ABSTRACT
Scaffold-cell interaction is one of the main concerns in bone tissue engineering. Scaffolds manufacture needs a high degree of control to define which parameters are correlated to a certain cell response. Silk fibroin (SF) porous scaffolds, obtained through salt leaching, have proven to drive osteogenesis during in vitro and in vivo experiments. These scaffolds, seeded with hMSCs, are used to grow bone-like tissue, and mineralised ECM formation is monitored using μCT imaging. The structure of the scaffold is not visible in μCT scans, while in cell culture condition, because of high water content in the protein. Therefore, a lot of information regarding cells-scaffold interaction, nutrient diffusion and local stresses is not directly measurable.

The aim of this study was to increase the radiopacity of the silk fibroin scaffold so that its architecture becomes visible in μCT imaging before and during cell culture. This is important in order to define where and how the mineralised matrix is formed in scaffold.

There are different methods on how one could increase the radiopacity in μCT, such as using contrast agents, stain the scaffold or add materials with high molecular weight and density to its structure. In this study, ceramic NPs were included in the SF matrix to provide a strong and uniform contrast. Hydroxyapatite and titanium dioxide (TiO₂) NPs were selected for their X-Ray attenuation properties and biocompatibility. It was observed that high concentrations of NPs included in SF matrix were necessary to increase the contrast of the structures above the noise of the background, and to be able to segment both scaffold and mineralized matrix with the same threshold. Comparison of CT images with histological images showed good agreement between the observed volume and trabecular thickness of the porous structure. This was true when the scaffold was imaged with a resolution of 10 μm before cell culture, but not at 18 μm as when using the bioreactor for cell culture. The SF-NP scaffolds, used for a 4-week static cell culture, proved to preserve cell viability and adhesion. Moreover, they enhanced osteogenesis and matrix formation compared to the original SF scaffold. During the culture was not possible to assess the exact position in the scaffold of matrix formation because of the reduced resolution. Nevertheless was still possible to monitor its the relative position by means of image registration. In conclusion, SF scaffold visualization in μCT was possible by means of ceramic NPs inclusion only before or after cell culture. Results suggest that, with different scanner or bioreactor set-up, the new SF-NP scaffolds could be used to monitor matrix formation in each pore, after each week of cell culture.