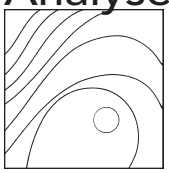
Long-Term Bony Integration and Resorption Kinetics of a Xenogenic Bone Substitute after Sinus Floor Augmentation: Histomorphometric Analyses of Human Biopsy Specimens



Marcus O. Klein, MD, DMD, PhD[1]
Peer W. Kämmerer, MD, DMD[2]
Hermann Götz, PhD[3]
Heinz Duschner, PhD[4]
Wilfried Wagner, MD, DMD, PhD[5]

In this case series, a systematic histomorphometric analysis of two human bone biopsy specimens 1 and 5 years after grafting with a xenogenic bovine bone substitute material (BSM) was conducted. While the 1-year specimen still showed extensive signs of an active desmal ossification, the specimen after 5 years mainly showed mature lamellar bone without bone turnover or remodeling. A completed bony integration without extensive resorption of the BSM particles could be detected. Altogether, a good integration in the bone with osteoconduction and a high biocompatibility was seen. (Int J Periodontics Restorative Dent 2013;33:XXX–XXX. doi: 10.11607/prd.1469)

[1]Associate Professor, Department of Oral, Maxillofacial and Plastic Surgery, University Medical Centre of the Johannes Gutenberg-University Mainz, Mainz, Germany; Private Practice, Düsseldorf, Germany.
[2]Assistant Professor, Department of Oral, Maxillofacial and Plastic Surgery, University Medical Centre of the Johannes Gutenberg-University Mainz, Mainz, Germany; Visiting Assistant Professor, Harvard Medical School, Boston, Massachusetts, USA.
[3]Medical Faculty, Institute of Applied Structure and Microanalysis, University Medical Centre of the Johannes Gutenberg-University Mainz, Mainz, Germany.
[4]Head and Director, Institute of Applied Structure and Microanalysis, University Medical Centre of the Johannes Gutenberg-University Mainz, Mainz, Germany.
[5]Head and Director, Department of Oral, Maxillofacial, and Plastic Surgery, University Medical Centre of the Johannes Gutenberg-University Mainz, Mainz, Germany.

Correspondence to: Dr Marcus O. Klein, Stresemannstrasse 7-9, Düsseldorf, Germany; fax: 06131/17-6602; email: marcusoklein@me.com.

In the oral and maxillofacial area, the use of alloplastic or xenogenic bone substitute materials (BSMs) is extensively documented and scientifically substantiated for numerous indications.[1] The following specific implant-related indications for a bone augmentation can be distinguished in general: augmentation of a vertical and/or horizontal bone deficit of the maxilla or mandible prior or simultaneous to implantation and treatment of peri-implant bony defects as well as structural maintenance after tooth extraction (socket/alveolar ridge preservation).[1] An established strategy for treatment of vertical deficits of the maxilla side regions is the internal or external elevation and augmentation of the maxillary sinus floor (sinus lift). For sinus lift procedures with a suitable BSM, the harvesting of autologous bone with associated donor morbidity can be avoided.[2] Numerous studies and systematic reviews confirm the equivalence of alloplastic substitution.[3–5] Current literature on the success rates of sinus lift procedures basically focuses (after complications such as membrane perforations or infections) on dental implant survival

rates and histomorphometric studies of specimens harvested during preparation of the implant bed. This examination generally reveals a sufficient bony integration of the BSMs. Due to current therapeutic modalities, which schedule a staged placement of dental implants no later than 6 to 8 months, as well as because of ethical considerations, there are basically no human long-term histologic investigations of BSM particles. Though, mostly because of the standardized surgical procedure of sinus lift preparation and the comparable advantageous osteoconductive environment, the "model" of the external sinus augmentation would suit well for the evaluation of long-term biologic properties of a BSM.

Bio-Oss (Geistlich, particle sizes of 0.25 to 1.0 mm and 1.0 to 2.0 mm), a xenogenic BSM, consists of the mineral phase of bovine bone. Organic ingredients are removed during manufacturing so that only the inorganic, natural hydroxylapatite-containing ultrastructure remains. For hydroxylapatite, prolonged resorption kinetics with a characteristic long-term stability in biologic environments are described.[6]

In general, for the assessment of the bony integration of BSM, histologic techniques based on cutting and grinding are employed. However, the interpretation of the clinically relevant three-dimensional topographic relationships of multiple BSM particles is limited when using two-dimensional histologic methods. By employing (semi-) automated analysis methods, the respective ratios of newly formed bone tissue as well as residual BSM particles of the specimen can be estimated. Micro-computed tomography (MCT) has been identified as a direct and tissue-preserving method for structural analysis of biologic hard tissues such as bone.[7–10] Both native alloplastic BSM specimens as well as biopsy specimens from augmented areas can be analyzed to estimate the healing and resorption behavior of various BSMs.[11–14]

This case series presents histomorphometric analyses (conventional histology, and MCT) of two human trephine biopsy specimens from sinus augmentations with bovine BSM. Because of exceedingly long healing periods (10.5 and 55.5 months), information on the biologic long-term behavior of the bovine BSM was obtained.

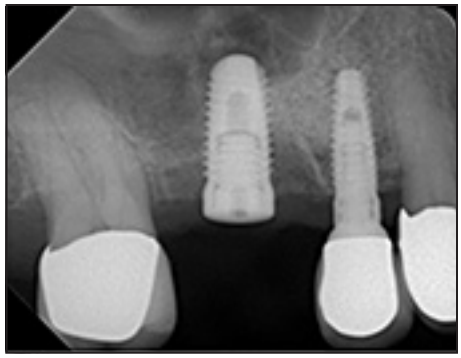
Patient 1

A 39-year-old, healthy man was introduced to the Department of Oral and Maxillofacial Surgery, University Medical Centre of the Johannes Gutenberg-University Mainz, for the functional rehabilitation of the edentulous regions of the maxillary left second premolar and first molar with dental endosseous implants. As the remaining bone height was less than 3 mm, a primary grafting procedure of the maxillary sinus with secondary insertion of implants was scheduled under local anesthesia. After preparation of a lateral window and elevation of the sinus membrane, the xenogenic bovine BSM (Bio-Oss) (particle size 1.0 to 2.0 mm), mixed with autologous blood and bone particles collected during preparation (BoneTrap, AstraTech) was applied. To protect the augmentation site, a collagen membrane (Bio-Gide, Geistlich) was placed over the lateral window. Due to personal circumstances of the patient, staged surgery with placement of dental implants (4.3 × 11 mm and 5 × 9 mm, Camlog Biotechnologies) could be performed no earlier than 10.5 months after the augmentation procedure. During preparation of the bony bed for the maxillary left first molar implant, a bone sample collected with a trephine bur was obtained through the alveolar crest. A transgingival healing mode was chosen. After an additional 3 months, prosthetic rehabilitation was provided.

Patient 2

A 45-year-old, healthy woman came to the Department of Oral and Maxillofacial Surgery, University Medical Centre of the Johannes Gutenberg-University Mainz, for functional rehabilitation of several missing teeth with dental implants. Although immediate insertion implants (3.3 × 13 mm; 3.8 × 13 mm; 4.3 × 13 mm, Camlog Biotechnologies) was performed, deficient vertical bone levels required a localized simultaneous external sinus lift for each site (surgical approach and materials analogous to patient 1). Grafting of the right maxillary sinus was extended apically to the

Fig 1 *Situation after dental implantation into the grafted sinus.*



roots of the maxillary right first molar [Au: Correct?]. After an additional 3 months, application of healing abutments was performed and further prosthetic rehabilitation was initiated.

Four years and 3 months after primary sinus grafting and placement of dental implants, the decayed tooth was removed and after an additional 4 months, a dental implant was inserted into the respective site (5 × 13 mm, Camlog Biotechnologies). During preparation of the implant site, a trephine bur bone biopsy specimen was harvested below the previous sinus augmentation (aged 4 years and 7.5 months) (Fig 1).

Embedding and histologic preparation

After fixation and dehydration through an ethanol gradient and defatting in xylene, the obtained trephine biopsy specimen was embedded without decalcification in methacrylate (Technovit 9100 New, HeraeusKulzer) according to the manufacturer's instructions. After appropriate trimming, the untreated block underwent microcomputed tomography (MCT). By using the cutting and grinding technique described by Donath and Breuner,[15] sections of a thickness of approximately 80 µm were made and stained conventionally with hematoxylin-eosin (HE).

Microcomputed tomography

Both a native, ungrafted Bio-Oss sample (particle size 1 to 2 mm) as well as a trephine biopsy specimen embedded in methacrylate were examined by high-resolution microcomputed tomography (MCT) (lateral resolution: 6 µm; MCT40 Scanco Medical AG) equipped with a microfocus x-ray source (70 kV, 114 µA).

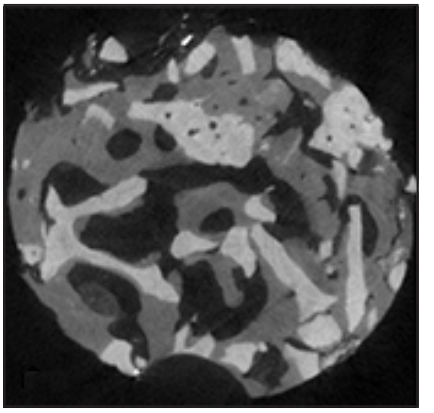
For the evaluation of the bone substitute material specimen in vitro, plexiglas cylinders (diameter, 5 mm) were loosely filled with particles of the BSM without application of axial pressure. At intervals of 6 µm, individual images were recorded first in the primary beam cone (0.18 deg; 2,000 settings/360 deg). To optimize the signals, 10 scans per setting were averaged (integration time 300 µs). The data were recorded automatically with a scan time of approximately 12 h/10 mm trephine height. The raw data were further processed for structural visualization and statistical analysis. The two-dimensional reconstruction of sections of approximately 80 µm thickness allowed demonstration of slices similar to the conventional thin section technique. The three-dimensional reconstruction provided visualization of the complex bone–biomaterial interaction. Furthermore, porosity was assessed.

For the ex vivo trephine biopsies, the volume ratios of the following compartments could be determined quantitatively through analysis of the two-dimensional phase distribution of the density (mg HA/cm^3), as follows:

- Nonmineralized soft tissue or bone interstices or pores (< 687 mg HA/cm^3),
- Newly formed (mineralized) bone (687 to 1,505 mg HA/cm^3),
- (Persistent) alloplastic bone substitute material (> 1,505 mg HA/cm^3).

Fig 2 (left) *MCT of native Bio-Oss particles (1 to 2 mm); three-dimensional reconstruction.*

Fig 3 (right) *MCT of the trephine bur biopsy specimen from patient 1. Representative primary images with newly formed bone tissue* (dark grey)*, residual BSM* (bright grey)*, and nonmineralized tissue* (black).



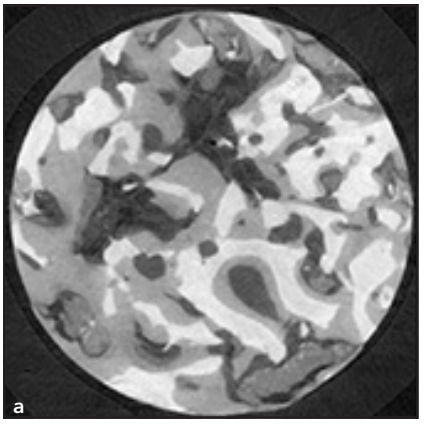
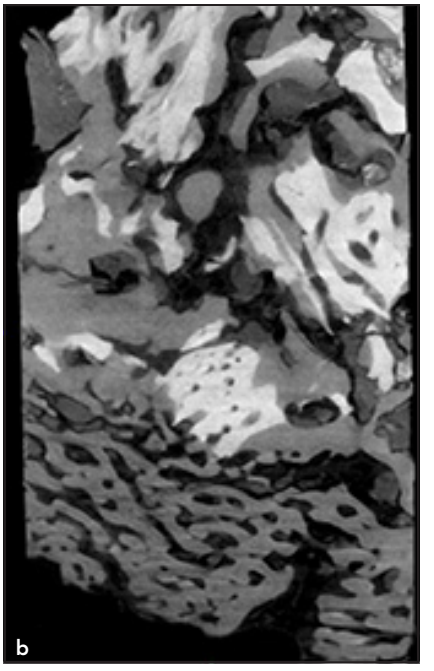


Fig 4 *MCT of the trephine bur biopsy specimen from patient 2.* (a) *Representative primary images with newly formed bone tissue* (dark grey)*, residual BSM* (bright grey)*, and nonmineralized tissue* (black). (b) *Reconstruction in the XZ-plane. Basally located is the original local lamellar bone of the maxilla* (floor of the maxillary sinus)*, more apically is the regenerated area after grafting with Bio-Oss.*



Results

Analysis of BSM in vitro

MCT examination of native BSM particles revealed the trabecular, highly porous structure of the substitute with large interparticle pore dimensions (Fig 2). In vitro, the mean pore size was 486 ± 194 µm with an overall porosity of 73.9%; accordingly, the ratio of solid BSM particles was 26.1%.[16]

Analysis of the biopsy specimen ex vivo

For both investigated specimens, MCT revealed formation of bone tissue along the whole sample length with good bony incorporation of the BSM showing tight contact zones between bone tissue and BSM particles. Almost all BSM particles were surrounded by a thin bony lamella.

In the scan, residual BSM appeared densest, followed by bone and soft tissue (Figs 3 and 4), so that the

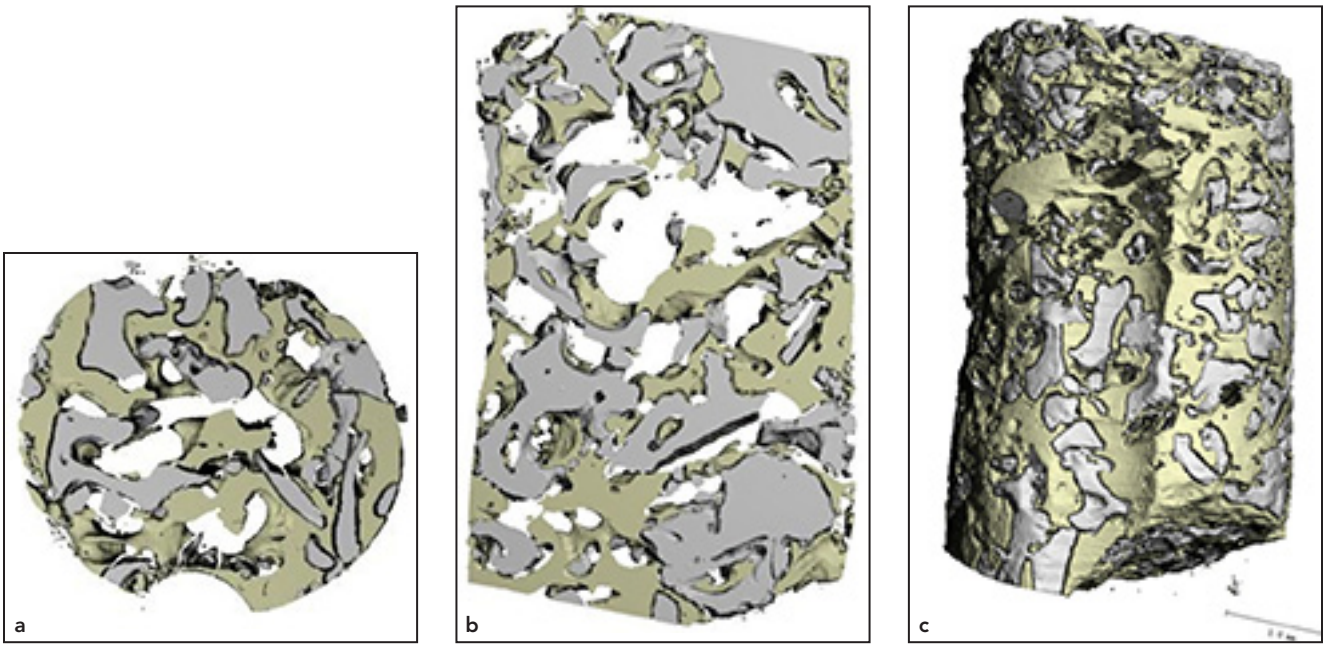


Fig 5 *MCT of the trephine bur biopsy specimen from patient 1.* (a) *Two-dimensional reconstruction* (XY-plane) *with color-encoded nonmineralized tissue* (white), *new formed bone* (ocher) *and residual BSM particles* (gray). (b) *Two-dimensional reconstruction* (XZ-plane). (c) *Three-dimensional reconstruction.*

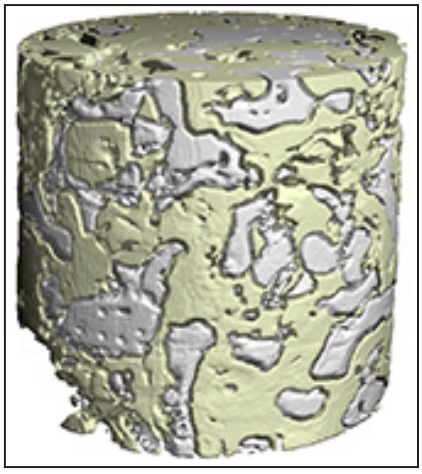


Fig 6 *MCT of the trephine bur biopsy specimen from patient 2. Three-dimensional reconstruction with color-encoded nonmineralized tissue* (white), *newly formed bone* (ocher), *and residual Bio-Oss particles* (gray).

following compartments could be color coded and recorded (Figs 5 and 6) as follows:

- White, nonmineralized (soft) tissue,
- Ocher, newly formed bone,
- Grey, residual BSM.

Accordingly, for the first specimen after 10.5 months, 30% of newly formed bone with 26.6% of remaining BSM was calculated. After 4 years and 7.5 months, 32.8% of new bone with 15.8% residual BSM was seen.

Histologic examination

In both cases, histologic preparations showed a sufficient bony integration of the BSM after sinus grafting along the complete length of the trephine biopsy samples.

Between the Bio-Oss particles, a directed, well vascularized bony tissue with close contact to indi-

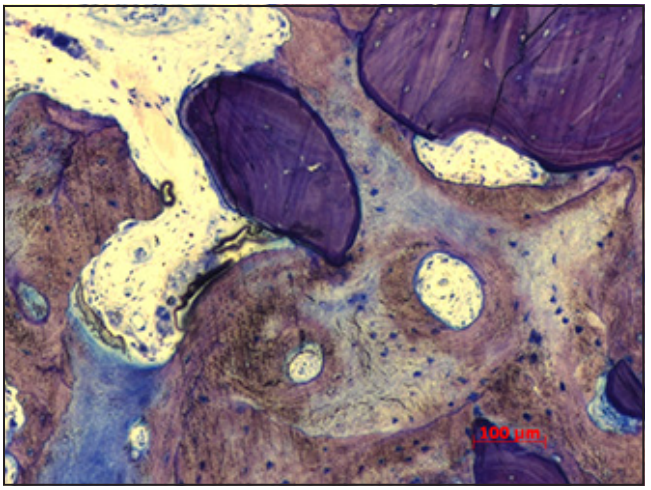


Fig 7 *Histologic examination of the trephine bur biopsy specimen from patient 1. The Bio-Oss particles appear violet, new formations of immature osteoid appear bluish; mineralized mature bony tissue appears brownish with clearly detectable osteocytes. Within the BSM particles, empty osteocyte cavities can be detected. Dark cement lines between bone tissue and BSM particles (original magnifications ×10).*

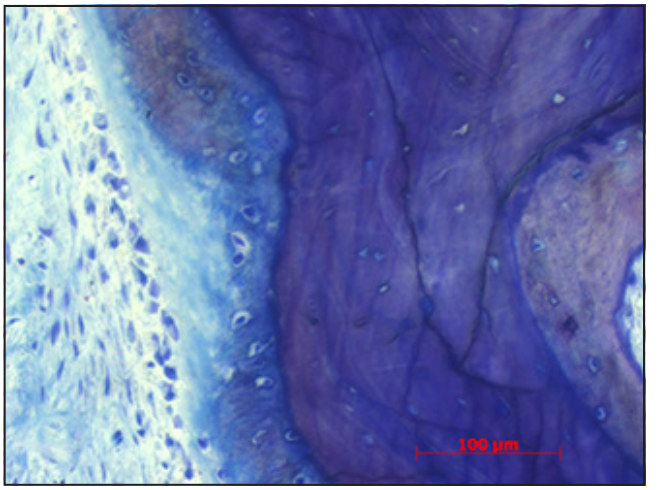


Fig 8 *Histologic examination of the trephine bur biopsy specimen from patient 1 with active bone regeneration activities around the Bio-Oss particle. On the left, the classical phases of desmal ossification with preosteoblasts and mature, osteoid- synthesizing osteoblasts directly adjacent to the BSM particle can be detected. Within the Bio-Oss particle, empty osteocyte cavities can be detected. On the right, mature, brownish bony tissue with entrapped osteocytes is seen (original magnification ×20).*

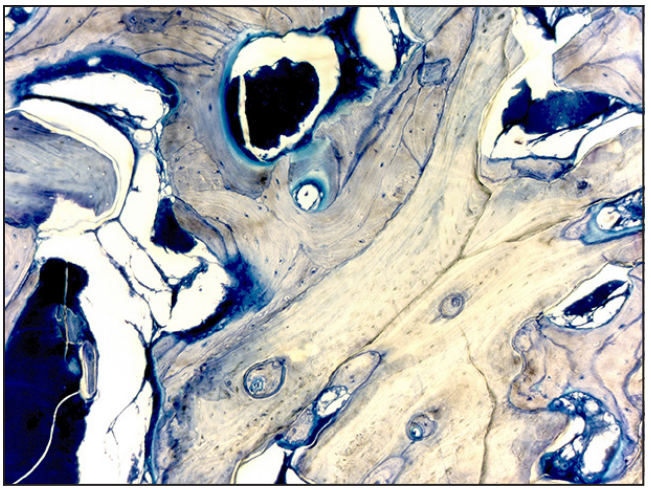


Fig 9 *Overview and detailed aspect of the histologic examination of the trephine bur biopsy specimen from patient 2. In the upper left corner of figure, directed mature lamellar bone is seen. Furthermore, osteones within pore structures are seen. Compared to patient 1, almost no signs of active bone formation can be detected (original magnification ×10).*

vidual BSM particles was seen; however, for both time points, only superficial resorptions could be detected.[17] A constant, clearly definable interface between bony tissue and BSM particles was seen (Figs 7 to 11).

An osteoconduction into small porous structures with establishment of osteons including central vessels, peri-vascular cells and osteogenic cells (osteoblasts, mature osteocytes) and newly formed bony tissue was observed.

However, the bony tissue surrounding the BSM particles showed considerable differences for the two investigated time points (10.5 mo vs 55.5 mo). While after 10.5 months, for various sites, an active bone regeneration with all signs of desmal ossification between the BSM particles could be observed, after 55.5 months a de facto completed ossification with uniformly directed lamellar bone was detected. Furthermore, numerous osteocyte lacunae with characteristic thin lamellipodiae were seen.

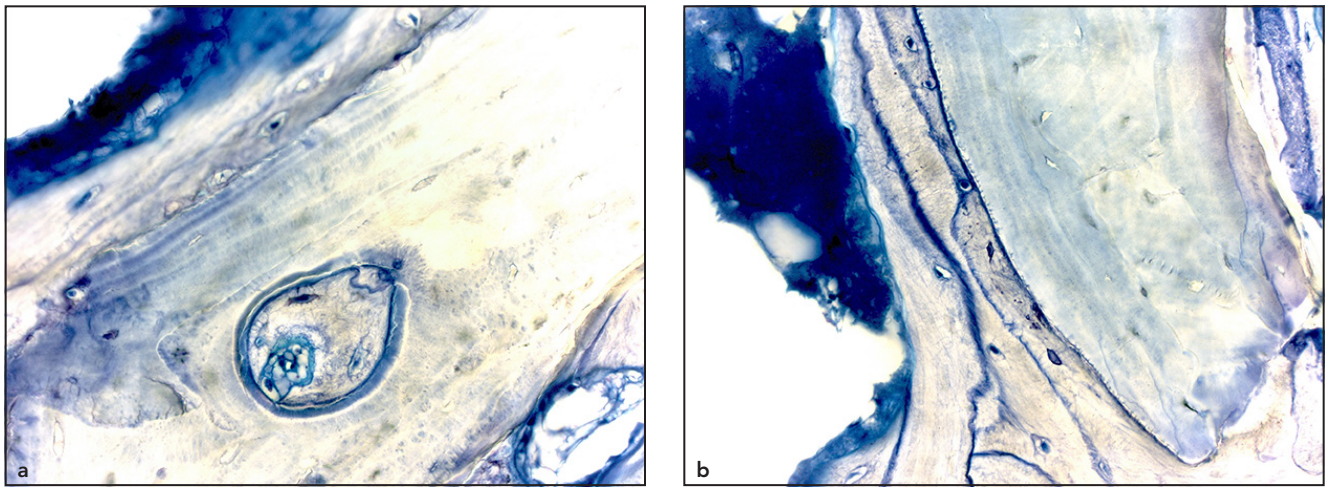
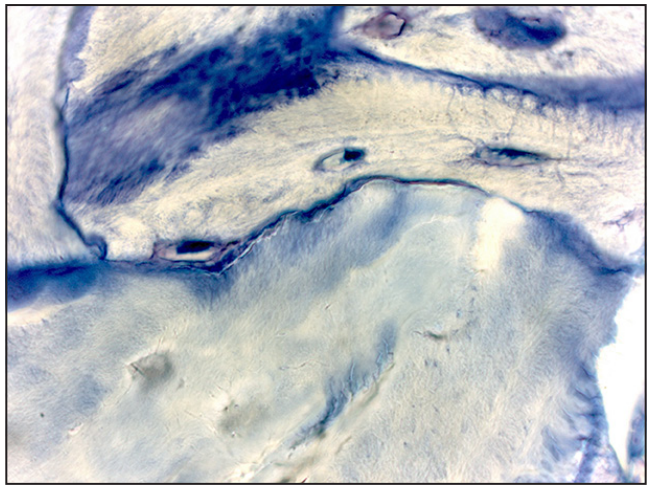


Fig 10 *High-resolution histologic examinations of the trephine bur biopsy specimen from patient 2.* (a) *Detailed view of Fig 9 shows an osteon within a BSM pore with central vessel, peri-vascular cells, and osteogenic cells (osteoblasts, mature osteocytes).* (b) *A straight interface between acellular BSM (right aspect of the image) and bone with osteoblasts and osteocytes. Furthermore, cement lines between the bone lamellae and adjacent to the BSM are seen (original magnifications ×40).*

Fig 11 *High-resolution histologic examination of the trephine bur biopsy specimen from patient 2. A very close contact between BSM (lower aspect of the image) and the adjacent bone is seen. On the left side, an osteoblast directly adjacent to the BSM is seen. On the right side, an osteocyte surrounded by mineralized bone matrix communicates with other osteocytes via thin processes (original magnification ×100).*



Discussion

Grafting of the maxillary sinus floor by employing BSM is a well documented approach to generate a stable bone volume for dental endosseous implants in case of insufficient bone supply.

Numerous animal studies, studies on human patients, as well as systematic reviews revealed high implant survival rates. Furthermore, corresponding histomorphometric data generally showed a bony regeneration with good integration of the grafting material.[1,18,19] Histomorphometric measurements with assessment of (areal) ratios of newly formed mineralized bone, residual BSM, or soft tissue compartments provide objective and reproducible information on the interactions of the grafting material with the adjacent biologic tissue (in terms of biocompatibility, osteoconduction, and resorbability). Table 1 displays a literature overview on clinical studies dealing with a larger patient number (> 20 treated individuals).

Table 1 **Literature overview on sinus floor augmentations with DBHA with or without the addition of AU**

Author (y)	Grafting material	Healing of the substitute (mo)	Newly formed bone (%)	Residual BSM (%)
Artzi et al[21] (2001)	DBHA	12	42.1	24.7
Cordaro et al[22] (2008)	DBHA	6 to 8	19.8 ± 7.9	37.7 ± 8.5
Felice et al[23] (2009)	DBHA	6	36.1 ± 4.6	33.4 ± 5.6
Ferreira et al[24] (2009)	DBHA	11.4	39 ± 11.9	8 ± 2.7
Galindo-Moreno et al[25] (2008)	DBHA + AU	6	31.02 ± 7.33	17.28 ± 1.32
Galindo-Moreno et al[26] (2010)	DBHA + AU	6	46.08 ± 16.63	37.02 ± 25.09
Hallman et al[27] (2002)	DBHA DBHA + AU	14 to 15.5 12 to 13	41.7 ± 26.6 39.9 ± 8	11.8 ± 3.6 12.3 ± 8.5
John et al[28] (2004)	DBHA DBHA + AU	3 to 8 3 to 8	29.5 ± 7.4 32.2 ± 6.7	14.9 ± 6.5 17.8 ± 6.7
Lindgren et al[29] (2009)	DBHA	8	41.6 ± 14	12
Mangano et al[30] (2007)	DBHA	6	36.2 ± 1.4	39 ± 2.9
Scarano et al[31] (2006)	DBHA	6	39 ± 1.6	31 ± 1.4
De Vincente et al[32] (2019)	DBHA + AU	9	29 ± 6.6	21 ± 7
Wallace et al[33] (2005)	DBHA	6 to 10	12.1 to 17.6	24.3 to 31.9

DBHA = xenogenicdeproteinized bovine hydroxyl apatite; AU = autologous bone.

In those studies, after sinus grafting with deproteinized bovine hydroxyapatite (DBHA, in most cases Bio-Oss) biopsy specimens were obtained in the course of secondary dental implantation and further histomorphometrically analyzed.

In this literature overview, the specimens were investigated after variable healing times, ranging from 3 to 15.5 months. The ratio of newly formed bone ranged from 12% to 46% and the ratio of residual BSM ranged from 8% to 39%. These heterogenic results can also be observed in many other publications; the ranges of the assessed ratios are similar to those of other BSM studies[1] as well as the results of the present case series. However, for interpretation and comparison of the results, different surgical techniques (eg, manual condensation of the BSM) as well as possible differences in the processing of the specimens and histomorphometric analyses have to be taken into consideration. In the two case reports, autologous blood and bone particles were added, and may have accelerated graft healing and new bone formation. Therefore, healing dynamics with the according histomorphometric data cannot be considered in the same time frame as the 100% DBHA cases presented in Table 1.

The present histomorphometric study offers the very rare possibility of comparing a relative young specimen to a human long-term biopsy specimen (10.5 mo and 55.5 mo after grafting of a xenogenic bovine BSM). Whereas the younger specimen still showed sites of active desmal ossification (new bone formation out of soft tissue), the older specimen constantly

revealed a completed ossification with consistent directed mature lamellar bone. In accordance with other investigations, both specimens showed no signs of excessive resorption of the bovine BSM. A long-term stability with further persistence of the grafting material has to be assumed, which correlates with other rare histomorphometric studies on the long-term performance of DBHA in the augmented sinus. In detail, Traini and coworkers showed a mean amount of newly formed bone of 46% and DBHA remnants of 16% after 9 years,[20] and Mordenfeld et al found a mean amount of newly formed bone of 45% and DBHA remnants of 17% after 11 years.[17]

It has to be considered that by exclusive interpretation of histomorphometric data, conclusions on the clinical value of a BSM can only be drawn to a certain degree, requiring additional information of clinical parameters such as survival rates of functionally loaded dental implants.

Conclusion

The sum of clinical complications, dental implant survival, and histomorphometric data give valuable evidence on the overall performance of a BSM.

Acknowledgment

The authors reported no conflicts of interest related to this study.

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