Utilizing micro-CT to evaluate bone structure surrounding dental implants: a comparison with histomorphometry

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ABSTRACT

Introduction

Although histology has proven to be a reliable method to evaluate the ossoeintegration of a dental implant, it is costly, time consuming, destructive, and limited to 1 or few sections. Micro-CT (µCT) is fast and delivers 3D information, but this technique has not been widely used and validated for histomorphmetric parameters yet. This study compared µCT and histomorphometry by means of evaluating their accuracy in determining the bone response to 2 different implant materials.

Materials and methods

Thirty-two titanium (Ti) and 16 hydroxyapatite (HA) implants were installed in 16 lop-eared rabbits. After 2 and 4 weeks, the animals were sacrified and the samples retrieved. After embedding, the samples were scanned with µCT, and analysed 3-dimensionally for bone area (BA) and bone-implant-contact (BIC). Thereafter, all samples were sectioned and stained for histomorphometry.

Results

For the Ti implants, the mean BIC was 25.25% and 28.86% after 2 and 4 weeks, respectively, when measured by histomorphometry, while it was 24.11% and 24.53% when measured with µCT. BA was 35.4% and 31.97% after 2 and 4 weeks for histomorphometry and 29.06% and 27.65% for µCT. For the HA implants, the mean BIC was 28.49% and 42.51% after 2 and 4 weeks, respectively, when measured by histomorphometry, while it was 33.74% and 42.19% when measured with µCT. BA was 30.59% and 47.17% after 2 and 4 weeks for histomorphometry and 37.16% and 44.95% for µCT.Direct comparison showed that only the 2 weeks BA for the titanium implants was significantly different between µCT and histology (P=0.008).

Conclusion

Although the technique has its limitations, µCT corresponded well with histomorphometry and should be considered as a tool to evaluate bone structure around implants.

INTRODUCTION

The success of osseointegration has brought in numerous benefits to the patients needing long-lasting rehabilitation of one's lost dentition [1-3]. While successful from the beginning of its practice, the quest for improvements in both short- and long-term host-to-implant response has resulted in substantial evolution of implant fixture design in the macro, micro, and nano surface levels. Then, these design changes are often tested in laboratory animal models for performance evaluation by histomorphometry or biomechanical testing.

However, as implant modifications become detailed and complex and studies become multifactorial in nature in an attempt to develop an informed platform for implant design optimization, it is sometimes difficult to capture detailed bone response in a timely manner and with enough resolution with the currently conventional methods. For example, the biomechanical removal torque testing may not be able to resolve small differences in biomechanical performance, caused by modifications of the implant surfaces at the nano level. Even histology, which has been proven to provide us with both qualitative and quantitative information, has a shortcoming due to the fact that it is based on one or a reduced number of sections through the sample cut, which is a limited subset of the entire specimen. Other key factors such as time to prepare the samples, destructiveness of the method, and cost must be taken into consideration when performing these studies. Thus, new evaluation techniques that increase evaluation throughput while providing at least the same resolution ability as current techniques are highly desirable.

Currently, one of the potential candidate tools as an adjunct for biomechanical testing is micro-computed-tomography (µCT), first described by Feldkamp et al. in the end of the 1980's[4], has been used in the past for quantification and characterization of bone architecture in relation to bone disorders. While clinical CT scanners typically produce images composed of 1 mm^3 volume elements (voxels), µCT produces improved resolution in the range of a few µm, or approximately 1,000,000 times smaller than regular CT scanning. It allows a non-destructive, 3D evaluation of the specimen in high resolution within a limited amount of time, and also provides 3D reconstructed images to obtain better understanding of bone architecture taking place within the region of interest. Several studies have validated the technique of

µCT by comparing it to histological sections of bone specimens and found a high correlation between both techniques [5-7]. However, one must keep in mind that the samples in these studies consisted only from trabecular bone, and did not have present metallic implantable devices in bone with the potential to metallic halation and beam hardening. These artifacts are created by dense objects and depend on the attenuation coefficient and geometry of the object [8]. Naturally, this make visualization of the bone within a 200 µm radius around the implant a challenge and thereby evaluation at the bone-implant interface difficult [9]. From a quantitative bone morphometric perspective, the ultimate goal in the future would be skipping the time consuming and costly histological section preparation while also decreasing the number of samples and/or subjects necessary for both biomechanical and morphometric components of the study. Ideally, the same sample providing bone morphometry should also be mechanically tested. However, in order to move towards this direction, it is important to prove the accuracy of 3D µCT based bone morphometry even at the bone-implant interface region. In this study, 3D µCT data was compared to 2D histology of titanium and hydroxyapatite implants, which were placed in the rabbit bone.

The aim of this study was to examen the validity of micro-CT as a tool for evaluation of implant osseointegration, by comparing 3-dimensional data with 2-dimensional histomorphometry, and to evaluate the effect of material density and geometry on beam hardening and micro-CT image quality.

MATERIALS AND METHODS

Implants and implant surgery

Two implant materials were used for the study, namely titanium (Ti) and hydroxyapatite (HA). For the Ti implants, thirty-six turned, commercially pure titanium (Grade 4) threaded implants of 8 mm length and 3.3 mm diameter were used. For the HA implants, sixteen polished non-threaded HA implants of length 9.0 mm and 4.2 mm diameter were used. The mass attenuation coefficient for titanium and hydroxyapatite at 55keV is 0.99 cm^2/g and 0.497 cm^2/g, respectively.

Thereafter, the implants were placed unicortically in the condyle of the distal femur (Ti) and proximal tibia (HA) of sixteen lop-eared rabbits (mean body weight, 3.9 kg). This study was approved by the Malmö/Lund regional animal ethics committee (approval number: M282-09).

Before surgery, the legs were shaved and disinfected with 70% ethanol and 70% chlorhexidine. The animals were anesthetized with intramuscular injections of a mixture of 0.15 mL/kg medetomidine (1 mg/mL Dormitor; Orion Pharma, Sollentuna, Sweden) and 0.35 mL/kg ketamine hydrochloride (50 mg/mL Ketalar; Pfizer AB, Sollentuna, Sweden). Lidocaine hydrochloride (Xylocaine; AstraZeneca AB, Södertälje, Sweden) was administrated as the local anesthetic at each insertion site at a dose of 1 mL. The implants were inserted with W&H implant unit (Elcomed, W&H SA-310, Burmoos Austria) at a rotation speed of 20 revolutions/minute.

Postoperatively, buprenorphine hydrochloride (0.5 mL Temgesic; Reckitt Benckiser, Slough, UK) was administered as an analgesic for three days.

After 2 and 4 weeks, the rabbits were sacrificed with an overdose (60 mg/mL) of pentobarbitalnatrium (Apoteksbolaget AB, Stockholm, Sweden). The retrieved samples were placed in 4 % formaldehyde for 24 hours and thereafter stored in 70 % ethanol.

Micro Computed Tomography and 3D Reconstruction

The 3D bone formations around the implants were examined using micro computed tomography (µCT 40, Scanco Medical, Basserdorf, Germany) with a slice resolution of 36 µm. Five hundred µCT slices were imaged at an X-ray energy level of 55 kV, and a current of 145 µA. Integration time was 200 ms with a total scanning time of 36.3 min (128 mAs).

All data were exported in Dicom-format and imported in Amira (Visage Imaging GmbH, Berlin, Germany) for evaluation. Since part of the implant was outside of the bone, the data were cropped along the implant axis to where the cortical bone started. Before segmentation, threshold levels for bone and implant were determined, based on visual inspection of the complete slices and on the gray-scale histogram (Figure 1). This was done by determining the upper and lower threshold levels for bone and implant in 5 samples for each implant type. Threshold levels of bone and implant did not overlap and allowed to make a clear distinction. The means were calculated and used for every sample. Threshold determination was repeated for intra-and inter-examiner repeatability evaluation.

Then, a region of interest (ROI) was defined, where the bone area (BA) would be calculated. Therefore, the implant was selected based on its threshold level and this region was circumferentially expanded, creating a 0.75 mm zone around the implant.

Subsequently, implant and bone in the ROI was differentiated based on their threshold levels, creating 3 volumes: implant, bone and soft tissue/empty spaces. These were converted into tetrahedral grid from triangular surfaces, to make area and volume measurements possible. Outcome variables were Bone Area (BA), being the percentage of bone that is present in the region around the implant and BIC (Bone Implant Contact), being the area-percentage of the total implant surface that is covered by bone.

Ground Section Preparation and Histological Analysis

After the µCT analysis, all samples were processed for undecalcified ground sectioning. In brief, after a series of dehydration and infiltration in resin, the samples were embedded in light-curing resin (Technovit 7200 VLC; Heraeus Kulzer Wehrheim, Germany). Thereafter, one central undecalcified cut and ground section

was prepared from each implant with the Exakt sawing and grinding equipment. The sections were ground to a final thickness of about 40 µm and stained with toluidine blue.

Histological evaluations were performed using a light microscope (Eclipse ME600, Nikon, Japan) and histomorphometrical data was analyzed by an image analysis software (Image J ver.1.43u; National Institutes of Health). Bone-Implant contact (BIC) percentage and Bone Area (BA) percentage were calculated along the entire implant and calculated with ×10 objective magnification and the amount of bone area in the same defined area performed in the 3D analysis was calculated.

Statistical Analysis

The statistical analyses was done using SPSS software (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk tests for normaility were not statistically significant. Non-parametric Wilcoxon signed rank test and Mann-Whitney U-Test with the significance level set at 0.05 were used for statistical comparison. Pearsson test was used to identify possible correlations between both methods.

RESULTS

Overall

In total, 48 implants were installed (32 titanium, 16 hydroxyapatite). Routine clinical inspections showed that healing after surgery progressed uneventfully, and there were no clinical signs of infection. At the time of sacrifice, all implants were already immobile suggesting osseointegration.

Histomorphometry

The histological sections presented newly formed trabeculae with deeply stained mineralized tissue after 2 and 4 weeks of healing.

Ti implants

The mean BIC after 2 weeks was 25.25% (SD 7.22; range 15.14 – 36.81), while the mean BA was 35.14% (SD 7.09; range 21.57 – 49.76). After 4 weeks, the mean BIC was 28.86% (SD 8.73; range 14.99 – 51.41) and the mean BA 31.97% (SD 10.48; range 14.71 – 50.55). There were no significant differences between both time periods in BIC (p=0.397) or BA (p=0.198) (Figure 2).

HA implants

The mean BIC after 2 weeks was 28.49% (SD 17.15; range 3.03 – 50.43), while the mean BA was 30.59% (SD 10.30; range 15.33 – 44.37). After 4 weeks, the mean BIC was 42.51% (SD 9.45; range 26.69 – 54.18) and the mean BA 47.17% (SD 14.81; range 28.28 – 71.50). There was no significant difference in BIC (p=0.074) between 2 and 4 weeks, but BA (p=0.036) increased significantly over time (Figure 3).

Micro-CT

The mean lower and upper threshold gray-levels for bone were 2902 (SD 187, range 2697 – 3135) and 7726 (SD 211, range 7566 – 8096). The threshold value for the titanium implant was 12881. The mean lower and upper threshold level for the HA implant were 8975 (SD 742, range 8203-9876) and 15480 (SD 568, range 14839-15937).

The intra-examiner repeatability on threshold level was high (90 % agreement within 5% deviation; Pearsson correlation coefficient: 0.998 - $p<0.001$; Wilcoxon signed ranks test: $p=0.139$), as was the inter-examiner reproducibility (80 % agreement within 5% deviation; Pearsson correlation coefficient: 0.997 - $p<0.001$; Wilcoxon signed ranks test: $p=0.059$)

Ti implants

After 2 weeks, mean BIC was 24.11% (SD 6.93; range 10.22 – 37.74) and mean BA 29.06% (SD 6.53; range 19.64 – 40.18). After 4 weeks, the mean BIC was 24.53% (SD 5.63; range 11.54 – 32.59), while the mean BA was 27.65% (SD 4.36; range 18.07 – 35.06). There were no significant changes in BIC ($p=0.826$) and BA ($p=0.397$) between both time points (Figure 2).

HA implants

After 2 weeks, mean BIC was 33.74% (SD 8.75; range 25.57 – 52.37) and mean BA 37.16% (SD 9.18; range 26.53 – 55.63). After 4 weeks, the mean BIC was 42.19% (SD 14.46; range 25.66 – 62.77), while the mean BA was 44.95% (SD 14.94; range 27.25 – 67.59). There were no significant changes in BIC ($p=0.172$) and BA ($p=0.294$) between both time points (Figure 3).

Micro-CT versus Histomorphometry

Micro-CT analyses and histomorphometry corresponded well (Figure 4 and Figure 5) and showed no significant differences at 2 weeks BIC ($p=0.594$), 4 weeks BIC ($p=0.085$) and 4 weeks BA ($P=0.058$) for the titanium implants. However, the 2 weeks BA was significantly different between µCT and histology ($p=0.008$). Both methods showed a non-significant increase in BIC and a non-significant decrease in BA between 2 and 4 weeks.

For the HA implants, no significant differences were observed in 2 weeks BIC ($p=0.327$), 2 weeks BA ($p=0.093$), 4 weeks BIC ($p=0.779$) and 4 weeks BA ($p=0.208$)

between histology and micro-CT. Histology showed a non-significant increase in BA over time, howver micro-CT did not.

Overall, there was a significant correlation for BIC ($p<0.001$) as well as for BA ($p<0.001$) between µCT and histomorphometry.

DISCUSSION

Micro-CT is fast, non-destructive and allows 3D-evaluation compared to histological sections. Therefore, this technology has been devoted significant interest over the last years. In this study, we evaluated the accuracy of this technique by comparing it with the histomorphometric results of the same samples, since very few studies have validated the use of µCT as a technique to evaluate bone structures around implants.

Although the absolute values for µCT were slightly different from histology, there was only a significant difference in the BA at 2 weeks for the titanium implants and histomorphometry revealed a significant increase in BA over time around the HA implants, while µCT did not. Possible differences between micro-CT and histomorphometry may depend on the cutting direction and slice thickness of the histological sections, as can be derived from the 3D images (Figure 6). As Sarve et al.[10] recently demonstrated, the results may vary with 30% depending on the cutting direction. On the other hand, the micro-CT data may be affected by artifacts, such as beam hardening.

Micro-CT allows evaluation of the total circumferential space while histomorphometry is limited to 1 or few slices. Therefore, one could assume that micro-CT is more accurate as the entire volume of the implant in bone sample is evaluated. in fact, a few studies have evaluated this method in relation to BIC and BA. Van Oosteryck et al.[11] concluded that micro-CT and histomorphometry were very alike, although this was based on an optical comparison of 1 sample. Similar to our study, Stoppie et al.[12] compared both techniques and concluded that the bone trabeculae were clearly visible, but became difficult to detect in proximity of the implant with µCT. These 2 studies, however, compared 2D µCT with 2D histomorphometry. Although there was a clear correlation between both methods, the authors reported that the results may vary depending on the treshold level, voxel size and cutting direction of the scan, and on the slice thickness and direction of the histological slide. Freilich et al.[13] compared bone height around 7 implants placed in rabbits by means of 3D µCT and histomorphometry, and found a similar outcome.

According to our results and also the literature in agreement, µCT appears to have a strong potential to become an alternative for histological examination. However, concerns have been expressed by multiple research groups. For instance, Schouten et al.[14] reported high discrepancy between µCT and histomorphometry regarding the bone volume around the implant. According to the authors, this was due to the noise around the implant and the threshold selection, which resulted in an overestimation of the µCT values. Also, several other authors described a blurred border around the implant due to metal artifacts caused by beam hardening, scatter and noise [12,14-16].

As this affects the voxels in immediate proximity around the implant, the size of the blurred zone will depend on the resolution of the image and can therefore easily range from 6 to 60µm. Therefore, it can be difficult to detect the bone structures in this area. Determining the treshold levels based on the total image facilitates the detection of the bony structures and might partially solve the problem.

Artifacts will also be influenced by the settings of the µCT and changes in energy-level may improve results As Van Oosterwyck et al. [11] pointed out, smaller bone trabeculae become invisible when the energy level is to high. In their study 90 and 130 kV were used, while the samples in our study were scanned at 55Kv. In addition, higher voltages will also induce more artifacts.

Although no filters were used in our study, some studies recommend the use of an aluminum filter[11,12] That might explain why some streak artifacts were present, although it did not interfere with the tresholding and quantification. Although some advocate additional software filtering to improve the image quality, we chose not to use any smoothening filters since this might change the result [17]. Of course, we could not exclude possible minor smoothening in the software's algorithm that was used to create the mesh that was used for quantification.

In the present study, 2 different materials were used, titanium and hydroxyapatite, respectively. The mass attenuation coefficient for hydroxyapite and titanium at 55keV is 0,497 cm^2/g and 0.99 cm^2/g, respectively. As a result, the X-rays will have different interactions with these two different materials. The higher density of titanium can result in a more blurred border around the implant due to beam hardening, scatter and noise, which can affect the measurement of BIC and BA. Secondly, the geometry of the two implants was different. The hydroxyapite inserts were cork shaped, while

the titanium inserts contained a screw-thread. As a result of the more complex structure of the titanium inserts, the delineation of the three volumes (implant, bone and soft tissue/empty spaces), necessary to calculate BIC and BA, will be more challenging. Figure 7 represents a colored version of the µCT slices, with each color representing a different attenuation level. While the HA implant demonstrates almost no halation, some halation can be seen around the TI implant, especially at the apical part. This was also confirmed by Stoppie et al.[12] and is probably related to the geometry of the implant apex.

It is, however, difficult to determine the precise effect of material composition on beam hardening, artifact creation and its effect on the measurements and extensive experimentation for appropriate set ups should be performed for each equipment. Although two different biomaterials were used, this study was not intended to compare both implants. However, in a dog model, Mouzin et al.[18] reported a significantly higher BIC for loaded HA implants compared to titanium implants, which is similar to the results in this study.

Although histology is still regarded as the “gold standard”, this technique also has limitations. For instance, cutting and grinding the implant in bone blocks may damage the interface and influence results. Also, Johansson & Morberg[19] showed that thicker slices results in an overestimation of measurements and therefore, sections over 30µm should be avoided. The biggest limitation, however, is that histology only presents 2D information. Therefore, one may question if one or few slices will accurately represent the total sample. Micro-CT will contain a lot more information about the bone structures and may therefore be a more accurate representation. On the other hand, µCT will have its limitations regarding the evaluation of bone pathologic conditions [20]. Whereas traditional histology will also deliver information regarding cells, µCT will not. Therefore, µCT will never fully replace histomorphometry. To a certain extent, however, it may serve as an alternative or addition to histology or as a high throughput tool for bone morphometry in industrial research and development scenarios.

CONCLUSION

Micro-CT corresponded well with histomorphometry and can therefore be a valuable tool to examine bone structures around implants in a 3-dimensional way. Implant

materal and geometry may affect halation and image quality, but do not necessarely prevent peri-implant bone analysis.

ACKNOWLEDGEMENTS

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FIGURES

Figure 1: Bone and implant were both defined as a region of interest, based on their threshold values. The yellow line marks a region of 0.75mm around the implant where the BA was measured. The data were cropped, leaving a region starting from the cortical bone to the apex of the implant (rectangular zone) in which the measurements were done. This procedure was similar for the titanium (a) and hydroxyapatite implants (b).

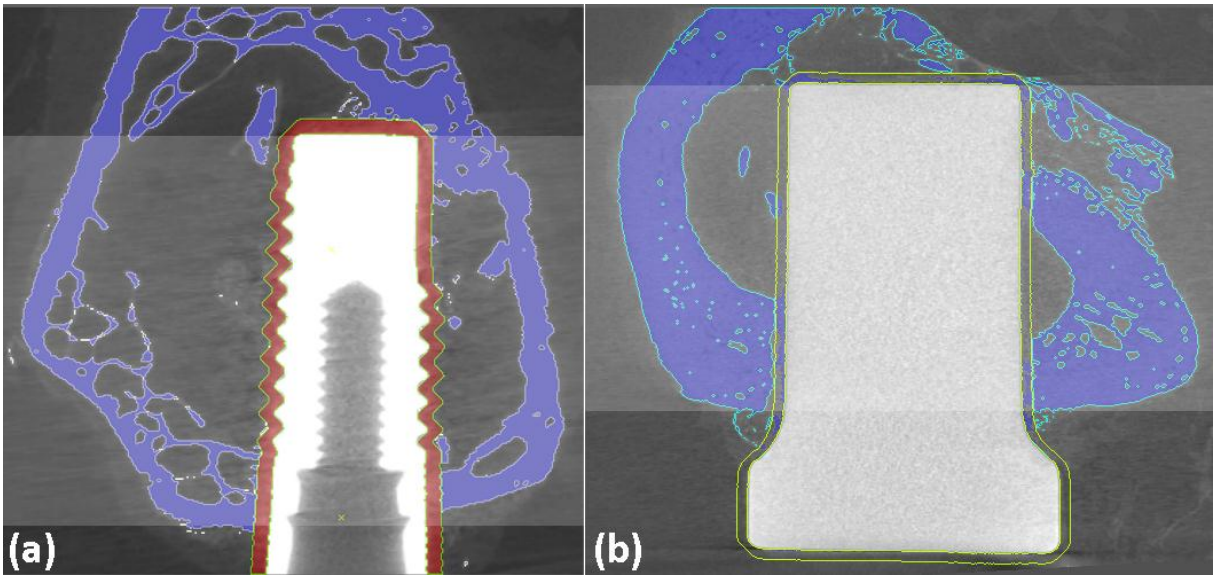


Figure 2: Boxplot representing the BIC and BA for the titanium implants, obtained by histomorphometry and micro-ct

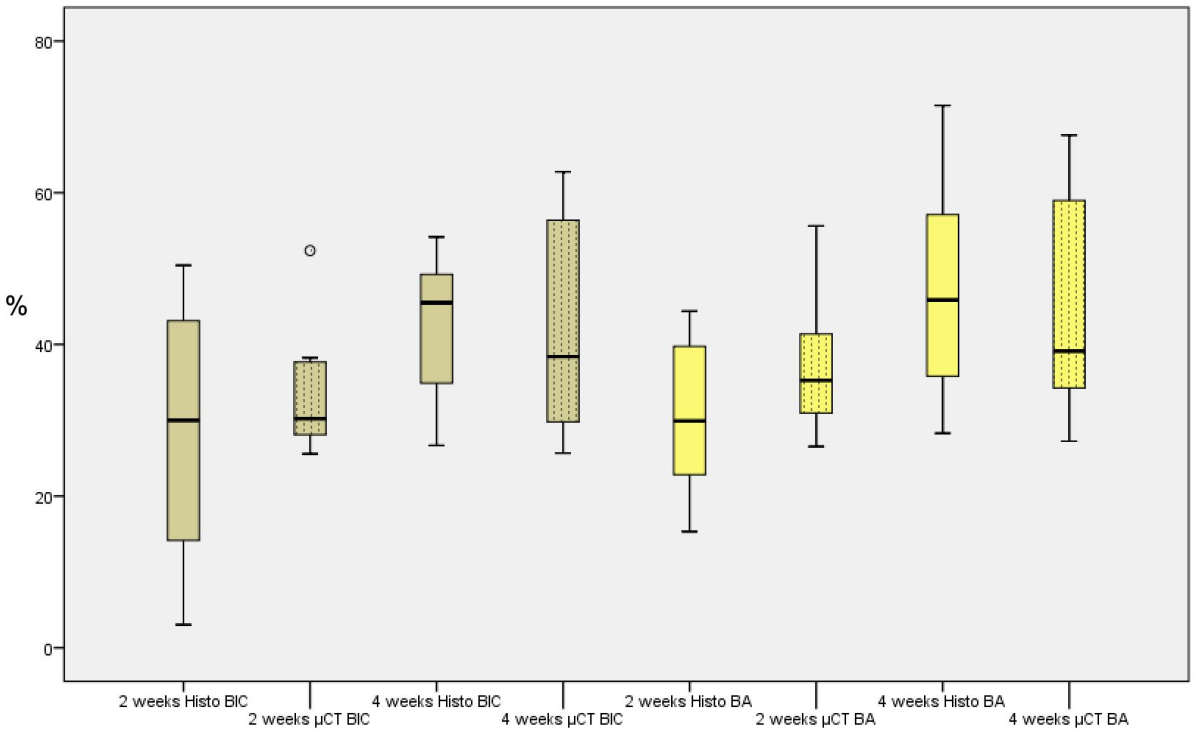


Figure 3: Boxplot representing the BIC and BA for the hydroxyapatite implants, obtained by histomorphometry and micro-ct.

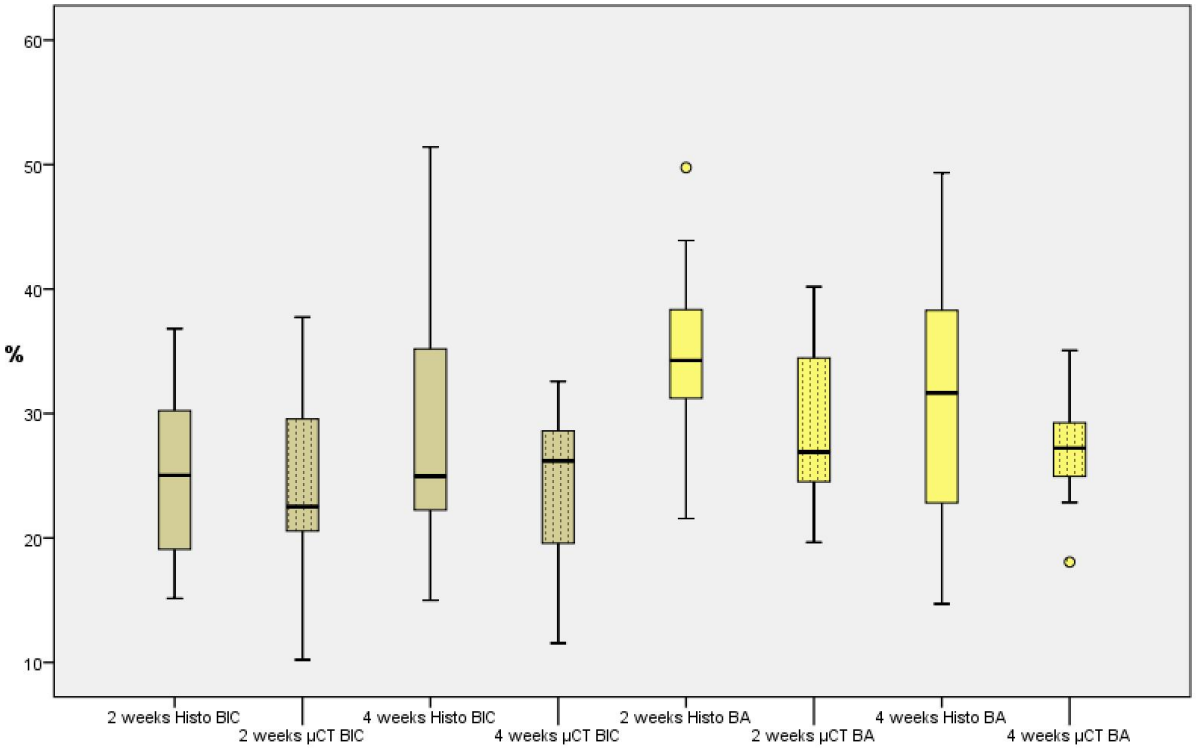


Figure 4: Figure representing the histological section (right) and the comparable layer from the micro-Ct image (left) for a Ti implant.

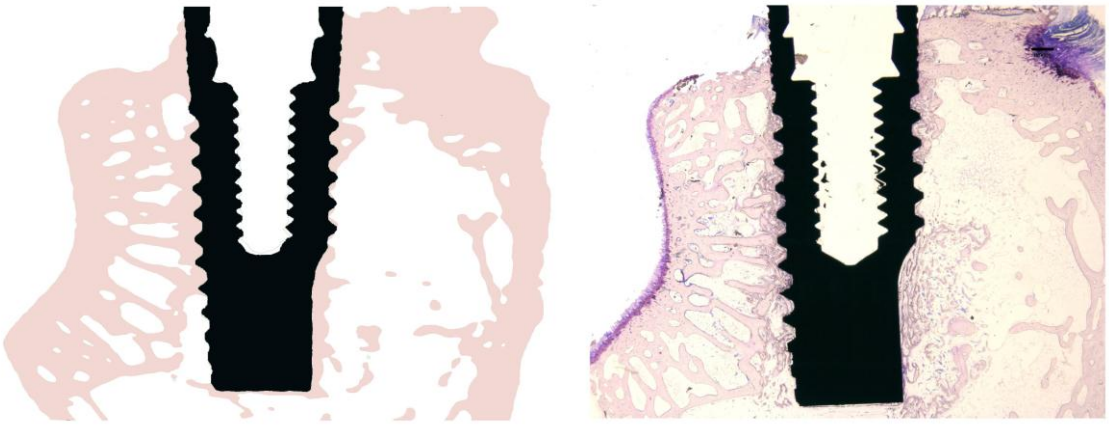


Figure 5: Figure representing the histological section (right) and the corresponding layer of the micro-CT image (Left) for a HA implant.

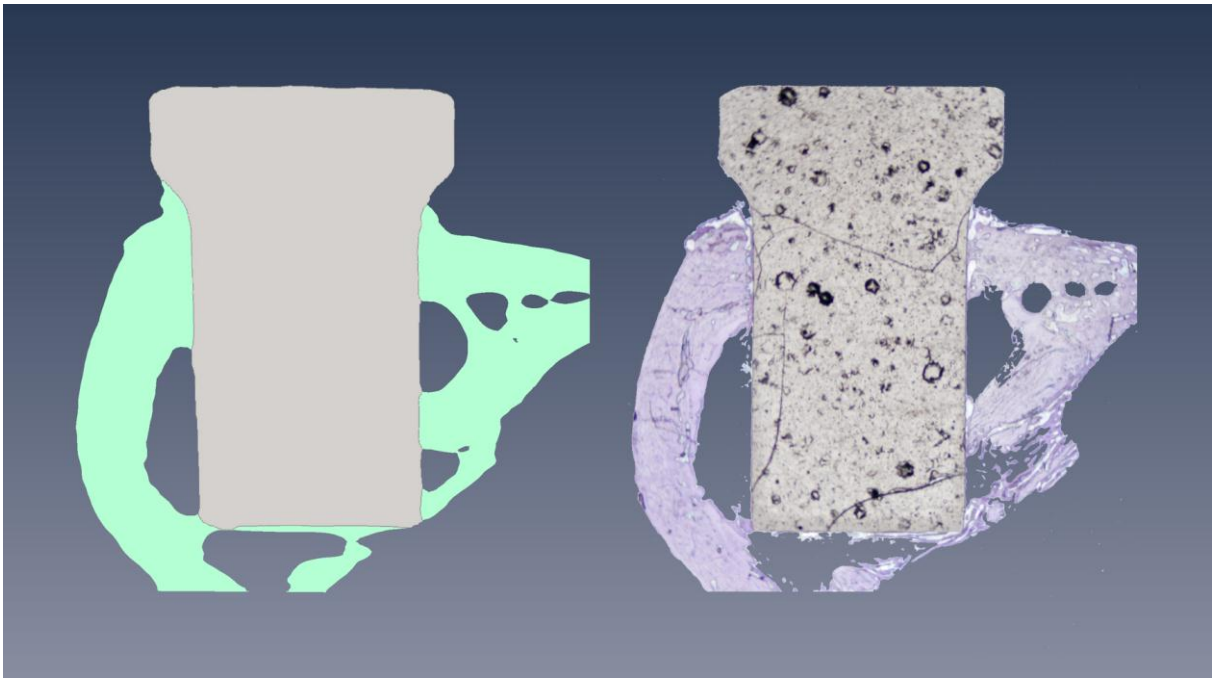


Figure 6: 3D representation of the titanium (left) and HA (right) implants in relation to the surrounding bone.

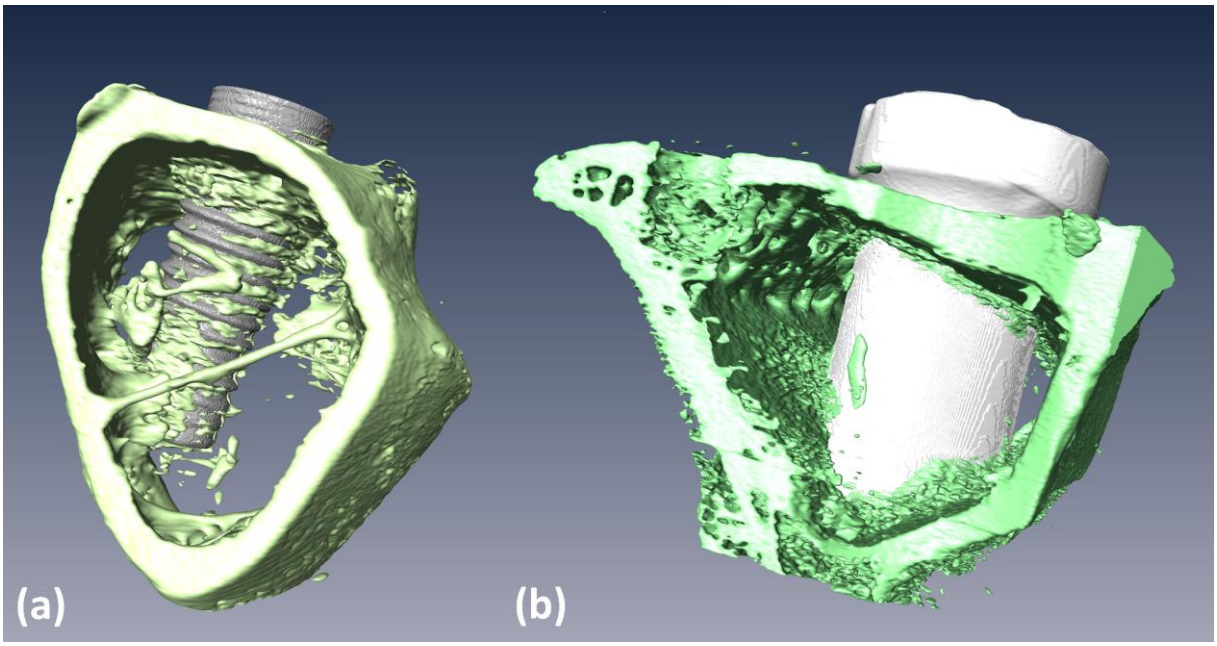
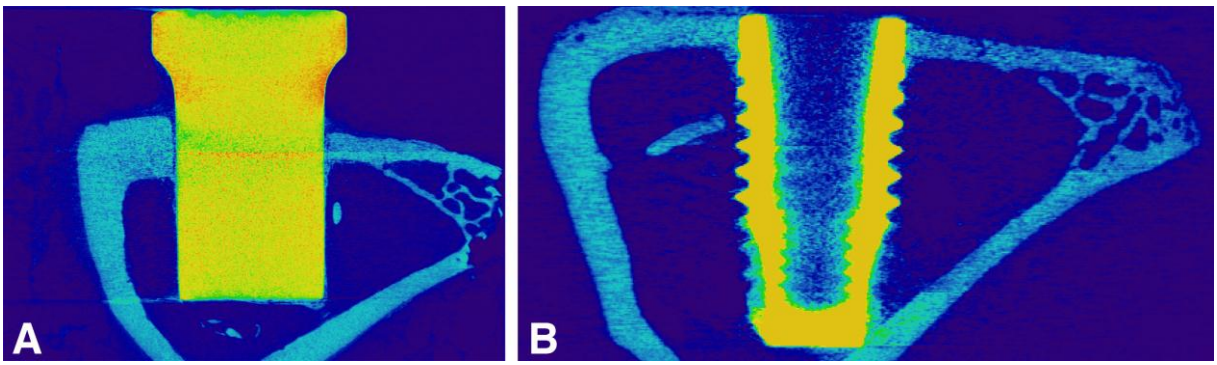


Figure 7: Color transformation of CT slices of a (A) hydroxyapatite and (B) titanium implant. The different color represent different attenuation levels. Almost no halation can be seen around the HA implant, while some halation can be seen around the Ti implant (light green), especially around the apical part.



REFERENCES

1. Attard NJ, Zarb GA. Long-term treatment outcomes in edentulous patients with implant-fixed prostheses: the Toronto study. Int J Prosthodont 2004;17(4):417-24.
2. Bergenblock S, Andersson B, Furst B, Jemt T. Long-Term Follow-Up of CeraOne Single-Implant Restorations: An 18-Year Follow-Up Study Based on a Prospective Patient Cohort. Clin Implant Dent Relat Res 2010.
3. Jemt T. Single implants in the anterior maxilla after 15 years of follow-up: comparison with central implants in the edentulous maxilla. Int J Prosthodont 2008;21(5):400-8.
4. Feldkamp LA, Goldstein SA, Parfitt AM, Jesion G, Kleerekoper M. The direct examination of three-dimensional bone architecture in vitro by computed tomography. J Bone Miner Res 1989;4(1):3-11.
5. Kuhn JL, Goldstein SA, Feldkamp LA, Goulet RW, Jesion G. Evaluation of a microcomputed tomography system to study trabecular bone structure. J Orthop Res 1990;8(6):833-42.
6. Muller R, Van Campenhout H, Van Damme B, Van Der Perre G, Dequeker J, Hildebrand T, Ruegsegger P. Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography. Bone 1998;23(1):59-66.
7. Uchiyama T, Tanizawa T, Muramatsu H, Endo N, Takahashi HE, Hara T. A morphometric comparison of trabecular structure of human ilium between microcomputed tomography and conventional histomorphometry. Calcif Tissue Int 1997;61(6):493-8.
8. Boas FE, Fleischmann D. CT artifacts: causes and reduction techniques. Imaging 2012;4(2):229-240.
9. Bernhardt R, Scharnweber D, Müller B, Thurner P, Schliephake H, Wyss P, Beckmann F, Goebbels J, Worch H. Comparison of microfocus- and synchrotron X-ray tomography for the analysis of osteointegration around Ti6Al4V implants. Eur Cell Mater 2004;7:42-51; discussion 51.
10. Sarve H, Lindblad J, Borgefors G, Johansson CB. Extracting 3D information on bone remodeling in the proximity of titanium implants in SRmuCT image volumes. Comput Methods Programs Biomed 2011;102(1):25-34.
11. Van Oossterwyck H, Duyck J, Vander Sloten J, Van der Perre G, Jansen J, Wevers M, Naert I. Use of microfocus computerized tomography as a new technique for characterizing bone tissue around oral implants. J Oral Implantol 2000;26(1):5-12.
12. Stoppie N, van der Waerden JP, Jansen JA, Duyck J, Wevers M, Naert IE. Validation of microfocus computed tomography in the evaluation of bone implant specimens. Clin Implant Dent Relat Res 2005;7(2):87-94.
13. Freilich M, Shafer D, Wei M, Kompalli R, Adams D, Kuhn L. Implant system for guiding a new layer of bone. Computed microtomography and histomorphometric analysis in the rabbit mandible. Clin Oral Implants Res 2009;20(2):201-7.
14. Schouten C, Meijer GJ, van den Beucken JJ, Spauwen PH, Jansen JA. The quantitative assessment of peri-implant bone responses using histomorphometry and micro-computed tomography. Biomaterials 2009;30(27):4539-49.
15. Butz F, Ogawa T, Chang TL, Nishimura I. Three-dimensional bone-implant integration profiling using micro-computed tomography. Int J Oral Maxillofac Implants 2006;21(5):687-95.
16. Ikeda H, Rossouw PE, Campbell PM, Kontogirogos E, Buschang PH. Three-dimensional analysis of peri-bone-implant contact of rough-surface miniscrew implants. Am J Orthod Dentofacial Orthop 2011;139(2):e153-63.
17. Dewulf W, Tan Y, Kiekens K. Sense and non-sense of beam hardening correction in CT metrology. CIRP Annals-Manufacturing Technology 2012.
18. Mouzin O, Soballe K, Bechtold JE. Loading improves anchorage of hydroxyapatite implants more than titanium implants. Journal of biomedical materials research 2001;58(1):61-68.

19. Johansson CB, Morberg P. Importance of ground section thickness for reliable histomorphometrical results. Biomaterials 1995;16(2):91-5.
20. Tamminen IS, Isaksson H, Aula AS, Honkanen E, Jurvelin JS, Kroger H. Reproducibility and agreement of micro-CT and histomorphometry in human trabecular bone with different metabolic status. J Bone Miner Metab 2010.