JO JOURNAL OF OSSEOINTEGRATION periodontics and prosthodontics

Alveolar ridge preservation with the use of deproteinized bovine bone mineral with collagen combined with a collagen matrix - histomorphometric outcome in humans

R. JUNKER[1], J. BEHRING[2]

[1]DDS, DR MED DENT, MSc, PhD, Professor and Head, Department of Prosthodontics and Biomaterials, Danube Private University, Krems, Austria
[2]DDS, DR MED DENT, MSc, Private Dental Office, Hamburg, Germany

TO CITE THIS ARTICLE
Junker R, Behring J. Alveolar ridge preservation with the use of deproteinized bovine bone mineral with collagen combined with a collagen matrix - histomorphometric outcome in humans technique. J Osseointegr 2023;15(2):113-123.

DOI 10.23805/JO.2023.568

KEYWORDS Allogenic block graft, Dental implant, Graft survival

ABSTRACT

Aim The objective was to get histological insight in the osteogenic potential of *de novo* formed tissue within extraction sockets grafted with a blend of granules of deproteinized bovine bone mineral and porcine collagen fibres (DMBB+C) combined with a collagen matrix (CMX) after healing times representative for ARP 1.1 or 2.1.
Methods Patients scheduled for ARP 1.1 or 2.1 with DMBB+C/CMX were included. At implant placement, as part of implant site preparation, after soft tissue flap elevation, core biopsies from the extraction sites were taken. Thereafter, descriptive histology and histomorphometric analysis was done.
Results Newly formed bone could be observed throughout the entire sections of all core biopsies. Less newly formed bone was located in the more coronal as compared to the more apical parts. Coronal bone bridging was not found in any section. Overall, after a mean healing time of 4.9 months (SD: 0.9 months; range: 4 - 6.5 months) a mean of 20.7% new bone (SD: 10.1%; range: 1.4% - 38.9%) was found.
Conclusions It can be concluded that, (1) the *de novo* formed tissue has an osteogenic potential throughout the entire grafted part of the former extraction site, (2) coronal bone bridging might not be expected, and (3) less new bone might form in coronal as compared to apical parts of the extraction sites.
Clinical significance De novo formed tissue after alveolar ridge preservation with DMBB+C combined with CMX has an osteogenic potential throughout the entire grafted part of the former extraction site and thus the collagen matrix used might be regarded as barrier membrane for guided bone regeneration.

INTRODUCTION

Nowadays, prosthodontic rehabilitation of missing teeth with dental implants has developed into a largely predictable treatment option (1-7).
However, in case of unassisted extraction socket healing, marginal alveolar bone reduction, which might hamper implant placement, has been shown in dog models (8-13) as well as in humans (14-25).
Remarkably, in dog models, grafting extraction sockets with a xenogeneic graft, a blend of granules of deproteinized bovine bone mineral (90%) and porcine collagen fibres (10%) (DBBM+C; Bio-Oss® Collagen; Geistlich Pharma AG, Wolhusen, Switzerland), modified bone modelling and counteracted marginal ridge contraction (9, 12, 13, 26).
These findings are paralleled in humans (17, 27-32). Thus, after 6 months of healing, Jung et al. (33) found by means of cone-beam computed tomography scans that socket treatment with DBBM+C at the bone level and application of either an autogenous, palatal, soft tissue graft or a collagen matrix (CMX; Geistlich Mucograft®; Geistlich Pharma AG, Wolhusen, Switzerland) at the soft tissue level resulted in less vertical and horizontal reduction of the alveolar ridge as compared with unassisted, spontaneous healing as control treatment. Like this, Araújo et al. (27) observed after 4 months of healing that socket treatment with DBBM+C covered with an autogenous, palatal, soft tissue graft markedly counteracted the reduction in the hard tissue component of edentulous sites. Along these lines, comparable results were reported by Scheyer et al. (30) and Cardaropoli et al. (31) for DBBM+C in combination with a barrier membrane (BioGide®; Geistlich Phar-

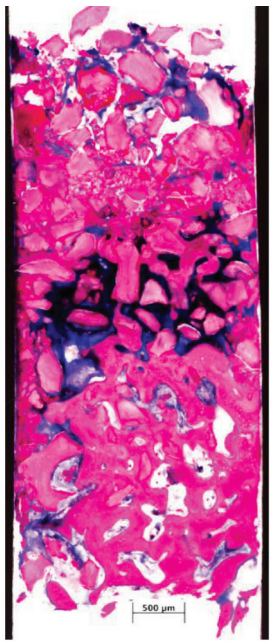

FIG. 1
Overview

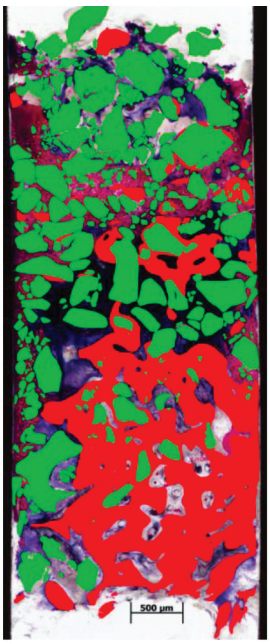

FIG. 2
Overview

Microphotographs of a core biopsy obtained in the premolar region of the maxilla after a healing time of 4.5 months.
Newly formed bone can be observed throughout the whole specimen.
Obviously, more newly formed bone is located in the more apical as compared to the more coronal part. Coronal bone bridging cannot be observed.
Overall, 23.4% new bone, 32.4% residual deproteinized bovine bone mineral as well as 34.1% soft tissue, bone marrow was found.

FIG. 1 Azur II und Pararosanilin staining; original magnification x50
FIG. 2 New bone coloured in red and deproteinized bovine bone mineral particles coloured in green: original magnification x50

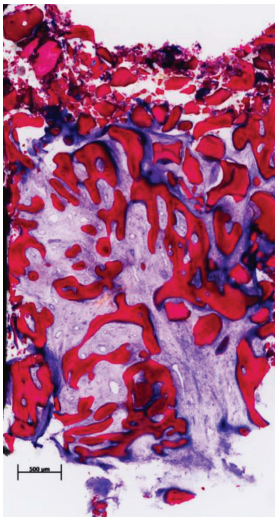

FIG. 3
Overview

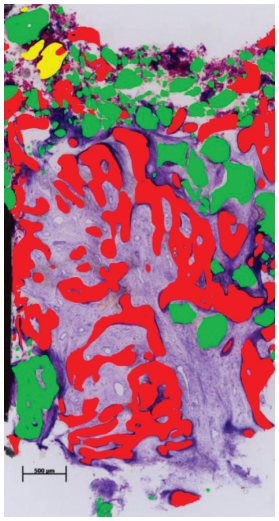

FIG. 4
Overview

Microphotograph of a core biopsy obtained in the premolar region of the maxilla after a healing time of 4 months.
Newly formed bone can be observed throughout the whole specimen.
However, in the most coronal part new bone formation seems to be less as compared to the more apical part. Coronal bone bridging cannot be found.
Overall, in this specimen 31.5% new bone, 9.9% residual deproteinized bovine bone mineral as well as 58.3% non-mineralized tissue was found.

FIG. 3 Azur II und Pararosanilin staining; original magnification x50
FIG. 4 Old bone coloured in yellow, new bone coloured in red and deproteinized bovine bone mineral particles coloured in green: original magnification x50

ma AG, Wolhusen, Switzerland) after 6 months (30) and 4 months (31) of healing as well as Maiorana et al. (29), Gabay et al. (32) and Cardaropoli et al. (28) for DBBM+C combined with CMX after healing periods of 6 (29, 32) and 4 months (28).
Moreover, human histomorphometric data for healing times between 3 and 6 months confirmed *de novo* bone formation in human extraction sockets grafted with DBBM+C (28-30, 32, 34-39). However, especially in the coronal parts of the grafted extraction sockets, neither at 3 months (34) nor at 6 months (29, 30, 36) *de novo* bone modelling and remodelling could be regarded as being completed.
Currently, the authors (RJ and JB) have a tendency to graft extraction sockets with DBBM+C at the bone level combined with the application of CMX at the soft tissue level and to install implants after a healing period of around 4 months, representing alveolar ridge preservation (ARP) with implant placement Type 3 (delayed; between 12 and 16 weeks; ARP 1.1) or Type 4 (standard placement in a healed ridge; after more than 16 weeks; ARP 2.1) (40).
However, to the best of our knowledge, human histological data for ARP 1.1 or 2.1 after grafting fresh extraction sockets with DBBM+C and CMX, evaluating the osteogenic potential of the *de novo* formed tissue, which might influence implant stability and bone to implant healing, are limited (28, 32, 36).
Therefore, the aim of the current clinical research project was to get more - histological and histomorphometric - insight in the osteogenic potential of de novo formed tissue within extraction sockets grafted with DBBM+C and CMX after healing times representative for ARP 1.1 or 2.1.

MATERIAL AND METHODS

Clinical Investigation design

This study was designed as a monocentric prospective case series in the private dental office of JB with histomorphometric analysis and descriptive statistics. Therefore, a case number calculation was not carried out, but a realistic consecutive number of includable patients was estimated.

Ethical considerations

The clinical investigation was performed according to ISO Standard 14155:2011, to the Swiss medical law (Heilmittelgesetz, HMG), to VKlin and to the Declaration of Hel-

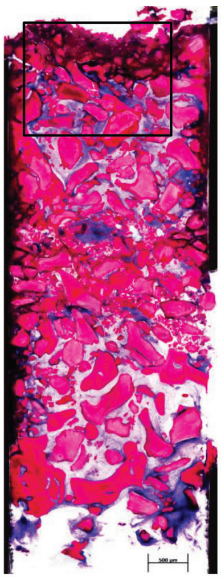

FIG. 5
Overview

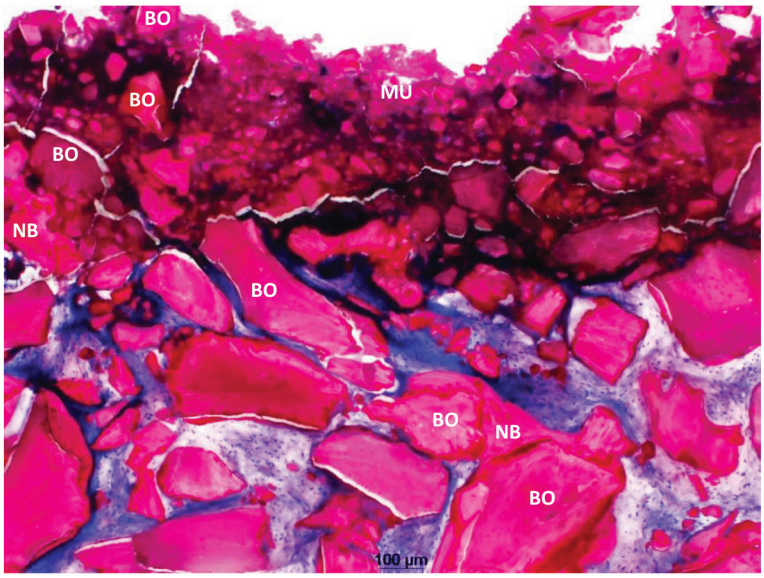

FIG. 6
Detail view of the framed part in Fig. 5.

Microphotographs of a core biopsy obtained in the premolar region of the maxilla after a healing time of 5 months.
In the coronal part of the specimen newly formed bone (NB), in close contact to residual deproteinized bovine bone mineral particles (BO), can be distinguished. However, most of the residual deproteinized bovine bone mineral particles are obviously not associated with ongoing bone formation and are found in non-mineralized tissue and remnants of the collagen matrix (MU). Moreover, coronal bone bridging cannot be found.
Overall, 16.2% new bone, 37.5% residual deproteinized bovine bone mineral as well as 36% non-mineralized tissue was found.

FIG. 5 AND 6 Azur II und Pararosanilin staining; original magnification x50

FIG. 7
Overview

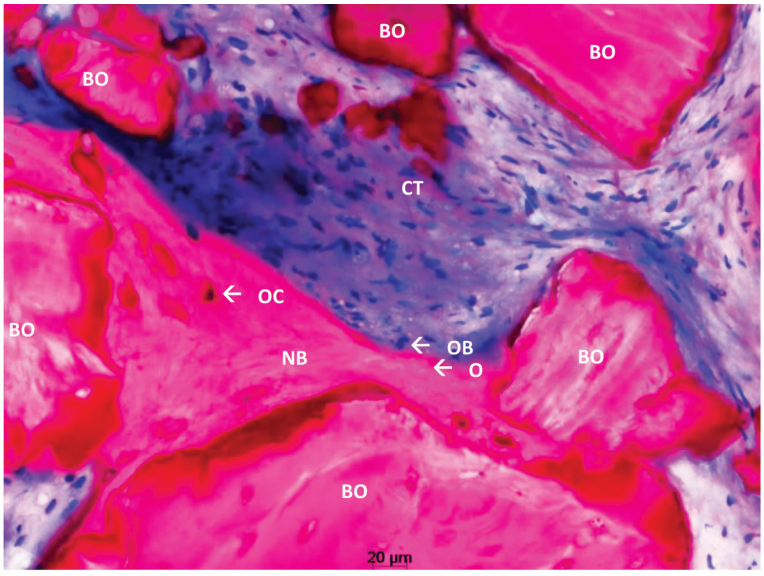

FIG. 8
Detail view of the framed part in Fig. 7.

Microphotographs of the core biopsy shown in Fig. 5. In the coronal part of the specimen ongoing new bone (NB) formation, in close contact to residual deproteinized bovine bone mineral particles (BO), can be seen (connective tissue (CT), osteoblast (OB), osteoid (O), osteocyte (OC)).

FIG. 7 AND 8 Azur II und Pararosanilin staining - FIG. 8 original magnification x50, Fig. 8 original magnification x200

sinki, 2013 (41). Prior to its initiation, this clinical trial was reviewed and approved by the German Federal Institute for Drugs and Medical Devices (Kurt-Georg-Kiesinger-Allee 3, D-53175 Bonn, Germany) on June 12th, 2015, as well as reviewed and approved by a legally competent ethics committee (Ethics Committee – Ärztekammer Hamburg, Weidestraße 122b, D-22083 Hamburg, Germany; EUDAMED CIV-14-10-012865) on November 27th, 2015. During the study the responsible authority (Freie und Hansestatt Hamburg, Behörde für Gesundheit und Verbraucherschutz, Amt für Verbraucherschutz, Pharmaziewesen und Medizinprodukte, Fachbereich Medizinprodukte – V42; Billstraße 80, D-20539 Hamburg, Germany) inspected the private dental practice of JB and checked

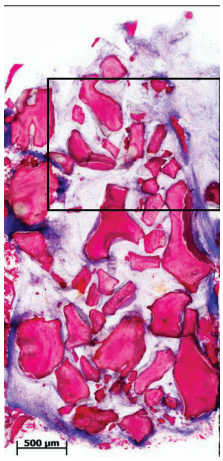

FIG. 9
Overview

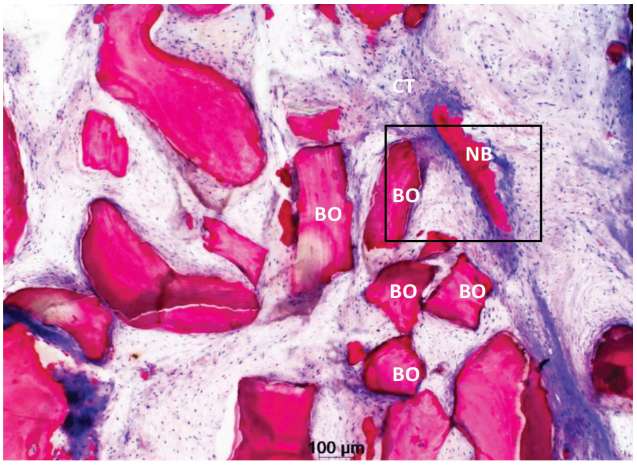

FIG. 10
Detail view of the framed part in Fig. 9.

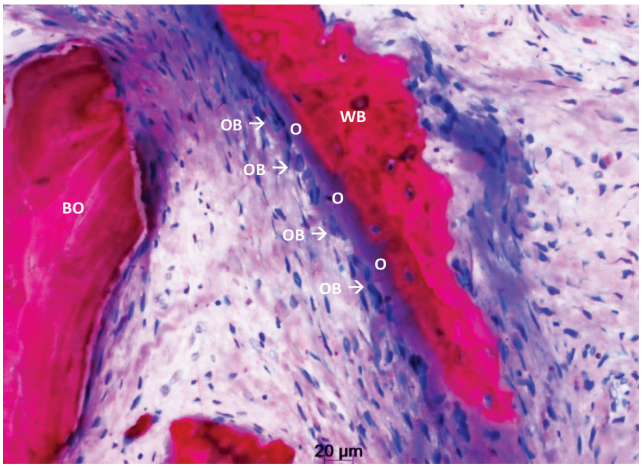

FIG. 11:
Detail view of the framed part in Fig. 10.

Microphotographs of a core biopsy obtained in the front region of the mandible after a healing time of 6 months.
In the coronal part of the specimen ongoing new bone (NB) formation within the connective tissue (CT) can be noticed (osteoblasts (OB), osteoid (O), woven bone (WB), residual deproteinized bovine bone mineral particles (BO)).
Overall, in this specimen 1.4% new bone, 40.5% residual deproteinized bovine bone mineral as well as 56.9% non-mineralized tissue was found.

FIG. 9, 10 AND 11 Azur II und Pararosanilin staining - FIG. 9 AND 10 original magnification x50, Fig. 11 original magnification x200

FIG. 12
Overview

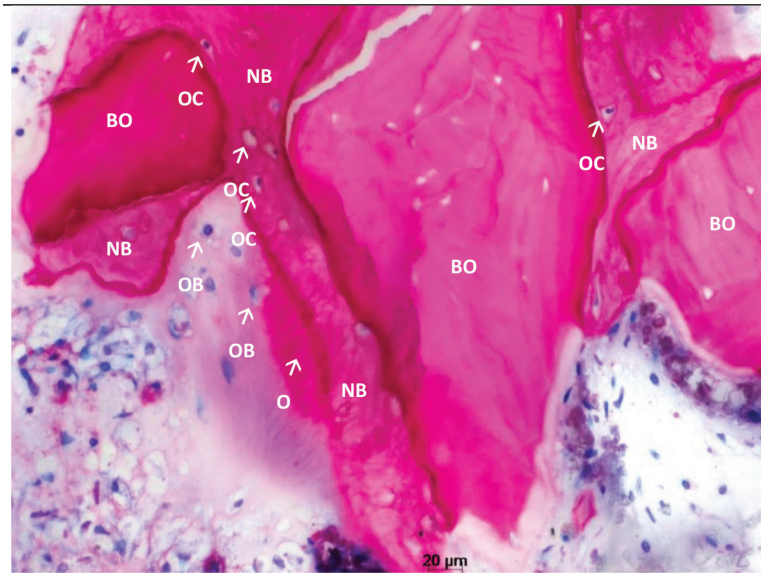

FIG. 13
Detail view of the framed part in Fig. 12.

Microphotographs of a core biopsy obtained in the premolar region of the maxilla after a healing time of 4.5 months.
Within the mid-section of the core biopsy ongoing new bone (NB) formation can be found. Seemingly, osteoid (O) is produced by osteoblasts (OB) onto newly formed bone (NB) already laid down on residual deproteinized bovine bone mineral particles (BO). Additional, within newly formed bone osteocytes (OC) can be detected.
Overall, in this specimen 28% new bone, 18% residual deproteinized bovine bone mineral as well as 45.7% non-mineralized tissue was found.

FIG. 12 AND 13 Azur II und Pararosanilin staining - FIG. 12 original magnification x50, Fig. 13 original magnification x200

the correct course of study execution on 29th August 2016. The study was registered in the German Register of Clinical Studies (DRKS00020451) and WHO listed (http://apps.who.int/trialsearch/).
Further, all patients were informed about the study and signed the applicable informed consent form before their enrolment.

Patient Population

Inclusion criteria

- Adult patients of sound mind (i.e. consent patients)
- Good general health
- Full-mouth plaque score (FMPS) ≤ 20%
- Full-mouth bleeding score (FMBS) ≤ 20%
- Absence of residual periodontal infection

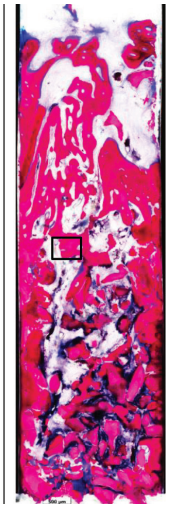

FIG. 14
Overview

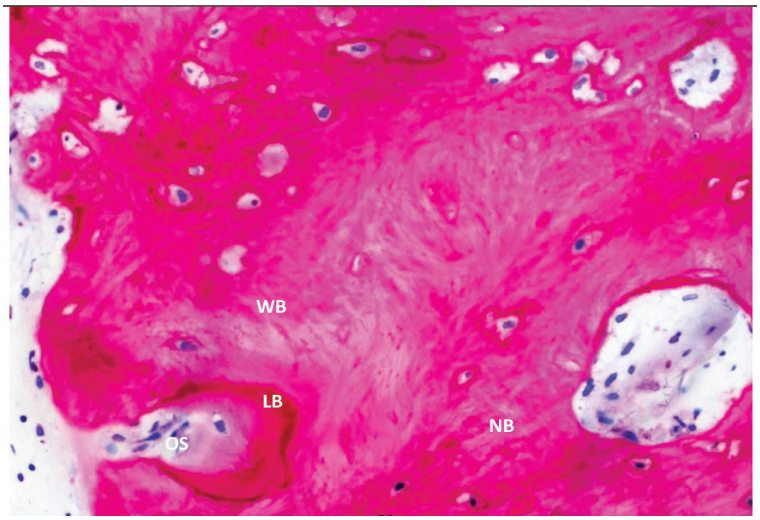

FIG. 15
Detail view of the framed part in Fig. 14.

Microphotographs of the core biopsy shown in Fig. 12.
Within the mid-section of the core biopsy ongoing remodelling of newly formed bone (NB) can be observed. Osteons (OS) can be identified and seemingly, woven bone (WB) is replaced by lamellar bone (LB).

FIG. 14 AND 15 Azur II und Pararosanilin staining - FIG. 14 original magnification x50, Fig. 15 original magnification x200

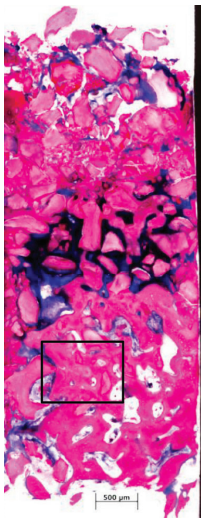

FIG. 16
Overview

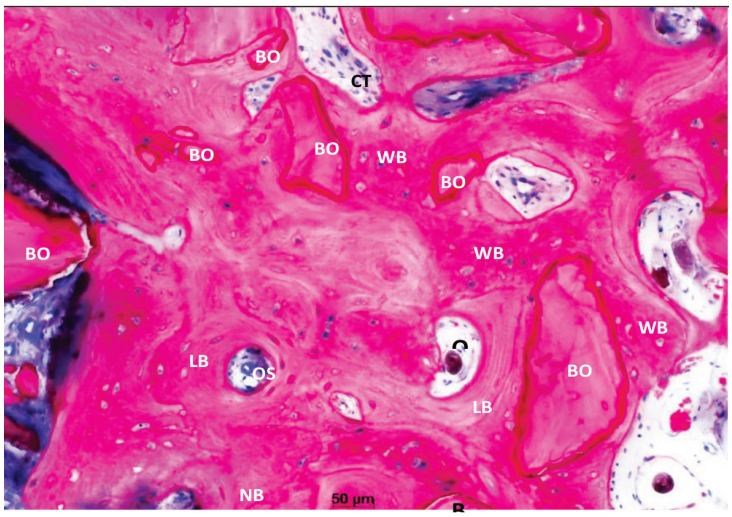

FIG. 17
Detail view of the framed part in Fig. 16.

Microphotographs of the core biopsy shown in Fig. 1.
In a more apical section residual deproteinized bovine bone mineral particles (BO) are embedded in newly formed bone (NB). Thereby, woven bone (WB) as well as lamellar bone (LB) can be distinguished. Furthermore, within the lamellar bone (LB) osteons (OS) are noticeable.

FIG. 16 AND 17 Azur II und Pararosanilin staining - FIG. 16 original magnification x50, Fig. 17 original magnification x100

- Scheduled for ARP 1.1 or 2.1 with DMBB+C and CMX before enrolment
- Willing to adhere to a strict Supportive Periodontal Therapy (SPT) protocol
- At least one primarily four-walled extraction socket

Exclusion criteria

- Minors
- Pregnancy / breastfeeding
- Patients taking medications that could influence bone metabolism
- Patients with uncontrolled or poorly controlled diabetes
- Patients using anticoagulants
- Patients requiring antibiotic prophylaxis
- Smokers > 5 cigarettes / day
- Patients with known allergic reactions/sensitivity against collagen products
- Patients presenting unstable or life-threatening conditions

Treatment

Eleven adult patients scheduled for tooth extraction, ARP 1.1 or 2.1 with DMBB+C / CMX and implant supported restoration in the maxilla or mandible with at least one primarily four-walled extraction socket were included.

Core biopsy Number	Region	Healing time [months]	New bone [%]	New bone [%]	New bone [%]
			Healing time 4 to 6.5 months N=12	Healing time 4 and 4.5 months N=8	Healing time 6 and 6.5 months N=3
1	14	4.5	23.4	23.4	
2	37	4.5	13.7	13.7	
3	25	5	16.2		
4	21	4.5	21.1	21.1	
5	15	4.5	28	28	
6	15	4	31.5	31.5	
7	45	4	21.7	21.7	
8	41	6	1.4		1.4
9	35	4.5	7.7	7.7	
10	36	6.5	38.9		38.9
11	37	6.5	16.1		16.1
12	45	4	29.2	29.2	
Mean (SD)		4.9 (0.9)	20.7 (10.1)	22 (7.5)	18.8 (15.4)
Minimum		4	1.4	7.7	1.4
Maximum		6.5	38.9	31.5	38.9

TABLE 1
New bone formation

The patients - 2 female and 9 male - were between 30 and 69 years of age and did not suffer from systemic diseases or taking medications affecting bone metabolism. Ten patients contributed with one tooth extraction site and one patient with two.

After applying local anaesthesia (adrenaline 1:100000; Ultracain® D-S forte; Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany) flapless tooth extraction with periotomes and elevators was performed with great care to preserve the buccal bone plate and the surrounding soft tissues. The extraction socket was thoroughly curetted, the completeness of the surrounding bone, in the sense of a primarily four-walled extraction socket, was determined with a periodontal probe and the adjacent gingival borders of the extraction socket were de-epithelized. Subsequently, the socket was, in accordance with the surgical guidelines of the manufacturer, filled with a xenogeneic graft, a blend of granules of deproteinized bovine bone mineral (90%) and porcine collagen fibres (10%) (DBBM+C; Bio-Oss® Collagen; Geistlich Pharma AG, Wolhusen, Switzerland). In brief, DBBM+C was moistened with sterile saline, cut to size, introduced into the socket with forceps and carefully packed with a plugger. Thereby, care was taken not to compress it too strongly. Thereafter, a collagen matrix (CMX; Mucograft® seal; Geistlich Pharma AG, Wolhusen, Switzerland) was applied according to the manufacturer's surgical protocol. Briefly, if necessary CMX was customised, to assure a tension-free, close adaptation to the de-epithelized marginal soft-tissue borders of the extraction socket and sutured with non-resorbable, single interrupted sutures (Prolene® suture 6-0, P3, EH7291H; Ethicon-Johnson & Johnson, Sommerville, USA). Care was taken that the compact CMX structure faced outwards and the pongy structure towards the extraction socket. Provisional restorations were place without pressure on the graft or causing tissue impingement. The patients were asked not to brush the surgical area until suture removal and were assigned for wound control and cleansing every second to third day. Ibuprofen (400 mg, 5 times per day for 1 day) and chlorhexidine solution (0.2%, 2 times per day, starting the day after surgery until suture removal) as well as systemic antibiotics (amoxicillin 500mg / 3 times per day for 3 days) were prescribed.

Sutures were removed between 10 and 14 days after the surgical procedure.

At implant placement, after a healing period of 4 to 6.5 months, representing ARP 1.1 or 2.1, a core biopsy from the extraction site was taken as part of implant site preparation. In brief, after soft tissue flap elevation, trephine drills with an internal diameter of 2.9 mm (Komet Dental; Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) were used to remove 5 to 8 mm long apico-coronal blocks of hard tissue. Thereafter, implant bed preparation was continued according to the manufacturer's surgical protocol, dental implants (BIOMET T3®; Zimmer BIOMET, Warsaw, USA) were installed, cover screws attached, and the soft-tissue flap replaced and secured with interrupted sutures. Subsequent to three months of submerged healing the restorative treatment was initiated.

The core biopsies were immediately inserted in 4% neutral buffered formalin solution and sent to a certified histological laboratory (CTA – Cell Tissue Analysis; Universitätsklinik für Zahn-, Mund- und Kieferheilkunde, Hugstetterstr. 55, Freiburg im Breisgau, Germany).

Histologic preparation, descriptive histology and histomorphometric analysis

TABLE 2 residual deproteinized bovine bone mineral

Core biopsy Number	Region	Healing time [months]	Bio-Oss® particles [%]	Bio-Oss® particles [%]	Bio-Oss® particles [%]
			Healing time 4 to 6.5 months N=12	Healing time 4 and 4.5 months N=8	Healing time 6 and 6.5 months N=3
1	14	4.5	32.4	32.4	
2	37	4.5	14.2	14.2	
3	25	5	37.9		
4	21	4.5	24.2	24.2	
5	15	4.5	18	18	
6	15	4	9.9	9.9	
7	45	4	31.7	31.7	
8	41	6	40.5		40.5
9	35	4.5	54.3	54.3	
10	36	6.5	19.3		19.3
11	37	6.5	28.3		28.3
12	45	4	20.7	20.7	
Mean (SD)		4.9 (0.9)	27.6 (12)	25.7 (13.1)	29.4 (8.7)
Minimum		4	9.9	9.9	19.3
Maximum		6.5	54.3	54.3	40.5

In brief, after complete fixation in 4% neutral buffered formalin solution, the core biopsy was dehydrated in alcohol solutions of increasing concentration, cleared in xylene, and embedded in polymethylmetacrylate (Technovit 9100; Kulzer, Wehrheim, Germany). Thereafter, serial sections including the central portion of the core biopsy were cut with diamond saws parallel with its long axis, glued onto special slides and ground to a final thickness of approximately 60 µm (modified from Schenk RK, University of Berne, Switzerland). Thereafter, the histological slides were stained with Azur II and Pararosanilin to allow a clear differentiation between older bones, newly formed bone, residual deproteinized bovine bone mineral as well as soft tissue. For qualitative evaluation, the sections were analysed by light microscopy (Axio Imager M1 microscope equipped with a digital AxioCam HRc; Carl Zeiss, Göttingen, Germany). In addition, for quantitative measurements an image analysing software (analySIS FIVE; Olympus, Ham-

Core biopsy Number	Region	Healing time [months]	New soft tissue [%]	New soft tissue [%]	New soft tissue [%]
			Healing time 4 to 6.5 months N=12	Healing time 4 and 4.5 months N=8	Healing time 6 and 6.5 months N=3
1	14	4.5	34.1	34.1	
2	37	4.5	71.7	71.7	
3	25	5	36		
4	21	4.5	42.5	42.5	
5	15	4.5	45.7	45.7	
6	15	4	58.3	58.3	
7	45	4	35.2	35.2	
8	41	6	56.9		56.9
9	35	4.5	38	38	
10	36	6.5	41.8		41.8
11	37	6.5	55.6		55.6
12	45	4	47.9	47.9	
Mean (SD)		4.9 (0.9)	47 (11,1)	47.8 (9.5)	48.2 (7.2)
Minimum		4	34.1	28.3	41.9
Maximum		6.5	71.7	62	58.2

burg, Germany) was used. By this means, the proportions of original bone, newly formed bone, residual deproteinized bovine bone mineral and soft tissue were determined on the total surface of the preparation and expressed in percent.

Statistical analysis

For histomorphometric analysis descriptive statistics with mean, standard deviation (SD), minimum (min) and maximum (max) were used.

RESULTS

In total, 11 patients (2 females and 9 males, 30 to 69 years of age), who had neither systemic diseases nor medications affecting bone metabolism, were included. Ten patients contributed with one tooth extraction site and one patient with two. Extraction sites included one incisor and four premolars in the maxilla as well as one incisor, three premolars and three molars in the mandible. Healing after tooth extraction and ARP was uneventful and each scheduled implant could be inserted. At implant placement, after a healing period of 4 to 6.5 months, all sites were covered with non-inflamed, attached keratinized mucosa.

Histologic findings

Overall, newly formed bone could be observed throughout the entire sections of all core biopsies. However, less newly formed bone was located in the more coronal as compared to the more apical parts. In addition, coronal bone bridging was not found in any section. Further, also residual deproteinized bovine bone particles of different size, not always in close contact to newly formed bone, could be distinguished all the way through all specimens of all core biopsies (Fig. 1 and 2; Fig. 3 and 4). Neither signs of deproteinized bovine bone particle resorption, nor inflammatory reaction were seen.

Though, in the coronal portions of all specimens newly formed bone was found (Fig. 5 and 6). Thereby, ongoing new bone formation onto residual deproteinized bovine bone particles (Fig. 7 and 8) as well as within the connective tissue (Fig. 9, 10 and 11) was observed. Further, most residual deproteinized bovine bone particles were not associated with ongoing bone formation and were found in soft tissue and remnants of the collagen matrix (Fig. 5 and 6, Fig. 9 and 10).

Also, within the mid- and apical sections ongoing new bone formation onto residual deproteinized bovine bone particles was seen (Fig. 12 and 13). Moreover, next to formation, remodelling of newly formed bone could be identified (Fig. 14 and 15, Fig. 16 and 17). In addition, deproteinized bovine bone particles, not associated with ongoing bone formation, were found within soft tissue fractions.

Histomorphometric findings

Newly formed bone

Overall, in primarily four-walled extraction sockets from the front, premolar and molar region (N=12), after a mean healing time of 4.9 months (SD: 0.9 months; range: 4 - 6.5 months) a mean of 20.7% new bone (SD: 10.1%; range: 1.4% - 38.9%) was found. It should be noted that in core biopsies representing healing times of 4 and 4.5 months (N=8) a mean of 22% new bone (SD: 7.5%; range: 7.7% - 31.5%) and in core biopsies representing healing times of 6 and 6.5 months (N=3) a mean of 18.8% new bone (SD: 15.4%; range: 1.4% - 38.9%) were found (Table 1).

Residual deproteinized bovine bone mineral graft, newly formed soft tissue

Further, after a mean healing time of 4.9 months (SD: 0.9 months; range: 4 - 6.5 months) a mean of 27.6% of residual deproteinized bovine bone mineral (Bio-Oss® particles; SD: 12%; range: 9.9% - 54.3%) and a mean of 47% of new soft tissue (SD: 11.1%; range: 34.1% - 71.7%) was found. In addition, it should be noticed that in core biopsies representing healing times of 4 and 4.5 months (N=8) a mean of 25.7% of residual deproteinized bovine bone mineral (SD: 13.1%; range: 9.9% - 54.3%) as well as a mean of 47.8% of new soft tissue (SD: 9.5%; range: 28.3% - 62%) was found. Moreover, in core biopsies representing healing times of 6 and 6.5 months (N=3) a mean of 29.4% of residual deproteinized bovine bone mineral (SD: 8.7%; range: 19.3% - 40.5%) as well as a mean of 48.2% of new soft tissue (SD: 7.2%; range: 41.9% - 58.2%) was found (Table 2 and 3).

DISCUSSION

Up to now, human histological data evaluating the osteogenic potential of *de novo* formed tissue, which might influence implant stability and bone to implant healing, for alveolar ridge preservation (ARP) with a blend of granules of deproteinized bovine bone mineral and porcine collagen fibres (DBBM+C) in combination with a collagen matrix (CMX), at the time points of delayed (12 - 16 weeks; ARP 1.1) or standard (> 16 weeks; ARP 2.1) implant placement, are limited. Therefore, the aim was to get more - histological and histomorphometric - insight in the osteogenic potential of *de novo* formed tissue within extraction sockets grafted with DBBM+C and CMX after healing times representative for ARP 1.1 or 2.1.

In general, after 4 - 6.5 months of healing, newly formed bone was found from marginal to apical portions of all core biopsies. Thereby, new bone formation onto residual deproteinized bovine bone particles of different sizes as well as within the connective tissue was observed. Seemingly, less new bone was located in coronal as compared to apical sections. Regarding new bone quantity marked differences were seen between the samples. Further, coronal bone bridging was never found. In addition, no ob-

vious signs of deproteinized bovine bone particle resorption were seen.

More particular, in the current investigation, within the newly formed tissue a mean of 20.7% new bone (SD: 10.1%; range: 1.4% - 38.9%) was found. Further, means of 27.6% residual deproteinized bovine bone mineral (SD: 12%; range: 9.9% - 54.3%) and 47% new soft tissue (SD: 11.1%; range: 34.1% - 71.7%) were seen.

Thereby, the found quantities and their variability, following grafting fresh human extraction sockets with DMBB+C, are in principle in line with others for different healing times and procedures of wound closure at the soft tissue level (28-30, 32, 34-39). For example, after a healing period of six months, Lindhe et al. (36) found, within human fresh extraction sockets filled with DBBM+C and covered with CMX at the marginal hard tissue wound, means of 39.9% mineralized bone (SD: 8.6%) and 1.6% osteoid (SD: 1.8%). In addition, means of 19.0% residual deproteinized bovine bone mineral (SD: 6.5%), 32.4% fibrous tissue (SD: 9.2%), 1.8% bone marrow (SD: 2.5%), and 5.5% residual tissue (SD: 2.9%) were discriminated. Likewise, Ramaglia et al. (39) investigated human fresh extraction sockets filled with DBBM+C and covered with CMX at the soft tissue level. After 16 weeks of healing a mean of 35.6% vital bone (SD: 15.6%) was found. Besides, means of 34.2% residual deproteinized bovine bone mineral (SD: 11.1%), and 30.2% connective tissue (SD: 9.6%) were published.

Further, in the present investigation in none of the specimen coronal bone bridging was seen. This observation deviates from previous findings. In a comparable study, Lindhe et al. (36) found in human extraction sockets filled with DBBM+C and covered with CMX after about six months of healing in five out of eleven sites a layer of mineralized bone. The reason for the dissimilarity is difficult to explain. Other clinical studies grafting fresh extraction sockets with DMBB+C with different healing times and procedures of wound closure at the soft tissue level do neither address coronal bone bridging (28-30, 32, 34, 35, 37-39), nor is it observable in complete coronal-apical sections presented in the publications (29, 30, 34, 37, 38). Further, the absence of coronal bone bridging in the current investigation is in contrast to findings in dog models (9, 10). The reason for this difference between the present findings and the observations reported from animal studies is difficult to understand, but may be related to species differences and/or the degree of socket fill with DBBM+C at placement (36). However, also unassisted healing of human extraction sockets is not consistently associated with coronal bone bridging (42, 43). Lindhe et al. (42)) evaluated tissue composition of fully healed human post-extraction sites in regions edentulous for five months to some years. In several but not all histological specimens, coronal bone bridging could be identified. Further, Bertl et al. (43) assessed the timeframe between tooth extraction and radiographically detectable socket cortication in humans. Completely corticated sockets were observed in 20% of the cases after 3–6 months, 40% after 6–9 months, and 95% after more than 9 months after tooth loss. Thus, both studies indicate the inter-individual timeframe variability for coronal bone bridging in humans. Hence, next to different healing periods, inter-individual healing differences might help to explain study dissimilarities. Nevertheless, newly formed bone was consistently found in coronal parts - even directly below remnants of the collagen matrix - of the core biopsies. A fact, that may point to the role of the collagen matrix as bioactive barrier membrane for guided bone regeneration (44).

In addition, in the present investigation newly formed bone was found all the way through all specimens of all core biopsies. This is in line with clinical investigations with comparable design (i.e. DBBM+C plus CMX) and duration (28, 36), with other clinical studies grafting fresh extraction sockets with DMBB+C with different healing times and procedures of wound closure at the soft tissue level (29, 30, 34, 35, 37-39) as well as with findings in dog models (9, 10). Moreover, in coronal parts of the current histological sections new bone formation onto residual deproteinized bovine bone particles was found. Additionally, in one histological section newly formed bone was observed within the connective tissue (i.e. without contact to deproteinized bovine bone particles or old bone). Also, in more central parts of the core biopsies ongoing bone modelling was found. Seemingly, osteoid was produced by osteoblasts onto residual deproteinized bovine bone mineral particles as well as onto newly formed woven bone already laid down on residual deproteinized bovine bone mineral particles. Besides, osteons, indicating remodelling of new woven bone into lamellar bone could be identified. Within more apical parts of the section's residual deproteinized bovine bone mineral particles were embedded in newly formed bone. Thereby, woven bone as well as osteons containing lamellar bone could be distinguished. Additionally, not on all residual deproteinized bovine bone particles new bone was found, less new bone was located in coronal as compared to apical sections and obviously, new bone formation started from the apical and lateral parts of the extraction sockets. These findings are in harmony with that of others (28-30, 34-39) and consistent with experimental findings on healing dynamics after incorporation of DBBM+C in the beagle dog-model (45).

Furthermore, in the current investigation means of 20.7% new bone (SD: 10.1%; range: 1.4% - 38.9%) and 27.6% residual deproteinized bovine bone mineral particles (SD: 12%; range: 9.9% - 54.3%) were found. As already indicated above, this fits into the line with other comparable studies (i.e. DBBM+C plus CMX) (28, 32, 36, 39) and studies with different healing times and procedures of wound closure at the soft tissue level (i.e. DBBM+C) (29, 30, 34, 35, 37, 38). Additionally, this is comparable to investigations using DBBM. For example Norton et al. (46) published after a mean healing time of 26 weeks (range: 15

– 44 weeks), means of 26.9% (range: 0 -52.8%) new bone and 25.6% of residual graft material. Above and beyond, no signs of deproteinized bovine bone particle resorption were seen. This result might be another reference on the validity of the hypothesis that deproteinized bovine bone mineral particles may not resorb but remain more or less unaltered at socket sites (36, 45). Interestingly, this "resistance to resorption" (36) has also been shown in a case report even after a period of 14 years (47). Further, remnants of the collagen matrix were found in a few histological sections. Also, this is in line with comparable studies using CMX (28, 39).
However, one should bear in mind that this study includes only a small number of patients and high interindividual variability was found. That should be considered a limitation and restricts generalisability.

CONCLUSION

With the limits of this prospective case series, it can be concluded that, after alveolar ridge preservation with a blend of granules of deproteinized bovine bone mineral and porcine collagen fibres in combination with a collagen matrix, at the time point of delayed (12 - 16 weeks) or standard (> 16 weeks) implant placement, (1) the *de novo* formed tissue has an osteogenic potential throughout the entire grafted part of the former extraction site, (2) coronal bone bridging might not be expected, (3) less new bone might form in coronal as compared to apical parts of the extraction sites, (4) healing of the extraction sites should not be regarded as being completed, and (5) the collagen matrix used might be regarded as barrier membrane for guided bone regeneration.
Data availability statement:
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding statement:
The current clinical investigation has been supported by a grant from Geistlich Pharma AG (Bahnhofstrasse 40, 6110 Wolhusen, Switzerland).

Conflict of interest disclosure:
The authors declare no conflict of interest.

Ethical approval statement:
This clinical trial was reviewed and approved by the German Federal Institute for Drugs and Medical Devices (Kurt-Georg-Kiesinger-Allee 3, D-53175 Bonn, Germany) on June 12th, 2015, as well as reviewed and approved by a legally competent ethics committee (Ethics Committee – Ärztekammer Hamburg, Weidestraße 122b, D-22083 Hamburg, Germany; EUDAMED CIV-14-10-012865) on November 27th, 2015.

Patient consent statement:
All patients were informed about the study and signed the applicable informed consent form before their enrolment.

Permission to reproduce material from other sources:
Materials from other sources are not used.

Clinical trial registration:
The study was registered in the German Register of Clinical Studies (DRKS00020451) and WHO listed (http://apps.who.int/trialsearch/).

Authors contributions:
R.J. and J.B. conceived the ideas, J.B. collected the date, R.J. and J.B. analysed the data, and R.J. led the writing.

Guideline:
STROBE – guideline was followed.

Acknowledgement:
The authors thank Annette Linder for preparing and analysing the histological sections.

REFERENCES

1. Alkan EA, Parlar A, Yildirim B, Senguven B. Histological comparison of healing following tooth extraction with ridge preservation using enamel matrix derivatives versus Bio-Oss Collagen: a pilot study. Int J Oral Maxillofac Surg. 2013;42(12):1522-8.
2. Araújo M, Linder E, Wennström J, Lindhe J. The influence of Bio-Oss Collagen on healing of an extraction socket: an experimental study in the dog. Int J Periodontics Restorative Dent. 2008;28:123-35.
3. Araujo M, Lindhe J. Ridge preservation with the use of Bio-Oss collagen: A 6-month study in the dog. Clinical Oral Implants Research. 2009;20:433-40.
4. Araújo MG, da Silva JCC, de Mendonca AF, Lindhe J. Ridge alterations following grafting of fresh extraction sockets in man. A randomized clinical trial. Clin Oral Implants Res. 2015;26(4):407-12.
5. Araújo MG, Liljenberg B, Lindhe J. Dynamics of Bio-Oss Collagen incorporation in fresh extraction wounds: an experimental study in the dog. Clin Oral Implants Res. 2010;21(1):55-64.
6. Araújo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol. 2005;32(2):212-8.
7. Araújo MG, Lindhe J. Ridge alterations following tooth extraction with and without flap elevation: an experimental study in the dog. Clin Oral Implants Res. 2009b;20(6):545-9.
8. Araújo MG, Lindhe J. Ridge preservation with the use of Bio-Oss collagen: A 6-month study in the dog. Clin Oral Implants Res. 2009;20(5):433-40.
9. Avila-Ortiz G, Chambrone L, Vignoletti F. Effect of alveolar ridge preservation interventions following tooth extraction: A systematic review and meta-analysis. J Clin Periodontol. 2019;46 Suppl 21:195-223.
10. Ayna M, Açil Y, Gulses A. Fateof a bovine-derived xenograft in maxillary sinus floor elevation after 14 years: histologic and radiologic analysis. Int J Periodontics Restorative Dent. 2015;35:541-7.
11. Bertl K, Kukla EB, Albugami R, Beck F, Gahleitner A, Stavropoulos A. Timeframe of socket cortication after tooth extraction: A retrospective radiographic study. Clin Oral Implants Res. 2018;29(1):130-8.
12. Cardaropoli D, Tamagnone L, Roffredo A, De Maria A, Gaveglio L. Alveolar Ridge Preservation Using Tridimensional Collagen Matrix and Deproteinized

Bovine Bone Mineral in the Esthetic Area: A CBCT and Histologic Human Pilot Study. Int J Periodontics Restorative Dent. 2018;38(Suppl):s29-s35.
13. Cardaropoli D, Tamagnone L, Roffredo A, Gaveglio L. Relationship between the buccal bone plate thickness and the healing of postextraction sockets with/without ridge preservation. Int J Periodontics Restorative Dent. 2014;34(2):211-7.
14. Cha JK, Song YW, Park SH, Jung RE, Jung UW, Thoma DS. Alveolar ridge preservation in the posterior maxilla reduces vertical dimensional change: A randomized controlled clinical trial. Clin Oral Implants Res. 2019;30(6):515-23.
15. Di Stefano DA, Orlando F, Ottobelli M, Fiori D, Garagiola U. A comparison between anorganic bone and collagen-preserving bone xenografts for alveolar ridge preservation: systematic review and future perspectives. Maxillofac Plast Reconstr Surg. 2022;44(1):24.
16. Fickl S, Zuhr O, Wachtel H, Bolz W, Huerzeler MB. Hard tissue alterations after socket preservation: an experimental study in the beagle dog. Clin Oral Implants Res. 2008;19(11):1111-8.
17. Fickl S, Zuhr O, Wachtel H, Stappert CF, Stein JM, Hurzeler MB. Dimensional changes of the alveolar ridge contour after different socket preservation techniques. J Clin Periodontol. 2008;35(10):906-13.
18. Gabay E, Katorza A, Zigdon-Giladi H, Horwitz J, Machtei EE. Histological and dimensional changes of the alveolar ridge following tooth extraction when using collagen matrix and collagen-embedded xenogenic bone substitute: A randomized clinical trial. Clin Implant Dent Relat Res. 2022;24(3):382-90.
19. Guarnieri R, Stefanelli L, De Angelis F, Mencio F, Pompa G, Di Carlo S. Extraction Socket Preservation Using Porcine-Derived Collagen Membrane Alone or Associated with Porcine-Derived Bone. Clinical Results of Randomized Controlled Study. J Oral Maxillofac Res. 2017;8(3):e5.
20. Heberer S, Al-Chawaf B, Jablonski C, Nelson J, Lage H, Nelson K. Healing of ungrafted and grafted extraction sockets after 12 weeks: a prospective clinical study. Int J Periodontics Restorative Dent. 2011;26:385-92.
21. Iocca O, Farcomeni A, Pardinas Lopez S, Talib HS. Alveolar ridge preservation after tooth extraction: a Bayesian Network meta-analysis of grafting materials efficacy on prevention of bone height and width reduction. J Clin Periodontol. 2017;44(1):104-14.
22. Iorio-Siciliano V, Blasi A, Nicolò M, Iorio-Siciliano A, Riccitiello F, Ramaglia L. Clinical outcomes of socket preservation using bovine-derived xenograft collagen and collagen membrane post-extraction: A 6-months randomized controlled clinical trial. Int J Periodontics Restorative Dent. 2017;37:e290-e6.
23. Jung R, Philip A, Annen B, Signorelli L, Thoma D, Hämmerle C, et al. Radiographhic evaluation of different techniques for ridge preservation after tooth extraction: a randomized controlled clinical trial. J Clin Periodontol. 2013;40(1):90-8.
24. Jung RE, Philipp A, Annen BM, Signorelli L, Thoma DS, Hammerle CH, et al. Radiographic evaluation of different techniques for ridge preservation after tooth extraction: a randomized controlled clinical trial. J Clin Periodontol. 2013;40(1):90-8.
25. Lang N, Salvi G. Implants in restorative dentistry. In: Lang, NP, Lindhe, J, editors Clinical periodontology and implant dentistry 6th ed Hoboken (NJ): Wiley. 2015:1156–63.
26. Lang NP. Oral Implants: The Paradigm Shift in Restorative Dentistry. J Dent Res. 2019;98(12):1287-93.
27. Lindhe J, Cecchinato D, Bressan EA, Toia M, Araujo MG, Liljenberg B. The alveolar process of the edentulous maxilla in periodontitis and non-periodontitis subjects. Clin Oral Implants Res. 2012;23(1):5-11.
28. Lindhe J, Cecchinato D, Donati M, Tomasi C, Liljenberg B. Ridge preservation with the use of deproteinized bovine bone mineral. Clin Oral Implants Res. 2014;25(7):786-90.
29. MacBeth N, Trullenque-Eriksson A, Donos N, Mardas N. Hard and soft tissue changes following alveolar ridge preservation: a systematic review. Clin Oral Implants Res. 2017;28(8):982-1004.
30. Machtei EE, Mayer Y, Horwitz J, Zigdon-Giladi H. Prospective randomized controlled clinical trial to compare hard tissue changes following socket preservation using alloplasts, xenografts vs no grafting: Clinical and histological findings. Clin Implant Dent Relat Res. 2019;21(1):14-20.
31. Maiorana C, Poli PP, Deflorian M, Testori T, Mandelli F, Nagursky H, et al. Alveolar socket preservation with demineralised bovine bone mineral and a collagen matrix. J Periodontal Implant Sci. 2017;47(4):194-210.
32. Morjaria KR, Wilson R, Palmer RM. Bone healing after tooth extraction with or without an intervention: a systematic review of randomized controlled trials. Clin Implant Dent Relat Res. 2014;16(1):1-20.
33. Nart J, Barallat L, Jimenez D, Mestres J, Gomez A, Carrasco MA, et al. Radiographic and histological evaluation of deproteinized bovine bone mineral vs. deproteinized bovine bone mineral with 10% collagen in ridge preservation. A randomized controlled clinical trial. Clin Oral Implants Res. 2017;28(7):840-8.
34. Norton M, Odell E, Thompson I, Cook R. Efficacy of bovine bone mineral for alveolar augmentation: a human histologic study. Clin Oral Implants Res. 2003;14:775-83.
35. Omar O, Elgali I, Dahlin C, Thomsen P. Barrier membranes: More than the barrier effect? Journal of Clinical Periodontology. 2019;46:103-23.
36. Pjetursson BE, Lang NP. Prosthetic treatment planning on the basis of scientific evidence. J Oral Rehabil. 2008;35 Suppl 1:72-9.
37. Pjetursson BE, Sailer I, Latyshev A, Rabel K, Kohal RJ, Karasan D. A systematic review and meta-analysis evaluating the survival, the failure, and the complication rates of veneered and monolithic all-ceramic implant-supported single crowns. Clin Oral Implants Res. 2021;32 Suppl 21(Suppl 21):254-88.
38. Ramaglia L, Saviano R, Matarese G, Cassandro F, Williams RC, Isola G. Histologic Evaluation of Soft and Hard Tissue Healing Following Alveolar Ridge Preservation with Deproteinized Bovine Bone Mineral Covered with Xenogenic Collagen Matrix. Int J Periodontics Restorative Dent. 2018;38(5):737-45.
39. Romeo E, Storelli S. Systematic review of the survival rate and the biological, technical, and aesthetic complications of fixed dental prostheses with cantilevers on implants reported in longitudinal studies with a mean of 5 years follow-up. Clin Oral Implants Res. 2012;23 Suppl 6:39-49.
40. Sailer I, Strasding M, Valente NA, Zwahlen M, Liu S, Pjetursson BE. A systematic review of the survival and complication rates of zirconia-ceramic and metal-ceramic multiple-unit fixed dental prostheses. Clin Oral Implants Res. 2018;29 Suppl 16:184-98.
41. Scheyer ET, Heard R, Janakievski J, Mandelaris G, Nevins ML, Pickering SR, et al. A randomized, controlled, multicentre clinical trial of post-extraction alveolar ridge preservation. J Clin Periodontol. 2016;43(12):1188-99.
42. Schulz MC, Kallweit MB, Kallweit S, Koch R, Lauer G, Mai R, et al. Autogenous bone and a bovine bone substitute for ridge preservation: preliminary clinical and histologic findings. Aust Dent J. 2016;61(1):62-70.
43. Spitznagel FA, Balmer M, Wiedemeier DB, Jung RE, Gierthmuehlen PC. Clinical outcomes of all-ceramic single crowns and fixed dental prostheses supported by ceramic implants: A systematic review and meta-analyses. Clin Oral Implants Res. 2022;33(1):1-20.
44. Tan WL, Wong TL, Wong MC, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res. 2012;23 Suppl 5:1-21.
45. Tonetti MS, Jung RE, Avila-Ortiz G, Blanco J, Cosyn J, Fickl S, et al. Management of the extraction socket and timing of implant placement: Consensus report and clinical recommendations of group 3 of the XV European Workshop in Periodontology. J Clin Periodontol. 2019;46 Suppl 21:183-94.
46. Van der Weijden F, Dell'Acqua F, Slot DE. Alveolar bone dimensional changes of post-extraction sockets in humans: a systematic review. J Clin Periodontol. 2009;36(12):1048-58.
47. World Medical Association Declaration of Helsinki Journal of American Medical Association. 2013;310(20).