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# 3d, Quantitative Image Analysis Of Poly(ε-caprolactone) Scaffolds With Bicontinuous Morphology

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# Introduction

It has been shown that scaffold microstructure impacts cell response.<sup>1</sup> Determining the relationship between scaffold structure and cellular response facilitates scaffold design. Some descriptors of scaffold morphology thought to be influential are pore volume, pore size distribution, connectivity, curvature, and surface area. In this work, we apply methods developed for 3-dimensional (3D) characterization of polymer blends<sup>2</sup> as bicontinuous structures to elucidate microstructural descriptors for a similarly derived poly( $\varepsilon$ -caprolactone) (PCL) scaffold. The results here will include pore volume, pore size distribution, and connectivity. Surface curvature, surface area and anisotropy will be discussed in future work.

## **Materials and Methods**

Details about the scaffold preparation process can be found in previous work.<sup>3</sup> In summary, the scaffold is prepared from a blend of PCL and polyethylene oxide at 50% mass fraction. The scaffold was placed in water for 5 h. to dissolve the PEO and then dried. The X-ray images were generated by a Skyscan 1072 micro-computed tomography ( $\mu$ -CAT) scanner. The data set was output as individual, 2D bitmap files.

## **Results and Discussion**

A subset of the X-ray  $\mu$ -CAT data was analyzed with the dimensions of (342±3)  $\mu$ m (mean ± std. uncertainty) along each side of the image cube. The scaffold/pore interface was found by

thresholding and then using a marching cubes algorithm.<sup>4</sup> A thinning algorithm<sup>5</sup> was then applied to the data cube to create a skeletonized network of the pore structure, which is necessary for the analysis. The X-ray results along with the skeletonization (red) are shown in Figure 1A. The skeletonized network only is displayed in Figure 1B. The pore volume was computed to be 54%.





Figure 1: X-ray image cube from PCL scaffold (grey) and skeletonized pores (red) (A), skeletonized network only (B).

Figure 1. X-ray image cube from PCL scaffold (grey) and skeletonized pores (red) (A), skeletonized network only (B).

The pore size distribution (Figure 2) is obtained by taking the shortest distance from a point on the skeletal network to the interface. This distance corresponds to the radius of the pore. The average pore size is  $(13.8\pm1.4) \mu m$  with a standard deviation of  $(6.1+0.6) \mu m$ .



# Figure 2: Pore size distribution in PCL scaffold.

Figure 2. Pore size distribution in PCL scaffold. Figure 3 displays a histogram of the coordination number at the junctions. present at each branch point. The three branch is dominant, which is similar to what is observed for spinodally phase separated polymer blends.



Figure 3: Histogram of coordination numbers for PCL scaffold. Figure 3. Histogram of coordination numbers for PCL scaffold.

#### Conclusions

We have computed several microstructural descriptors of a PCL scaffold that exhibits a bicontinuous morphology. Other descriptors computable with this method that are not described here are also of interest. From the skeletonization, the pore tortuosity can also be extracted when the linear and path length are measured between junction points.

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