QUANTITATIVE ANALYSIS OF A CANDIDATE POROSITY REFERENCE SCAFFOLD: TYPE 1

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INRODUCTION

Porosity of tissue scaffolds is universally accepted as an important parameter. However, there is significant variability concerning which aspects of porosity are important, or how best to measure them. We have initiated a collaborative project of testing and analysis through ASTM (F04.42.06) to establish a reference material for scaffold porosity. There are currently three candidates for consideration for the reference scaffolds. Image analysis will be the primary characterization method for reference materials. Preliminary collaborative work will lead to the establishment of parameters for image analysis, establishment of ancillary test methods, and selection of a manufacturing method for fabrication of reference scaffolds.

In this work, we are performing three-dimensional quantitation on a candidate reference scaffold image set. The Type 1 scaffold is designed by Case Western Reserve University (CWRU) and manufactured by 3-D Systems, Inc. (Valencia, CA) using stereolithography.[1] We present an analysis of the four types of unit cells that exist within the Type 1 scaffold and theoretical values for total pore volume, pore volume distribution and pore size distribution. We also extract these descriptors from image analysis of the original computer aided design (CAD) model and from X-ray micro-computed tomography (μ-CT) image data for the scaffold designated as Part #2.

EXPERIMENTAL

Scaffold Fabrication

The scaffolds were fabricated using a 3D Systems' Viper SLA machine which consists of a servomechanism to control the beam of a solid-state UV laser in the X and Y horizontal axis, a tank containing a liquid monomer resin, and a motorized build table that travels vertically in the Z-axis up and down in the tank of resin. The CAD model is first sliced into layers by pre-processing software (3D Systems' Lightyear) and the data is downloaded to the Viper SLA machine. The layer profile data is used to control the laser and when the beam focuses on the resin surface it causes the resin to polymerize. The build table is lowered into the liquid Accura si 10 resin one layer thickness at a time after each layer has been polymerized.

X-Ray Micro-Computed Tomography

The μ-CT images were generated by a Skyscan 1072 micro-computed tomography scanner. The data set was output as individual, 2D bitmap files. In this work, the error is a type B standard uncertainty from the resolution limit of the instrument and is 3.2%.

Image Analysis

A 3-dimensional image analysis package called Blob3D was used to extract pore size distribution and pore volume distribution for the CAD model scaffold and for the μ-CT imaging data of Part #2. Blob3D was written by R. Ketcham and his group using Interactive Data Language (IDL, Research System, Inc.).[2] Blob3D is designed for processing large three-dimensional data sets and extracting morphological information. We used it extract unit cells (UCs). For our data, pore volume (PV) was found from each unit cell. Then, each unit cell was fit with an ellipsoid to extract the long and short axis lengths. The long axis was used as the pore length (PL).

RESULTS

The CAD model of reference scaffold Type 1 is shown in Figure 1A along with the μ-CT imaging results from Part #2 in Figure 1B. The image in Figure 1B selected for quantitative analysis is a subset of the whole image and was cropped to eliminate the mounting medium from the images. In both images, pores are represent as white and scaffold as gray or black. From the design specifications, the scaffold is 7.1 mm on each side and consists of 6 pores along each side. Each square-shaped pore is designed to be 600 μm on a side. The distance from the surface to a pore and between the pores is 500 μm.

To further understand the μ-CT imaging results, a theoretical analysis was performed on the scaffold using only the specified dimensions to extract the quantities of interest. The Type 1 scaffold has 216 pores distributed into 4 distinct UCs found by Blob3D which are shown in Figure 2. There are 64 UCs shown in Figure 2A found in the interior of the scaffold. These UCs have a PV of 0.756 mm^3 and PL of 1.1 mm. Figure 2B displays a UC found on each face of the cube with 1 long axis. There are 94 of these UCs that have a PV of

0.846 mm^3 and a PL of 1.35 mm. The UC in Figure 2C has 2 long axes and a PV of 0.936 mm^3. There are 48 of these UCs in the scaffold with a PL of 1.35 mm. Lastly, there are 8 UCs shown in Figure 2D that are found on each corner of the scaffold. These unit cells have a PV of 1.026 mm^3 and a PL of 1.35 mm. From this analysis, the theoretical total pore volume is 51.1 volume %. The PV distribution and PL distribution will be shown in the Figures 3 and 4 with other data.

The Blob 3D image analysis software was used to extract the aforementioned quantities from the Type 1 scaffold constructed from the CAD model. This was done as a comparison to the μ-CT results and for insight into any bias introduced by the image processing software itself. From the Blob3D analysis of the model CAD data, the total pore volume is 53.4 volume %. An identical analysis was performed on the Part #2 imaging data which resulted in a total pore volume of (48.7 $\pm$ 1.6) volume %.

Figure 3 displays the PV distribution (A) and PL distribution (B) for the theoretical, CAD model and Part #2 scaffolds. The CAD pore volumes and diameters are $\approx$5 to 10 % larger than the theoretical results while the distribution between the theoretical and CAD results are virtually identical. The results extracted from the μ-CT images of Part #2 have a broad distribution when compared to the theoretical and CAD results. For the pore size distribution, the average PL for the theoretical values is 1.28 mm, for the CAD model is 1.35 mm, and for the Part #2 data is (1.45 $\pm$ 0.05) mm. For the PV distribution, the average for the theoretical values is 0.849 mm^3, for the CAD model is 0.889 mm^3, and for the Part #2 data is (0.800 $\pm$ 2.6 x 10^{-5}) mm^3.

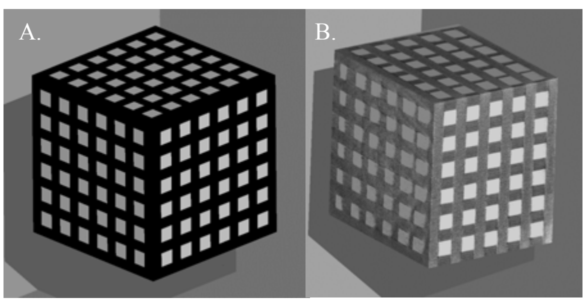

Figure 1: Type 1 reference scaffold. A) Reconstructed from CAD, B) Partial data set from X-ray images of Part #2.

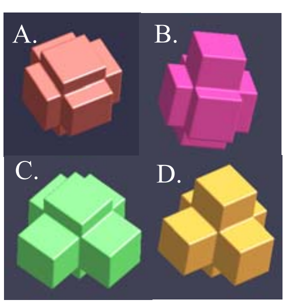

Figure 2: Four types of unit cells found in Type 1 scaffold. A) No long axis, B) 1 long axis, C) 2 long axes, D) 3 long axes.

CONCLUSIONS

We have established a method to understand the structure of a candidate reference scaffold. This is done through analysis of the theoretical and CAD derived quantities and by comparison to μ-CT data. Total pore volume, pore volume distribution and pore size distribution are the initial quantities of interest. This approach can be consistently applied to the four scaffolds under consideration.

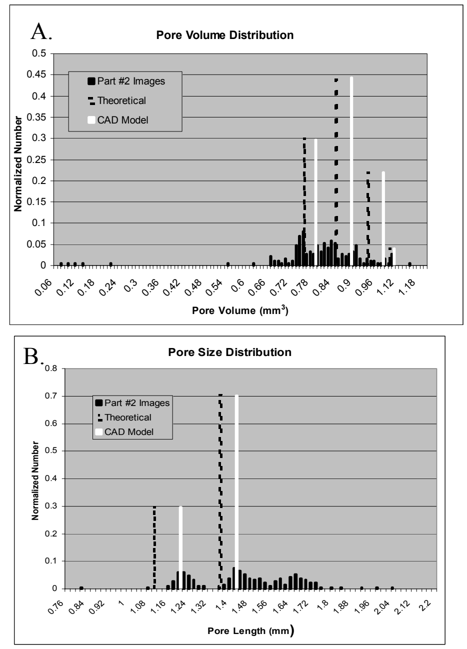

Figure 3: A) Pore volume distribution and B) pore size distribution for the Type 1 reference scaffold.

REFERENCES

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