Characterization of a novel bioactive composite using advanced X-ray computed tomography

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Abstract

A novel bioactive, porous silica–calcium phosphate nanocomposite (SCPC) that can be used to treat large bone defects in load-bearing positions has been tested and has shown great potential for applications in tissue engineering. Porosity is essential to the performance of the composite material as a tissue engineering scaffold, as porous scaffolds provide a physical, 3-D template to support new tissue formation. However, porosity characterization using conventional techniques such as porosimetry or scanning electron microscopy requires extensive preparation of samples and may destroy important features during preparation and analysis stage. In this work, the new composite is characterized using an advanced high resolution X-ray computed tomography, which is a non-destructive testing technique that allows construction of the 3-D topology of the microstructure. The results clearly show the effectiveness and versatility of this technique in characterizing the porous architecture of the novel composite biomaterial. The pore distribution, morphology and interconnectivity in the composite scaffolds were found to be ideal for use in tissue engineering applications.

Keywords: X-ray computed tomography; Porosity; Bioceramics; Bone-graft

1. Introduction

Tissue regeneration in large bone defects caused by pathological or traumatic conditions is a major challenge in orthopedic and maxillofacial surgeries. Recently, a novel, porous, resorbable silica–calcium phosphate nanocomposite (SCPC) has been proposed as a tissue engineering scaffold for drug delivery and bone regeneration [1,2]. The porosity of SCPC mimics that of trabecular bone as it possesses a wide pore-size distribution varying from nano- to micrometer range. Moreover, cell colonization and vascularization of SCPC graft were enhanced by the interconnectivity of the pores which also provided channels for tissue-fluid recirculation and removal of metabolic waste products [1].

Characterization of the porous architecture in scaffolds is essential in predicting their performance in vivo as well as for understanding the dynamic changes occurring in the texture and pore morphology during resorption. Porosity characterization of scaffolds has traditionally involved the use of mercury intrusion porosimetry for assessing the pore-size distribution, and gas adsorption for specific surface area analysis [3,4]. Hg porosimetry offers a distinct advantage as a wide pore-size distribution (800 μm–3 nm) can be determined, however, it is a destructive technique and analysis is limited by the quantity and/or shape of the samples. High pressures (up to 60000 psi) are used to force mercury into small pores and the pore-size...
distribution is determined from the volume intruded at each pressure increment. Use of such high pressure may destroy the initial porous architecture and thus the results may not be reflective of the original texture of the scaffolds. Moreover, data is strongly affected by the geometry of the pore-necks which introduces an error known as the “ink-bottle effect” [4–6].

Another technique commonly used to quantify the surface area in porous scaffolds is gas adsorption which is based on the volume of gas adsorbed (mostly N₂). An advantage of gas adsorption is that porosity in intricate-shaped samples can also be analyzed. It is useful in precisely determining the diameter of nanoporous materials in the range of 0.3–300 nm [7]. However, the technique needs extensive pre-analysis preparation and involves cooling the samples to extremely low temperatures (−196 °C) which may damage the samples. Similar to Hg porosimetry, gas adsorption technique provides information about the entire sample and can neither detect isolated pores in the scaffold nor discriminate connections between pores based on their diameters [7].

Scanning electron microscopy (SEM) can be also used to analyze matrix microstructure, pore morphology and interconnectivity. Though SEM images provide a high resolution, highly detailed views of the surface topology, an inherent weakness with SEM analysis is that it is limited to two-dimensional measurements on relatively small fields of view. Thus, only a fraction of the sample may be viewed at once and the analysis is more qualitative than quantitative. Moreover, SEM is also a destructive technique involving extensive sample preparation, and the analysis is limited by the shape and size of the sample. The aforementioned shortcomings associated with traditionally used techniques have prompted researchers to examine and develop other techniques to accurately characterize the porous architecture in scaffolds. X-ray micro-computed tomography (XRT), a non-destructive technique, is being increasingly used to provide structural information within an object by mathematically reconstructing its 3-D image from a series of projections [6]. It has been used previously to assess the porous microstructure of composites in materials science [6] as well as in medical applications to demonstrate 3-D bone microstructure [8], and to demonstrate the porous architecture in biomaterials [7,9–11]. The image analysis provides the ability to make quantitative measurements on complex structures. The material quantity and size limitations are also eliminated and the images can be taken at any required section of the sample with no extensive pre-imaging sample preparation. Though high resolution images can be obtained revealing distinct pore morphology and pore interconnectivity, an inherent disadvantage of this technique is that the minimum pore-size that can be measured is dependent upon the resolution and sensitivity of the machine. Although, with high resolution imaging techniques, pixels in the size range of 2 μm can be easily resolved [11]. Nonetheless, high resolution XRT analysis provides valuable complementary information that can be used independently or along with mercury intrusion porosimetry, gas adsorption and SEM analysis techniques to provide a holistic characterization of the scaffolds. In this study, we have used high resolution X-ray tomography to characterize the porosity in the 3-D architecture of SCPC composite scaffolds.

2. Materials and methods

SCPC powders were prepared as reported elsewhere [1,2]. Briefly, powders of three different compositions with varying amounts of silica [S1C9 (3.31 mol% SiO₂), S3C7 (10.74 mol% SiO₂) and S5C5 (19.49 mol% SiO₂)] were mixed with 20 and 40 vol.% polyethylene glycol (PEG). The particle size of the SCPC powder was between 45–150 μ and that of PEG was between 90–450 μ, with the PEG particle sizes being equally distributed in 90–250 μ and 250–425 μ range. The powders were thoroughly mixed and compressed under a uniaxial load of 360 MPa into green pellets 12 mm in diameter and 8 mm in height. The samples were then heated at 350 °C for 24 h to remove the porogen, and subsequently sintered at 800 °C for 1 h.

The porous scaffolds were then analyzed using a 3rd generation X-ray computed tomography machine. A 225 keV micro-focus tube at 0.1 mA current, with a theoretical maximum resolution of 5 μm/pixel, was used as the source. The source–detector and the source–sample distances were critical in determining the magnification of the Fig. 1. Advanced X-ray tomography has been used to reconstruct the 3-D image of composite S1C9 using multiple angle projections. The technique can be used to recreate the porous architecture of intricate-shaped composite scaffolds.
sample, and thus the resolution of the scan. The closer the sample was to the source, and the further away from the detector, the higher the magnification. Once the sample was loaded, a conical X-ray beam scanned the total height of the sample, which was then rotated a fraction of a degree and scanned again. This procedure was then repeated until a full rotation of 360° was achieved, and enough raw images were then obtained to be used in the 3-D profile generation.

The data was acquired using Data Acquisition System V2.1.4 and then processed in an 8 CPU server using Data Processing System Version 1.3.10. A 3-D volume of the ceramic was generated using FlashCT-VIZ Version 2.4.2 Build 4. Cross-sectional slice were selected in the samples and the images obtained were analyzed using Image Pro Plus Version 5.0.2.9. The selected images were then transferred to the ImagePro software, where they were converted to grayscale images, and the pores were characterized based on the differences in the grayscale values.

3. Results

X-ray tomography was used to reconstruct the 3-D images of the composites using projections from multiple angles. Fig. 1 illustrates the reconstruction of S1C9 prepared with 40 vol.% PEG. Fig. 2 shows high resolution scans obtained for SCPC composites prepared with 40 vol.% PEG. Imaginary cross-sectional slices were obtained in the middle region of the scaffolds. The maximum resolution for the scans was 19 µm/pixel. The pores appeared as dark spots on a white background that represented the composite matrix. The pore-size distribution amongst larger pores (100–1000 µm) was also analyzed for S1C9 and S3C7 as shown in Fig. 3. As the maximum resolution achieved was about 19 µm/pixel, the smaller pore-sizes could not be analyzed with accuracy, hence they were not included in the result.

Fig. 4 shows the XRT scans for S1C9 prepared with 20 vol.% PEG. As expected, the porosity in the composite...
matrix was reduced significantly. Fig. 5(a) shows the 3-D orthogonal XRT scans for composite S1C9 prepared with 20 vol.% PEG. Obtaining an orthogonal scan for the scaffold matrix is essential as it shows the pore morphology in the 3-D architecture of the scaffold. It can be observed that the pores were uniformly distributed in the horizontal as well as vertical sections of the scans. Further breakdown of the orthogonal scan is shown in Figs. 5(b) and (c) which show pore distribution in the $x$–$y$ and $y$–$z$ plane, respectively. These images again show a homogenous distribution of pores in all the representative areas of the composites. Fig. 6 shows an in situ density analysis for S1C9 prepared with 40 vol.% PEG. The density profile which has been superimposed on the image shows a zero value at the location of the pores, and peaks of equal height for the various positions in the ceramic matrix indicating a uniform density of the composite matrix.

4. Discussion

X-ray tomography provides a non-destructive way to reproducibly create 3-D representations and analyze the structural characteristics of composite materials. A 3-D reconstruction of the sample profile as shown in Fig. 1 can be extremely useful in analyzing the topological features of scaffolds with intricate shapes designed to mimic the complex three-dimensional features of bone. The flexibility of selecting any section in the bulk of the material and to analyze the pore morphology and distribution gives a distinct advantage to X-ray tomography as compared to other techniques like mercury porosimetry or gas adsorption. All SCPC composite scaffolds showed presence of homogeneously distributed pores in a wide pore-size range (Fig. 2). The amount of microporosity in SCPC composites appeared similar, and this was consistent with a previous analysis of porosity in the composite scaffolds using mercury porosimetry which had indicated similar percentage of micropores present in all the porous SCPC samples prepared with 40 vol.% PEG [12]. Pore-size distribution for S1C9 and S3C7, calculated from XRT images showed a similar trend (Fig. 3), and this was in agreement with previous porosity analyses using Hg porosimetry and gas adsorption techniques [12]. However, we are cognizant of the fact that porosimetry and XRT results would not match exactly, as the mercury porosimetry measures only those pores that are accessible to the pressurized mercury where as XRT measures both connected and isolated pores contributing to the total pore volume. The lower estimations of XRT can be related to the resolution of the imaging system that will not be able to calculate the submicron and nano-scale pores, and hence ignore their contribution.

As the porogen concentration was reduced from 40 to 20 vol.% PEG, there was a significant reduction in the final porosity of the scaffolds. As shown in Fig. 4, the pore density and interconnectivity in the composite matrix reduced substantially. However, the pores were homogeneously distributed and exhibited a wide size range within the scaffold. Three-dimensional orthogonal scans of S1C9 prepared with 20 vol.% PEG (Fig. 5) also demonstrated that the pores were uniformly distributed in the entirety of the scaffolds. Moreover, the pores were shown to possess a 3-D morphology that would help in the three-dimension bone ingrowth in the composite matrix when these scaffolds are used for tissue engineering applications. Presence of a wide pore-size range would also help in a controlled resorp-
tion and mechanical behavior of the scaffolds in vivo. XRT also provides an extremely useful feature of an in situ assessment of the density profiles (Fig. 6) of the composite scaffolds. This would give a distinct advantage to researchers in analyzing hard bone samples as it would allow for an assessment of bone density even when the porous architecture of the hard tissue is being characterized.

5. Conclusion

SCPC composite scaffolds contained a homogeneous distribution of interconnected pores with 3-D morphology within a resorbable, bioactive matrix, making it ideally suitable for applications in tissue engineering. Image analysis using high resolution X-ray tomography provided a detailed characterization of the porous architecture in the scaffolds. XRT provides a non-destructive way to analyze the total porosity, pore-size distribution and pore morphology in tissue engineering scaffolds. A homogenous distribution of pores in a wide pore-size range was observed in all the SCPC scaffolds. Three-dimensional orthogonal scans of scaffolds helped to characterize the morphology and porosity in the scaffold matrix. This method of porosity characterization is not afforded by other conventionally used porosity analysis techniques. Moreover, X-ray tomography offers a unique capability to analyze the tissue ingrowth and monitor the dynamic changes occurring in pore morphology of resorbable scaffolds in vivo. Thus, high resolution X-ray tomography can be an excellent complementary tool to the existing technologies for characterization of porosity in composite materials.

Currently, work is underway to analyze the changes that occur in the pore morphology and pore-size distribution in porous SCPC scaffolds subjected to different stress patterns that simulate real-life load bearing conditions in human adults.

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Fig. 6. In situ density profile assessment for S1C9 prepared with 40 vol.% PEG. The density profile has been superimposed on the XRT sectional image. The base line (indicated by arrows) represents zero density. The density profile consists of equal intensity peaks for locations of the composite matrix and shows a zero density value at the pore locations.

References