Validation of micro-CT as an imaging tool for the quantification of bone formation in and around explanted bone tissue engineering scaffolds

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Introduction: At present, histomorphometric analysis is the golden standard for the quantification of tissue formation in and around implants and scaffolds. This technique is however labour-intensive, time-consuming and only provides 2D quantitative information. X-ray microfocus computed tomography (micro-CT) is recently put forward as a non-destructive, 3D imaging tool for the quantification of bone formation in and around bone scaffolds and implants. However, since it is known that artefacts are inherently present in micro-CT images and since segmentation possibly causes errors in the structural analysis, the accuracy and reliability of the technique needs to be assessed. Additional errors arise related to the limited difference in contrast between bone and scaffold material. This study provides a protocol for 2D as well as 3D comparison between micro-CT and histology and assesses the effect of the sample material and threshold on the accuracy and reliability of the binarized micro-CT data. The goal of the work is to validate the use of micro-CT as a partial replacement of histomorphometry, taking into account the visualization and binarization errors present in the micro-CT images.

Materials and Methods: Three types of scaffolds are assessed: inert titanium (Ti) scaffolds, biodegradable hydroxyapatite (HA) scaffolds and biodegradable polyester-based scaffolds containing alpha-tricalcium-phosphate particles (PH-αTCP). After a 10 weeks implantation period in a 2 cm diaphysal defect in the right tibia of New Zealand White rabbits, the scaffolds were explanted and prepared for micro-CT and subsequently histological analysis. Prior to implantation a micro-CT dataset was rendered for each scaffold.

- 2D comparison of micro-CT vs. histology

In 2D both the amount of bone as well as the spatial distribution was verified by matching interpolated micro-CT images of the explants to the corresponding histological sections[1]. An optimal threshold was defined at the maximum in coinciding and minimum in non-coinciding solid pixels (bone or scaffold) in the overlay images. The visualization and binarization error in the micro-CT images was then defined by the percentage overlap and total mismatch.

- 3D comparison of micro-CT vs. histology

To assess the accuracy of micro-CT to quantify the total bone volume formed in and around the scaffolds and the spatial distribution of the newly formed bone, a 3D comparison was made with histology. Therefore, an extrapolation of the 2D histological data was performed. To determine the volume of newly formed bone in and around the scaffold using the micro-CT data, Mimics v9.0 (Materialise NV, Leuven, Belgium) was applied. First, a cylinder defined by the outer scaffold boundaries was fitted to the micro-CT dataset of the explant and, after segmenting bone, scaffold and pore space, the volume of bone inside and surrounding the scaffold within the defect region was determined. The correlation between the micro-CT and the histological data was then assessed.

Results and Discussion: Explants with PH-αTCP scaffolds show a mean overlap of 91.9 ± 2.4 %. This overlap is however only defined for the newly formed bone since the polymeric structure can at present not be visualized by micro-CT when combined with bone. For explants with Ti scaffolds, the overlap and mismatch both for bone and scaffold can be defined.
Figure 1 shows the correlation between the bone volume calculated from micro-CT and histological data for a limited series of ceramic and polymeric bone scaffolds. It can be seen that micro-CT overestimates histology. However, it should be noted that the bone volume calculated from the histological data is only based on an extrapolation of 2D data. Figure 1 also shows that, for explants with HA scaffolds, a larger overestimation is found than for PH-αTCP scaffolds. This can be explained by the difficulty in segmentation between bone and HA scaffold since both have a comparable X-ray attenuation.

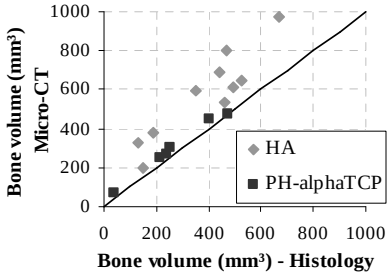

Fig.1: Correlation between the bone volume calculated from micro-CT and from histological data for ceramic and polymeric scaffolds.

Conclusion: This study shows that the accuracy of the micro-CT images does not only depend on the inherently present artefacts, but also the sample material influences the image quality, the choice of the optimal threshold values for bone, scaffold and pore space, and hence the final quantification of bone formation. It can be concluded that micro-CT can partially replace histomorphometry when taking into account the visualization and binarization errors present in the micro-CT images.

References: [1] Kerckhofs G., Schrooten J., Van Marcke P., Van Cleynenbreugel T. and Wevers M., Standardisation and Validation of Micro-CT for the Morphological Characterization of Porous Structures, Proceedings of the ECNDT 2006, Berlin, 25-29 September 2006