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Synchrotron computed microtomography as a tool for the assessment of cell proliferation in polymer scaffolds for tissue engineering

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Background, Motivation and Objective. Tissue engineering is commonly defined as the interdisciplinary field that combines cells, biomaterials and different types of stimuli to repair or replace damaged tissues and organs. Such biomaterials are usually employed in the form of scaffolds, structures that should provide initial support for cell adhesion and proliferation, and then degrade as the neotissue develops. One of the key factors for tissue formation is the scaffold's porosity, which must allow cell penetration and a proper diffusion of nutrients and oxygen. While 2D assessment of both microstructure and tissue growth is considered routine, 3D visualization is more complicated and less accessible. Among the currently adopted techniques, computed microtomography (microCT) stands out for being non-destructive and suitable for a wide variety of materials, but it has not been sufficiently explored and is far from being standardized (DOI:10.1242/jcs.179077). This work aimed to test the ability of microCT to assess cell proliferation in fibrous polymer scaffolds, using two different contrast agents, osmium tetroxide (OsO_4) and potassium iodide (KI).

Methods. Fibrous mats were obtained using the solution blow spinning technique. Poly(□-caprolactone) (PCL) solution (6% m/V in chloroform) was loaded into a commercial airbrush connected to an air compressor, and pressure was set at 30 psi. Aluminum foil was used as a target and the distance from nozzle to collector was held constant at 10 cm. Biological assessment was conducted with Vero cells from the Adolfo Lutz Institute. For cell morphology analyses, 3.0 x 10^5 cell/ml were inoculated on the samples in 199 medium with 10% fetal calf serum (FCS). After 24h of incubation, the samples were fixed in 3% glutaraldehyde in phosphate buffer 0.1M at pH 7.2 for 45 minutes at 4°C, and stained with 1% OsO_4 or, alternatively, with 5% KI solution for 2hs at 4°C. The specimens were then dehydrated with an ethanol series and critical point dried. Samples from the unseeded and cell seeded mats were imaged by scanning electron microscopy (SEM) and microCT. For SEM (Quanta 250, FEI), all samples were gold coated; voltage and magnification are shown in the SEM images. For microCT [from the Brazilian Synchrotron Light Laboratory (LNLS) at Centro Nacional de Pesquisa em Energia e Materiais (CNPEM) – white beam, peak energy 11keV, 10x objective], 360º images were acquired and smaller areas of interest were cropped. Software ImageJ was employed for fiber diameter measurement using SEM images and 3D reconstruction of microCT images.

Results. Average fiber diameter was 335.39 nm ± 107.03, but there are larger fiber bundles and beads in the micrometric scale (Fig. 1A). Since the resolution limit of the microCT equipment was of 1.2 µm, it was not possible to visualize all fibers nor entirely reconstruct the microstructure (Fig. 2A). Cells seeded on the samples spread and formed more than one semiconfluent layer, in different depths (Fig. 1B). It was not possible to distinguish fiber bundles/defects from cells in the microCT images, however the obvious filling shown in Figures 2B and 2C (compared to Fig. 2A)

most likely represents this cell proliferation in more than one plane, as two distinguished layers can be seen. For this particular construct, there were no differences for the different dyes.

Discussion and Conclusions. Current microCT limitations hinder a complete reconstruction of nanofibrous scaffolds, but, hopefully, future advancements in the field (especially with the Sirius Project, one of the first fourth-generation synchrotron lightsources) will resolve this matter. As for cell visualization, this technique was considered satisfactory as qualitative analysis. Despite the lack of contrast between PCL and the stained cells, which prevents an accurate identification of the components, it is possible to better understand cell-material interaction. Clearly, microCT results must always be supported by other techniques, such as SEM, or confocal fluorescence microscopy, for example. In terms of contrast agents, since both successfully stained the cells, and, apparently, not the material, KI seems to be the best option in this case, considering OsO_4's high toxicity.

Figures and Tables

Figure 1. SEM images of (A) unseeded scaffold and (B) scaffold seeded with Vero cells, forming semiconfluent layers.

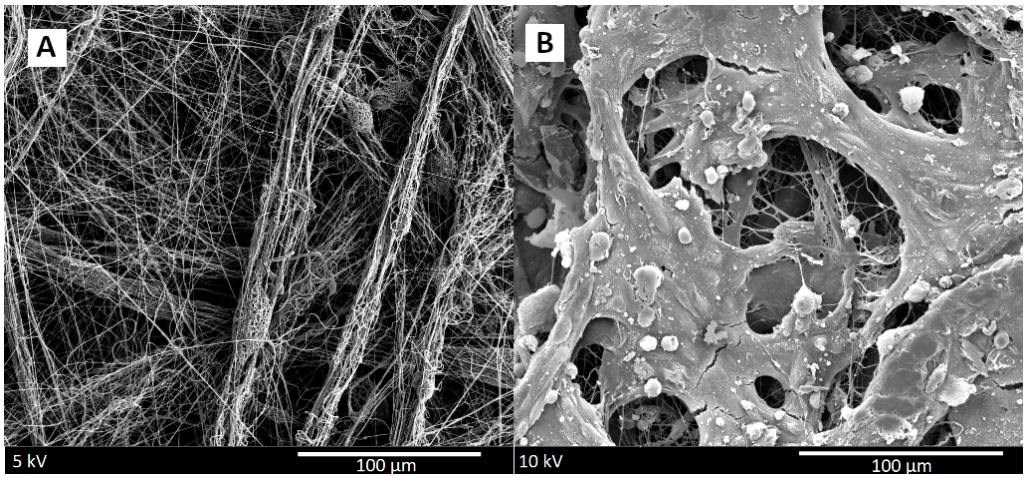
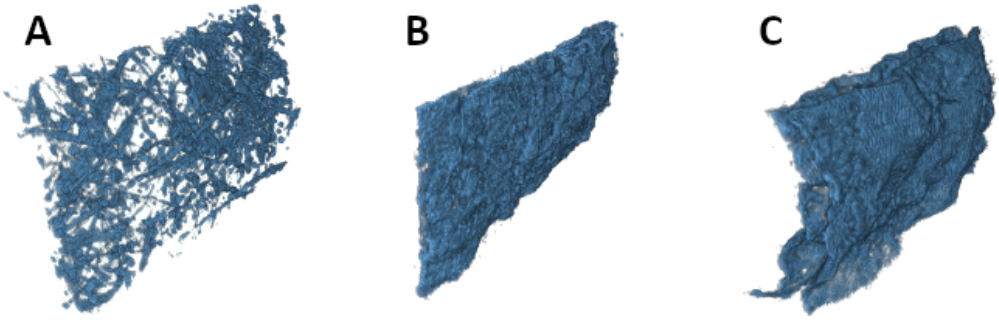


Figure 2. 3D reconstruction of (A) unseeded scaffold; (B) scaffold seeded with Vero cells and stained with KI; (C) scaffold seeded with Vero cells and stained with OsO_4.



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Keywords. Fibrous scaffolds; microtomography; Vero cells; cell proliferation.