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Morphology and mechanical properties of 3D printed PCL-PLA-ZnO nanocomposite scaffolds for bone regeneration

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ABSTRACT

Nowadays, to solve the problems of implants, damaged bone is repaired using biocompatible scaffolds. In this research, PCL/PLA/ZnO scaffolds were fabricated by melt extrusion 3D printing. Polycaprolactone is one of the well-known biocompatible and bioactive materials for bone scaffolds, but it is hydrophobic and has a low degradation rate. Therefore, polylactic acid was used to increase degradability and hydrophilicity. Also, zinc oxide was used to increase mechanical and biological properties. For this purpose, PCL or PCL containing 1, 2 and 3 wt% ZnO nanoparticles and PLA were printed layer by layer. The morphology and mechanical properties were studied by SEM images and compression test, respectively. The SEM images showed that the printed scaffolds had a regular structure with a pore size between 300 and 400 micrometers. Adding PLA and ZnO to PCL scaffolds significantly improves mechanical performance and PCL/PLA/ZnO composites have a favorable compressive strength for bone tissue engineering applications. By adding PLA to PCL, the stiffness increased from 52 MPa to 214 MPa. The addition of zinc oxide nanoparticles up to 3% by weight has increased the stiffness from 214 MPa to 273.48 MPa in the PCL/PLA/3%ZnO sample. Results have demonstrated that PCL/PLA/ZnO scaffolds prepared by melt extrusion 3D printers are a highly promising solution for bone tissue engineering.

KEYWORDS: *3D printing, polycaprolactone, polylactic acid, zinc oxide nanoparticles, bone tissue engineering, morphology, mechanical properties.*

1. Introduction

Bone defects are one of the main challenges in universal medicine, which are caused by trauma, osteoporosis, birth defects, arthritis and neoplasms, etc. [1]. When bone tissue is damaged, the cells may be able to regenerate the defect. When a bone defect is large, a bone graft is used [2]. Bone graft is a very expensive procedure and may result in significant donor site damage and disability, disfigurement, and scarring, and is associated with surgical risks such as bleeding, inflammation, infection, and chronic pain [3]. Therefore, the development of artificial bone is necessary to overcome these limitations and improve the results of bone repair and regeneration [2].

Bone tissue engineering uses scaffolds, cells, and growth factors to make artificial bones for implantation in patients' bodies. Scaffolds, as one of the most important components of this treatment, need special features. Ideally, tissue engineering scaffolds should have several properties like biocompatibility, biodegradability, and a three-dimensional porous structure that can provide sufficient space for cell migration, adhesion, and new bone

tissue growth. In addition, the material of the scaffold must be able to be processed into a three-dimensional structure and have sufficient mechanical strength Based on the properties required for bone scaffolds, various polymers have been considered for the fabrication of bone scaffolds, such as polycaprolactone (PCL), polylactic acid (PLA), poly-lactic acid (PLLA) and polyvinylidene fluoride (PVDF) [4-6]. Polycaprolactone is a semi-crystalline linear aliphatic polyester. Polycaprolactone is widely used as a scaffold in bone tissue engineering due to its biocompatibility, biodegradability, high flexibility, structural stability, and favorable mechanical properties. Also, polycaprolactone has favorable rheological properties such as low glass transition temperature (-60°C) and low melting temperature (59 to 64°C). However, polycaprolactone has low biodegradability, bioactivity and surface energy (high hydrophobicity), which causes a decrease in cell adhesion and tissue regeneration rate [6-9]. To solve these problems, additives can be added to polycaprolactone. Polylactic acid or polylactide is the most widely biodegradable aliphatic polyester that is widely used as a biological material for medical applications. Polylactic acid is a thermoplastic polymer with high mechanical strength and Young's modulus. The glass transition temperature of polylactic acid is 60 °C and its melting point is 160 °C [9-14]. The previous researches show that the composite of PCL/PLA has better mechanical properties, higher swelling ratio and cell-scaffold interactions compared to PCL scaffold [15]. Also, the use of zinc oxide nanoparticles in biomedical applications, especially in bone tissue engineering, is very effective due to mechanical properties, biocompatibility, biodegradability, low cost, antibacterial, and antifungal properties [16,17]. The results of the research show that the addition of zinc oxide nanoparticles to the scaffold significantly increases the Young's modulus. Also, adding zinc oxide to the PCL/PLA scaffold improves antibacterial properties and increases cell proliferation [18].

There are several methods for the fabrication of bone scaffolds, like phase separation, gas foaming, solvent casting/particulate leaching, freeze drying, electrospinning, and 3D printing. 3D printing is based on the principle of layer fabrication in which materials are stacked layer by layer. This technology can be used to rapidly fabricate components of any complex shape using computer-aided modeling (CAD) or computed tomography (CT) scanning. The 3D printing industry has recently grown due to the reduction in fabricating costs, controlled shape and porosity, improved accuracy, and speed of printing, which enabled huge advances in medical devices, implant materials, and cellular printing. The printed porous structure increases the circulation of body fluid inside the implant and promotes the internal growth of new bone tissue. In addition, architecturally porous implants minimize stress-shielding effects compared to an all-solid stem of the same geometry. Therefore, 3D printing allows the printing of personalized bone scaffolds for the patient and specific clinical conditions [19-21].

Considering the advantages of the 3D printing method, fabrication of PCL-PLA-based scaffolds with this method has received less attention. So in this research, layered PCL/PLA scaffolds containing ZnO nanoparticles were fabricated by 3D printing, and their physical, and mechanical properties were investigated. The presence of a PLA layer can increase biodegradability and hydrophilicity. The inclusion of zinc oxide nanoparticles in the PCL layer can also improve the mechanical and biological properties of the scaffolds.

2. Experimental details

To fabricate PCL/PLA/ZnO composites by 3D printing, PCL (Aldrich with an average MW of 80,000 Da), ZnO with a purity of 99+% and a particle size range of 10-30 nm (US Research Nanomaterials, Inc) and PLA (Hisun, REVODE190 (L130)) were used as the starting materials. Chloroform (Merck) with a purity of 99% was used as the solvent. According to the desired shape, the G Code file was designed with Simplify3D software. According to previous studies, adding more than 3 wt% ZnO can cause toxicity in the body [22]. As a result, this limit was considered in adding ZnO nanoparticles. As shown in Figure 1. PCL or PCL containing 1, 2 and 3 wt% ZnO nanoparticles and PLA were fed into separate steel syringes, placed in the 3D printer instrument and printed layer by layer using 3DPL N2 Plus Bioprinter (3DPL, Iran). The printing conditions were considered as follows: temperature and rate 105 °C and 40 mm/min for PCL and 210 °C and 700 mm/min for PLA, 0.25 cm layer height, 400 µm nozzle diameter, pressure 2-3 bar, 50 % porosity and 0:90° orientation. The temperature and rate were optimized to achieve an acceptable appearance of the printed scaffolds. In the samples containing ZnO nanoparticles, To ensure that nanoparticles were distributed uniformly

in the PCL, ZnO nanoparticles were first dispersed in chloroform and sonicated for 30 min. Next, the PCL granule was added to the ZnO/Chloroform colloid, stirred for 2 hours until the PCL was completely dissolved, and then sonicated for 30 min. Subsequently, the composite mixture was cast on a Petri dish. The cast film was dried, cut into small pieces and fed into a syringe.

The morphology, filament diameter and pore size of the 3D-printed samples were examined by scanning electron microscopy (TESCAN-Vega 3). The ImageJ software was used to measure the filament diameter and pore size. The mechanical properties of the samples were examined by compression tests performed using a compression test instrument (SANTAM, Iran) at a 5 mm/min crosshead speed. The test was repeated three times for each scaffold.

3. Results and discussion

Porous scaffolds of PCL, PCL/PLA 2:1, PCL/PLA 1:1, PCL/PLA/1%ZnO, PCL/PLA/2%ZnO and PCL/PLA/3%ZnO were fabricated successfully using a 3D printer. Morphology, pore shape and filament diameter were investigated using SEM images that are shown in Figure 2. As can be seen, the samples have a regular, interconnected and porous structure according to the pattern

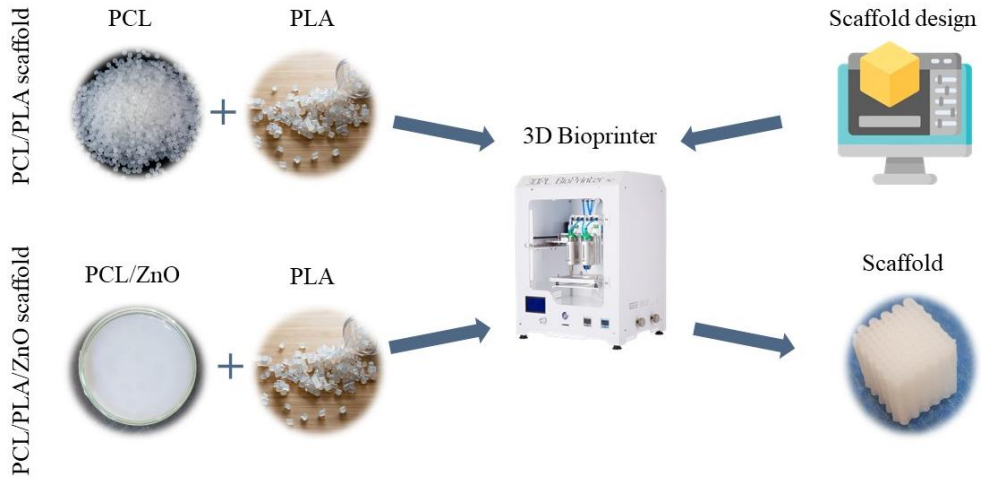

Fig. 1- Schematic of the overall procedure for the fabrication of 3D printed samples.

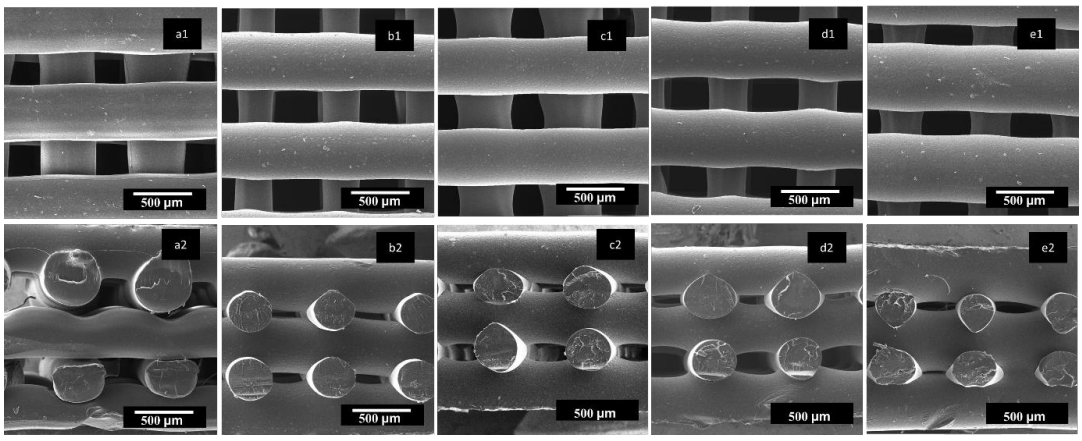

Fig. 2- SEM images of PCL/PLA 2:1, PCL/PLA 1:1, PCL/PLA/1%ZnO, PCL/PLA/2%ZnO and PCL/PLA/3%ZnO scaffolds. A1,b1,c1,d1 and e1) surface of scaffold , a2,b2,c2,d2 and e2) scaffold cross section.

designed before printing. All filaments have been printed uniformly, without cracks or deformation, and have kept their shape after leaving the nozzle and being placed on the print bed. Since the lower layers have not been deformed and shifted, it can be concluded that the selected speeds are suitable for PCL and PLA printing and the layers have dried enough to keep their shape. In the samples where PLA filaments are visible in the bottom layer, it is clear that the PCL surface is slightly wrinkled and the PLA surface is almost smooth. This is because PLA solidifies quickly and has a low shrinkage rate during the solidification process, but the PCL filament solidifies a little later and the surface becomes wrinkled.

The cross-sectional images show that the filaments have a circular geometry without severe distortion and they are parallel to each other in successive layers. There is good adhesion between the filaments of each layer and the underlying layer, except for the PCL/PLA 2:1 sample, which is related to the printing method of this scaffold and the very fast solidification of the underlying layer.

Pore size and average diameter of PCL and PLA filaments were calculated using ImageJ software and the results are given in Table 1. All samples have square or rectangular porosity with length and width in the range of 300 to 400 micrometers. The three-dimensional structure and pore size of the scaffolds play an important role in cell infiltration. In general, in the scaffolds with pore sizes of 80 to 190 micrometers, the aggregation of cells was observed and cell infiltration did not happen well. A pore size of at least 300 micrometers is necessary

Table 1- Pore size and average diameter of PCL and PLA filaments

Sample	Pore size (µm)	Average filament diameter of PCL (µm)	Average filament diameter of PLA (µm)
PCL	389	346	-
PCL/PLA 2:1	304	491	-
PCL/PLA 1:1	378	432	248
PCL/PLA/1% ZnO	281	454	436
PCL/PLA/2% ZnO	435	448	318
PCL/PLA /3% ZnO	437	525	368

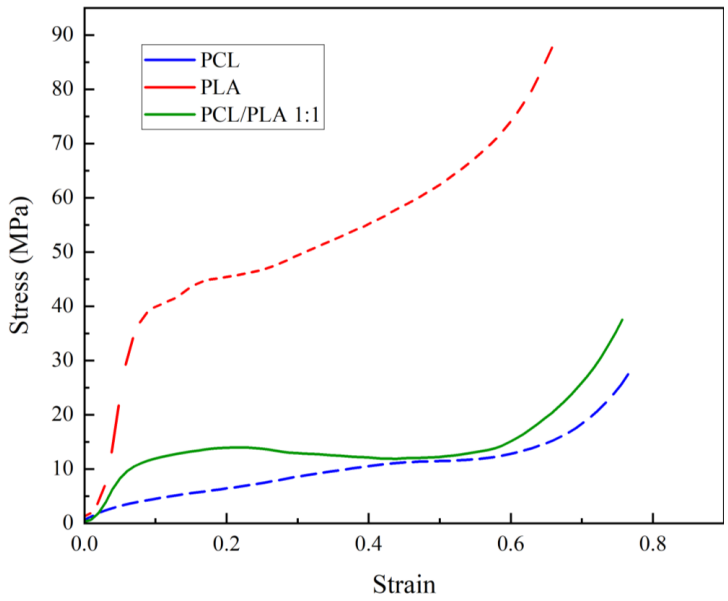

Fig. 3- Engineering stress-strain diagram of PCL, PLA and PCL/PLA 1:1 samples.

for cell infiltration, which according to the range of 300 up to 400 micrometers of sample porosity, scaffolds can be suitable for cell infiltration. Also, the diameter of polycaprolactone filaments was almost equal to polylactic acid filaments. In the samples containing zinc oxide nanoparticles, the diameter of the PCL filaments increased with increasing ZnO content. It can be attributed to the increase in viscosity with increasing ZnO content [23].

Cuboid samples of PCL, PLA, PCL/PLA/1%ZnO, PCL/PLA/2%ZnO and PCL/PLA/3%ZnO were printed with dimensions of 8*8*10 mm^3 and the mechanical properties of the samples were investigated under compression. The obtained engineering stress-strain curves are presented in Figures 3 and 4. The stress-strain curves of porous composite scaffolds show three distinct regions, including linear elastic, plateau and densification regions. A flow-softening region is observed before the plateau region. It can be attributed to the stress concentration around zinc oxide nanoparticles or friction between polymer chains that decreases as the chains begin to move.

The stiffness (slope of the linear region), onset strain of the densification region, the pore wall fracture stress and the length of the plateau region were calculated and the results are given in Table 2. As can be seen, the PLA sample has the highest stiffness among all polymer samples, and by adding PLA to PCL in the PCL/PLA 1:1 sample, the stiffness increased from 52 MPa to 214 MPa. The addition of PLA to PCL increased the wall fracture stress, but did not have a significant effect on the onset strain of the densification region. The addition of ZnO nanoparticles to the PCL/PLA 1:1 sample has increased the stiffness up to 273.48 MPa

Table 2- The stiffness (slope of the linear region), onset strain of the densification region, the pore wall fracture stress and the length of the plateau region

Sample	Stiffness (MPa)	Onset strain of the densification region (ε_d)	The pore wall fracture stress (σ_d)	Length of the plateau region
PCL	52.39	0.67	18.02	-
PLA	762.68	0.57	71.05	-
PCL/PLA 1:1	214.66	0.68	22.83	0.36
PCL/PLA/1%ZnO	225.64	0.71	23.07	0.48
PCL/PLA/2%ZnO	233.62	0.70	24.34	0.49
PCL/PLA/3%ZnO	273.48	0.70	34.07	0.52

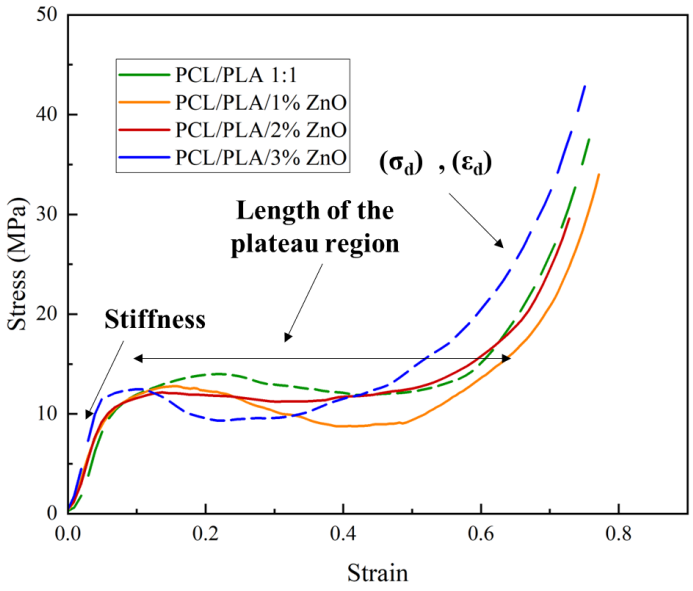

Fig. 4- Engineering stress-strain diagram of PCL/PLA 1:1, PCL/PLA/1%ZnO, PCL/PLA/2%ZnO and PCL/PLA/3%ZnO samples.

in the PCL/PLA/3%ZnO sample. This result is expected due to the higher strength of ceramic particles. In the samples containing ZnO nanoparticles, the length of the plateau region, onset strain of the densification region and the wall fracture stress have increased. The longer the plateau region, the stronger the walls and the walls fail later. In other words, the walls tolerate more stress and reach the densification region later. As a result, the life of the sample is longe

Since the compressive strength of cortical bone is between 100 and 200 MPa and trabecular bone is between 2 and 20 MPa [24], the fabricated scaffolds can be used for cortical and trabecular bones.

4. Conclusions

PCL/PLA/ZnO scaffolds were fabricated by 3D printing and the morphology and mechanical properties were investigated. The following results were obtained:

- SEM images of the samples showed that all the samples have square or rectangular porosity with length and width in the range of 300 to 400 micrometers which is suitable for the infiltration of osteoblast cells.

- In all the samples, except the PCL/PLA 2:1 sample, there is adhesion between the layers.

The diameter of polycaprolactone filaments was almost equal to that of polylactic acid filaments.

- By performing a compression test, it was found that the addition of PLA and zinc oxide nanoparticles to PCL increased the compressive strength and among the composite samples, the PCL/PLA/3% ZnO sample has the highest strength.

- In the presence of ZnO nanoparticles, the length of the plateau region, onset strain of the densification region and the wall fracture stress have increased.

- Considering the 50% porosity of the bone composite scaffold (PCL/PLA/ZnO), it has a favorable compressive strength for bone tissue engineering applications.

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