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Polymer-ceramic nanocomposites of polycaprolactone-nano hydroxyapatite (PCL-nHA) for bone tissue engineering

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ABSTRACT

Natural bone consists of calcium phosphate with nanometer-sized needle-like crystals of approximately 5-20 nm width by 60 nm length. Nanophase calcium phosphate ceramics like hydroxyapatite can mimic the dimensions of constituent components of natural tissues; can enhance osteoblast adhesion and resorption with long-term functionality of tissue engineered implants. Nanosized hydroxyapatite (HA) possesses exceptional biocompatibility and bioactivity properties with respect to bone cells and tissues. Polymer-ceramic nanocomposites fabricated from electrospun nanofibrous scaffold coated/incorporated with nanoceramic material prove to be an excellent scaffold for bone tissue engineering. The morphology of nanofibres mimics the extracellular matrix of natural tissue organization which further increases the adhesion and proliferation of bone cells. Polycaprolactone (PCL) is a bioresorbable polymer with potential applications for bone and cartilage repair. Nanocomposites can also be prepared by sintering polymeric nanospheres to make a scaffold and then coat it with the hydroxyapatite. In the present study nanofibrous scaffolds of polycaprolactone are prepared with electrospinning technique with 10wt%, 15 wt% concentration using chloroform as organic solvent. The characterization was done with scanning electron microscope (SEM). The polycaprolactone microspheres were prepared using water in oil-in-water emulsions. The characterization was carried out using scanning electron microscopy. Nanophase hydroxyapatite was prepared by wet precipitation route with calcium hydroxide and orthophosphoric acid as precursors. Characterization was done using transmission electron microscope (TEM) and X-Ray diffraction (XRD). The combination of (PCL-nHA) nanocomposite will prove to be an excellent scaffold for bone regeneration and repair.

KEYWORDS

Bone tissue engineering;
Nanocomposite;
Hydroxyapatite;
Electrospinning.

INTRODUCTION

Filling of bone defects is a significant questions in every day clinical work. Autogeneous bone is still the most effective bone graft substitution material (''gold standard''), fullfilling essential physicochemical and biological properties, despite its inherent limitations (availability, post-operative pain)[1]. The most common alternative to the autograft material are (human) allografts or (animal, e.g. bovine) xenografts. Allografts have the

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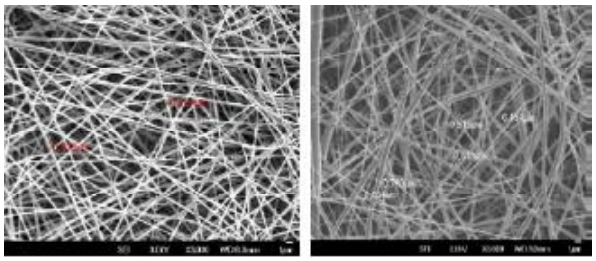

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Figure 1: Influence of PCL solution concentration on fibers morphologies observed under SEM with A) 10wt% B) 15wt%.

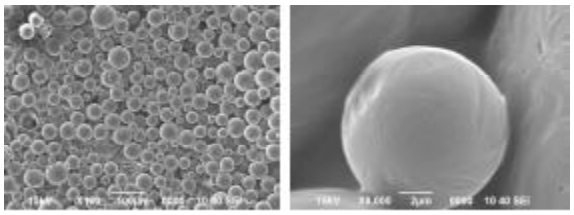

Figure 2: Morphology of PCL microspheres observed with scanning electron microscope at different magnification

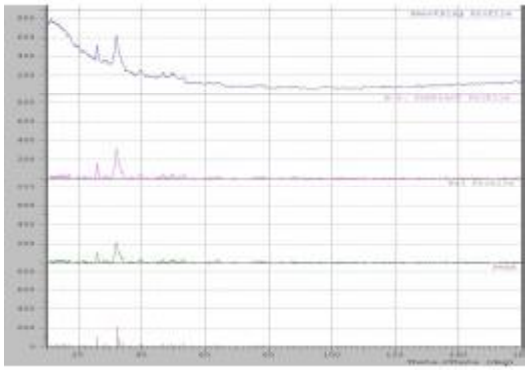

Figure 3 : The peak of XRD observed at 31.6 and 45.1 represents the Braggs reflection of HA

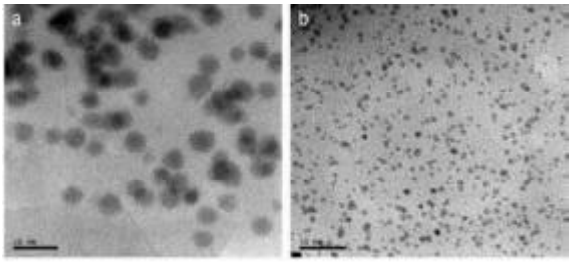

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Figure 4: HR-TEM images of HA showing spherical shaped particles with size in the range of 25-50 nm

disadvantages of limited supply and potential infectivity (e.g. HIV, Hepatitis). With xenografts there are the questions of unfavorable immune response and also of infectivity. Approximately fifteen years ago, biologists and engineers merged to form a new field called tissue engineering[2]. Bone tissue engineering involves growing osteoblasts or cells that can become osteoblasts on porous permanent or temporary scaffolds. The cells would come directly from the patient, eliminating problems of immunological rejection, pathogen transfer, and lack of donor cells. The scaffolds provide physical supports on which the cells can grow in three-dimensional constructs, and it is hoped that the cells will develop into new tissue with the same mechanical and chemical properties as native bone. If engineered bone can be developed successfully in the laboratory, scaffolds, autologous cells, and bone tissue engineering could then be used to treat patients with bone injuries or diseases in the clinical setting.

Various polymer composites have been used to make bone tissue engineering scaffolds.Kulkarni *et* al. reported that both PLA and PGA (Polylactic acid & polyglycolic acid) are biocompatible polyesters belonging to the family of poly-á hydroxyl acids. PLA and PGA materials degrade by hydrolysis of ester bonds[3]. Specifically, PLA degrades into lactic acid, which is a part of the body and can be excreted as water and carbon dioxide. No significant accumulation of degradation products of PLA have been reported in vital parts[3]. Engelberg et al. reported that LPLA has a weight average molecular weight ranging from 50 to 300 kDa and a number average molecular weight of 19.6 to 150 kDa. They also stated that DL-PLA has a weight average molecular weight ranging from 21 to 550 KDA and a number average molecular weight from 13.4 to 163 kDa[4].

Wang et al. reported that PCL is a biocompatible, semi-crystalline polymer that has been used to make scaffolds. PCL has a slower degradation rate than most biopolymers, a glass transition temperature of -60ºC, a melting temperature of 58-60ºC, and a decomposition temperature of 350ºC. Its mechanical properties include a tensile strength of 16 MPa, tensile modulus of 400 MPa, flexural modulus of 500 MPa, elongation at yield of 7%, and elongation at failure of 80% (Wang et al.)[5]. Ceramic materials have also been used in scaffolds[5].

Tancred et al. made scaffolds using HA, TCP, and HA/TCP (Tricalcium phosphate and hydroxyapatite) in a ratio of 3:1. HA and TCP are macroporous calcium phosphates that are biocompatible and have excellent

osteoconductive properties, allowing for bone ingrowth and formation[6]. Tancred et al. described a new process in which they poured a ceramic slip into a wax negative mold, removed the wax, and fired the ceramic scaffold. They found that this method allowed for the accurate macrostructural reproduction of cancellous bone (Tancred et al., 1998). Chu et al. did a study to characterize the mechanical and in vivo performance of HA implants. Thirteen HA implants with orthogonal channels at 40% porosity were tested on an Instron machine. The authors reported a compressive strength and compressive modulus of 30 ± 8 MPa and 1.4 ± 0.4, respectively[7].

METHODS

In the present study two different kinds of nanostructured scaffolds were prepared to use it as a matrix for seeding bone cells.

Electrospinning of PCL nanofibers

Firstly, nanofibrous scaffolds of polycaprolactone were prepared with electrospinning[8] technique with 10wt%, 15 wt% concentration using chloroform as organic solvent. The applied dc voltage was kept at 10 KV, tip target distance was maintained at 10cm and the flow rate was kept at 0.01ml/min. The characterization was done with scanning electron microscope (SEM).

Preparation of PCL microspheres

PCL microspheres were prepared by w/o/w emulsions[9]. 10wt% PCL was dissolved in chloroform by stirring the solution at 2000rpm for 5 minutes. The polymeric solution was added slowly to polyvinyl alcohol and stirred at 4000 rpm for 150 minutes at 30^0C resulting in the formation of water in oil-in water emulsion. Solvent was evaporated and the microspheres were washed in deionized water and were freeze dried. The morphology was observed using scanning electron microscope (SEM).

Preparation of nanophase hydroxyapatite

Nanophase hydroxyapatite was prepared by wet precipitation route with calcium hydroxide and orthophosphoric acid as precursors[9]. Orthophosphoric acid was added to calcium hydroxide solution under constant stirring conditions and the whole experiment was carried out at the constant temperature of 40^0C. The pH was maintained at 7 by adding ammonium hydroxide. Characterization was done using transmission electron microscope (TEM) and X-Ray diffraction (XRD).

RESULTS AND DISCUSSION

The diameters of nanofibres of PCL range from 200nm-300nm for 10wt% concentration and from 500nm-900nm for 15wt% concentration. The morphology was smooth and uniform

The diameter of PCL microspheres were in the range of 1-2μm. The morphology of spheres is smooth and they are uniform in size.

Nanophase hydroxyapatite was characterized with XRD and the peaks of XRD observed at 31.6 and 45.1 represents the Braggs reflection of HA.

Nanophase crystals of HA were in the range of 25-50nm.

DISCUSSION AND CONCLUSIONS

The combination of (PCL-nHA) nanocomposite will prove to be an excellent scaffold for bone regeneration and repair.

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