

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

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Statement of Purpose: We have investigated the ability of X-ray microcomputed tomography (μ CT) 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, μ CT generates 3D images, can penetrate deep into the scaffold interior and is quantitative [1]. For these reasons, we have investigated μ CT 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 μ CT. 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 μ CT, scaffolds were fixed, stained with osmium tetroxide [1] and imaged (Scanco μ CT 40, 55 kVp, 145 μ A, 8 μ m voxel resolution, 0.3 s integration, 325 slices, sigma 1.2, support 1.2, threshold 34). Osmium staining increases cell

contrast in μ CT. 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.

Results: A scaffold that has been removed from the 96-well plate is shown in Fig. 1a. SEM shows that large pores (≈ 300 μ m) were present in scaffolds (Fig. 1b). Fluorescence microscopy, Picogreen assay and μ CT 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 μ CT image of 400 000 cells cultured 1 d on a scaffold are shown at the same size scale. The micrograph has better resolution but the μ CT image is 3D and its cell volume can be quantified. μ CT 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 μ CT image of cells distributed in a scaffold is shown in Fig. 2.

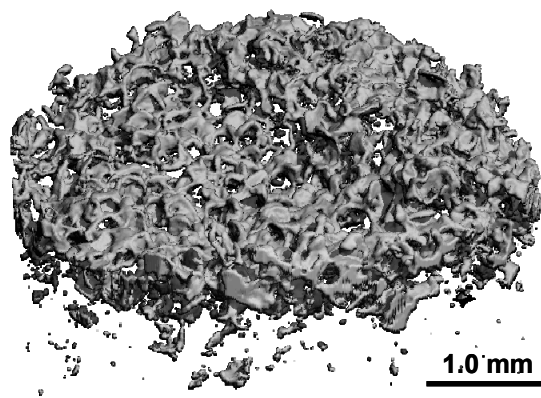


Fig. 2. μ CT image of 400 000 cells cultured 1 d on a scaffold. Note: threshold was set so that 95% of the voxels shown are attributable to cells and not the scaffold.

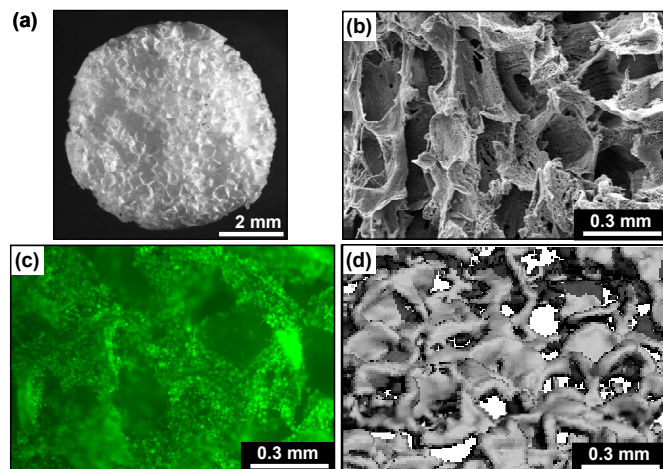


Fig. 1. Stereomicrograph (a) and SEM (b) of a scaffold. Fluorescence microscopy (c) and μ CT (d) image of 400 000 cells cultured 1 d on scaffold. (d) Fluorescence micrograph of 400 000 cells cultured 1 d on a scaffold.

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

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