Effect of hydroxyapatite on biodegradable scaffolds fabricated by SLS

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Abstract.

Selective laser sintering (SLS) has the potential to fabricate bioresorbable polymer / ceramic composite scaffolds with pre-designed external and internal architecture that can be used for bone tissue engineering applications. Scaffolds were fabricated using poly-c-caprolactone as the base material. The effect of 15 and 30 wt% of hydroxyapatite (HA) addition was investigated in terms of compressive properties, accuracy, surface topology, and wettability. Fabricated dimensions of PCL microstructures showed great deviations from their nominal values. Average surface roughness was found to be Ra=25±4 gm. Increased HA content had no statistically significant effect on accuracy and surface roughness. However the addition of HA had a significant influence on compressive properties, hydrophobicity and wettability of the samples. Addition of 30 wt% HA improved initial compressive modulus of pure PCL scaffolds from 1.31±0.08 MPa to 1.58±0.18 MPa. Yield strength values increased from 0.14±0.07 MPa to 0.17±0.01 MPa by adding 15 wt% of HA, but decreased with further HA addition. Yield strain for all compositions was over c=0.06. Increased HA content decreased hydrophobicity and increased wettability of scaffold surfaces. The study demonstrated the ability of SLS to fabricate tissue engineering scaffolds, and the positive effect of HA particle reinforcement in terms of compressive mechanical properties and surface characteristics.

Introduction

Selective Laser Sintering (SLS) is a promising technology for fabricating complex scaffold geometries with pre-designed, intricate internal architectures for applications in tissue engineering. SLS is a powder based solid freeform fabrication technology. 3D models are created in CAD environment and sliced into thin layers. The geometry is reconstructed layer-by-layer using a CO_2 laser beam to sinter the layers according to the slice data. In the scanned regions the sintering process is characterized by particle coalescence resulting from the delivered heat and energy. As a result of particle coalescence, reduction in surface free energy is the main driving force for the sintering process [1].

Bone tissue engineering scaffolds aim to temporarily replace the functions of the extracellular matrix of natural bone, to which cells can attach, proliferate and form tissues. Mechanical and microstructural characteristics and surface morphology have been shown to strongly influence cell adhesion, growth and differentiation [2-3]

Several papers reported successful selective laser sintering of bone scaffolds using biodegradable polymer/ceramic composites [4-5], however quantitative characterization of accuracy, surface and mechanical properties is less investigated. In this study poly-c-caprolactone (PCL) and hydroxyapatite (HA) were selected for selective laser sintering hard tissue scaffolds. Accuracy, surface morphology and mechanical properties of the fabricated PCL scaffolds are examined as a function of increasing HA content. Addition of HA is expected to enhance surface morphology including bioactivity and wettability of the scaffolds and to improve their mechanical behavior.

Materials and Methods

Materials. Poly-c-caprolactone (PCL, Sigma Aldrich Chemical Co.) pellets were cryogenically grinded and sieved. The used powder batch had an average particle size of 125 μm with particle size distribution where 80 % of all particles are between 70-160 μm as measured by Malvern Mastersizer particle size analyzer. The hydroxyapatite (HA) powder used in this experiment is sold under brand name Captal 60-1 (Plasma Biotal Ltd.) and has an average particle size of 38 gm. Mixtures of PCL/HA powders were produced by physical blending. PCL was used as the base material and HA was added to produce blends with 0, 15 and 30 wt% HA content.

Geometry. Test specimens for mechanical testing, surface roughness and contact angle measurements were designed to be circular solid discs with diameter and thickness of 12 mm and 3.5 mm, respectively. Additionally, for mechanical testing and accuracy measurements lattice structures with a relative density of 0.33, strut size of 0.6 mm and pore size of 1.2 mm were created. All specimens were designed using SolidWorks and were exported to STL file format.

Scaffold Fabrication. All specimens were fabricated on a DTM Sinterstation 2500 ^{Plus} (3D Systems) using the same process parameter settings - layer thickness of 0.15 mm, part bed temperature of 35 °C, laser fill power of 10 W, outline laser power of 5 W and scan spacing of 0.15 mm.

Accuracy. Dimensions of struts manufactured in different building directions were obtained from scanning electron micrographs (Zeiss EVO LS15).

Surface characteristics. Surface topology and accuracy of the fabricated specimens were examined using scanning electron microscopy (SEM). Surface profile was obtained using optoNCDT laser profiler. Obtained data was analyzed in Matlab7.1 and surface roughness (Ra) was calculated.

Mechanical properties. Compressive mechanical properties of the fabricated specimens were tested to ISO 604 standards using a 5 kN load cell at crosshead speed of 1 mm/s and applying 1 N preload. Compressive modulus, yield strength and yield strain values were reported. Scaffolds were tested in a way that loading direction was parallel to struts fabricated in the X-building direction.

Contact Angle. The contact angle of water on the sintered surfaces was measured using ArtCAM 130 MI BW monochrome camera, and FTA200 contact angle analyzer software. Static approach was used to analyze initial contact angle, and contact angle evolution after 1, 2 and 3 s.

Statistics. All measurements were performed on 7 replicates. Result data was expressed as mean±standard deviation. Comparisons between groups were made using one-way ANOVA analysis. Statistical significance was set at $P<0.05$.

Results and discussions

Accuracy. The struts within the scaffolds showed great dimensional variance in the three manufacturing directions. Struts built in the X, Y and Z direction were measured to be 582±242, 977±36, 730±101 pm, respectively. High standard deviation values are the results of manufacturing induced deformations. All struts were designed to have a square cross section of 600 x 600 μm, however, the cross section of a single, randomly selected strut built in the X direction was reproduced as 411 x 750 pm rectangle with fillets on the corner. This is the result of the different scanning patterns in different building directions. Poor accuracy of micro-features fabricated by commercial SLS machines is also due to the fact the laser beam diameter (0.4 mm) is in the range of the fabricated feature sizes (0.6 mm).

The addition of HA had no statistically significant effect on the accuracy of the samples, however standard deviations increased with higher HA content, indicating decrease in reproducibility.

Surface properties. It has been shown that rough surfaces and high surface to volume ratio is favorable for cell attachment [2]. In terms of tissue engineering, an advantage of powder based manufacturing technologies including SLS, is that fabricated part surfaces are rough. Surface roughness is dependent on the particle size of the processed powder. Surface roughness (Ra) of PCL scaffold was 25±4 inn and was not significantly altered by the addition of HA. SEM images (Fig. 1, Fig. 2a) show neck formation between PCL particles indicating that the final densification stage of sintering where pores are elmininated from the designed solid regions was not reached. Therefore accuracy and roughness of the scaffolds were mainly determined by the particle size (125µm) of the PCL that was greater than that of HA (38 µm).

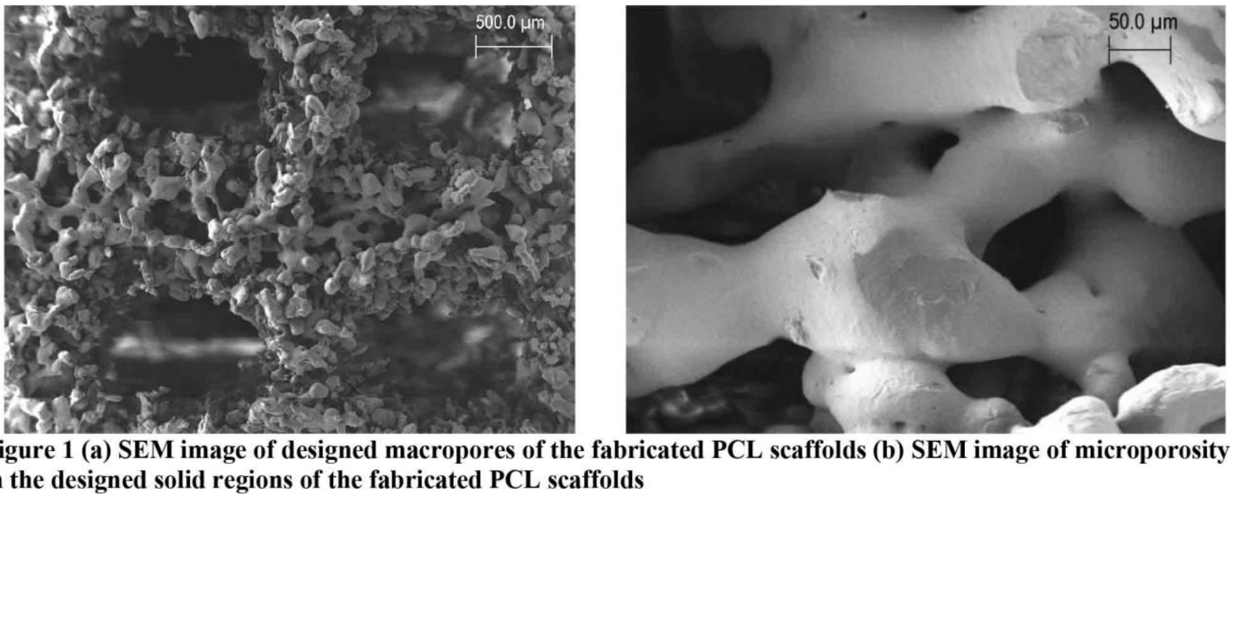

Figure 1 (a) SEM image of designed macropores of the fabricated PCL scaffolds (b) SEM image of microporosity in the designed solid regions of the fabricated PCL scaffolds

Contact angle. Initial contact angle of pure PCL scaffolds was 104±5.5° and was constantly decreased by the addition of HA. The initial contact angle for composites with 15 wt% of HA decreased to 98±13.6° and with 30 wt% HA to 83±16.9°. Results demonstrated the ability of added HA powder to reduce hydrophobicity of the PCL scaffolds. Contact angle of water on the surface of parts with 0 and 15 wt% HA did not change significantly in the examined 3 s interval; however specimens with 30 wt% of HA were completely wettable as the water drop was soaked in within 3 s. The hydrophobicity of the scaffolds was decreased and wettability was increased by the addition of HA making them more attractive for cell attachment [3].

Mechanical properties. It has to be noted that microporosity was present both in the designed solid disks and in the designed solid regions of the sintered scaffolds. Average measured relative density of the disks was 0.47. For the scaffolds the overall measured and designed values were 0.3 and 0.33, respectively, indicating that microporosity of the struts is lower than that of the disks. Reduction in porosity within the designed solid regions for parts with smaller feature sizes is the result of higher energy density being delivered to a given surface area due the more frequent outline scans.

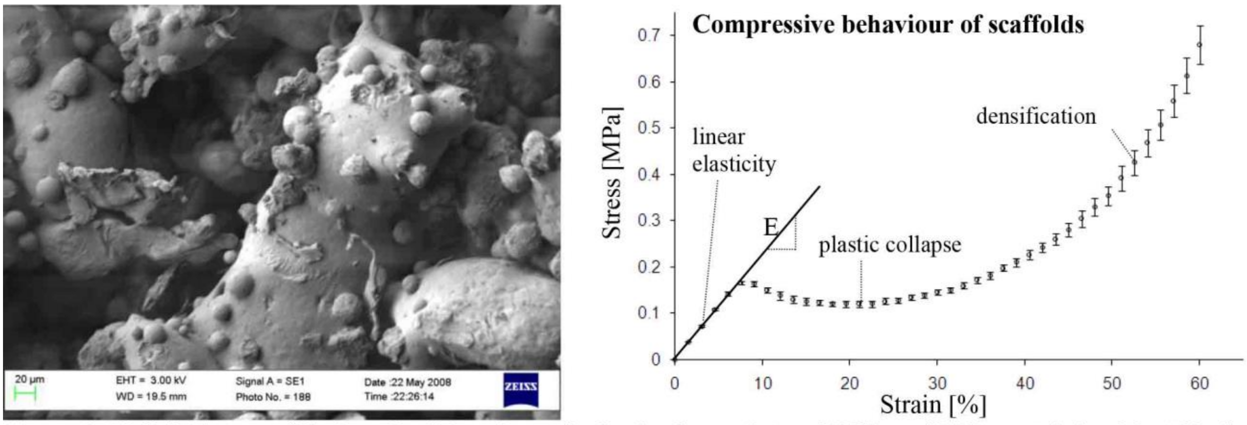

Figure 2 (a) SEM image of designed solid regions of selective laser sintered PCL scaffolds containing 30 wt% of HA, (b) Master curve of stress strain behavior of porous microstructures as exhibited by the fabricated scaffolds containing 15 wt% of HA

For the designed solid disks increase in HA content resulted in a linear increase in compressive modulus from 3.75±0.38 MPa for pure PCL scaffolds to 5.58±0.62 MPa for scaffolds with 30 wt% of HA content. Although in particle reinforced composites mainly the matrix bears the loading, HA particles inhibit the movement of molecular chains in the matrix [6], explaining the observed improvement of mechanical properties.

However, HA particles would agglomerate at higher concentrations, resulting in deterioration of compressive properties. In the compressive behavior of fabricated scaffolds three distinct regions can be identified [7]. As shown in Fig. 2b, the first region was a linear elastic slope characterized by elastic compression of the struts, followed by a plastic collapse region that marks the buckling of struts and formation of plastic hinges, finally further stress compressed the solid material itself, resulting in steep densification (Fig 2).

	PCL	15 wt% HA	30 wt% HA
E [MPa]	1.31±0.08	1.50±0.05	1.58±0.18
σ_y [MPa]	0.14±0.07	0.17±0.01	0.13±0.01
ε_y [%]	6.32±0.53	7.41±052	7.58±0.32

Table 1 Compressive mechanical properties (E, compressive modulus; σ_y, yield strength; ε, compressive strain) of selective laser sintered scaffolds with different HA content

Compressive properties for the scaffolds are summarized in Table 1. Addition of 15 wt% HA significantly improved their mechanical properties. With addition of 30 wt% HA yield strength decreased, however it must be noted that yield strain for all materials was over 6 % that is significantly greater than typical strains during physiological loading. Nevertheless the observed compressive properties are not sufficient for load bearing bone scaffolds but can be further improved by post processing.

Conclusions

The study demonstrated that bone scaffolds with predesigned external and internal architectures can be manufactured using SLS. The addition of HA made the PCL scaffold more suitable for tissue engineering applications by increasing mechanical properties and decreasing hydrophobicity. Compressive properties of scaffolds should be further improved by post processing of sintered samples.

Acknowledgments

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