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

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Aims.
It is clear that through its variety of functions in the body a change in bone structure by trauma or disease will highly influence the quality of life. The options for orthopaedic surgeons to heal bone defects have evolved at lot, but still patients with large load bearing bone defects cannot be treated optimally. The existing clinical therapies require a long and in most cases painful healing period. Also a complete restoration of the skeletal function is not guaranteed in all cases. Tissue engineering (TE) can, by combining porous carriers in combination with osteogenic cells, provide a solution for this problem. These carriers, also called bone tissue engineering scaffolds, are seeded with (patient own) cells. These cells should, with the proper stimulation, synthesize bone matrix. In order to test potential cell-scaffold combinations both in vitro and in vivo experiments are used. For the evaluation of in vivo experiments, namely animal experiments, in general the scaffold together with the newly formed tissue are explanted after a defined implantation period and investigated by histological analysis among others to identify and quantify the newly formed tissue. This procedure is however time consuming, labor intensive and does only provide 2D quantitative information. This study investigates the possibility of using microfocus X-ray computed tomography (micro-CT) as an alternative for histomorphometry. If it can be shown that micro-CT is complementary to histology with regard to bone formation it is the goal to develop a protocol that can be used to replace the histological analysis by micro-CT analysis to investigate bone ingrowth in and around bone scaffolds quantitatively in a non-destructive and fast 3D method. To investigate the complementarity, this study provides a protocol for 2D as well as 3D comparison between micro-CT and histology and assesses the effect of the scaffold material on the accuracy and reliability of the binarized micro-CT data.

Materials and methods.

Materials.
Explants with three types of scaffolds were assessed: uncoated inert metallic, biodegradable composite (polymeric network + ceramic grains) and biodegradable ceramic ones. All porous structures had struts ranging from 20 to 100 µm, pores ranging from 50 to 500 µm and a global porosity of about 70 to 80 %.
In vivo experiments
After a 10 weeks implantation period in a 2 cm diaphysal defect in the right tibia of New Zealand White rabbits, explants were cut out 1 cm below and above the implanted scaffold after which they were prepared for micro-CT and subsequently histological analysis. Prior to implantation a micro-CT dataset was rendered for each scaffold.

Micro-CT equipment
For this study, a Philips HOMX 161 X-ray system with AEA Tomohawk CT software was used. Characteristics of the device can be found in Ref.[1]. In this study the thickness, width and height of the image voxels both for the explants and the naked scaffolds ranged between 20 and 30 µm.

Validation and quantification protocol
The flow chart of the protocol is shown in figure 1. First, in 2D both the amount of bone and/or scaffold as well as their spatial distribution was verified by matching interpolated micro-CT images of the explants to the corresponding histological sections. A more elaborated description of this part of the protocol can be found in Ref.[1]. The visualization and binarization error in the micro-CT images was defined by the percentage overlap, over- and underestimation.

Bone volume calculated from histomorphometry is determined by interpolation of the 2D data. Therefore, we defined this bone volume not as being 3D, but as being 2D+. Since micro-CT renders actual 3D information, an exact comparison with histology, as was done in 2D, was not possible and hence the same interpolation approach was applied to the micro-CT data. These 2D+ results were mutually compared by taking into account the visualization and binarization error defined in 2D. When proven that the 2D+ bone volume derived from the corrected micro-CT data corresponded to the 2D+ histological data, it was concluded that the 2D visualization and binarization error could also be applied on the 3D micro-CT data.

Finally, the amount of bone formation in and around the different types of bone tissue engineering scaffolds was determined from the micro-CT data corrected for the visualization and binarization error.

Figure 1: Flow chart of the proposed validation protocol. First a 2D comparison was made between micro-CT and histology from which a 2D mismatch was defined. This mismatch was applied on the 2D+ data to verify the applicability in 3D. When proven its applicability, the 2D mismatch was applied on the 3D micro-CT data where a true 3D bone and/or scaffold volume was defined.
Results and discussion.

2D comparison
The visualization and binarization error in the micro-CT images for explants with the three types of scaffolds was determined. Table 1 summarizes the results and figure 1 shows that after correction for the mismatch, a good correlation between micro-CT and histology was found. It was seen that the denser the material, the larger the visualization and binarization error (= 2D mismatch).

Table 1: Overlap, overestimation, underestimation and mutual 2D mismatch for the micro-CT images of the explants with the three types of scaffold.

<table>
<thead>
<tr>
<th></th>
<th>Metallic</th>
<th>Ceramic</th>
<th>Polymeric</th>
</tr>
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<tbody>
<tr>
<td>Overlap</td>
<td>81.4 ± 9.1 %</td>
<td>91.6 ± 3.3 %</td>
<td>94.6 ± 3.0 %</td>
</tr>
<tr>
<td>Overestimation</td>
<td>58.3 ± 13.7 %</td>
<td>30.6 ± 10.7 %</td>
<td>18.6 ± 5.2 %</td>
</tr>
<tr>
<td>Underestimation</td>
<td>18.6 ± 9.1 %</td>
<td>8.4 ± 3.3 %</td>
<td>5.4 ± 3.0 %</td>
</tr>
<tr>
<td>Mutual mismatch</td>
<td>39.7 ± 14.0 %</td>
<td>21.2 ± 8.8 %</td>
<td>14.8 ± 7.1 %</td>
</tr>
</tbody>
</table>

Figure 2: A) Correlation between the bone surface measured in the histological sections and the corresponding micro-CT images and B) the same correlation, but the bone surface measured in the corresponding micro-CT images was corrected for the 2D mismatch.

2D+ comparison
For 2D+ comparison, the bone volume from the micro-CT images was determined as it was done for the histological section. The same amount of images as for the histomorphometric calculations was analysed and the amount of bone in the 2D sections was multiplied by the thickness of the histological sections (400 µm) and multiplied by three, since only one third of the histological sections was analysed. Then, both results were mutually compared. As can be seen in figure 3, it was concluded the 2D mismatch can also be applied on 2D+ or 3D data.

3D quantification
After 2D mismatch correction, the actual 3D newly formed bone volume within the scaffold was determined and the results are shown in figure 4. It could be concluded that ceramic scaffolds induce more bone formation than metallic. This was indeed confirmed by histomorphometry.
Conclusion.
This study showed that the accuracy of the micro-CT images does not only depend on the inherently present artefacts, but also the scaffold material influences the image quality, the visualization and binarization error, and hence the final quantification of bone formation. It could be concluded that micro-CT can replace histomorphometry for the 3D quantification of bone formation in and around bone tissue engineering scaffolds when taking into account the visualization and binarization errors. Hence, scaffolds can now be characterized quantitatively to a high degree via micro-CT in order to select suitable materials and architectures and the biological outcome can be correlated to the scaffold properties.

References