Introduction

A main field in biomaterials research is the surface modification of titanium implants to improve the implant healing behaviour. There are various methods to confirm the success of such bio-functionalizations.

Beside histological investigations, in this work we have used x-ray microtomography with synchrotron radiation (SR µCT) as a non-destructive method to detect and analyse the osteointegration of titanium implants in all three dimensions. The aim of this work was the comparison of bone detection in the SR µCT-volume to filtered images from histological slices with similar analysis procedures.

Materials and Methods

From a mini-pig experiment samples containing bio-functionalised Ti-implants with surrounding tissue after a healing time of 6 months in the jaw were selected for a comparative investigation.

The SR µCT experiments were performed at HASYLAB BW5 (DESY, Hamburg, Germany). Formalin fixed cylindrical samples were prepared with a diameter of about 8 mm, containing the implant nearly centred within the bony tissue. With a photon energy of 70 keV and an image size of 1536 x 1536 pixels from each sample 720 projections were recorded. A filtered back projection algorithm was used to obtain the three-dimensional data of x-ray absorption for the samples. The visualization of the reconstructed data was done with a volume rendering software (VGStudio, Volume Graphics, Germany). After SR µCT measurements the samples were histologically prepared (embedding: Technovit 9100 neu, slice spacing: 500 µm, staining: Masson-Goldner). Automatic and semi-automatic analysis procedures were created to record the amount of mineralized bone around titanium in the SR µCT volume and the histological images.

Results

The rendering procedure of the x-ray absorption values for bony tissue from SR µCT resulted in a detailed visualization of bone morphology with a spatial resolution of about 9 µm (Fig. 2). Also a sharp implant/bone interface is visible. To find bone thresholds in microscopical images from histological slices (Fig.1, bottom), we had to use colour splitting and histogram arrangements. The cavity analysis for an exemplary sample shows no significant difference in the mean bone value between microtomography and histology (Fig. 3). Slicing the whole CT volume (281 slices) with the same orientation used for histology we detected bone amounts in the cavity of 17 % - 54 % compared to 23 % - 38 % from 6 histological slices. The standard error of the mean bone value (SE) was 0.4 % using the µCT volume and 2.7 % with the histological images.
Discussion

Related to the detailed three-dimensional visualization of bony tissue around titanium implants, the SR µCT measurements have proven their reliability in the field of osteointegration analysis. Taking the wide range of detected bone amounts with SR µCT into account, the results in histology depend on a much too limited number of randomly positioned slices with a large slice distance. A further problem for an automated histological image analysis is the non-uniform staining of the bony tissue, which makes distinction between tissues sometimes difficult. This also concerns the geometrical setting of reference areas in the images. The advantage of histological imaging is still the superb lateral resolution and the visualization of biochemical tissue properties. But with a view to a statistical relevant analysis of a large tissue volume and high samples number, visualization and quantification is more practicable with SR µCT. Considering all these aspects, a combination of classical histology with SR µCT will be a powerful instrument for an improved understanding of biological reactions around bio-functionalized implants.

This study will be continued with different bio-functionalized implants using strategies of contrast enhancement in SR µCT in conjunction with bone mineralization.

Acknowledgments

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![Figure 1: top: implant design; bottom: images from histological slices inside the marked region of interest (ROI)](image1)

![Figure 2: 3D view of bony tissue inside the ROI from SRµCT at HASYLAB BW5 (implant transparent)](image2)

![Figure 3: Analysis of bone values inside the ROI from SR µCT and histological data](image3)