffolds in vitro

1.5 × SBF was prepared based on an established protocol developed by Varma [18]. Briefly, NaCl, KCl, $CaCl_2$, $MgCl_2 \cdot 6H_2O$, $NaHCO_3$, $K_2HPO_4 \cdot 3H_2O$, Na_2SO_4 were dissolved in deionized water. Solution pH value was adjusted with HCl and the final pH of the solution was 7.3. The ion concentrations of the 1.5 × SBF were 213.0 mM Na^+, 3.8 mM Ca^{2+}, 2.3 mM Mg^{2+}, 7.5 mM K^+, 221.9 mM Cl^-, 1.5 mM HPO_4^{2-}, 6.3 mM HCO_3^- and 0.7 mM SO_4^{2-}. Subsequently, the PLGA/MWNTs scaffolds (22 mm × 22 mm) were immersed in 1.5 × SBF at 37 °C for 7, 14 and 21 days. The solution was replaced every 24 h. At the end of the incubation time, the samples were rinsed with deionized water and then dried under vacuum at room temperature. The morphology of the samples was observed with SEM (JSM-5900LV, JEOL, Japan) at an accelerating voltage of 20 kV. X-ray diffraction (XRD) measurements were performed to investigate the components of the mineral grown on the scaffold by means of a diffractometer (X'Pert Pro MPD, Philips, Holland).

3. Results and discussion

SEM images of the electrospun pure PLGA and PLGA/MWNTs scaffolds are shown in Fig. 1a and b, respectively. It can be seen that long and continuous fibers were successfully prepared by electrospinning in both groups. Both the scaffolds had a three-dimensional network structure, which mimicked the structure of the natural extracellular matrix. The average diameter of the PLGA fibers was about 750 nm, which was much thinner than that of the PLGA/MWNTs fibers (2150 nm). This may be due to the viscosity effect. After addition of MWNTs into the PLGA matrix, the polymer viscosity increased from 49.6 to 80.3 mPa s. The surface tension of the fluid emerging from the tip of the needle increased as the viscosity increased. This means under the same voltage, the elongation of the PLGA/MWNTs fibers would be slower and the jet stream has already reached the collector electrode before it further split into thinner streams, and thus thicker fibers were collected on the collector electrode. The phenomenon was common and it confirmed that viscosity is an important process parameter during electrospinning [19]. Raman spectrogram of the PLGA/MWNTs scaffolds in Fig. 1c confirmed the existence of MWNTs in the composite scaffolds. Two strong peaks at 1580 cm^{-1} and 1355 cm^{-1} were assigned to the characteristic G-band and D-band of MWNTs, respectively.

As shown in Fig. 2, mineral crystals were observed on the electrospun fibers after only 7 days of incubation. The deposits were made up of small spherical and hemispherical crystals. In higher magnification images, it can be seen that the small spherical and hemispherical deposits were composed of smaller crystals. After 14 days of incubation, spherical and hemispherical crystals fused with each other; the mineral layers became thicker and almost covered the entire surface of the scaffolds. After 21 days of incubation, the crystals increased significantly and the mineral layers totally covered the surface of the scaffolds.

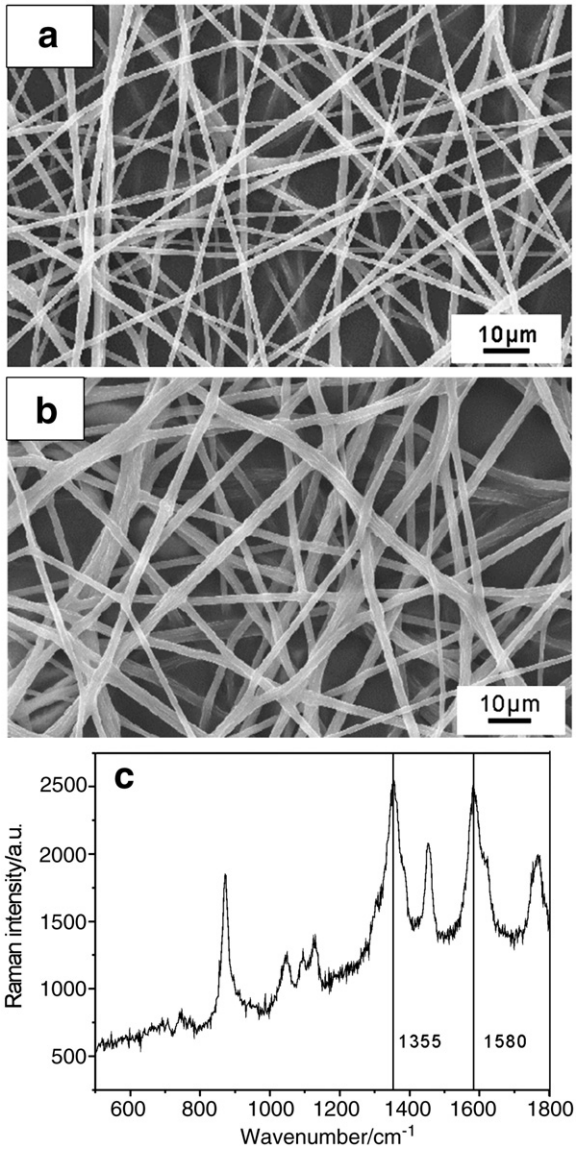

Fig. 1. SEM images of the electrospun (a) PLGA and (b) PLGA/MWNTs scaffolds and (c) Raman spectrogram of the PLGA/MWNTs scaffolds.