MECHANICAL TESTING METHODS OF CUBIC SUB-SIZED COMPRESSION SAMPLES FOR POLYMERIC TISSUE-ENGINEERED SCAFFOLDS

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INTRODUCTION

The work presented here details a method for testing polymeric tissue-engineered (TE) scaffold samples in compression and the errors encountered when measuring the stress-strain response. Biodegradable, polymeric scaffolds have shown promise for use in the repair of damaged tissues such as arteries, cartilage, bone, and skin, for examples see [1-3]. The engineered tissue, for a time at least, consists of the scaffold with cells grown in/on it. The engineered tissue when implanted must endure the mechanical stresses applied by the body. Therefore, the mechanical properties of the engineered tissue must be known. Straightforward as the mechanical testing may seem, care must be taken in order to produce reliable data. These same methods used to produce the reliable data can also be used to ensure the quality of the scaffold before it is implanted. This work presents a method for accurately measuring the compression properties of polymeric scaffold materials to the precision that the materials will allow.

It has long been known that using the crosshead displacement to calculate the strain (termed crosshead strain here) in tension or compression testing can lead to errors because of deflection of the tensile machine or imprecisely machined specimens [4]. However, crosshead strain can be used to make comparisons between materials if the materials have about the same stiffness. Thus, crosshead strain can be used for quality control. Crosshead strain is, in fact, the standard method for measuring the compressive properties of rigid cellular plastics [5] and has been used to measure the properties of TE scaffolds [6-8].

Here we used image correlation techniques to measure strain directly during compression tests of a copolymer scaffold. The errors inherent in testing these materials in compression are discussed within the limitations of these types of materials and the difficulties of sample preparation.

METHODS

A table-top, 5000 N capacity servo-hydraulic, materials-testing machine was outfitted with 20 mm diameter platens machined flat and parallel to 0.005 mm with a surface finish of 4×10^{-4} mm. The displacement of the hydraulic actuator was measured with an LVDT with 0.04 mm rms absolute error. The load was measured with a ±5000 N load cell calibrated at 10 % of its range (±500 N). The absolute error for the load cell is less than 0.4 N. A video microscope imaged one face of the specimen during the test at about 75x. The samples were tested in displacement control with the crosshead velocity set to 3×10^{-3} mm/s for a nominal strain rate of 1×10^{-3}/s. The video images were captured every 2.14 s.

The material tested here was made by co-extruding poly(e-caprolactone) (PCL), a biodegradable polyester, and poly(ethylene oxide) (PEO) as described in [9]. The samples were machined into cubes nominally 3 mm on a side, then agitated in distilled water to dissolve away the water soluble PEO. The samples were dried in a vacuum desiccator, and to increase the contrast of features on the sample, the side of the sample facing the microscope was striped with about 12 nm of gold-palladium. The samples were again soaked in water and the tests were conducted with the specimens wet (as they would have to be if tissue were grown in the scaffold). Each pixel represented about 8 μm of the specimen.

The average strain in the sample was calculated from the displacement of the data provided by the LVDT as well as image correlation of the video images. The image correlation was done by selecting a region of 384 x 288 pixels in the center of the specimen. The region was then subdivided in 32 x 32 pixel subimages. A displacement vector was estimated for each subimage by maximizing the correlation between the base image and the deformed image using bi-cubic splines to interpolate between pixels. Lines were then fit to each column (or row) of squares that were vertically (or horizontally) adjacent. Assuming that the strain was uniform, the slopes of these lines were averaged to estimate the normal strains in the sample. To keep the displacements to a few pixels or less the base image was updated every tenth frame or 21 s. The absolute displacement was maintained by adding the displacement of subsequent frames to the displacements that were already calculated for the new base image.

RESULTS

The microstructure of the samples showed irregular, interconnected pores 50 to 150 μm in diameter (Figure 1). The pores were clearly visible on the video images collected from the tests (Figure 1). The gold-palladium raised the contrast of the pores, but the subimages from the gold-palladium free (whiter) areas gave about the same correlation as from the coated areas. An image of the displacement field superimposed on the subimages is shown in Figure 2. The stress-strain curve is typical of a cellular polymer (Figure 3).

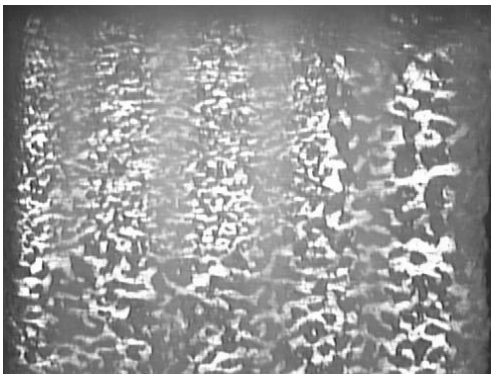

Figure 1. Video microscope image of the sample collected during the compression test.

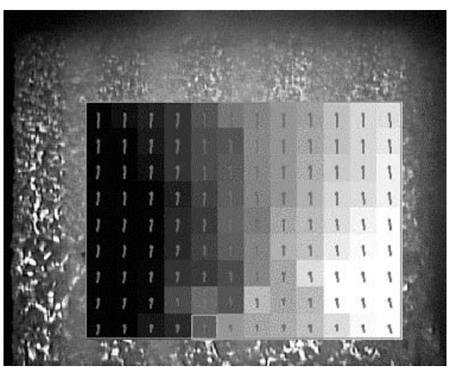

Figure 2. The displacement vectors superimposed on the subimages.

The largest error is in the measurement of strain. The stress measurement consists of the measured force divided by the cross sectional area of the sample. Even with the oversized load cell we used, the force is measured to 1% at peak load. If the sample is not machined perfectly, a large error in the strain can occur when the platen first comes into contact with the sample. (Because tissue will be grown into these samples, we do not expect perfect sample geometry, flat and parallel to 0.005 mm.) However, after a short time the small area of the sample where the platen makes contact will deflect and the full face of the sample will come into contact. The stress curve will have a steep section at low stress but the resulting modulus should not be affected dramatically.

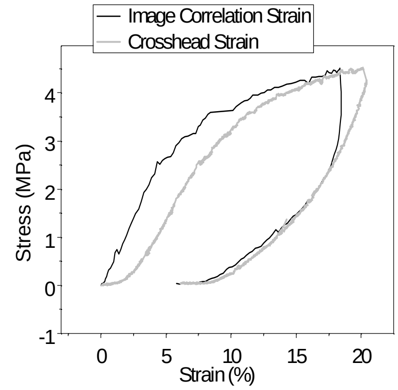

Figure 3. Typical stress strain curve for the material.

The difference between the strain measured from the image correlation and the crosshead strain for sample 4 (Figure 3) is about 2 %; this illustrates the large errors in strain that can occur by using crosshead strain. Using image correlation, we can also measure Poisson's ratio directly (measured for the material tested here as 0.33 ± 0.06).

SUMMARY

Small (3mm cubic) samples can be used for compression testing of TE scaffold constructs. Care must be taken to measure the strain accurately using a separate sensing method rather than measuring strain from crosshead displacement. We successfully imaged the sample and used image correlation to measure the strain.

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