# Quantification of bone tissue regeneration employing $\beta$-tricalcium phosphate by three-dimensional non-invasive synchrotron micro-tomography — A comparative examination with histomorphometry


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Introduction:

The aim of this study was to evaluate the validity of two dimensional histomorphometric measurement of bone biopsy specimen after sinus floor evaluation by means of **high contrast, high resolution, three-dimensional and non-destructive synchrotron micro-tomography (SCT)**. Histomorphometry and immune-histology or three-dimensional x-ray method micro computer tomography is still the gold standard for evaluating bone specimen. In this study evaluation was done by non-destructive synchrotron micro-tomography (SCT) and compared to standard histology in order to demonstrate the potential of this new approach for the evaluation of bone biopsy samples.

Material and methods:

Unilateral sinus grafting was carried out in 37 patients using a combination of $\beta$-tricalcium phosphate ($\beta$-TCP) and autogenous bone chips. Cerasorb, Cerasorb-M and CEROS-TCP granulate was used with porosity of 35% and 60%. At implant placement, 6 months after sinus grafting, a cylindrical specimen was biopsied from the augmented area. Subsequent to the histological embedding in resin the specimens were imaged using a SCT facility resulting in three-dimensional (3-D) images with approximately 4 $\mu$m spatial resolution (1.5 $\mu$m pixel size) for each patient's specimen. Subsequent to the SCT acquisition, tissue sections were prepared for histomorphometric analysis.


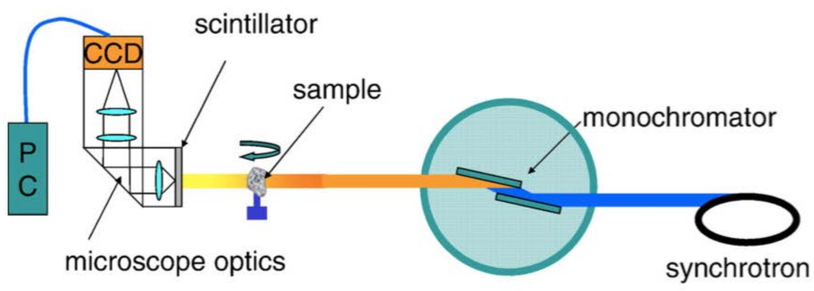


Fig. 1: Sketch of a typical set-up for synchrotron micro-tomography: the radiation coming from the synchrotron light source passes a monochromator and then the sample.


Results:

First, the bone biopsy specimen were analysed in SCT and then cut into slices from histologic sections. The histologic sections were searched manually in the three-dimensional data of SCT to have a direct comparison between these different procedures. The bone samples were classified into groups from one to three depending on the integerity of their bone pillar, the quality of tomography analysis and the analogy between the two-dimensional SCT section and the histology. The first group contained samples of best quality bone specimen and the third group samples of lowest quality specimen.

The SCT images picture all details of the samples on a grey scale, depending of their mass density. In order to calculate the bone mass, SCT images needed to be converted into black-and-white boolean data sets. All grey-scales expected to be bone were pictured white and all those that were expected not to be bone, such as unmineralized osteoid or blood, were pictured black. The bone mass was determined by adding of all white pixel.

For every sample the bone mass was calculated three times. First, the histologic section was measured manually, second the equivalent section of three-dimensional SCT data set. Third, the bone mass was measured on the whole three-dimensional (3D) pillar which is the new opportunity of SCT analysation. The 2D data represent the bone mass in a randomly chosen histologic section in contrast to the 3D data which represent the real bone mass of the whole bone pillar. The deviation between these two results shows the possible error of measurement while using 2D analysis instead of 3D for complex situations. **In total the deviation is ±4,48% but up to 18%**.


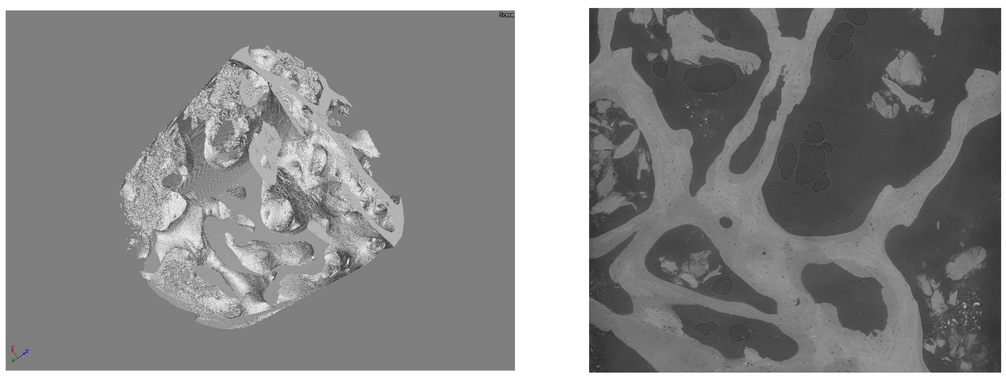

Fig. 2: 3D visualization of whole bone biopsy (left) and the histologic area inside of cylindrical sample (right). The development of the bone can be read off the mineralisation lines which are represented by different grey scales in the bone depending on their mass density.

| sample group | | deviation 2DHisto-2DCT | deviation 2DHisto-3DCT | deviation 2DCT-3DCT |
|---|---|---|---|---|
| 1 | arithmetic mean in % | **2,9** | 6,49 | **3,59** |
| | number N | 13 | 13 | 13 |
| | standard deviation. | 2,26 | 1,6 | 4,74 |
| | statistical significance | 1 | 0,002 | 0,018 |
| 2 | arithmetic mean in % | **2,69** | 7,6 | **4,9** |
| | number N | 17 | 17 | 17 |
| | standard deviation. | 2,32 | 7,66 | 6,86 |
| | statistical significance | < 0,001 | 0,001 | 0,009 |
| 3 | arithmetic mean in % | **2,82** | 7,95 | **5,12** |
| | number N | 7 | 7 | 7 |
| | standard deviation. | 2,49 | 5,99 | 4,78 |
| | statistical significance | 0,024 | 0,013 | 0,03 |
| 1 & 2 | arithmetic mean in % | **2,78** | 7,12 | **4,33** |
| | number N | 30 | 30 | 30 |
| | standard deviation. | 2,25 | 6,82 | 5,98 |
| | statistical significance | < 0,001 | < 0,001 | < 0,001 |
| total | arithmetic mean in % | **2,79** | 7,27 | **4,48** |
| | number N | 37 | 37 | 37 |
| | standard deviation. | 2,27 | 6,6 | 5,72 |
| | statistical significance | < 0,001 | < 0,001 | < 0,001 |

Fig. 3: Compilation of all bone deviations between histologic section, 2D and 3D synchrotron data set.

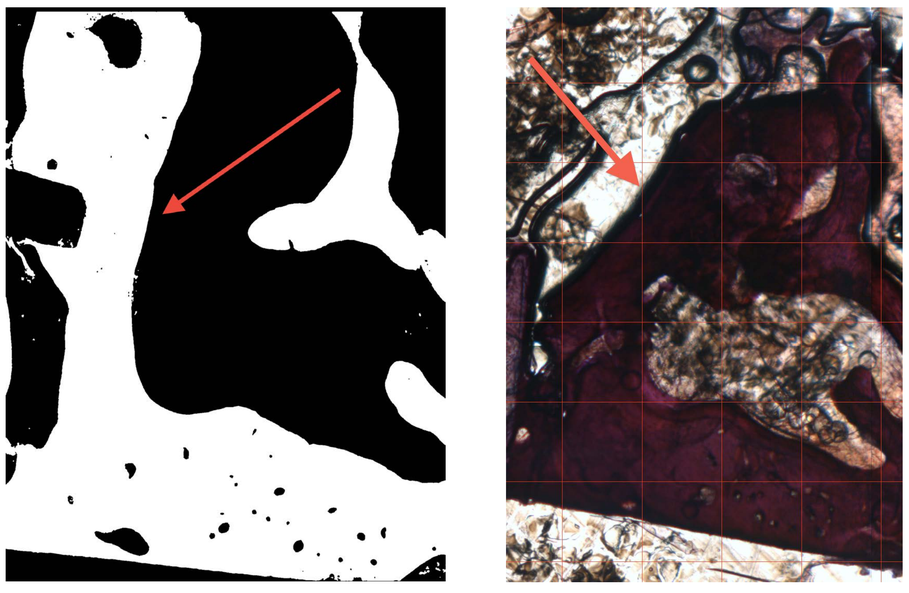

Fig. 4: Fragment dislocation, effect of histologic sectioning. In the left image intact osseous trabecular meshwork is pictured after 3D scanning. After histologic processing bone is invoiced, rotated and displaced.


Conclusion:

The evaluation of biomaterials, with differing biodegradability and the characterization of the biological response to regenerative procedures which are subject to multifactorial influences, requires non-destructive diagnostic tools to elucidate the dynamics of these processes in a three-dimensional (3-D) manner. Conventional hard tissue histologic and histomorphometric methods with their complex sectioning procedures have the disadvantage of either damaging the 3-D structure of the tissue sample under investigation, i.e. the bone biopsies, or revealing only limited information regarding the dynamics of regenerative processes in highly complex anatomic structures.

The SCT data reveals new quality of bone images. Not just two or three sections of a sample can be analysed, but the whole specimen with all kinds of complex structures. The development of the bone can be read off the mineralisation lines which are represented by different grey scales in the bone. On cellular level there are still set boundaries for SCT. Immune-histology has the advantage of different antibody staining methods to distinguish between cell types and their activity. But due to better optics and higher photon flux the resolution of SCT is getting higher, meaning that it is already possible to picture cellulare structures. In this reagard one should not forget that biological materials can be altered by too high a dose of photon flux.

This study showed that even samples of lower quality bone specimen (group 3) can be analysed by SCT giving valid results for bone mass. Samples of lower quality are hard to evaluate by histologic sectioning because of mechanical instability and smaller areas to measure.

The beta-tricalcium phosphate used in all samples showed distinct formations of newly built woven bone. An application in oral surgery can be recommended. **To summarise, histological analysation can remain standard for big homogeneous samples but for smaller and heterogeneous samples three-dimensional analysation with SCT is more accurate and offers a better representation of bone structures.**

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