PREPARATION AND BIOLOGICAL PROPERTIES OF BIOACTIVE CARBON-BASED MATERIALS

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Introduction

Carbon nanomaterials including carbon nanofibers (CNFs) and carbon nanotubes (CNTs), due to their large surface area and energy, carbon nanomaterials show great potential in promoting osteoblast adhesion and proliferation [1-3]. However, carbon materials are inherently bioinert, only having osteoconductivity but no osteoinductivity [4]. On the other hand, continuous ultra-long CNFs produced by polyacrylonitrile (PAN) electrospinning and carbonization normally can not be effectively degraded in vivo and eliminated from living body by bio-solubilization, aerobic bio-oxidation, phagocytosis and/or excretion functions like biodegradable β-tricalcium phosphate (β-TCP) and polylactide-based polyesters [5]. Therefore, the fabrication of biodegraded CNFs decorated with bioactive materials may be extended the use of CNFs in biomedical areas. In this study, inorganic nanoparticles (tricalcium phosphate (β-TCP) or Bio-glass) were embedded to get biodegradable and bioactive CNFs for bone tissue engineering.

Experimental

Using triethylphosphate (TEP), calcium nitric tetrahydrate (CN) and tetraethyl orthosilicate (TEOS) as precursors, PAN/TEP-CN or PAN/TEP-CN-TEOS nanofibers were prepared via sol-gel/electrospinning technique in combination of polyacrylonitrile (PAN). The composite nanofibers were subjected to calcinations under selected conditions and CNF/β-TCP or CNF/bio-glass nanofibers were resulted. Briefly, the CNF/β-TCP membranes were prepared as follows: Firstly, TEP (2.28 ml) (Aldrich, USA) was mixed with 10 ml of distilled water, stirred at 353 K for 48 h to obtain a hydrolyzed TEP solution. CN (4.72 g) (Aldrich, USA) (Ca/P=1.5) was dissolved into the hydrolyzed TEP solution, stirred at room temperature for 120 h to generate a calcium-phosphorous complex. Then, 1 ml of the prepared solution was added to 10 ml of N,N-dimethylformamide (DMF, analytic pure, 99.5%,Tianjin Fine Chemical Co.) containing 10 wt% PAN (Mw =100,000 g/mol, 93.0 wt % acrylonitrile, 5.3 wt % methylacrylate, and 1.7 wt % itaconic acid, Courtaulds Co., UK), and stirred for 6 h at room temperature to obtain a homogeneous solution. This solution was fed into 20 ml syringe and electrospun through 0.5 mm diameter needle using an electrostatic spinning apparatus. The flow rate of spinning solution was maintained to 0.3 ml h-1 using a syringe pump (TOP 5300) and an applied voltage (DW-P303-1AC, China) was kept at 15 kV. The as-spun nanofibrous membranes were collected on an aluminum roller with a diameter of 50 mm at a rotating speed of 3000 rpm. The distance between the needle tip and the roller was 15 cm. And then the as-spun nanofibrous membranes were stabilized at 533 K for 30 min. in air, and carbonized at 1373 K for 2 h in N_2 surrounding to obtain the hybrid nanofibers [14]. Thermal treatment at high temperature converted PAN to carbon with concomitant formation of β-TCP from calcium-phosphorous complex, resulting in the production of CNFs/β-TCP. CNF/bio-glass hybrid membranes were fabricated in a similar way.

Methods including FE-SEM(Hitachi S-4700), HR-TEM (Hitachi H-800)XRD, (Rigaku D/max 2500) and FTIR (), as well as biomineralization with simulated body fluid (SBF) soaking and in vitro cell culture were used to characterize the chemical composition, microphase structure, surface morphology, biocompatibility and bioactivity etc. of the CNFs/β-TCP and CNF/bio-glass hybrids.

## Results and Discussion

As shown in Fig. 1(A), CNFs/β-TCP membranes are shown a millimeter-length-scale fibrous morphology with a partial alignment along the rolling direction and an average diameter ~200-400 nm. Fig. 1(B) demonstrates that some nanoparticles are exposed on the surface of CNFs, and also parts of nanoparticles are dispersed in CNFs. The nanoparticles has been identified as β-TCP by XRD analysis. From Fig. 1(C), it exhibits a series of sharp characteristic diffraction peaks at 2θ=25.7°, 27.7°, 31.0° and 34.3°, corresponding to the crystal planes of (1010), (214), (0210) and (220), respectively, suggesting the formation of β-TCP (JCPDS No. 09-79 0169) single crystalline phase with an rhombohedral (R3c) structure. The strongest characteristic peak (0210) can be used to measure the average crystallite size by applying the Scherrer equation: D=κλ/βcosθ. Here, D is the average diameter of the crystals in angstroms, κ is the shape factorwhich is often assigned a value of 0.89 if the shape is unknown, X-ray wavelength λ is 1.54056, β is the full width at half-maximum of the strongest characteristic peak in radians, and θ is the Bragg angle in degrees. The average diameter of β-TCP nanoparticles was calculated approximately to 28.9 nm. TEM-affiliated energy dispersive spectroscopy (EDS) results indicate that the content of the β-TCP nanocrystallines in the CNFs is about 15 wt%.

After being treated with HCl solution, the continuous ultra-long CNFs/β-TCP nanofibers with lengths approaching to 10 cm become to be short segments with a narrowed length distribution of 2 to 8 μm (Fig. 2).

The shortened CNFs and the release of calcium cations and phosphate anions as nutrient mineral salts may be advantageous to improve the physiochemical compatibility and degradation of CNFs-based scaffold. On the contrary,

nothing has happened to the pure CNFs after HCl treatment, which is similar to the results reported by RH Hurt, who found that no degradation occurred to normal carbon materials under the acid treatment [6].
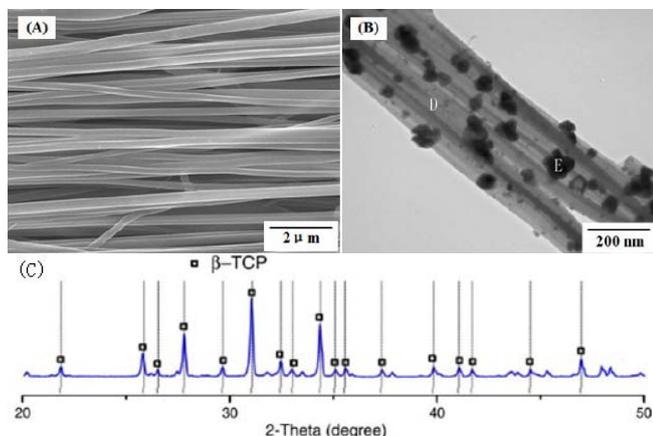

Fig. 1 (A) SEM, (B) TEM images and (C) XRD spectrum of the CNFs/β-TCP.
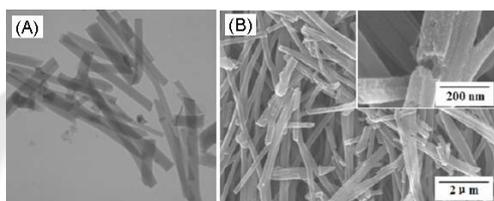
Fig. 2 TEM and SEM image of the degraded CNFs/β-TCP after immersion in HCl for 24 h.

Human periodontal ligament cells (PDLCs) were cultured on CNFs and CNFs/β-TCP membranes, and evaluated their proliferation on the membranes by MTT assay and SEM observation. The results show that both the CNFs and CNFs/β-TCP membranes have good biocompatibility, favor the PDLCs to adhere and proliferate preferencely along the aligned longitudinal direction of nanofibers due to contact guidance effect. In comparison to pure CNF membranes, however, PDLCs demonstrate fast proliferation rate and high extracellular matrix production on CNFs/β-TCP membranes with prolonged culture time. This behavior is thought attributed to the more adhesion sites for PDLCs provided by the β-TCP nanoparticles on the nanofiber.
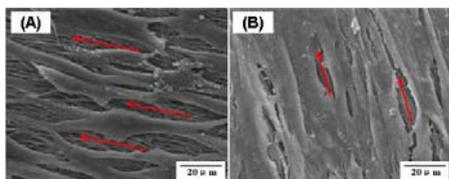
Fig.3 SEM images of PDLCs cultured on pure CNF (A) and CNFs/β-TCP (B) membranes for 7 days.

CNF/bioglass hybrid membranes also show prospectful bioactivity. As shown in Fig.4, the CNF/bioglass hybrid nanofibers fabricated from sol-gel/electrospinning exhibit similar fiber morphology (Fig. 4) to that of CNFs/β-TCP. The CNF/bioglass composite nanofibers were immersed in five-timed stimulated body fluid (5SBF) to induce biomineralization and a layer of carbonated hydroxyapatite was detected on fiber surface with apatite depositing initially from the sites where the bio-glass nanoparticles were present. The results suggest this kind of CNF/bioglass hybrid can be considered as scaffold material for bone regeneration.
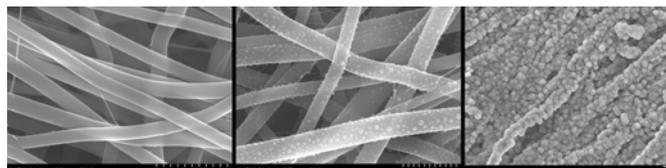
Fig. 4 SEM images for CNF/Bioglass nanofibers: as-spun, after carbonization and mineralization for 30 h.

Conclusion

By incoporating bioactive β-TCP or bio-glass nanoparticles into CNFs via sol-gel/electrospinning technique had been proved a convenient and efficient way to ameliorate the biological properties of CNFs. The results showed the decorated carbon nanofibers having good biocompatibility and improved bioactivity. Moreover, the continuous composite CNFs could crack into short segments owing to the dissolution of β-TCP in aqueous environment to favor the clearance from body to minimize toxicity, which showed advantages over conventional CNFs for biomedical applications. In addition, the inorganic particles decorated CNFs may also serve as gradient reinforcement for other kind of biodegradable bone tissue engineering scaffolds. These features make CNFs/ β-TCP and CNF/bioglass potential choices as cell scaffold material.

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