

## RESEARCH

# Evaluation of demineralized bone and bone transplants *in vitro* and *in vivo* with cone beam computed tomography imaging

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**Objectives:** The objective of this study was to determine the ability of two flat panel cone beam CT (CBCT) devices to identify demineralized bone and bone transplants *in vivo* and *in vitro*.

**Methods:** Datasets from patients with autologous bone grafts ( $n = 9$ , KaVo 3DeXam (KaVo, Biberach, Germany);  $n = 38$ , Accuitomo 40 (Morita, Osaka, Japan)) were retrospectively evaluated. Demineralized and non-demineralized porcine cancellous bone blocks were examined with the two CBCT devices. A SawBone<sup>®</sup> skull (Pacific Research Laboratories, Vashon, WA) was used as a positioning tool for the bone blocks. Descriptive evaluation and image quality assessment were conducted on the KaVo 3DeXam data (voxel size 0.3 mm) using the OsiriX viewer as well as on the Morita Accuitomo data (voxel size 0.25 mm) using proprietary viewer software.

**Results:** Both *in vivo* and *in vitro*, the descriptive analysis of the images of the two devices showed well-visualized bone transplants with clearly defined cancellous bones and well-defined single bone trabeculae in all cross-sections. *In vitro*, demineralized samples showed lower radiographic opacity but no significant loss of quality compared with fresh bone ( $P = 0.070$ ). Single cancellous bone trabeculae were significantly better visualized with the Morita 3D Accuitomo device than with the KaVo 3DeXam device ( $P = 0.038$ ).

**Conclusion:** Both the KaVo 3DeXam and Morita 3D Accuitomo devices produce good-quality images of cancellous bones in *in vivo* remodelling as well as after *in vitro* demineralization.

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**Keywords:** cone beam computed tomography; bone augmentation; implantology

## Introduction

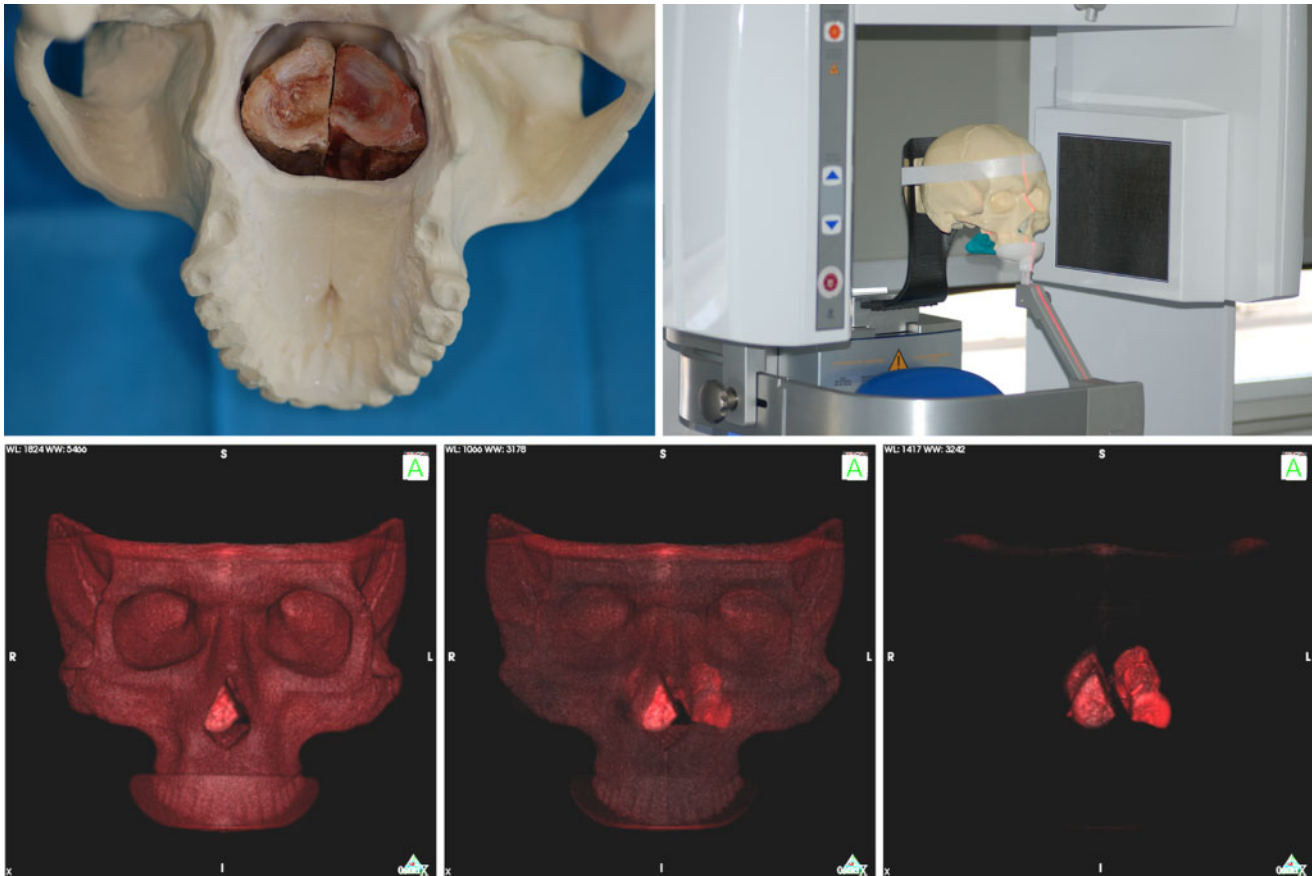
Cone beam CT (CBCT) is an imaging technology based on the axial cross-sectional reconstruction of radiographic images of cylindrical or spherical volumes.<sup>1</sup> CBCT is primarily used as an alternative to multi-detector CT (MDCT) to generate planar cross-sections and three-dimensional reconstructions of bone situations with a lower radiation dose. A further advantage of the CBCT device is its general availability in dental practices.<sup>2–5</sup> Multidimensional imaging technologies are crucial for meeting the requirements of modern dental implantology and oral surgery.<sup>2</sup>

CBCT has been assumed to be inappropriate for the imaging of partly demineralized bone, including bone transplants in the remodelling phase after augmentation.<sup>3</sup>

However, CBCT devices have improved significantly in recent years. Several manufacturers have developed more advanced image intensifiers, and flat panel technology has become a further detector type in sectional radiography.<sup>4–7</sup> Current flat panel volume CT devices in dentistry include, for instance, the Accuitomo (Morita, Osaka, Japan), NewTom VG (Quantitative Radiology, Verona, Italy), ProMax 3D (Planmeca, Helsinki, Finland), Picasso (E-Woo, Giheung-gu, South Korea), KaVo 3DeXam (former i-CAT) (KaVo, Biberach, Germany), and several Orange Dental devices including Picasso Master (Orange Dental, Bora-dong, South Korea), Scanora 3D (Soredex, Subang Jaya, Malaysia) and Iluma (Kodak Dental Systems, Rochester, NY).

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**Figure 1** Experimental set-up for the *in vitro* experiments. Top left: position of both samples in the maxillary sinus of the SawBone®. Top right: position of the SawBone® equivalent to the position in a patient (here the KaVo 3DeXam). Lower left to right: OsiriX 3D imaging of the experimental set-up with the SawBone® fading away to show the samples in the correct position

This study further evaluates cancellous bone imaging during the remodelling phase by testing two CBCT devices, *i.e.* the Morita 3D Accuitomo 40 and the KaVo 3DeXam, in an *in vitro* model of demineralized bone. Data from patients with autologous bone grafts were evaluated retrospectively.<sup>8</sup> Data from patients were used to provide imaging results of cancellous bone with both devices. The *in vitro* model presented here is one method to standardize the imaging of demineralized bone.

## Materials and methods

### Study design

The first part of the study retrospectively evaluated patient data for both CBCT devices. The second part evaluated freshly frozen and demineralized porcine spine bone specimens ( $n = 20$  per group) for both CBCT devices; a SawBone® skull (Pacific Research Laboratories, Vashon, WA) was used as a positioning tool for the bone specimens.

### Patient data

We retrospectively evaluated CBCT data of patients from the University of Mainz who had autologous bone

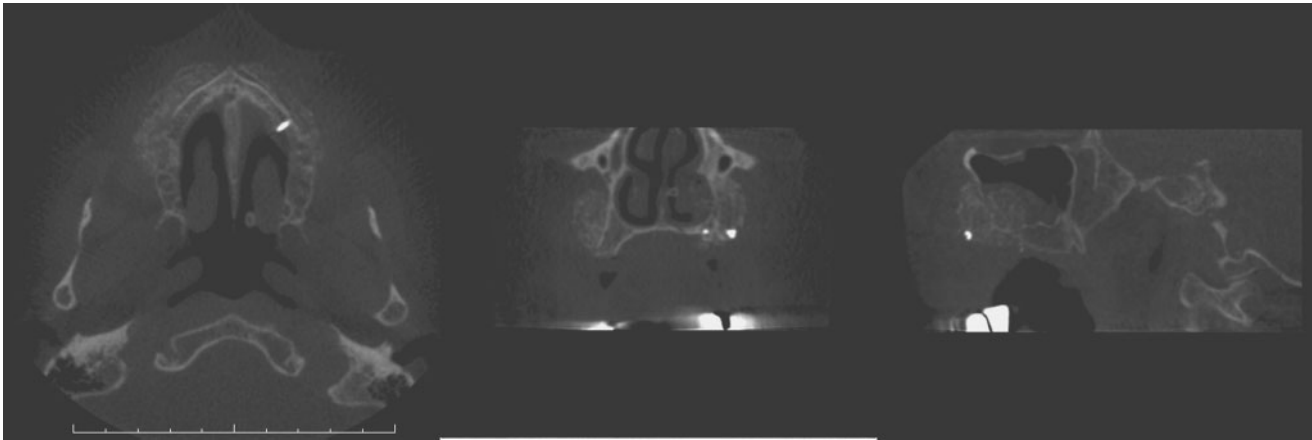
grafts but no additional alloplastic ceramic material ( $n = 9$  for the KaVo 3DeXam and  $n = 38$  for the Morita Accuitomo). The KaVo 3DeXam group consisted of 6 women aged 49–77 years and 3 men aged 48–73 years; the Morita Accuitomo group consisted of 26 women aged 44–79 years and 12 men aged 43–75 years. Patient data were used in compliance with current ethics guidelines and laws.

### SawBone® based skull model

A SawBone® skull was modified by cutting off the dorsal wall of the maxilla to make room for half a porcine spine vertebra. We did not choose a real bone skull to avoid imaging interference with the samples. Other commercially available models, such as the full skull RANDO® phantom, were not appropriate for the purpose of this study since no samples can be placed in this model. The SawBone® skull allowed bone grafts to be placed at the correct positions equivalent to those in patients (Figure 1).

### In vitro testing of porcine bone material

Porcine spines, already cut in half, were obtained from a Munich slaughterhouse. Half pieces of vertebral bone



**Figure 2** Example from the KaVo 3DeXam device the three radiological imaging cross-sections show the bone transplant in the area of the left alveolar rim in a patient (100 days after transplantation from the iliac crest)

were mechanically separated and freed from connective tissue. 20 pieces were freshly frozen at  $-30^{\circ}\text{C}$ ; 20 other specimens were demineralized in 10% ethylenediamine-tetraacetic acid/phosphate-buffered saline (EDTA-PBS; pH 7.4) for 7 days, and the solution was changed daily as previously described.<sup>9</sup> The demineralized porcine bone pieces showed 75% mineralization in densitometry measurements. The pieces were then defatted with acetone. CBCT scanning was carried out with a SawBone<sup>®</sup> skull as a positioning device (Figure 1). Specimens were packed in a latex cover for hygiene reasons.

#### CBCT imaging data analysis

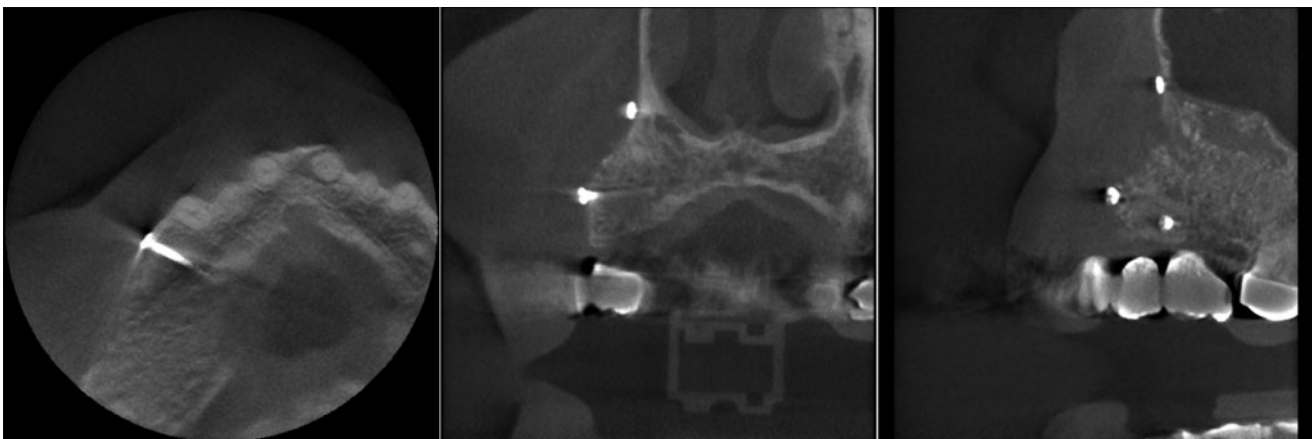
The devices used in this study were the 3D Accuitomo 40 and the KaVo 3DeXam. Data from both study groups were evaluated in the same manner. KaVo 3DeXam data (voxel size 0.3 mm; 120 kV and approximately 5 mAs) were descriptively evaluated by means of multiplanar reconstruction images of each dimension, which were produced with the OsiriX software using a bone window setting (OsiriX version 2.7.5

(OsiriX Foundation, Geneva, Switzerland); window level: 300.00; window width: 1500.00; resulting in a window between  $-450$  and  $+1050$  Hounsfield units).<sup>10</sup> Data from the Morita Accuitomo (voxel size 0.25 mm; approximately 85 kV and 3.5 mAs) were analysed with proprietary viewer software. All image-processing steps were of clinical standard and did not result in contrast or pixel loss.

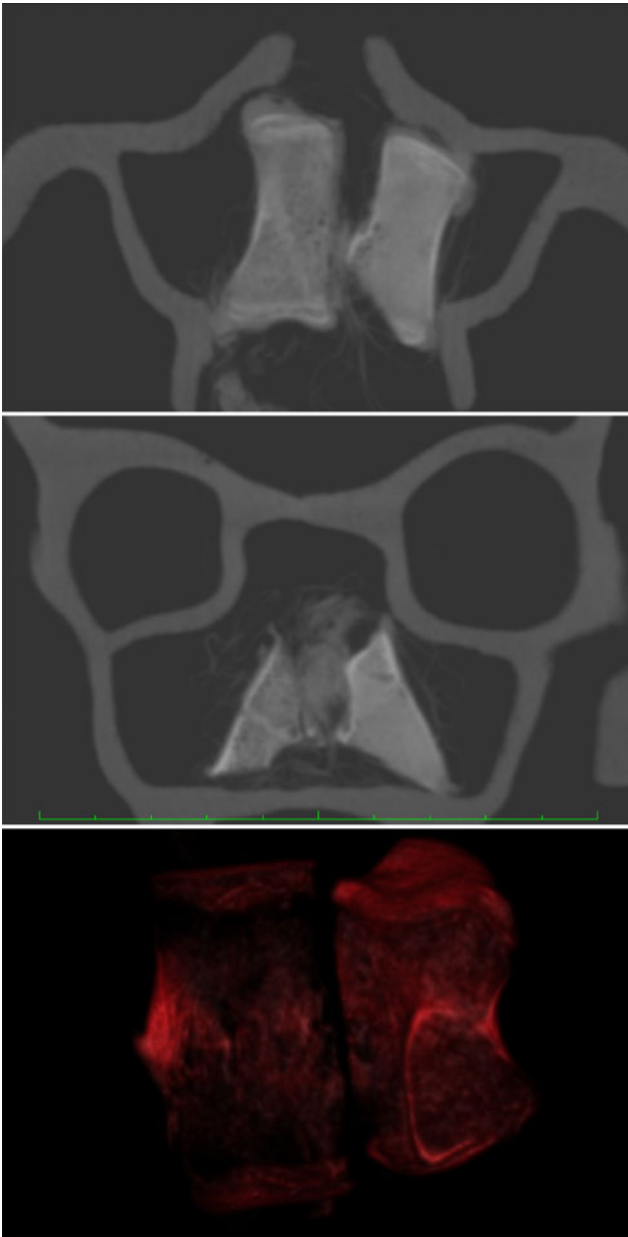
#### Image quality assessment

Two equal sets of images of patient data and of both *in vitro* experiment groups were evaluated by four independent observers (dentists certified by the German Board for CBCT imaging). Images such as those demonstrated in Figures 2–5, including two orthogonal displays, were presented to the examiners. Demineralized samples from each device were compared with fresh ones with the following standardized rating:

- 0 = cancellous bone not circumscribed
- 1 = cancellous bone circumscribed



**Figure 3** Example from the 3D Accuitomo device: the three radiological imaging cross-sections show the bone transplant in the frontolateral right alveolar rim in a patient (130 days after transplantation from the iliac crest)



**Figure 4** The *in vitro* test with the KaVo 3D shows both the freshly frozen and the partly demineralized bone sample in the OsiriX program. Top, axial view; middle, coronal view; bottom, 3D reconstruction

Clinical images of the Morita were compared with the KaVo 3DeXam with the following rating:

- 0 = augmentation circumscribed
- 1 = augmentation imaging completely satisfying

Results were statistically analysed with the unpaired *t*-test for patient data analysis and one-way ANOVA for the *in vitro* experiment. Statistical significance of differences between results was assumed if  $P \leq 0.05$ .

## Results

### *First part of the study: evaluation of patient data*

The descriptive analysis of the images showed well-visualized bone transplants in all cross-sections. The cancellous bone was clearly circumscribed and separated from the surrounding connective tissue. The images of the single bone trabeculae were of good quality. The images produced with the KaVo 3DeXam device showed softer borders and margins, whereas the Morita Accuitomo device showed better contrast and brightness (Figures 2 and 3). All images were sufficient for clinical use and implant planning.

### *Second part of the study: in vitro testing of porcine bone material in CBCT devices*

EDTA treatment reduced mineralization by 25% in cancellous bone samples. These samples were compared with the non-demineralized freshly frozen bone samples, as described above (Figures 4 and 5). Images of each CBCT device showed good visualization in both sample groups. Demineralized samples showed lower radiographic opacity but still enabled visibility of large bone areas. Cancellous bone trabeculae were more easily seen with the Morita 3D Accuitomo device than with the KaVo 3DeXam device. However, contrast and brightness adjustments in the viewer program OsiriX allowed a similar overall definability of the entire bone block.

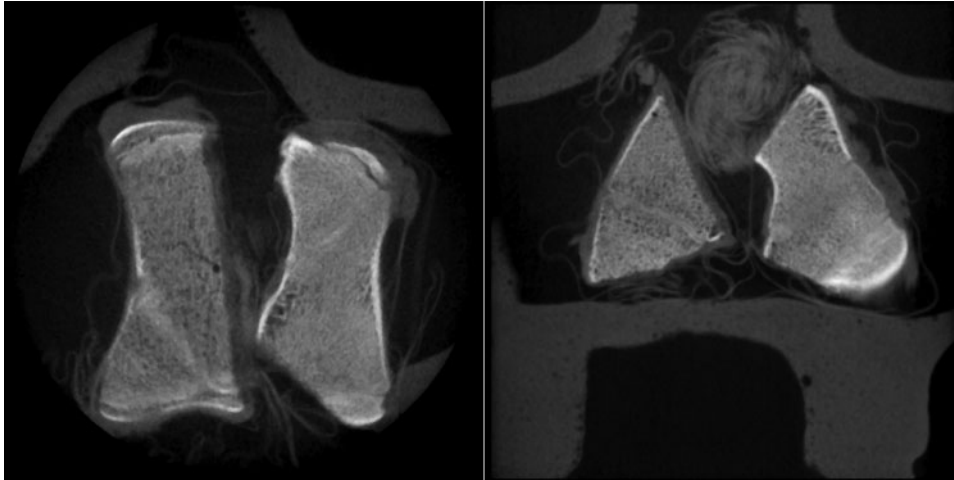
### *Image quality assessment*

The analysis of patient data shows a significant difference between the Morita Accuitomo and the KaVo 3DeXam devices in the unpaired *t*-test ( $P = 0.038$ ) (Figure 6). Data comparison of the *in vitro* image assessment by one-way ANOVA shows no difference between the data ( $P = 0.070$ ).

## Discussion

Images of dentomaxillofacial structures were evaluated with CBCT.<sup>3,7,10–15</sup> Bone transplants are important in dental implantology, but only a few reports on CBCT imaging quality have been published.<sup>14–17</sup> A retrospective study examined the imaging quality of autologous bone grafts with the NewTom® 9000 CBCT device, describing a limited definition of cancellous bone in the remodelling phase.<sup>14,16</sup> As a sequel to this study, we evaluated the imaging of bone transplants with the Morita 3D Accuitomo 40 and the KaVo 3DeXam CBCT devices in a two-part study. The examination included retrospective patient data and a new *in vitro* model with porcine spine bone blocks.

We applied a usual evaluation procedure for both CBCT devices. These methods of analysis were combined with a voxel size of 0.3 mm<sup>3</sup> for the KaVo 3DeXam and 0.25 mm<sup>3</sup> for the Morita Accuitomo to achieve



**Figure 5** The *in vitro* test with the 3D Accuitomo device shows better contrast and sharper details at the bone trabeculae. Left, axial; right, coronal

the same standard of quality as in our clinical practice. Equivalence of the images with the OsiriX DICOM viewer compared with the primary software of the manufacturer was validated for the KaVo 3DeXam data.

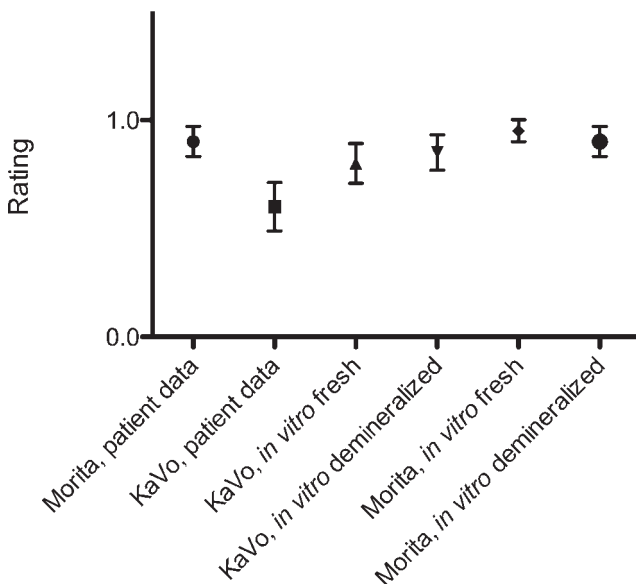
Evaluation of skull bone imaging in CBCT devices has been described previously.<sup>18</sup> The descriptive evaluation presented here is a common methodology.<sup>19</sup> Some authors based quality assessments on density measurements.<sup>3</sup> We did not consider Hounsfield values because of their inconsistency between the different CBCT devices; additionally, voxels are not always isotropic, as has been shown by MDCT.<sup>2</sup> We combined

the clinical descriptive results with a reproducible *in vitro* model that can be easily applied in other studies, which provides an interesting experimental tool in addition to those already available.

This retrospective study indicated that the Morita 3D Accuitomo and the KaVo 3DeXam devices provide appropriate imaging techniques for the imaging of cancellous bone grafts in *in vivo* remodelling. Both devices allowed a clear definition of the graft border towards the soft tissue. The Morita 3D Accuitomo showed sharper contrast and a clear definition of single bone trabeculae. The descriptive results were significant in the related image quality assessment. We assume that the wide range of manual adjustments available with the Accuitomo are the main reason for this advantage, whereas the KaVo settings are widely automated for faster processing. These results correspond to a previous study on the NewTom® 9000 CBCT device.<sup>3</sup>

The *in vitro* results show analogous outcomes. However, no significant difference existed between the two devices or between fresh and demineralized samples. The good overall visualization of freshly frozen and demineralized samples indicates the suitability of both devices for the imaging of cancellous bone transplants in remodelling and for the related planning of implant procedures. The difference between the two CBCT devices allows an estimation of quality differences in the detail resolution. These results indicate that the Morita Accuitomo device might be more appropriate for the imaging of particular trabecular bone structures. Thus, a larger number of patients were examined *in vivo* ( $n = 38$ ) compared with the number of patients examined with the KaVo 3DeXam ( $n = 9$ ).

In conclusion, it was shown that both the KaVo 3DeXam and the Morita 3D Accuitomo devices allow good visualization of cancellous bones, in *in vivo* remodelling and after *in vitro* demineralization.



**Figure 6** Image quality assessments. Patient data: 0, cancellous bone not circumscribable; 1, cancellous bone circumscribable. *In vitro* study: 0, augmentation definable from soft tissue; 1, augmentation imaging completely satisfactory

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