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Mechanical Properties of 3D Printed Reinforced Polycaprolactone Composite Scaffolds

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Objectives This paper describes the fabrication of a new porous 3D-printed scaffold composed of polycaprolactone (PCL) and polyether-ether ketone (PEEK) micro-particles for bone tissue engineering (BTE) applications.
Methods In order to improve the compatibility of the reinforcing PEEK powder with polycaprolactone, the PEEK powder was surface-modified by an amino-silane coupling agent. After modification, Fourier-transform infrared spectrometry (FTIR) and differential scanning calorimetry (DSC) were used to investigate the chemical reaction between PEEK and silane coupling agent. In order to increase the compressive modulus of the 3D printed PCL scaffold, 10% silane-modified PEEK was incorporated into the PCL polymeric matrix. Scanning electron microscopy (SEM) was used for cell morphology and attachment evaluation.
Results The results indicated that the silane coupling agent was successfully grafted onto the particle surface. The compressive modulus of PCL scaffold increased by incorporating the silane-modified PEEK, despite having higher porosity, compared with the pure PCL scaffolds. Addition of amino-silane had a positive impact on cell response, and that surface modification led to improved particle dispersion.
Conclusion In conclusion, it seems that the incorporation of surface-modified PEEK micro-particles into the PCL porous scaffold could enhance its mechanical properties, and may be applicable for the management of large bone defects.
Keywords Polyetheretherketone; Polycaprolactone; Amino-propyl-triethoxysilane; Tissue Scaffolds; Printing, Three-Dimensional

Introduction

Tissue engineering scaffolds should be able to provide functional and structural support for tissue repair. The integrity of the scaffold frame is the foundation of structural support for the embedded cells, and is necessary for the cells to regenerate and proliferate into the required shape and size of the presumptive tissue. An ideal scaffold should also contain appropriate porosities, which in case of bone engineering, may lead to a decrease in mechanical properties.[1] One-component scaffolding cannot meet all the requirements for physicochemical characteristics of the tissue scaffolds, especially from the mechanical standpoint.
Polycaprolactone (PCL) is a biodegradable, thermoplastic polymer with a low melting point (around 60°C) and a low degradation rate, making it much more suitable for long-term application in specific anatomical sites.[2-4] Biodegradation of PCL takes 2-3 years and bone-like tissue takes over a year to fully heal. As a result, PCL is a suitable candidate for bone tissue repair.[5]
Polyether ether ketone (PEEK) is a semi-crystalline thermoplastic polymer known for its outstanding biocompatibility and mechanical properties. This polymer has become a very interesting biomaterial with remarkable potential to become a bone-repair material and therefore was chosen to be incorporated into polycaprolactone as a reinforcing phase.[6-9]
Incorporation of PEEK micro-particles into the PCL matrix will possibly result in unique properties in the final material. A composite of these two biopolymers may be collectively manipulated physically, chemically, and biologically to provide a promising polymer platform for mechanically active tissues or critical bone defects in order to fulfill the mechanical functions of the target tissues.
Nevertheless, the encountered problem is that the reinforcing particles tend to aggregate within the matrix, and the phase-separation phenomena caused by this agglomeration could hinder the mechanical properties of the composite.[10] Thus, it is necessary to modify the surface of PEEK particles with organic molecules. A uniform dispersion and strong interface may be achieved by using a reactive modifier such as silane coupling agent. It is believed that amino-terminated organo-silanes provide significant bioactive properties.[11-14] Amino functional groups carry a positive charge and form electrostatic interactions with negative charges on the cell surface.[15] Among the amino-silanes, 3-aminopropyltriethoxysilane (APTES) with three hydrolysable ethoxy groups, is one that allows robust anchoring of the silane network. In addition, the reactive amines have a major role in immobilization and self-assembly of cell-adhesive proteins, such as fibronectin or vitronectin that support cell adhesion.[16-19] These amino groups are nontoxic to the cells and tissues, and not only decrease the surface hydrophobicity, but also neutralize the acid generated during the scaffold degradation, reducing the inflammation around the implanted scaffold. As a result,

amine groups have a positive effect on improving cytocompatibility.[17]
In this study, aminosilane-modified PEEK fillers were fabricated as a reinforcing phase to improve the mechanical and biological properties of 3D-printed PCL scaffolds for applications in bone tissue engineering.

EXPERIMENTAL PROCEDURE

Surface treatment of filler phase:
PEEK (VICTREX® -150UF10) fine powder with a particle size of 10 μm and density of 1.30 gr.cm-3 was used as the filler material. Equal amounts of PEEK and APTES (Sigma Aldrich, Munich, Germany) (4 g each) were dispersed in 12 mL of isopropyl alcohol and stirred for 4 h under a nitrogenous atmosphere; then, they were desiccated in a vacuum drying chamber at 90°C for 6 h to remove the solvent. The obtained precipitates were adequately washed with ethanol and were subsequently separated from the washing solution by filtration. Finally, the powders were dried at 60°C for 8 h. This treatment created a hydrophilic surface, promoting PCL/PEEK adhesion, and reduced agglomeration of the particles.

Preparing the ink for printing:
PCL (Mw = 48-90 kDa; Sigma Aldrich) was used as the polymer matrix, and the silane-coated PEEK micro-particles were blended homogenously with the PCL at 60°C. An ultrasonic bath was also employed to allow particles to be uniformly distributed in a ratio of 0.1:1 when comparing PEEK to PCL. Briefly, pure PCL pellets were heated to ensure all materials are in a molten state. Then, silanized PEEK powder was added at the desired concentrations. The materials were mixed for 10 min to ensure uniform dispersion of the particles. After cooling down, the blended material was chopped into small pellets to be loaded to the 3D printing system.

Preparation of PEEK/PCL scaffolds using the 3D-printing technique:
The scaffolds were fabricated through the melt extrusion technique using a 3D-bioplotter system (Envision TEC, Germany). ACAD/CAM software was used to design square block models with the dimensions of 5×5×5 mm3. The optimal pressure of 2.5 bar and the plotting speed of 4 mm/s were applied. The scaffolds were printed layer-by-layer with a plotting needle with an internal diameter of 400 μm (Nordson, USA) to obtain 0.6-0.8 mm distance between the strands and a 90° shift between the layers. With the nozzle temperature set to 130°C, the syringe pump extruder then purged and pressurized the composition with nitrogen stream.

CHARACTERIZATION

Fourier-transform infrared spectroscopy (FTIR):
FTIR is a technique which is used to obtain infrared spectrum of absorption, emission, and conductivity of materials and detect different functional groups. FTIR spectrum is recorded between 4000 and 400 cm−1.
The functional group vibrations of the silane-coated PEEK surfaces were examined using FTIR (Nicolet iS5 Thermo Scientific™; OMNIC™, USA) over the range of 500-4000 cm–1 at a resolution of 4 cm–1. Four scans were collected from each sample to ensure that the silane coupling agent coated the PEEK surface.

Differential scanning calorimetry (DSC):
DSC is a thermal analysis instrument measuring how physical properties of a sample change along with temperature against time.
The glass transition and melting properties of the samples were investigated by DSC (NETZSCHDSC404F1; Pegasus, Selb, Germany) operating under argon flow; 10 mg of each sample was first heated up to 200°C at a temperature rate of 10°C min-1, followed by cooling to room temperature at a rate of 5°C min-1, and then heated up to 450°C at a heating rate of 10°C min-1. The glass transition and melting temperatures were taken at the peak onset in the calorimetric curves, respectively, and the ostensible enthalpies were calculated as normalized integrals of the corresponding peaks.

Contact angle measurement:
The contact angles were measured by the sessile drop method. The sessile drop method is used for direct measurement of the contact angle to determine the wetting of a solid by oil or water.[18] A Dino-Lite digital microscope camera (Dino-Lite, Hsinchu, Taiwan) captured the image of 5 μL drop of deionized water that deposited on the samples, and subsequently the image was analyzed using the Image J software.

Morphology, porosity and mechanical properties:
A 3D laser scanning microscope (LEXT OLS 4000, Olympus, Japan) was used to study the morphology of the PCL/PEEK scaffolds. The porosity was measured using the liquid displacement method.[20] Dry scaffolds were weighed, and then immersed in ethanol. Ethanol penetrated through the scaffolds without any subsequent dimensional changes of the scaffold matrix. The porosity was calculated using the following equation:
$$\text{Porosity} = (W2 - W1 / W2 - W3) \times 100$$
Where W1 is the weight of the sample in air, W2 is the weight of the sample with liquid in their pores, and W3 is the weight of the sample suspended in ethanol.
In order to characterize the effectiveness of the incorporation of PEEK particles and the APTES silane coating, a compression test was conducted on pure PCL scaffold and silane-coupled PEEK/PCL scaffolds, utilizing a mechanical testing machine (Shimadzu, Japan) with a cross-head speed of 1 mm.min−1 and a 5 kN load cell at room temperature. The compressive modulus was calculated as the slope of the initial linear section of the

stress-strain curve. The compression tests were performed according to the ASTM D695-08 protocol and the reported data were the mean of the three samples.

Cell morphology and attachment:

The dental pulp stem cells (DPSc) were isolated from a normal impacted third molar and cultured in an osteogenic medium. The scaffolds were disinfected by immersion in 70% ethanol and rinsing with phosphate-buffered saline (PBS). The cells were seeded (1×105 cells/cm2) on the scaffold and cultured for time periods of 3, 7 and 14 days. The culture medium was changed every 2 days. After incubation, the cells were fixed with glutaraldehyde (4% in PBS) for 45 min; The scaffolds were gradually dehydrated in graded ethanol concentrations (30%, 50%, 70%, 90%, 95%, and 100%); each grade was used for 15 min, and the scaffolds were then dried. Finally, cell adhesion was observed using a scanning electron microscope (SEM; JSM-6510LV; Jeol, Tokyo, Japan) after gold (Au) coating. Exempt status was granted by Marquette University IRB.

Statistical analysis

For mechanical properties and contact angle, three samples were tested per group. An independent t-test was utilized for data analysis. Data were presented as mean±standard deviation. P values< 0.05 were considered to be statistically significant.

Results

Phase separation is the Achilles heel in regard to organic/inorganic composites. Modifying the surface of filler particles is an effective approach to improve interfacial compatibility between the biopolymer and the reinforcing phase.[21, 22]

In order to confirm amino-silane functionalization of PEEK particles, the FTIR spectrum was used to study the new interactions. There was an appearance of wide absorption bands in the wavenumber region 1000-1200 cm-1, corresponding to the stretching modes of Si–O–C bonds of the aminopropyl group. The N–H stretching vibrations were reported at approximately 3300-3400 cm-1. Moreover, it could be found that the new bands appeared at about 2800-2900 cm-1 for the -CH3 group of amino-silane. The results implied that the APTES was successfully grafted onto the surface of PEEK particles (Fig. 1a).

The DSC results showed that the introduction of an amino-silane to the PEEK causes an endothermic peak shift. This shift confirms that the organofunctional group on the silane reacts with the PEEK polymer. The covalent bonds between the APTES and the surface of PEEK polymers cause higher energy requirements to achieve the glassy state before reaching the Tg, due to less chain mobility (Fig. 1b).

The wettability of the surface was enhanced by reducing the contact angle from 77.5° ± 3.2° to 69.4° ± 2.4° which is in favor of cellular attachment (P< 0.05) (Fig. 2).

The 3D laser scanning micrographs of the 3D-printed PCL/PEEK scaffold confirmed that the designed scaffolds had a well-defined and interconnected pore in the range of 200-600 µm with micro-sized struts (Fig. 3). The interconnected porous structure, along with the mechanical properties provides the basic requirements for load-bearing bone tissue engineering applications.

An ideal bone tissue engineering scaffold possesses pores of about 200–900 µm and porosity of 60–95%. The porosity features influence the biological properties, but regular fabrication methods encountered difficulties in designing biomimetic scaffolds with optimum pore shape, size and inter-connectivity. The 3D printing manufacturing methods enable accurate and precise manufacturing of porous construction.[23, 24]

The compressive modulus of PCL/PEEK scaffold with 74% porosity was 76±4 MPa that was not statistically different from the pure PCL scaffold with 50% porosity (P>0.05). Therefore, higher porosity did not influence the mechanical properties of the scaffolds negatively

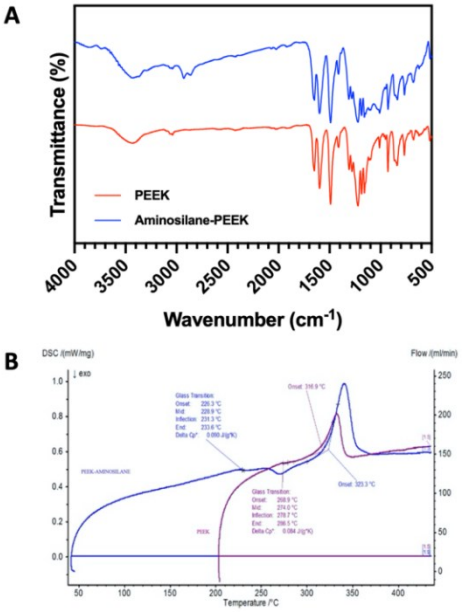

Figure 1- (a) FTIR spectra and (b) DSC graphs of PEEK and APTES grafted PEEK particles

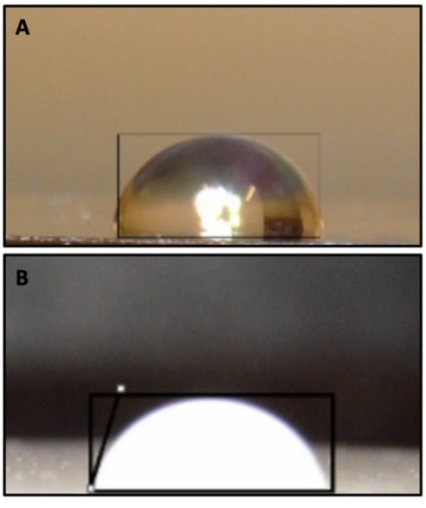

Figure 2- Contact angle of (a) PCL and (b) PCL/PEEK

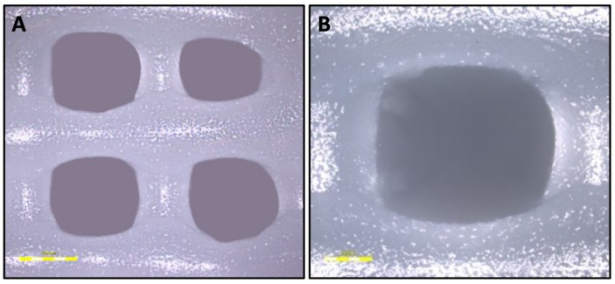

Figure 3- Morphology of 3D printed scaffold using laser microscope; (a) low magnification and (b) high magnification

Discussion

Composite scaffolds have been developed to overcome the limitations of one-component scaffolds. In this study, a novel microhybrid structure was obtained consisting of PCL/PEEK in order to construct scaffolds with improved mechanical properties.

In a porous scaffold, the pore volume will be filled with the newly formed bone tissue and the degradable biomaterials will be absorbed by the human body. Therefore, the scaffold provides a negative template for bone regeneration.[25-27] Incorporation of high strength biocompatible fillers such as PEEK into the polymeric matrix is a promising method to improve the mechanical behavior of the resultant composite. The compressive modulus of PCL/PEEK hybrid constructs were compared with the PCL scaffolds. The porosity of the composite scaffolds resembles proximal tibial trabecular bone, which has typically 50-90% porosity. Studies regarding the role of scaffold porosity on bone ingrowth have shown that typically higher porosity scaffolds (>70% porosity) have superior ingrowth compared with low porosity scaffolds (<70% porosity).[23, 24] High mechanical properties, despite high degree of porosity, suggested that the addition of PEEK micro-particles was able to enhance the mechanical properties of the PCL scaffolds. Some studies reported that the loadings of reinforced phase led to a reduction in the mechanical properties of the scaffolds, due to the aggregation of nanofillers. Efficient surface modification of filler surface provides a network of covalent links, characterized by superior mechanical properties.[28, 29]

Silane treatment greatly improved the dispersion of PEEK micro-particles within the PCL matrix without any agglomeration. APTES is the silane coupling agent commonly used to create a charged surface for protein and biomolecule immobilization and the adhesion of inorganic particles to polymer matrix.[12] Hence, the compressive modulus of the PCL/PEEK scaffolds increased compared with the scaffolds without the additional treatment.

DPCs provide osteoinductive bone factors and differentiate to osteoblasts in vitro and in vivo. Therefore, they have shown great capacity for biomimetic bone regeneration. DPCs have higher colony forming ability compared with bone marrow stromal cells under similar conditions.[30]

The SEM micrographs of cultured DPCs onto 3D printed PCL/PEEK scaffold after 14 days are shown in Fig. 4.

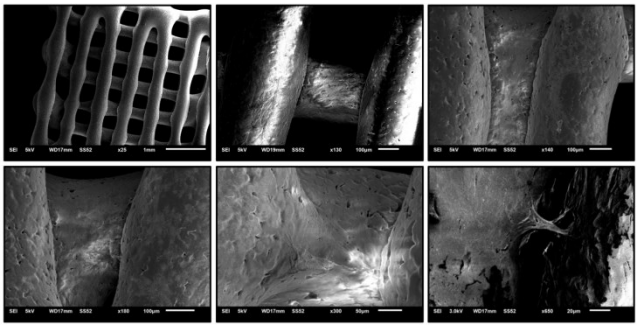

Figure 4- SEM micrographs of the cells attached on the 3D printed composite scaffolds at different magnifications

As can be seen, the DPCs were well attached to the scaffold. This observation implies that the highly interconnected porosity in the construct has allowed for the culture systems to supply adequate oxygen and nutrient flux within the 3D constructs to support ongoing tissue formation. Furthermore, the silane coating of the PEEK micro-particles and introduction of NH2 groups did not jeopardize cytocompatibility. Also, it should be noted that the increase in surface wettability of the samples helped improve the cytocompatibility of the PCL scaffolds. It has been confirmed that positively charged amine groups improve osteoblast-specific gene expression, alkaline phosphatase enzymatic activity, cell adhesion and spreading rate that play important roles in the application of tissue engineering.[31] Meanwhile, surface wettability has a major role in the cell attachment phenomenon and cell behavior.

According to the obtained results, designing a PCL scaffold with higher porosity along with adequate mechanical properties can be achieved by incorporation of organo-silane modified PEEK micro-particles.

Conclusion

The purpose of this study was to develop composite PCL-PEEK scaffolds for bone tissue engineering applications. An ideal BTE scaffold should have proper mechanical properties and bioactivity to facilitate the proliferation and differentiation of cells and resist dynamic remodeling during tissue repair.

After the successful silanization of PEEK micro-particles, PCL-PEEK scaffolds were developed through 3D printing techniques. The addition of amino-silane to the surface of reinforcing particles improved hydrophilicity and bioactivity, and furthermore, enhanced the mechanical properties of the scaffold, indicating a strong compatibility and dispersibility between the polymer matrix and PEEK micro-particles. According to our data, PCL-10wt% nanocomposite PEEK scaffold could be a favorable candidate for BTE application.

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Conflict of Interest

No Conflict of Interest Declared ■

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