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3-D CHARACTERIZATION OF FIBROBLAST CULTURES ON PET-TEXTILES

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INTRODUCTION: Cell culture experiments are typically performed as *in vitro* studies based on 2-D seeding and characterization. Here, the procedures can be kept relatively simple. With respect to the *in vivo* situation, however, 2-D models are often inappropriate due to the 3-D character of living tissue in nature. Consequently, 3-D *in vitro* systems should better resemble the natural situation. For the 3-D *in vitro* studies suitable scaffolds have to be chosen and methods for 3-D characterization have to be adapted. Micro-tomography using X-rays (µCT) belongs to the most promising techniques for 3-D characterization. Using synchrotron radiation sources, the spatial resolution can be extended down to the sub-micrometer range and individual cells can be made visible. Since the samples consist mainly of light elements, the cells have to be labelled by the use of highly absorptive agents, well known from electron microscopy, to obtain enough contrast in the most frequently used absorption contrast modus. Contrary to electron microscopy, µCT does not need vacuum conditions making experiments in the hydrated state possible. The aim of the current study is the optimisation of µCT to uncover cell shape and cell distribution on porous scaffolds, which can be even opaque, in the hydrated environment.

METHODS: Tomography is a technique to non-destructively reconstruct a 3-D image of a solid state from a set of filtered 2-D projections. Using the parallel beams of a synchrotron radiation source, the sample is precisely rotated stepwise to 180°, and after each step a projection is recorded. Because the contrast mechanism is X-ray absorption, one gets a representation of the local absorption coefficients.[1] This means that the constituents have to differ in X-ray absorption to allow segmentation.

The samples, rat tendon fibroblasts seeded on texturized poly-ethylene-teraphtalate (PET) multi-filament yarns (30 filaments with a diameter of 20 µm each) embedded in a hydrated matrix, were held in low absorbing glass or plastic containers. Since the fibroblasts show low X-ray absorption as the surrounding embedding and scaffold material it is inevitable to stain the cells with a higher absorbing contrast agent. For first experiments, OsO_4 has been used. The sample preparation procedure was identical to that used for scanning electron microscopy, just interrupted before dehydration: The rat tendon fibroblasts were fixated in 3% glutaraldehyde in phosphate buffered saline (PBS) overnight. After washing them once they were post-fixed with 0.1% and 0.01% OsO_4, respectively, for 1 h. Subsequently, the samples were washed twice with PBS. At HASYLAB at DESY the samples were transferred into glass capillaries (diameter 0.7 mm, wall thickness about 10 µm) and PBS was added to keep the samples in a hydrated environment. The tomograms are generated from 720 projections obtained at the photon energy of 9 keV with a spatial resolution of 1.4 µm.

RESULTS: First, the porosity of the scaffold (textile) is extracted from tomograms of the PET-textiles without any cell. The advantage of µCT with respect to well-established techniques for porosity determination is the length scale accessible ranging from about 1 µm to 1 mm. In addition, one does not only obtain mean values but the whole 3-D micro-architecture necessary to study phenomena like cell migration and supply. Current limitation is the huge amount of data (Gigabytes) to be treated.

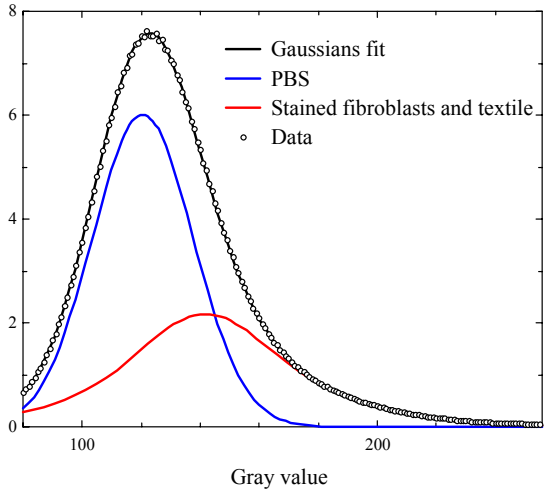

Fig. 1: Histogram for sample with high stain concentration; data, multiple Gaussians fit, gray level distributions (from fit) for PBS as well as for stained fibroblasts and the textile are shown.

The samples with the different OsO_4 concentrations were reconstructed with success, although bubble formation due to X-ray exposure was sometimes present.

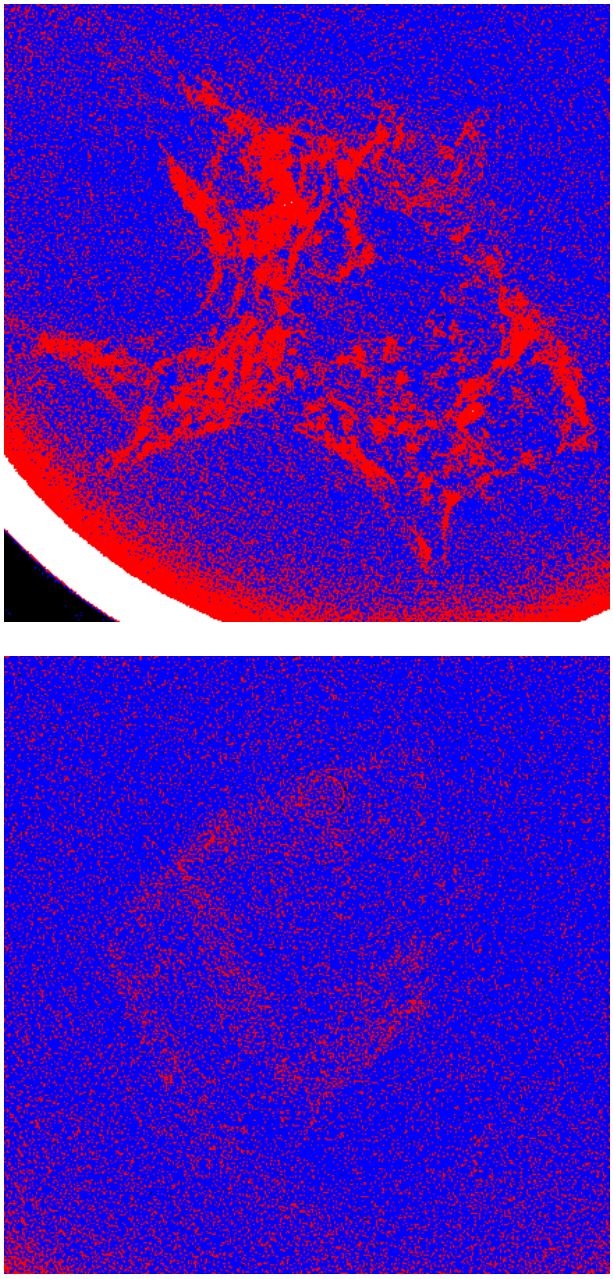

Fig2.: Image sizes: 631 µm x 644 µm
Top: Slice of the sample with high OsO_4 concentration, segmented using two colours, blue (grey levels 70-131) and red (132-246). In the lower left corner, a part of the sample container is visible (white). Bottom: Slice of the sample with low OsO_4 concentration, segmented using two colours, blue (60-120) and red (121-246).

The obtained tomograms reveal that the higher staining concentration results in sufficient contrast and gives rise to images with similarities to micrographs from electron microscopy. For the lower staining concentration, however, the image contrast was insufficient to segment the fibroblasts, since the grey values for the stained cells strongly overlap with those of the background. The two slices presented in fig. 2 were coloured according to the histogram of the individual datasets. The threshold between red and blue corresponds to the mean value of the two peak positions (cp. fig: 1).

DISCUSSION & CONCLUSIONS: µCT, a non-destructive technique is used in materials science to uncover the microstructure of composites avoiding surface preparation artefacts. The same holds for composites consisting of porous materials and biological matter. Only recently µCT has been applied to samples containing soft tissue cells. Their visualization was successful. Although many details as detected by electron microscopy are not uncovered, the shape of individual cells can be made visible. Therefore, it is possible to investigate the in-growth of cells into porous scaffolds in all 3 spatial directions. The cell behaviour can be adjusted by the choice of scaffold porosity and microstructure. Consequently, µCT is a valuable visualization technique to optimise scaffolds for the desired application. By the use of appropriate contrast agents and concentrations µCT may even be used for *in vivo* investigations. This requires the coordination of the composite materials, the photon energy and label concentration, not yet optimised for the present study. The availability of synchrotron radiation sources with high coherence enables us to take advantage of different contrast mechanisms.[2] For example diffraction enhanced imaging helps to determine internal and external interfaces, as we show by PET-textiles measured at ESRF in Grenoble. Here, changes of the refractive index are mapped. The adaptation of synchrotron-radiation-based µCT to biological samples on the cellular level opens up a new possibility to study cell-substrate interactions in 3-D even on opaque porous scaffolds.

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