

MANUFACTURE AND CHARACTERIZATION OF NOVEL 3D POROUS SCAFFOLDS FOR BONE TISSUE ENGINEERING

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INTRODUCTION

Methods used currently to create porous polymer scaffolds include salt leaching, phase separation, and emulsion freeze-drying. These methods are capable of producing high porosity but tend to create structures with low mechanical integrity. In order to create structures possessing appropriate mechanical properties for load-bearing bone tissue engineering applications, a new approach involving solvent coating and a porogen was examined here. The polymer used for the scaffolds was a form of poly(lactic acid), which has an appropriate polymeric structure for osteoblasts to grow and deposit bone matrix making it suitable for bone applications [1]. The purpose of this study was to determine effects of porogen concentration on the microstructural morphology and mechanical properties of the resulting porous PLDL scaffolds. The ability of osteoblast-like cells seeded onto the polymer scaffolds to attach and remain viable *in vitro* was also assessed.

MATERIALS AND METHODS

Medical grade poly(L-lactide-co-DL-lactide 70:30) [PLDL] with IV = 4.86 dl/g was obtained from Purac America (Lincolnshire, IL). Azodicarbonamide, the pore-forming agent, was obtained from Aldrich (Milwaukee, WI). These were combined in dry mixes of 5%, 10%, 15%, and 20% azodicarbonamide and solvated in acetone. The solutions were coated on 316 stainless steel wires (California Fine Wire, Grover Beach, CA) of 89 μm diameter using a solution coating process. A long length of wire was threaded into a syringe and needle with the needle-end closest to the end of the wire. PLDL-azo solution was injected into the syringe so that as the syringe and needle were slowly pulled across the length of wire, a coating of the solution was deposited on the wire by the needle orifice. Several coats were applied to achieve an end weight of 60-90 mg PLDL-azo per meter of wire. The wire was cut into lengths of about 100 mm, and approximately sixty coated wires were then bundled in heat shrink tubing. The bundles were oven heated at 120°C for 25 minutes to activate the tubing and anneal the coated wires together. After removal of the tubing, the samples were submerged in peanut oil at 260°C for 25-30

seconds to decompose the azodicarbonamide, thus creating a porous microstructure surrounding the wires. They were immediately quenched in water and then hexane to cool the polymer and remove oil. The wires were subsequently removed from the structures, revealing axially oriented channels running through the micropores created by the porogen.

The samples were cut to about 5 mm in height for analysis first with micro computed tomography (microCT, Scanco USA) to nondestructively assess microstructural morphology and anisotropy. The samples (n=10 for each group) were scanned with the microCT at 35 μm voxel resolution with an integration time of 120 ms. They were then evaluated to quantify architectural parameters. The samples were also tested using an electromechanical testing device in compression at a rate of 20% strain/minute to determine compressive strength and modulus.

Scaffolds about 3 mm thick were tested for biocompatibility using mouse osteoblast-like MC3T3-E1 cells. Scaffolds were sterilized with gamma irradiation at a dose of 25 kGy. Each construct was seeded with 1 million cells and grown in culture medium containing α -MEM with 10% FBS and 1% Pen. Strep. The constructs were stained after 1 week with calcein and ethidium bromide for live and dead cells, respectively, to be examined using a laser scanning confocal microscope.

RESULTS

Data for 3D samples were obtained by the microCT in the form of image slices, which were then compiled to reconstruct whole volume 3D images such as the one shown in Figure 1. These images can also be manipulated by rotation or slicing. Figure 2 shows a section of the scaffold sliced longitudinally. Some axial channels as well as microporosity can be seen here.

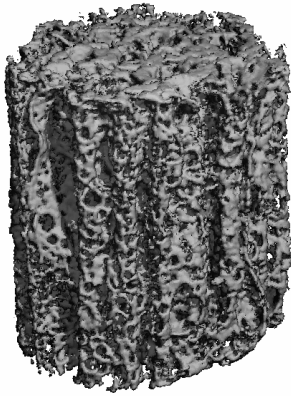


Figure 1. Porous PLDL scaffold (15% azo formulation).

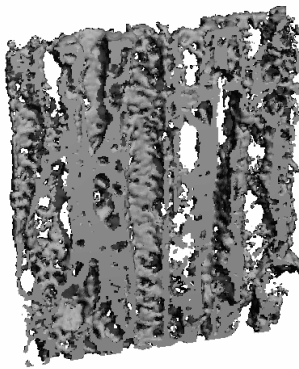


Figure 2. Slice of porous PLDL scaffold.

The architectural parameters calculated included volume fraction (VF, %), trabecular plate number (TbN, 1/mm), trabecular thickness (TbTh, μm), trabecular spacing (TbSp, μm), and degree of anisotropy (DA). These parameters are shown in Table I. Table II shows the compressive strength and modulus values determined for each group.

Table I. Scaffold architecture.

Group	VF	TbN	TbTh	TbSp	DA
5%	41.3 \pm 3.8	6.33 \pm 0.24	103 \pm 5.2	166 \pm 8.3	1.40 \pm 0.03
10%	37.7 \pm 6.5	5.41 \pm 0.85	92.7 \pm 4.2	212 \pm 34.3	1.31 \pm 0.07
15%	32.4 \pm 4.5	4.22 \pm 0.44	96.1 \pm 3.9	264 \pm 31.2	1.36 \pm 0.04
20%	28.8 \pm 2.7	3.48 \pm 0.21	100 \pm 3.0	324 \pm 23.4	1.35 \pm 0.03

Table II. Mechanical properties.

Group	Strength (MPa)	Modulus (MPa)
5%	11.5 \pm 1.9	161.6 \pm 45.7
10%	6.5 \pm 1.8	139.1 \pm 49.5
15%	6.1 \pm 1.3	109.9 \pm 24.8
20%	5.8 \pm 0.4	86.3 \pm 16.0

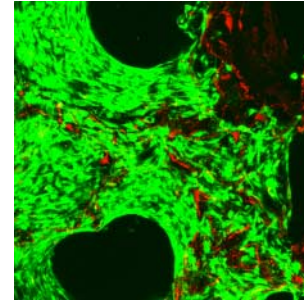


Figure 3. Confocal image of 1 week seeded PLDL scaffold.

Increasing the amount of porogen creates a decrease in volume fraction. Also, as porogen concentration is increased, trabecular plate number decreases and trabecular spacing increases. Trabecular thickness remains fairly constant for scaffolds of varying porogen amounts. The degree of anisotropy is also fairly uniform amongst the four groups. Compressive strength and modulus both decrease with an increased concentration of porogen.

The scaffolds have been shown to be viable for MC3T3-E1 mouse osteoblastic cells. Figure 3 shows live cells present on the surface of the construct. Green indicates live cells. Red typically indicates dead cells, but the ethidium bromide also stained the PLDL, thus producing some red autofluorescence.

DISCUSSION

Volume fraction for the scaffolds consistently decreases with increasing porogen concentration. Also consistent is the decrease of trabecular number and increase in trabecular spacing with an increase in porogen concentration. With these results, it is not unexpected that compressive strength and modulus follow trends of decrease as well. The mechanical properties exhibited by the scaffolds fall in the normal ranges of strength and modulus for trabecular bone, which are about 1-10 MPa for strength and 50-250 MPa for modulus [2].

The scaffolds were designed to have longitudinal channels as well as microporosity with the intent of promoting cell attachment and revascularization within the center of the constructs. The high surface to volume ratio of the porogen-created microporosity was conducive to osteoblast-like cell attachment. The combination of the axial orientation and the microporosity may therefore simultaneously promote cell attachment and revascularization within the scaffold.

In the future, more extensive mechanical testing will be considered. To better compare these structures to trabecular bone, characterization of fracture toughness and the effects of fatigue should be examined. Also, scaffolds reinforced with ceramic or glass constituents will be produced and tested. Ongoing studies will evaluate the influence of scaffold architecture on the biological responses *in vitro* and *in vivo*.

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