Micro-Computed Tomography Technique to Study a Porous Biphasic Scaffold

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Statement of purpose: Various techniques have been utilized to characterize the structural properties of scaffolds used in tissue engineering including theoretical assessments, scanning electron microscopy (SEM), porosimetry, and microcomputed tomography (µCT). Micro-CT works well with scaffolds made of dense materials such as ceramics. However, previous attempts to use standard µCT techniques on polymeric scaffolds have suffered from poor imaging contrast, even with the use of image enhancing agents, e.g. ferrocene. The evaluation of pore interconnectivity has proven to be especially challenging. In this study, high resolution µCT analysis was carried out on a biphasic PLGA/TCP and PLGA scaffold to characterize scaffold properties such as porosity, pore size distribution, and interconnectivity (Figure 1). Theoretical assessments and SEM analysis were utilized to corroborate the µCT results.

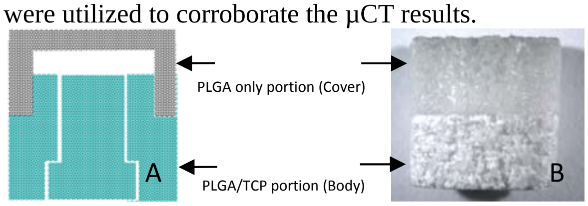

Figure 1. (A) Schematic representation of the biphasic scaffold with a PLGA cover and a PLGA/TCP body. (B) Macroscopic image of the biphasic scaffold.

Methods: The scaffolds were prepared by a solvent merging/particulate leaching process (Liao C. J Biomed Mater Res. 2002;59:676-681.). Scaffolds were analyzed using SEM, a manual theoretical estimation, and µCT. SEM: The general pore size and material structure were evaluated by SEM. The diameter of surface pores were measured from the digital SEM image (Figure 3B). Theoretical Calculations (TC): The scaffold was cut into two sections; a PLGA-only section (Cover), and a composite section comprised of a 2:1 ratio of PLGA to TCP (Body) (Figure 2A). Porosity was calculated using physical mass and volume measurements together with the bulk density properties of the materials. µCT Analysis: The samples were scanned on a high-resolution, volumetric µCT scanner (µCT40, ScanCo Medical, Zurich, CH). The image data was acquired at 6 µm isotropic voxel resolution. 2D analyses and 3D reconstruction of the scaffolds were performed utilizing a proprietary algorithm (Numira, Salt Lake City, Utah). To correlate µCT results with the manual (theoretical) porosity calculation method, two distinct regions of interest (ROI's) were defined in the µCT reconstructions (Figure 2B). Details of the pore size distribution, porosity and the number of disconnected pores were provided by the µCT analyses.

Results: SEM analysis reveals that the scaffold contains many pores with a wide range in sizes from 57 – 392 µm (Figure 3). The porosity of both the cover and body of the scaffold from the theoretical assessment were consistent across both samples (Table 1). As seen with SEM, micro-CT confirmed that the pores have a wide range in sizes with Dn10, Dn50, and Dn90 of 8.2, 59.9, and 246.4 µm respectively (Table 2). The porosities calculated using two different methods were very similar. Micro-CT analysis also revealed that the majority of the porous volume of the scaffold connects to exterior surfaces (Table 1).

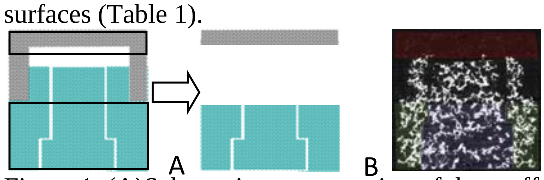

Figure 1. (A)Schematic representation of the scaffold preparation for theoretical assessment. (B)MicroCT image representing the areas used to calculate the porosity of the scaffold to mimic the theoretical assessment.

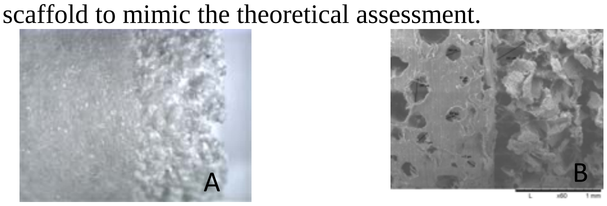

Figure 3. (A) Macroscopic image of the biphasic scaffold. (B) SEM image of the biphasic scaffold with visible pores ranging in size from 57 – 392 µm.

Table 1. Summary of analyses from µCT scans and theoretical calculations (TC).

Parameter	Body-1	Cover-1	Body-2	Cover-2
Volume of disconnected pores	0.277 mm^3	0.004 mm^3	0.254 mm^3	0.001 mm^3
# of disconnected pores	1591	33	1840	21
%Connected porosity	99.8	99.9	99.8	99.9
Porosity (µCT)	71.0	87.1	61.4	88.1
Porosity (TC)	75.0	90.3	75.8	91.1

Table 2. Summary data of the number distribution width based on diameter.

Distribution Width	Diameter (µm)
Dn10	8.2
Dn50	59.9
Dn90	246.4

Conclusions: Porosity results obtained with µCT generally agreed with SEM and theoretical assessments. This proprietary µCT technique also provides insight into the interconnectivity of the pores. Future work will be carried out to increase the sample size. Micro-CT analysis is a viable method to evaluate scaffold features with the added benefit of being a non-destructive test.

Reference:

(Liao C. J Biomed Mater Res. 2002;59:676-681.)