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Preparation and characterization of polycaprolactone/forsterite nanocomposite porous scaffolds designed for bone tissue regeneration

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ABSTRACT

Biocomposite scaffolds made from polymers and bioceramics can provide the mechanical structure necessary for osteoinductivity in the growth of new bone. The aim of this research was to investigate the properties of a novel nanocomposite scaffold made from a combination of polycaprolactone (PCL) and forsterite nanopowder which could find use in bone tissue engineering applications. The scaffold itself was fabricated by a method of solvent casting and particle leaching. The effect of forsterite content on the mechanical properties, bioactivity, biodegradability, and cytotoxicity of the scaffolds was investigated. Significant improvement in the mechanical properties was observed in the nanocomposite scaffolds as compared to that seen in the pure PCL scaffolds. Bioactivity was also observed in the nanocomposite scaffolds, a trait which was not present in the pure PCL scaffolds. Biodegradation assay indicated that the addition of forsterite nanopowder could modulate the degradation rate of PCL. In vitro tests of cytotoxicity and osteoblast proliferation showed that the nanocomposite scaffolds were non-cytotoxic, thereby allowing cells to adhere, grow, and proliferate on the surface of these scaffolds. The results obtained in this experiment suggest that the combination of PCL with forsterite nanopowder can be used to form scaffolds suitable for use in bone tissue engineering. The exact material behavior required can be adjusted through variation of the ratio between PCL and forsterite nanopowder used to form the scaffold.

1. Introduction

Tumors, trauma, disease, and a number of other ailments are responsible for the presence of defects in bones. The main challenge in reconstructive bone surgery is the repair of these bone defects. Conventional treatment methods rely on autogenic and allogeneic approaches, both of which necessitate a secondary surgical operation for the removal of donor bone from the patient's body [1]. Tissue engineering offers an alternative approach which eliminates the need to perform a secondary surgery and can greatly improve the safety and efficiency of the medical procedure. Instead of removal through a secondary surgical operation, the implanted material can be designed to dissolve naturally after its purpose in stimulating new tissue growth has been served.

Tissue engineering is an interdisciplinary field which strives to generate replacement tissues to repair and improve the function of damaged organs. One conventional method involves the implantation of osteoblasts (cells responsible for new bone growth) onto three-dimensional scaffolds. The scaffolds prove a physical framework to which the cells can attach and ultimately proliferate to form new tissue. The scaffold's critical role in tissue engineering thereby places high demands on the structures physical and biological properties. The scaffold must provide the sites necessary for new tissue growth in an in vivo environment without agitating an immune system response [2].

Biocompatability, bioactivity, the interconnectivity of a porous structure, adequate mechanical properties, and an appropriate degradation rate must all be taken into consideration in the design of a successful scaffold. The creation of a suitable porous structure is particularly challenging as it should possess both macroscopic passages to facilitate cell ingrowth and migration in addition to a microscopic network for the delivery of nutrients and the removal of cellular waste products [3].

Biodegradable polymer scaffolds have been widely researched and developed for tissue regeneration utilizing a variety of different polymers in their fabrication. Polycaprolactone (PCL) has emerged as a preferred polymer material due to its combined advantages of biocompatibility and a bioresorption rate appropriate for bone tissue regeneration. However, the scaffolds made from

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PCL do not exhibit the necessary mechanical properties and bioactive behavior [5]. Furthermore, PCL suffers from an intrinsic hydrophobic nature which inhibits surface wetting and interaction with biological fluids, both of which are prerequisites for cell adhesion and proliferation. These problems observed in pure PCL scaffolds can be overcome with polymer matrix composites with a PCL matrix incorporating other bioactive phases, such as hydroxyapatite or Bioglass [6,7].

Forsterite (Mg_2SiO_4) is a new bioceramic that has demonstrated good bioactivity for nanoscale structures in preliminary studies. Forsterite also possesses mechanical properties superior to those of hydroxyapatite and Bioglass [8–10]. An in vitro biocompatibility study on nanoscale–forsterite suggests that a superior controlled release of Mg and Si into the biological environment is achievable with forsterite nanopowder than that of bulk-form forsterite [9]. It is therefore expected that forsterite-nanopowder-incorporated PCL scaffold forsterite nanopowdercould simultaneously lead to enhanced biodegradation, improved bioactivity, and better mechanical properties.

Nanocomposite scaffolds based on forsterite nanopowder distributed in a PCL matrix have previously been fabricated and studied by our research group using the salt leaching/solvent casting method. The current research aims to investigate the properties of these nanocomposite scaffolds from the perspective of the mechanical properties, in vitro degradation behavior, bioactivity, and several other biological considerations. A correlation between forsterite nanopowder content and each of the above mentioned properties will be determined through this research.

2. Materials and methods

2.1. Mechanical properties of nanocomposite scaffolds

Nanocomposite scaffolds with different amounts of forsterite nanopowder (10–50 wt.%) were prepared by a salt leaching/solvent casting technique. Details of the preparation method are stated elsewhere [11]. Briefly, PCL pellets were dissolved in chloroform with a concentration of 0.1 g/ml and mixed with forsterite nanopowder. After the forsterite nanopowder is completely dispersed with the aid of an ultrasonic bath, NaCl particles were added to the suspension and the final dispersion was cast into cylindrical Teflon moulds. The samples were air-dried for 48 h and soaked in deionized water for a period of 3 days in order to leach out the salt particles. Salt-removed samples were freeze-dried and stored under vacuum. Apure polymer scaffold was prepared without forsterite nanopowder as a reference. The weight percentages of the PCL, forsterite nanopowder and NaCl along with the porosity percentage and pore size of the prepared scaffolds are presented in Table 1 Table 2 [11]. Following the suggestion of ASTM F451-86, disk-shaped specimens of nanocomposites and pure PCL scaffolds were prepared and tested to evaluate the mechanical properties. The sample discs had a height-to-diameter ratio of 1:1 (Height = 10 mm, Diameter = 10 mm) in order to reduce the effect of friction hills and improve stability against buckling [12,13]. The compression strength of the scaffolds was measured by a dynamic testing machine (HCT 400/25, Zwick/Roell, Germany) at room temperature with a constant displacement rate of 0.01 mm/s. At least four specimens were tested for each sample.

Table 1
Preparation parameters, porosities and pore size of the neat polymer and nanocomposite scaffolds.

Sample	Forsterite (wt.%)	PCL (wt.%)	NaCl (wt.%)[a]	Porosity (%)	Pore size (μm)
1	0	100	80	92.65	193 ± 55
2	10	90	80	92.14	172 ± 60
3	20	80	80	91.86	119 ± 45
4	30	70	80	91.38	113 ± 46
5	40	60	80	91.03	109 ± 50
6	50	50	80	90.94	98 ± 41

[a] The percentage of NaCl is to the total weight of PCL and forsterite nanopowder [11].

2.2. Biodegradability assays

A short-term degradation study was set up to monitor the in vitro behaviour of the samples. For degradation experiments, samples of the pure PCL and nanocomposite scaffolds were placed into phosphate buffered saline solution (PBS) at pH = 7.4 and 37 °C. Each of the buffer solution was refreshed every 3 days.This test was performed up to 30 days and at the selected time points, three samples of each scaffold were removed from the buffer and weighed wet after surface wiping. Afterwards, they were rinsed with deionized distilled water and dried in a vacuum oven at 37 °C for 24 h. Water absorption and weight loss were calculated according to Eqs. (1) and (2), respectively:

$$\text{Water absorption } (\%) = 100 \times (W_a - W_0)/W_0 \quad (1)$$

$$\text{Weight loss } (\%) = 100 \times (W_0 - W_t)/W_0 \quad (2)$$

where W_0 is the starting dry weight, W_a is the wet sample weight after removal from the solution, and W_t is the dry sample weight after removal. Furthermore, pH values of the solutions during scaffold soaking were recorded.

2.3. In vitro bioactivity assays

In vitro bioactivity of nanocomposite scaffolds was studied by soaking samples in Simulated Body Fluid (SBF) solution. The SBF was prepared as described by Kokubo et al. [14] and the scaffolds were immersed in it at 37 °C for specified periods up to 28 days.

Scanning Electron Microscopy (SEM) and Electron Dispersive Spectrometry (EDS) were used to evaluate the formation of apatite on the surface of both the pure PCL and nanocomposite scaffolds. SBF solutions were collected at regular intervals to determine the ion concentrations of Ca, Mg and P by inductively coupled plasma atomic emission spectroscopy (ICP) (AES; Varian, USA). In addition, pH values of the solution during scaffold soaking were recorded.

2.4. Cell attachment assays

The in vitro biocompatibility of the scaffolds was tested using SaOS-2 (Sarcoma estrogenic) cell line from the National Cell Bank of Iran at the Pasteur Institute. The line was kept in continuous culture in Delbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). The samples were placed in 48 well culture plates to investigate their capacity to support cell adhesion and proliferation. Prior to cell seeding, the scaffolds were sterilized for 20 min under ultraviolet light. Aliquots of 100 μL containing 5×10^5 SaOS-2 cells were seeded on top of the scaffold samples pre-soaked in 70 μl of DMEM supplemented with 10% FBS medium and allowed to proliferate for 2 days at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell morphology was investigated by means of SEM. For SEM analysis, samples were washed twice with PBS and the cells were fixed. For fixation, the samples were soaked in solution of 2.5% glutaraldehyde in 0.1 M PBS for 2 h, post-fixed with 0.1% osmium tetroxide in 0.1 M PBS for 30 min, dehydrated through acetone series, dried in a freeze dryer at −80 °C for 12 h, and kept dry with silica gel. Then, samples were sputtered with a thin gold layer and analyzed under SEM.