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Evaluation and Fabrication of 3D-Printed Bioactive β-TCP/PCL Scaffolds for Bone Tissue Engineering

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Abstract

Bone is one of the tissue types that is transplanted the most worldwide. Each year, there are over four million procedures that incorporate the use of bone grafts or substitutes to help heal bone deficiencies. These treatments are still limited greatly, and there is always a demand for help because of trauma, cancer, infection, and arthritis. Because of this, researchers are targeting improvement in developing bioactive three-dimensional (3D) scaffolds that favor the process of bone regeneration. Natural bone is a complex and well-designed natural material, and a successful bone substitute material has two challenges to meet, being both biocompatible and bioactive to allow the growth and differentiation of cells and having adequate mechanical stability after implantation. A hybrid PCL/β-TCP scaffold has been developed to meet these demands. The blended PCL enhances mechanical properties and biocompatibility of the scaffold compared to the scaffold made of PCL alone. In addition, the incorporated β-TCP fortifies the scaffold and can boost the osteogenic potential while the polymer elaborates the mechanical stability of the ceramic scaffolds. We investigated Polycaprolactone (PCL)/beta-tricalcium phosphate (β-TCP) composites based on application mechanical stimulus, as a support framework for engineered bone tissue. 3D structures based on PCL by solvent free 3D printing technique.

Keywords: Bioactive scaffolds, composite biomaterials, 3D scaffold, 3D printing, PCL, TCP, bone tissue

INTRODUCTION

Tissue engineering focuses on repairing, replacing or renewing damaged or dysfunctional soft or hard tissues because of trauma or disease. Allografts and xenografts are less painful however, trauma, tumor removal or infection often require autografts which follow a long and painful surgical process. Because bone defects are common – affecting 96% of people at the end of their life, caused primarily by trauma, bone tumors, congenital deformities and bone infections [1]. There is an enormous clinical need for the use of bone grafts. Various biomaterials have been explored for use as scaffolds for tissue engineering [2]. Successful tissue repair requires three major factors: scaffolds, cells and proper environmental conditions. Polycaprolactone (PCL): It is one of the most widely used biomaterials in the form of a bone scaffold as it is biocompatible and biodegradable [3].

To address the current challenges associated with treatment, significant effort is being invested in research for the engineering of bone tissue (BTE) to create innovative substitutes for standard bone grafts [4]. Several techniques have been developed to create 3D porous scaffolds using different biomaterials for aiding and guiding the process of bone regeneration [5]. There is still no known optimal scaffold material, and the clinical translation of 3D scaffolds is still limited.

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Recently, scaffolds 3D polymeric with good mechanical and biocompatibility properties have been proposed as bone gates for large bone defects repair [6–7]. These polymers can be natural or synthetic, biodegradable and non-biodegradable. Among them, the use of Poly-ε-caprolactone (PCL), a synthetic biodegradable polymer, in the field of bone regeneration is well known because of its good biocompatibility [8–9]. PCL exhibits exceptional mechanical capabilities and a high degree of toughness at room temperature due to its melting temperature of 59–64°C and glass transition temperature of 60°C [10]. Recently, Fuchs and associates created PCL scaffolds for oral bone repair via melt electrospinning writing. However, PCL scaffolds are not as good as bone implants they are highly hydrophobic and have no biological activity on their surface [11].

The primary inorganic component of natural bone is believed to be β-TCP. Its great bone conduction property and high binding to the host bone tissue make it very effective in promoting bone formation [12, 13]. Clinical applications for bone regeneration date back to 1980, and it has been thoroughly studied and utilized for decades [14]. β-TCP is osteoconductive and osteoinductive in nature and naturally resorbable which allows the material to be replaced by new bone. While the mechanical strength of the protein β-TCP is high, it is brittle [15–16]. Combining β-TCP with synthetic polymers can enhance scaffold mechanical properties. Similar reinforcement strategies have been reported in polymer composite systems, where fillers improve stiffness, thermal stability, and structural performance of polymer matrices [17].

Bone regeneration continues to be a significant hurdle in tissue engineering, where synthetic scaffolds are increasingly employed as substitutes for conventional bone grafts. Bioactive ceramics – like hydroxyapatite and β-tricalcium phosphate (β-TCP) – are commonly used due to their resemblance to natural bone and excellent properties for supporting bone growth. Nevertheless, their brittleness necessitates the integration with biodegradable polymers – like polycaprolactone (PCL) – to enhance mechanical strength. The creation of polymer–ceramic composite scaffolds, especially through 3D printing methods, permits precise manipulation of scaffold structure. Composite reinforcement approaches using fibers and particulate fillers have been extensively explored to improve structural integrity and mechanical performance [18]. Despite these improvements, issues, such as mechanical constraints and inadequate blood vessel formation persist, underscoring the necessity for refined designs of bioactive scaffolds.

Developing an ideal scaffold design continues to be one of the key obstacles in tissue engineering. Recently, scaffolds that are 3D-printed and made from polymers, particularly polycaprolactone (PCL) combined with hydroxyapatite (HA), have garnered considerable interest because of their promising effectiveness in vivo for bone regeneration. These scaffolds are characterized by their biodegradability and strong chemical interaction with natural bone tissue. Additionally, β-tricalcium phosphate (β-TCP) is vital for boosting osteogenic activity by facilitating the formation of osteoblasts and releasing calcium ions that encourage bone differentiation [19]. Hydroxyapatite, which is a fundamental mineral component found in natural bone and a type of calcium phosphate (CaP), is extensively utilized in bone tissue engineering due to its superb biocompatibility and osteoconductive characteristics.

MATERIALS AND METHODS

Materials

Chitosan (medium MW), PCL (Mn = 80,000 g/mol for receiving the material from standard supplier) and the beta-syntax (TCP; particle size < 0.063 um) were bought from standard suppliers. DMEM was used as a culture medium. For the cell viability assays, the reagents were MTT, fetal bovine serum, acetic acid, and formic acid which were purchased from reliable suppliers and used without further modification. Deionized water and 0.10PBS were used to prepare all the solutions.

Scaffold Fabrication

Both polymers (β-TCP and PCL (Mw 46 000)) were purchased from commercial companies and were used as they were. We produced PCL/β-TCP composite scaffolds using a 3D customized

bioprinting system [20]. To get the uniform mixed, the powders with similar particle sizes were milled together using mini ball mill. No solvent had been added to the composite. The combined mixture was fed into a heated dispenser that melted the polymer at an optimum temperature. The molten material was forced through a nozzle under controlled pressure of air building the scaffold layer by layer. We prepared several compositions by changing the content of the beta carboxy phosphate (β-TCP): pure PCL, PCL/β-TCP ~12 wt% of β-TCP and PCL/β-TCP ~32 wt% of β-TCP.

Characterization of Scaffolds

We tested the scaffold porosity and attachment of cells by scanning electron microscopy (SEM). Prior to the imaging process, samples were fixed in 10% formalin for 30 min and washed with PBS several times. The samples were dehydrated next using a series of graded concentrations of ethanol (50, 60, 70, 80, 90, 100 percents for 2 min each) with overnight drying in air. After they were dry, we sputter coated the samples with a thin layer of gold and imaged them under an SEM which was operating at 10kV. A scanning electron microscope with field emission (FE-SEM) was used to get more detailed information on surface morphology and surface composition. Energy-dispersive X-ray spectroscopy (EDS) was used to obtain qualitative and quantitative compositional data.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR (Fourier Transform Infrared Spectroscopy) was used to examine the functional groups and chemical structure of composite scaffolds produced. This technique is widely employed for evaluating chemical bonding, crystallinity, and structural integrity in polymer composite materials [21, 22]. The spectra were recorded using an FTIR-ATR spectrometer (PerkinElmer, USA) and wavenumbers between 4000 and 650 cm^{-1}. The purpose was to find out what effect the heat and pressure of 3D printing had on the bonding and crystallinity. We searched for characteristic peaks of both polycaprolactone (PCL) and beta-tricalcium phosphate (β-TCP) to verify the ceramic phase was successfully incorporated into the polymer. We also analyzed the spectra to assess polymer–ceramic interactions and the structural integrity of the scaffolds after fabrication.

Cell Culture Study

Scaffolds were cut to the same size, and their surface was sterilized with UV light for approximately 30 min to eliminate any contamination on the surface. Before seeding the cells, we pre-treated the surfaces with fibronectin to stimulate initial cell adhesion [23]. Human mesenchymal stem cells (hMSCs) were cultured in 10% fetal bovine serum (FBS), 10% antibiotics in the growth medium (DMEM) at 37°C, in a humidified incubator and 5% atmosphere of CO_2. Cells were seeded on each of the scaffolds at a concentration of 1×10^5 cells and allowed to adhere for a specific period. After the first cell attachments, the culture medium was switched to an osteogenic differentiation medium with dexamethasone, β-glycerophosphate, and ascorbic acid to encourage the formation of bone. The cell-seeded scaffolds were incubated for specific periods to assess cell viability, proliferation, and the potential for osteogenic differentiation.

ALP Activity Assay

The osteogenic differentiation of cells grown on the engineered scaffolds was evaluated by quantifying alkaline phosphatase (ALP) activity at predetermined time intervals. At each time point, the samples were collected and delicately washed with phosphate-buffered saline (PBS) to remove any leftover culture medium [24]. The scaffolds were then treated in an assay buffer and subsequently centrifuged to obtain the clear supernatant for further analysis.

The activity of ALP was measured by employing p-nitrophenyl phosphate (pNPP) as a substrate. The reaction mixture was kept under controlled conditions for incubation, and a stop solution was introduced to halt the enzymatic reaction. Absorbance was measured at 405 nm with a microplate reader [25]. The enzyme activity was then determined using a standard calibration curve and presented in suitable units, indicating the extent of osteogenic differentiation.

RESULT

Scaffold

Three scaffold groups have been produced by the bioprinting system; pure PCL; PCL with 12 wt% of β-TCP; and PCL β-TCP with 32 wt% β-TCP. The percentages represent the mass fraction of the β-TCP in the polymer matrix. The average diameter of the strand of all groups was between 405 and 450 μm. The pores were roughly square in shape. The length of side of each pore was between 355 to 400 micrometers. These dimensions were verified by measuring at least 10 locations in each group of scaffolds ensuring uniformity in design. Such a size of the pores is appropriate for efficient infiltration and growth of cells. The porosity measurements were as follows: 59.92 ± 1.32% for the pure PCL; 60.48 ± 1.29% for the PCL/β-TCP with 12 wt%; and 61.88 ± 1.23% for the PCL/β-TCP with 32wt%. A higher content of β-TCP resulted in slightly higher porosity which is consistent with material expectation.

Energy-Dispersive X-Ray Spectroscopy (EDS)

FE-SEM images of the scaffold surface and cross-sections revealed the presence of the particles of β-TCP as being embedded in the polymer and rougher surface because of their incorporation. Energy dispersive X-ray spectroscopy (EDS) was then employed to analyze the elemental composition. EDS was performed by scanning six random regions on each of the samples. Calculated Ca/P ratios were found to be 0.80 (as) 0.07 for PCL/Thompson's group β-TCP 12 (as) wt% and 1.50 (as) 0.06 for PCL/β-TCP 32 (as) wt%. Pure PCL scaffolds revealed the presence of carbon and oxygen, proving that there are no ceramic elements. EDS elemental mapping further revealed that the particles of the used organic precursor (β-TCP) are distributed uniformly across the entire composite scaffolds, indicating the successful blending and dispersion of the particles in the polymer matrix.

Fourier Transform Infrared Spectroscopy (FTIR)

We analyzed the chemical nature of our sample fabricated scaffolds and raw PCL using Fourier Transform Infrared Spectroscopy (FTIR-ATR). The resulting spectra for all samples were very similar, and it would be expected that this would mean the chemical structures were close to each other. Strong absorption features were observed at 1732 centimeters cm^{-1} (C=O stretch), at 2945 and 2866 cm^{-1} (asymmetric and symmetric CH2 stretches), at 1295 cm^{-1} (C–O/C–C stretches in the crystalline regions), and at 1242 cm^{-1} (asymmetric C–O–C stretch). These peaks appeared unchanged, confirming that the chemical structure of the PCL was stable during fabrication. The extrusion temperatures and pressures did not cause any degradation.

SEM Analysis

SEM (scanning electron microscopy) revealed uniformly smooth, bead-free fibers. Similar SEM investigations have been employed to assess surface morphology, filler dispersion, and interfacial interactions in composite materials [26, 27]. This demonstrates that most important process variables, such as applied voltage, needle–collector distance, and polymer flow rate, were effectively optimized. Energy-dispersive X-ray analysis (EDAX) confirmed the presence of calcium and phosphorus from β-TCP. Increasing the content of the compared peptides (β-TCP) have increased the concentration of Ca and P. Elemental mapping revealed that the particles spread evenly on the scaffold surface. SEM was also used to measure the diameters of the fibers and the pore sizes that are important to osteoblast attachment. Diameters were between 200 and 500 nm where the average was around 291 nm which is a nanoscale size favorable to interaction between cells. Pore size was also evaluated. Increased levels of β-TCP reduced the mean pore size. Of the above samples, PGC formed the largest pores and PGCT5 formed the smallest. These architectural differences have a major impact on bone regeneration and cellular behavior.

CONCLUSION

In the present study, bioactive PCL/β-TCP composite scaffolds were successfully fabricated using a solvent-free 3D-printing approach. The scaffolds exhibited a well-defined interconnected porous architecture with pore sizes ranging from 355 to 400 μm and strand diameters between 405 and 450 μm,

providing a favorable environment for cell infiltration and tissue ingrowth. The incorporation of β-TCP resulted in a modest increase in scaffold porosity while preserving the overall structural integrity of the printed constructs. SEM and EDS analyzes confirmed the homogeneous distribution of β-TCP particles throughout the polymer matrix and demonstrated an increase in calcium and phosphorus content with increasing ceramic loading. FTIR results indicated that the characteristic chemical structure of PCL remained unchanged during the printing process, suggesting that the fabrication conditions did not induce detectable thermal degradation. In vitro investigations demonstrated that the scaffolds supported cellular attachment and osteogenic differentiation, as evidenced by ALP activity. The presence of β-TCP is expected to contribute to the bioactivity of the scaffold through the release of calcium-containing species, which are known to play an important role in bone formation and regeneration. Collectively, the findings indicate that the developed PCL/β-TCP scaffolds possess suitable structural, physicochemical, and biological characteristics for bone tissue engineering applications. Similar studies have shown that β-TCP incorporation can enhance osteogenic performance and improve the biological response of 3D-printed PCL-based scaffolds, supporting the potential of such composite systems for regenerative medicine.

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