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Published in:
Journal of Cranio-Maxillofacial Surgery

DOI:
https://doi.org/10.1016/j.jcms.2017.05.019

Publication date:
2017

Document Version
Version created as part of publication process; publisher's layout; not normally made publicly available

Citation for published version (APA):
Synchrotron radiation µCT and histology evaluation of bone-to-implant contact

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Abstract

The purpose of this study was to evaluate bone-to-implant contact (BIC) in two-dimensional (2D) histology compared to high-resolution three-dimensional (3D) synchrotron radiation micro computed tomography (SR micro-CT). High spatial resolution, excellent signal-to-noise ratio, and contrast establish SR micro-CT as the leading imaging modality for hard X-ray microtomography. Using SR micro-CT at voxel size 5 μm in an experimental goat mandible model, no statistically significant difference was found between the different treatment modalities nor between recipient and reconstructed bone. The histological evaluation showed a statistically significant difference between BIC in reconstructed and recipient bone (p < 0.0001). Further, no statistically significant difference was found between the different treatment modalities which we found was due to large variation and subsequently due to low power. Comparing histology and SR micro-CT evaluation a bias of 5.2% was found in reconstructed area, and 15.3% in recipient bone. We conclude that for evaluation of BIC with histology and SR micro-CT, SR micro-CT cannot be proven more precise than histology for evaluation of BIC, however, with this SR micro-CT method, one histologic bone section is comparable to the 3D evaluation. Further, the two methods complement each other with knowledge on BIC in 2D and 3D.

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1. Introduction

Bone-to-implant contact (BIC) in loaded implants is proven to be 60–70% when using light microscopy, and 10% less in unloaded implants (Albrektsson, 2008). However, histology studies in minipigs, pigs, and dogs show comparable BIC in unloaded implants ranging between 49% and 77.2% after healing periods of 2–4 months (Stadlinger et al., 2009; Bressan et al., 2012; Botzenhart et al., 2015). Attainment of osseointegration and primary stability is essential for implant success. A high BIC is a prerequisite for implant stability and is vital in order to generate secondary stability (Brånemark, 1983; Albrektsson, 2008).

Osseointegration and bone structure are classically investigated in a 2D manner by histology (Brånemark et al., 1969). Three-dimensional (3D) evaluation of bone quantification, structure, and

* Sunstar Suisse SA, Etoy, Switzerland, is thanked for the financial support for the histological preparation of specimens, and for the tuition for the Ph.D. school. The authors and the Company were by signed contract kept out of conflict of interests.

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http://dx.doi.org/10.1016/j.jcms.2017.05.019
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Please cite this article in press as: Neldam CA, et al., Synchrotron radiation µCT and histology evaluation of bone-to-implant contact, Journal of Cranio-Maxillo-Facial Surgery (2017), http://dx.doi.org/10.1016/j.jcms.2017.05.019
mineralization has been performed with micro computed tomography (micro-CT) and with synchrotron radiation micro-CT (SR micro-CT) (Bonse et al., 1994; Britz et al., 2010; Peyrin et al., 2010; Sarve et al., 2011, 2013; Bernhardt et al., 2012; Stalder et al., 2014; Neldam et al., 2015). Micro-CT and SR micro-CT are non-destructive techniques, providing 3D images of bone in vitro (Nuzzo et al., 2002; Jung et al., 2003; Bernhardt et al., 2004).

However, beam hardening is inevitable with polychromatic beams used in micro-CT, which is not an issue with SR micro-CT, where a monochromatic beam is used (Nuzzo et al., 2002). SR micro-CT exploits the parallel-beam geometry, offers high spatial resolution, excellent signal-to-noise ratio, and, due to narrow bandwidth illumination, reduced artefacts and increased contrast. The strength of SR micro-CT lies in its 3D evaluation of the entire implant surface and, hence, the information it provides, including the geometry and distribution of peri-implant tissues (Bernhardt et al., 2012). Titanium implants have a higher X-ray absorption than bone. However, newly formed bone with a low mineral content can be difficult to detect at the implant interface due to its reduced absorption signal (Bernhardt et al., 2012). Furthermore, the partial volume effect (PVE), which is dependent on the CT resolution expressed by voxel size, provides challenges when materials with very different absorption coefficients are sampled in the same voxel, resulting in a measured beam attenuation proportional to only the average value of the two (Hohne and Bernstein, 1986; Ito et al., 2003; Bernhardt et al., 2012).

Histology is two-dimensional (2D), but can be transformed into 3D using stereology. The availability of a wide range of contrast agents offers superb sensitivity, but histology is basically a highly destructive technique (Bonse et al., 1994). SR micro-CT acquisition is presented in a 2D, slice-by-slice manner and subsequently reconstructed into 3D X-ray images. The methods are used to evaluate, in a non-destructive way, the implant contact with the surrounding tissues, including: bone, marrow spaces, grafting material, and fibrous tissue (Stiller et al., 2009; Rack et al., 2011; Neldam and Pinholt, 2014, Neldam et al., 2015).

In preparation for SR micro-CT scanning, and in contrast to preparation for histology, it is not necessary to reduce the size specimens in our study by cutting and grinding. Polishing of the bone specimens for histology may result in titanium particles dispersed throughout the specimen and embedding material, hence making BIC evaluation difficult. However, histology does offer important information on bone vitality, because it is not possible to visualize cells or osteoid in X-ray tomography.

Tooth loss is associated with bone loss; on average 40–60% bone loss is evident within the first 3 years after extraction (Tallgren, 1972; Schropp et al., 2003). Consequently, alveolar ridge augmentation can be necessary prior to implant installation. Although autologous bone is considered gold standard for augmentation, it has some inherent disadvantages, including limited quantities, and donor site morbidity (Ono et al., 2011). Consequently, bone augmentation procedures using synthetic bone substitute materials have become significantly more common. However, not many studies exist concerning SR micro-CT evaluation of different augmentation materials and titanium dental implants. Alloplastic materials such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) are promising materials due to their synthetic nature and their osteoconductive (HA, β-TCP) abilities and unlimited quantities (Kim et al., 2012; Leventis et al., 2016).

In this study, a goat model with a critical-sized defect (CSD) was used for evaluation of osseointegration in the mandible after immediate vertical bone augmentation. 35 bone specimens with five different augmentation procedures were used for evaluation. Additionally, a 3D imaging technique was applied and complemented with a 2D histological evaluation. The aim of the study was to evaluate BIC using 2D histology and 3D SR micro-CT after immediate vertical bone augmentation.

2. Material and Methods

2.1. Experimental animals and critical size defects

Seven healthy female adult goats (land-bred), 4-years of age, and weighing 40–50 kg, whose metabolism and bone formation are comparable with those of a human (Anderson et al., 1999), were chosen for this experimental study. A standardized CSD at the mandibular base of the goat bicortical, 25 mm in length, was chosen to represent the atrophic defect (Viljanen et al., 1996; Gao et al., 1997; Anderson et al., 1999) within the skeletal envelope (Lundgren et al., 1995; Slotte and Lundgren, 2002). The goats were housed and operated on at the research facilities of the Foulum Agricultural Center of Research, University of Aarhus, Denmark. National guidelines for use and care of laboratory animals were followed, and the study was approved by the Danish Veterinary Ethical Committee (permit no: 2006/561-1130).

2.2. Experimental design

Under general anesthesia, using Ketamine (Pfizer Aps., Ballerup, Denmark) and Streptocillin, pre- and post-operatively, i.m. 5 mL × 5 days (Boehringer Ingelheim A/S, Copenhagen, Denmark), five bone defects mimicking the atrophic alveolar process were created at the base of the mandible via an external bilateral approach. CSDs were created by reduction of the cortical basal part of the mandible, measuring 25 mm in length, 4 mm in height, and bicortical in width (Anderson et al., 1999). Insertion of 3.5 mm × 8 mm dental titanium implants (Astra Tech OsseoSpeed, ST Molndal, Sweden) with cover screws was performed centrally into each defect ad modum Astra, leaving 3.5 mm ejection into the defect (Fig. 1).

Randomized, immediate, vertical, peri-implant augmentation was performed in each defect (n = 35) using one of the following reconstructive methods:

i. Synthetic, resorbable, in situ hardening β-TCP, 0.4 mL (GUIDOR easy-graft CLASSIC, Sunstar Suisse SA, Etoy, Switzerland).

ii. Synthetic, partially resorbable, in situ hardening biphasic calcium phosphate (ratio HA/β-TCP: 60/40), 0.4 mL (GUIDOR easy-graft CRYSTAL, Sunstar Suisse SA, Etoy, Switzerland).

Fig. 1. Goat mandible with the critical sized defects and reconstruction material.
iii. Autologous bone augmentation by bone chips of between 0.5 \times 3 \times 1 \text{ mm}^3 and 0.5 \times 5 \times 1 \text{ mm}^3 in size, processed in bone mills (Liebinger, Freiburg, Germany; Quinten, Leimen, Germany). The defect was covered by a titanium membrane (Riemser Arzten mittte AG, Greifswald, Insel Riems, Germany), fixed with titanium alloy osteosynthesis self-tapping screws, 2.4 mm in diameter and 6 mm in length (Synthes, Solothurn, Switzerland).

iv. Autologous bone chips of between 0.5 \times 3 \times 1 \text{ mm}^3 and 0.5 \times 5 \times 1 \text{ mm}^3 in size, processed in bone mills (Liebinger, Freiburg, Germany; Quinten, Leimen, Germany), without a membrane.

v. Empty defect covered by a titanium membrane (Riemser Arzten mittle AG, Greifswald, Insel Riems, Germany), fixed with titanium alloy osteosynthesis self-tapping screws, 2.4 mm in diameter and 6 mm in length (Synthes, Solothurn, Switzerland).

2.3. Bone graft substitutes

Synthetic \( \beta \)-TCP (GUIDOR easy-graft CLASSIC, Sunstar Suisse SA, Etoy, Switzerland) and synthetic biphasic calcium phosphate (BCP) (GUIDOR easy-graft CRYSTAL, Sunstar Suisse SA, Etoy, Switzerland), with particle diameters of 0.5–1 mm and 0.45–1 mm respectively, were used. The BCP comprises 40\% \( \beta \)-TCP and 60\% HA, with every granule composed of a sintered mixture of both materials. The calcium phosphate granules are coated with a thin layer of resorbable polymer (poly(lactide-co-glycolide)). Prior to application, the granules were mixed with N-methyl-2-pyrrolidone (NMP) according to the company’s instructions, which plasticizes the coating, making the mass of granules formable. This hardens upon contact with blood and forms a stable scaffold after rinsing with sterile saline.

2.4. Harvesting and preparation of bone specimens

After 20 weeks, the goats were anesthetized with isoflurane (Baxter, Allerød, Denmark) and euthanized by an overdose of pentobarbital i.v. (100 mg/kg, Le Vet B.V. Oudewater, The Netherlands). The specimens were harvested, fixed in 10\% formaldehyde (Righospitalets Apotek, Rigshospitalet, Denmark), and dehydrated in graded alcohol solutions, for non-decalcified specimen preparation. The specimens were finally embedded in a cylinder, 12 mm in diameter, with methylmethacrylate (Technovit 9100 neu\®, Heraeus Kulzer, Wehrheim, Germany) ad modum Donath (Donath and Breuner, 1982). This represented a dental implant – 3.5 mm in diameter – surrounded radially by 4 mm of bone. Before cutting and grinding for microscopic slide preparation the specimens were scanned in SR micro-CT. Finally, the specimens were cut and ground for microscopic slide preparation in the Department of Biomaterials, Sahlgrenska Academy, University of Gothenburg, Sweden to produce non-decalcified specimens.

2.5. Synchrotron-based microtomography

Tomographic data were acquired at the ID19 beam line of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. As described in previous work (Neldam et al., 2015), high photon energy of 67.4 KeV was chosen in order to sufficiently transmit titanium implant material (Grodzins et al., 1983). A 2048 \times 2048 pixel indirect scintillator detector, with a pixel size of 5 \text{μm}, acquired tomographic scans of only the region of interest (ROI) – i.e. not the complete sample was scanned (local microtomography). Reconstruction was performed onsite using a filtered projection algorithm via the ESRF in-house developed software PyHST (Mirone et al., 2014). Full-size tomograms were 2048 \times 2048 \times 1024 pixels and the scanning was performed at 1999 angles (for complete descriptions see Neldam et al., 2015).

In order to evaluate BIC, a Matlab (MathWorks Inc., Massachusetts, USA) program was developed to semi-automatically segment the tomograms into implant, bone, and cavity, and to derive statistical data on these segments. For each tomogram, the following were applied: 1) a combined segmentation and bias correction, 2) identification of a coordinate system defined by the central axis of the implant, and 3) analysis of the volume of bone and cavity as a function of height and distance to the implant surface. In detail, each step was as follows:

1. Due to the large amount of data, the original tomograms were down-sampled by a factor of 4 in the x-, y-, and z-directions in the tomogram’s coordinate system, which resulted in isotropic voxel size of 20 \text{μm} (Fig. 2). Subdivision of the tomograms to avoid down-sampling was not possible due to the noise reduction in proximity to the implant. Subsequently, the segmentation was performed by manually identifying the location of an implant, a cavity, and a bone voxel, where both the cavity and bone voxels were outside

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![Flowchart illustrating the acquisition of data, from down sampling to BIC analysis.](image-url)
but near the implant surface. The threshold was found using the average intensity value between bone and implant, and was used to segment the implant. Furthermore, 12 seed location voxels, spread throughout the full tomogram, were manually identified. The down-sampled tomogram was smoothed, and the bias field was fitted to these values. The bias field, comprising an inhomogeneity in the CT, caused different levels of gray, representing, for example, bone. In the column to the right (Fig. 2), the bias field was subtracted from non-implant voxels, and the average bias-corrected intensity values between the initial bone and cavity locations were used as threshold values to segment bone and cavity. The segmented bone locations were then taken as seed locations, and the procedure was repeated until convergence.

2. Since the tomograms were given in non-standardized coordinate frames, an implant-centered coordinate system was identified as follows. Firstly, the centers of mass of the segmented implant voxels were used as origins. Then four points on the surface of the implant were manually identified (the direction from macro to micro thread is denoted up): i. The end point of the valley of the micro thread in the first ridge of the macro thread (Fig. 3) denoted the reference point. ii. The micro thread above the reference point. iii. The peak of the 13th micro thread above the reference point. iv. The ridge of the first macro thread below the reference point.

Since the threads of the implants have the shape of a torus, it was not possible to define a plane that separated the micro and macro threads. Subsequently, zones were defined by the implant’s vertical axis: the micro thread area was initiated from ii) to iii), and the macro thread area was demarcated by ii) and iv). To analyze the BIC, the bone and cavity volumes were evaluated in a 3D volume band from 0 to 10 μm perpendicular to the implant surface in both micro and macro thread areas.

2.6. Definition of region of interest

The region of interest (ROI) was defined as the peri-implant bone covering the implant surface. The implant was divided into two vertical zones (Fig. 4):

1. The micro thread area: comprising the different treatment modalities.
2. The macro thread area: comprising recipient bone at the two first macro threads, serving as measures for recipient bone.

2.7. Definition of BIC

Histologically, BIC is defined as bone in direct contact with the implant surface. Furthermore, marrow, graft, and fibrous tissue were also evaluated when in contact with the implant surface. Additionally, BIC was evaluated by SR micro-CT within the nearest two voxels of the surface.

2.8. Histology evaluation

After scanning at the ESRF the undecalciﬁed bone specimens were cut and stained in the Department of Biomaterials, Sahlgrenska Academy, University of Gothenburg, Sweden ad modum Donath (Donath and Breuner, 1982). The tissue blocks were cut into sections after random rotation around the vertical axis of the implant center. The sections with a thickness of approximately 100 μm were cut and ground down to a thickness of 10 μm (Donath and Breuner, 1982) and surface-stained with toluidine blue.

BIC was measured using a microscope (Nikon ECLIPSE 80i, Tokyo, Japan) equipped with a motorized Proscan 11 stage (Prior, Cambridge, UK), a MT1201 microcator (Heidenhain, Traunreut, Germany), and a DP72 digital camera (Olympus, Tokyo, Japan) connected to a PC with the stereological software newCAST (version 3.4.1.0, Visiopharm, Hørsholm, Denmark). The measurements were performed in the Department of Rheumatology, Aarhus University, Aarhus, Denmark.

BIC was evaluated using cycloid test lines parallel to the vertical axis, generating isotopic, uniformly random, intersection points with the structure of interest (Baddeley et al., 1986). Full-length grid lines were used to evaluate the intersections between the different tissues and the surface of the dental implant. The evaluation was performed at a magnification of ×462, with a line spacing of 30 μm, and sampling fraction of 200% in the two ROIs. Quantitative measurement of BIC was the primary outcome variable and measured as bone in direct contact with the implant surface. Furthermore, direct contact between the implant surface and graft, bone marrow, and fibrous tissue was measured.

2.9. Statistics

The results from the 2D histology tests were presented as mean and standard deviation (SD) for each bone specimen. The analyses were performed using Excel version 2010 (Microsoft Corporation, WA, USA). The statistical model used for comparison of data from the different treatment modalities (n = 5), and a comparison of peri-implant tissue volume in micro and macro thread areas, was performed, using mixed-model analyses, with the statistical software SAS (SAS Institute, Inc., NC, USA). p-values <0.05 were considered significant. Furthermore, in order to measure the difference in BIC between the gold standard treatment – autologous bone with a membrane – and easy-graft CLASSIC and easy-graft CRYSTAL, post-hoc analyses in both the micro and macro thread areas, using https://www.stat.ubc.ca/~rollin/stats/ssize/n1.html,
were performed. Coincidence between the two methods was determined by correlation analysis, and presented as a Bland–Altman plot (Altman and Bland, 1983). In our case, histology was considered gold standard and thus the difference between the two methods was calculated by subtracting the SR micro-CT results from the histologic measurements.

3. Results

The animals revealed normal wound healing and the observation period was uneventful, no implants were lost, and all implants healed submerged, without exposure. In the micro thread area, four samples had to be excluded due to off-center cutting of the samples. In the macro thread area three samples were excluded.

3.1. Histological evaluation of BIC

In the micro thread area (augmented area) four specimens had to be excluded due to off-center cutting, hence 31 specimens were evaluated. In the macro thread area (recipient bone) three specimens had to be excluded due to off-center cutting, hence 32 specimens were evaluated. Histological measures concerning mean BIC, SD, and \( p \)-values can be found in Table 1. The 2D evaluation showed a statistically significant difference in BIC between micro and macro thread areas \( (p < 0.0001) \). No statistically significant difference was found between the different treatment modalities in either the micro or macro thread areas, when evaluating the different tissues. In general, high variation was found in both micro and macro thread areas.

Power calculations between easy-graft and autologous bone with a membrane revealed a power of 59%, in the micro thread area, with this study’s sample size \( (n = 12) \). For a power of 80% a sample size of 20 would have been required. In the macro thread area, a power of 9% was found \( (n = 12) \). Again, for a power of 80% a sample size of 245 would have been required. When comparing easy-graft CRYSTAL with autologous bone with a membrane, a power of 16% was found in the micro thread area \( (n = 14) \). For a power of 80% a sample size of 118 would have been required. In the macro thread area, a power of 79% was found \( (n = 14) \), but for a power of 80% a sample size of 15 would have been required.

The coefficient of variation (CV%) in the micro thread area, for five randomly chosen bone sections, 1 year after the first evaluation, was 5.1%.

3.2. SR micro-CT evaluation of BIC

SR micro-CT measures concerning mean BIC, SD, and \( p \)-values can be found in Table 2. The 3D evaluation showed no statistically significant difference between the micro and macro thread areas \( (p = 0.1530) \). No statistically significant difference was found
and 0.39 in the micro and macro thread areas, respectively (Fig. 5).

15.3% in the micro- and macro thread areas, respectively, for SR micro-CT when compared with histology (Figs. 6 and 8). However, this was not evident in the SR micro-CT evaluation. Nevertheless, the histological evaluation revealed a statistically significant difference in BIC between the different treatment modalities in the micro thread area. This resulted in evaluation of 1
to 2 macro threads, because the implant shape was a helix. Since the histological sections were rotated in an unbiased way, it was decided to evaluate one complete macro thread and the peak of the next thread for approximation of comparable ROIs in 2D and 3D. Evaluation of the Bland–Altman plot revealed a lower bias when BIC was <20% compared with higher BIC values. This indicates that an uncertainty exists when evaluating large amounts of bone in close proximity to the implant surface or maybe the single bone section evaluated is not representative of the entire bone sample.

4. Discussion

The aim of this study was to compare the histomorphometric evaluation of BIC, which has been used for decades, with more recent 3D SR micro-CT technology. High BIC is considered a prerequisite for implant stability and survival, and a functional dental reconstruction. The use of a CSD with immediate implant installation and bone augmentation was chosen. In this study, 35 bone specimens with five different augmentation procedures were used. No statistically significant difference in BIC was found between defects reconstructed with autologous bone and with the different grafting materials easy-graft CLASSIC and easy-graft CRYSTAL. Nevertheless, the histological evaluation revealed a statistically significant difference between the micro and macro thread areas; this was not evident in the SR micro-CT evaluation.

This study is, to our knowledge, the first to document and evaluate BIC after immediate vertical bone augmentation consisting of autologous bone, β-TCP (easy-graft CLASSIC), and the BCP bone graft material easy-graft CRYSTAL (β-TCP and HA), comparing SR micro-CT quantitative results with conventional histology. Histological evaluation revealed that cavities reconstructed with autologous bone with a membrane had the highest mean BIC, with 30% in the grafted area. Differences in healing resulting from autologous bone, which is considered the gold standard for bone augmentation (Ono et al., 2011), were not statistically significant when compared with the other treatment modalities in this study. The lack of statistically significant differences could be due to large variability and subsequently low power. Intra-observer variation of 5.1% (CV%), evaluated 1 year after the initial histological analysis, was acceptable.

Evaluation of BIC with SR micro-CT has been performed in several studies. However, a comparison is difficult because of a large variability in methods used, and none of the evaluated studies are in reconstructed bone (Bernhardt et al., 2004, 2012; Sarve et al., 2013). In our study, a comparison in the micro thread area of SR micro-CT and histology presented a bias of 5.2%, which is considered minor. Bernhardt et al. (2012) found a comparable, non-significant difference of 4.9% when comparing four bone sections with 3D SR micro-CT scans. Furthermore, evaluation of the Bland–Altman plot revealed a lower bias when BIC was <20% compared with higher BIC values. This indicates that an uncertainty exists when evaluating large amounts of bone in close proximity to the implant surface or maybe the single bone section evaluated is not representative of the entire bone sample. Furthermore, in this study a bias of 15.3% in the macro thread area was found, which might be due to differences in size of the macro thread area in 2D and 3D (Fig. 4). The ROI in the macro thread area was demarcated at the lower border by the next macro thread in accordance with the transition between micro and macro thread. This resulted in evaluation of 1 to 2 macro threads, because the implant shape was a helix. Since the histological sections were rotated in an unbiased way, it was decided to evaluate one complete macro thread and the peak of the next thread for approximation of comparable ROIs in 2D and 3D. Evaluation of the Bland–Altman plot in the macro thread area revealed large variability between SR micro-CT and histology, which we believe is caused by the differences in size of the evaluated macro thread area, in this study.

Table 1

<table>
<thead>
<tr>
<th>Treatment mean (SD)</th>
<th>Bone Micro</th>
<th>Bone Macro</th>
<th>Marrow Micro</th>
<th>Marrow Macro</th>
<th>Fibrous tissue Micro</th>
<th>Fibrous tissue Macro</th>
<th>Graft Micro</th>
<th>Graft Macro</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Easy-graft CLASSIC</td>
<td>21.3% (13.7%)</td>
<td>42.5% (30.3%)</td>
<td>27.2% (14.2%)</td>
<td>56.9% (30.1%)</td>
<td>51.5% (18.4%)</td>
<td>0.7% (1.5%)</td>
<td>0% (0%)</td>
<td>0.8470</td>
<td>Bone 0.8470</td>
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<tr>
<td>Easy-graft CRYSTAL</td>
<td>23.4% (23.1%)</td>
<td>38.6% (20.6%)</td>
<td>47% (17.4%)</td>
<td>58% (14.3%)</td>
<td>29.3% (23.7%)</td>
<td>3.3% (8%)</td>
<td>24.8% (16.2%)</td>
<td>0% (0%)</td>
<td>Bone 0.8470</td>
</tr>
<tr>
<td>Autologous bone with a membrane</td>
<td>30% (18.5%)</td>
<td>47.7% (24.3%)</td>
<td>45.2% (22.6%)</td>
<td>52.3% (24.3%)</td>
<td>0.7% (1.5%)</td>
<td>0% (0%)</td>
<td>0.6724</td>
<td>Graft 0.5132</td>
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<td>Autologous bone without a membrane</td>
<td>19.7% (18.1%)</td>
<td>31% (25.7%)</td>
<td>28.1% (24.3%)</td>
<td>63.2% (32%)</td>
<td>52.2% (36.5%)</td>
<td>5.8% (14.2%)</td>
<td>Marrow 0.2499</td>
<td>Graft MV</td>
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<tr>
<td>Empty</td>
<td>18.4% (15%)</td>
<td>42.4% (19.3%)</td>
<td>32.4% (17.2%)</td>
<td>51.3% (21.6%)</td>
<td>33.4% (28%)</td>
<td>6.3% (13.5%)</td>
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Table 2

<table>
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<th>Treatment mean (SD)</th>
<th>Bone Micro</th>
<th>Bone Macro</th>
<th>Cavity Micro</th>
<th>Cavity Macro</th>
<th>p-value</th>
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<td>80.5% (12.4%)</td>
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<td>Easy-graft CRYSTAL</td>
<td>25.7% (14.8%)</td>
<td>22.8% (14.2%)</td>
<td>74.3% (14.8%)</td>
<td>77.2% (14.2%)</td>
<td>Bone 0.2499</td>
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<td>Autologous bone with a membrane</td>
<td>14.8% (4.9%)</td>
<td>19.2% (6.4%)</td>
<td>85.2% (4.9%)</td>
<td>80.8% (6.4%)</td>
<td>Marrow 0.2499</td>
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<tr>
<td>Autologous bone without a membrane</td>
<td>21.5% (14.9%)</td>
<td>25.7% (18%)</td>
<td>78.5% (14%)</td>
<td>74.3% (18%)</td>
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<td>12.1% (8.2%)</td>
<td>21.2% (14.6%)</td>
<td>87.9% (8.2%)</td>
<td>78.8% (14.6%)</td>
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Please cite this article in press as: Neldam CA, et al., Synchrotron radiation μCT and histology evaluation of bone-to-implant contact. Journal of Cranio-Maxillo-Facial Surgery (2017), http://dx.doi.org/10.1016/j.jcms.2017.05.019
According to Bernhardt et al. (2004, 2012) and Sarve et al. (2013), the PVE influences the BIC in the SR micro-CT scan because the evaluation is performed 1–2 pixels away from the surface, while in the histologic evaluation the BIC is measured at the implant surface, resulting in an allegedly different BIC with the SR micro-CT scans compared with histology. Furthermore, thin, separated regions of mineralized tissue in proximity to the implant surface might be underestimated in SR micro-CT but included in histology (Bernhardt et al., 2004). However, we believe that refraction, which is an artefact in CT scans, influences the BIC.

Fig. 5. The BIC correlation analysis between histology and SR micro-CT in the micro thread area (augmented area).

Fig. 6. The agreement analysis showed a bias of 5.2% in the micro thread area for SR micro-CT compared with histology.
evaluation. Refraction effects can disturb absorption tomography images. Refraction is strongest at interfaces between materials with very different refractive indexes. At interfaces between two materials of very different densities, e.g. the interface between a titanium implant and the much less dense surrounding tissue, refraction will typically enhance the contrast difference between the two materials, as demonstrated by (Liu et al., 2009), leading to edge enhancement in the image. Ito et al. (2003) evaluated bone samples with micro-CT and SR micro-CT and found that the PVE was not evident on the SR micro-CT evaluation (voxel size 6 \( \mu m \)), whereas the micro-CT revealed a blurred border between bone and cavity. In our study, when evaluating the titanium implant in the center of a bone sample, a bright line was visible at the edge of the implant (Fig. 9). The algorithm used in this study aimed to compensate for refraction, and with a difference of 5.2% between histology and SR micro-CT in reconstructed bone, this was considered acceptable.

The segmentation of bone in histological specimens can be hindered by preparation artifacts. Small titanium particles were evident in some of the specimens, but not in close proximity to the implant surface. Minor movements and preparation artefacts (cutting and polishing) in histology are excluded when using SR micro-CT. Ring artifacts and star-like structures are evident in the SR micro-CT scans, and the high absorption of implants compared with bone provides reconstructive challenges (Bernhardt et al., 2004). Nevertheless, the parallel beam of the SR micro-CT provides a slice-by-slice reconstruction of the tomograms, with absorption coefficients giving a Gaussian shape, which consequently makes it rather simple to determine the threshold for the different components such as bone, implant, and embedding material, even if the related peaks overlap (Bernhardt et al., 2004).

Generally, bone was underestimated using the 3D evaluation. Non-mineralized or low-mineralized tissue is invisible with SR micro-CT when using high voltage for evaluation of highly absorbent titanium implants in close proximity to bone. The BIC in this study was evaluated as volume in a band 0–10 \( \mu m \) from the implant surface, and the threshold in proximity to the implant was amended to adjust for refraction. Our findings are in accordance with Bernhard et al. (2004), and the BIC found in this study was low compared with that found by Bernhardt et al. (2012) and Sarve et al. (2013).

A disadvantage of 3D evaluation for BIC is the low lateral resolution compared with histology. In our study a 5 \( \mu m \) pixel size was used, and the BIC evaluation in 3D was applied to a volume 0–10 \( \mu m \) perpendicular to the implant surface, hence not a ‘true’ BIC. This was comparable to previous studies in which BIC was measured from 10 to 18 \( \mu m \) from the surface (Bernhardt et al., 2004, 2012; Sarve et al., 2013). The PVE can hinder histological measurements if the slides are too thick (Johansson and Morberg, 1995). Sections with a thickness over 30 \( \mu m \) were found to overestimate true bony contacts due to over-projection (Johansson and Morberg, 1995). However, the sections used in this study were 10 \( \mu m \) thick. The challenge when evaluating BIC, which is a 2D factor, is that we want to obtain those data using 3D measurements. Histologically, BIC is measured as tissue intersections at the implant surface and thus not in 3D. Furthermore, down sampling of the tomograms — which is necessary for handling the large amount of data, in this study — makes it challenging to determine exactly which type of tissue is in direct contact with the implant surface. Nevertheless, the algorithm used revealed a difference between histology and SR micro-CT of 5.2% when comparing 3D with one bone section.

**Fig. 7.** The BIC correlation analysis between histology and SR micro-CT in the macro thread area (recipient bone).
5. Conclusion

In conclusion, 3D evaluation is effective when applied to bone micro architecture due to its non-destructive nature. However, when it comes to assessing BIC, this technique has shown limitations. Generally, SR micro-CT underestimates bone compared with histology and cannot be considered more effective than histology for evaluation of BIC. However, the SR micro-CT method used in this

Fig. 8. The agreement analysis showed a bias of 15.3% in the macro thread area for SR micro-CT compared with histology.

Fig. 9. Tomographic slice of an implant with surrounding bone (left). The magnified area (right) shows bright lines visible at the edge of the implant, verifying that the refraction effect enhances the contrast edges of the implant.
study was highly comparable to histological evaluation. No statistically different differences were found between the different treatment modalities, which we found were due to large variability and subsequently low power. When comparing 2D and 3D, a bias of 5.2% was found in reconstructed bone, quite in agreement with previous studies (Müller et al., 1998; Stiller et al., 2009; Bernhardt et al., 2012). One important issue when evaluating in 2D or 3D is the spatial resolution—distinguishing bone, graft, and cavity is dependent on the voxel size, so a higher resolution might provide more information. The resolution is improving at the synchrotron source, but the limiting factor could be the capacity to handle the enormous amounts of data that come with high-resolution imaging.

Conflicts of interest
None.

Acknowledgement
We would like to thank the staff at the ESRF, beamline ID19, Grenoble, France: Paul Tafforeau and Elodie Boller for performing the scans, the reconstructions and their generous help during our visits to the research facilities. Sunstar Suisse SA, Etoy, Switzerland is thanked for the financial support for the histological preparation of specimens, and for the PhD tuition.

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