Measuring Cell Adhesion and Proliferation in Polymer Scaffolds by X-Ray Microcomputed Tomography

Shauna M. Dorsey[1,2], Sheng Lin-Gibson[1], Carl G. Simon, Jr.[1]
[1]Polymers Division, National Institute of Standards and Technology, Gaithersburg, MD, USA
[2]Biochemistry Department, University of Maryland, College Park, MD, USA

Statement of Purpose: We have investigated the ability of X-ray microcomputed tomography (μCT) to make three-dimensional (3D) measurements of cell adhesion and proliferation in polymeric tissue engineering scaffolds. Standard methods for measuring cell number in scaffolds include fluorescence microscopy and soluble assays for enzymes, protein or DNA. Microscopy yields two-dimensional images of cell distribution but is not quantitative. Confocal microscopy can generate 3D images of cell distribution, but cannot penetrate opaque scaffold struts to image the interior of scaffolds. Soluble assays are quantitative but do not yield images of cell distribution. In contrast, μCT generates 3D images, can penetrate deep into the scaffold interior and is quantitative [1]. For these reasons, we have investigated μCT for imaging cells in tissue scaffolds.

Methods: Poly(ε-caprolactone) (mass averaged relative molecular mass 80 000) (PCL) scaffolds with 97 % porosity were made in 96-well plates by salt-leaching as described [2,3]. Scaffold structure was characterized by scanning electron microscopy (SEM) and gravimetrics. MC3T3-E1 osteoblasts (Riken, Japan) were seeded on scaffolds in 96-well plates at several concentrations (cells per scaffold: 0, 5000, 10 000, 25 000, 100 000, 400 000) and cultured for 1 d, 7 d or 14 d as described [2,3]. Six scaffold replicates were used for each treatment. Cell adhesion and proliferation on scaffolds were assessed using microscopy, a soluble assay and μCT. For microscopy, cells were fixed and nuclei stained with Sytox green as described [2,3]. For a soluble assay, cells were lysed and the Picogreen DNA assay was used according to manufacturer's instructions (Invitrogen). For μCT, scaffolds were fixed, stained with osmium tetroxide [1] and imaged (Scanco μCT 40, 55 kVp, 145 μA, 8 μm voxel resolution, 0.3 s integration, 325 slices, sigma 1.2, support 1.2, threshold 34). Osmium staining increases cell contrast in μCT. Threshold 34 was used because at this value the signal from cells was 20X higher than background when comparing voxel intensity histograms from scaffolds with cells to scaffolds without cells.

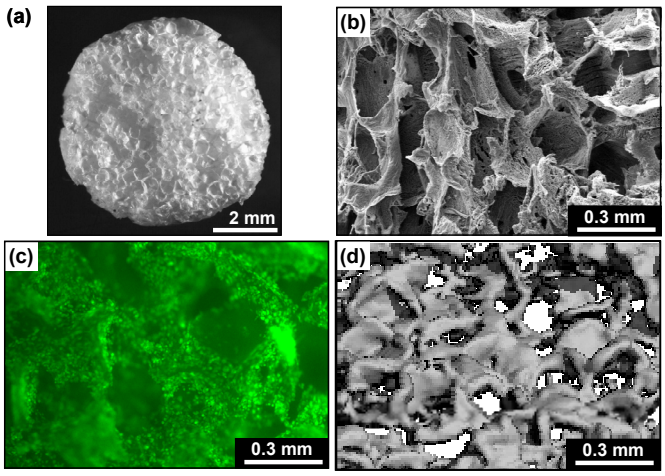

Fig. 1. Stereomicrograph (a) and SEM (b) of a scaffold. Fluorescence microscopy (c) and μCT (d) image of 400 000 cells cultured 1 d on scaffold.

Results: A scaffold that has been removed from the 96-well plate is shown in Fig. 1a. SEM shows that large pores (≈300 μm) were present in scaffolds (Fig. 1b). Fluorescence microscopy, Picogreen assay and μCT were in good agreement with one another showing that cells adhered and proliferated in the scaffolds under all conditions (not shown). In Fig. 1c,d, a fluorescence micrograph and μCT image of 400 000 cells cultured 1d on a scaffold are shown at the same size scale. The micrograph has better resolution but the μCT image is 3D and its cell volume can be quantified. μCT volume analysis of osmium-stained cells on scaffolds showed that greater than 25 000 cells must be seeded on a 96-well scaffold in order for the cell volume to be significantly different from background (not shown). A 3D μCT image of cells distributed in a scaffold is shown in Fig. 2.

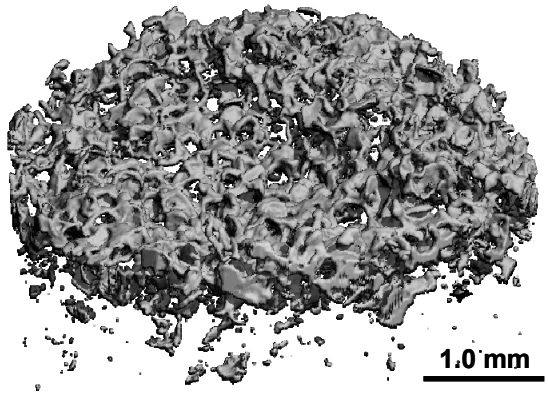

Fig. 2. μCT image of 400 000 cells cultured 1 d on a scaffold. Note: threshold was set so that 95% of the voxels shown are attributable to cells and not the scaffold.

Conclusions: μCT can measure cell adhesion and proliferation in tissue scaffolds yielding quantitative volume analysis and 3D images of cell distribution.

Acknowledgements: We acknowledge Nancy Lin, Jirun Sun, Yanyin Yang, Diana Zeiger (NIST) and Morgan Alexander (U. Nottingham) for critical input. S.M.D. acknowledges the NIST-NSF summer undergraduate research fellowship (SURF). This work was supported by NIST and NIH/NIBIB R21 EB006497-01. This article, a contribution of the NIST, is not subject to US copyright. Identification of instruments and materials in this paper does not imply recommendation by NIST, nor does it imply the materials are the best available for the purpose.

References:

1. Barry JJA, Howard D, Shakesheff KM, Howdle SM, Alexander MR (2006) Adv Mater 18, 1406.
2. Simon Jr CG, Stephens JS, Dorsey SM, Becker ML (2007) Rev Sci Instrum 78, 072207-1.
3. Yang Y, Becker ML, Bolikal D, Kohn J, Zeiger DN, Simon Jr CG (2008) Adv Mater 20, 2037.