Materials Science and Engineering C 46 (2015) 177–183

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Socket augmentation using a commercial collagen-based product — an animal study in pigs

Christiane Kunert-Keil [a,*], Tomasz Gredes [a], Friedhelm Heinemann [b], Marzena Dominiak [c], Ute Botzenhart [a], Tomasz Gedrange [a]

[a] Department of Orthodontics, Carl Gustav Carus Campus, Technische Universität Dresden, Fetscherstr. 74, D-01307 Dresden, Germany
[b] Department of Prosthodontics, Gerodontology and Biomaterials, Medical University of Greifswald, Rotgerberstr. 8, D-17487 Greifswald, Germany
[c] Department of Dental Surgery, Silesian Piast Medical University, 26 Krakowska st, 50-424 Wroclaw, Poland

ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 25 August 2014
Accepted 8 October 2014
Available online 16 October 2014

Keywords:
Bone regeneration
Bone substitute
Bone resorption
Collagen
Histology

ABSTRACT

The aim of the present study was to identify properties of pure collagen for augmentation techniques and compare to a proved xenogenic material and natural bone regeneration. For that the osteogenesis of extraction alveoli after augmentation with a collagen cone covered with an absorbable collagen membrane in a single product (PARASORB Sombrero®, Resorba) was evaluated in a pig model. Extraction alveoli were treated with the collagen cone and the collagen membrane in a single product (test group; n = 7) or demineralized bovine bone mineral and a collagen membrane (two separate products; positive control; n = 7). Untreated alveoli were used (n = 6) as negative controls.[1] Bone specimens were extracted 1 and 3 months after teeth extraction. Serial longitudinal sections were stained with Masson Goldner trichrome. Furthermore, bone specimens were examined using X-ray analyses. Significant differences of bone atrophy were detected 12 weeks after material insertion using X-ray analyses. The bone atrophy was reduced by approximately 32% after insertion of the positive control (P = 0.046). Bone atrophy reached 37.6% of those from untreated alveoli (P = 0.002) using the test group. After 4 weeks, bone formation was noticeable in most sites, whereas after 12 weeks of healing, specimens of all groups exhibited nearly complete osseous organization of the former defected area. The mandibulary bone texture showed typical spongious bone structures. Histomorphometric analyses revealed after 4 and 12 weeks significant higher levels of bone marrow in test and negative control than in positive control. Quantification of bone tissue and osteoid does not show any significant difference. The present study confirms reduced bone resorption following socket augmentation with an absorbable collagen membrane with collagen cone while the resulting bone structure is similar to natural bone regeneration. Pure collagen can be used for bone augmentation, and shows over other xenogenic materials, a clear advantage with respect to the bone density and structure.

1. Introduction

Fresh extraction sockets of the alveolar ridge represent a special challenge due to the fact that the normal healing process of an extraction socket is one of regressive remodeling. After tooth extraction alveolar bone resorption is accelerated most significantly over the first several months [1,2]. Today, teeth replacement with implant-supported prostheses is a predictable option of therapy. The buccal wall is particularly important for implant placement and its loss can lead to un-esthetic gingival discoloration, peri-implantitis, thread exposure, and implant failure [3,4]. Thus, there is a high interest in minimizing tissue resorption after tooth extraction and maintaining the contour of the alveolar crest. Bone resorption generally and especially at the buccal wall has been reduced but not prevented when using bone augmentation materials [5]. Using post-extraction ridge preservation procedure, 85% of the initial ridge dimensions could be preserved [6].

Autogenous bone still is the gold standard for the reconstruction of bone defects and augmentations, due to its osteoconductive and osteoinductive properties. However, the amount of autogenous graft that can be harvested is limited. Therefore, various bone substitution materials have been used for studying ossification and bone formation in order to enhance alveolar ridge dimensions after tooth extraction.

Different types of biomaterials, such as minerals and non-mineral based materials as well as natural and artificial polymers have been introduced. Allogenic, xenogenic, or alloplastic bone grafts are mostly static, inert materials and often found un-resorbed in graft sites. For a long time, the goal of many studies on the bone healing was completely

* Corresponding author at: Fetscherstr. 74, Haus 28, D-01307 Dresden, Germany.
E-mail addresses: christiane.kunert-keil@uniklinikum-dresden.de (C. Kunert-Keil), tomasz.gredes@uniklinikum-dresden.de (T. Gredes), friedhelmheinemann@web.de (F. Heinemann), marzena.dominiak@umed.wroc.pl (M. Dominiak), ute.botzenhart@uniklinikum-dresden.de (U. Botzenhart), tomasz.gedrange@uniklinikum-dresden.de (T. Gedrange).

[1] Abbreviations: CC = collagen cone; DBBM = demineralized bovine bone mineral; NC = negative control; PC = positive control.

http://dx.doi.org/10.1016/j.msec.2014.10.033
0928-4931/

resorbable biomaterials. Though, it has been shown that a high rate of resorption could have an effect on new bone formation as the bone substitute was degrading faster than a new bone tissue was built up [7,8]. Furthermore, residual graft particles that do not resorb can interfere with stress- and strain-induced bone remodeling. For that, a number of natural and synthetic biodegradable polymers are in use as tissue scaffolds. Natural polymers used in bone tissue engineering include collagen, fibrin, alginate, silk, hyaluronic acid, and chitosan [9].

Collagen is physiologically ubiquitous, and the most abundant extracellular matrix protein and component of connective tissue in the human body. In the form of elongated fibers, collagen is found in tendon, ligament and skin, as well as in cartilage and bone. This natural polymer is commonly used in medicine, dentistry, pharmacology, cosmetology and tissue engineering applications because of its excellent biocompatibility, low antigenicity, high biodegradability, and good hemostatic as well as cell-binding properties [10,11]. It is well known that collagen undergoes rapid degradation upon implantation within 4–5 weeks [12]. Collagen can be used in various forms, e.g. gels, sponges, membranes, scaffolds or powder [10]. The diversity of forms allows collagen to be efficient in various fields, including wound healing, and soft and bone tissue augmentation. Recently, mesenchymal stem cell osteogenic differentiation as well as alveolar ridge augmentation was demonstrated using collagen scaffolds [12,13].

Histological outcome of augmented areas has been proven to be very unsteady and has to be classified into stages of bone regeneration even in exact time controls [14–16]. The reasons for that could be age, genetics, and metabolism, and have to be identified in the future to ease the choice of augmentation procedure and time decision for the second surgery of each patient.

The aim of the present study was to identify properties of collagen for augmentation techniques and compare to a proven xenogenic material and natural bone regeneration. For that, osteogenic potential of a collagen cone covered with a collagen membrane in a single product was examined in fresh extraction sockets of a well-documented animal model. The osteogenic potential of the collagen cone was compared with those of un-augmented sockets and deproteinized bovine bone mineral (DBBM) treated extraction sockets covered with a collagen membrane. The histological analysis focused on alveolar bone resorption as well as on amount and quality of new bone formation around the different grafting materials.

2. Material and methods

2.1. Bone substitution materials

2.1.1. PARASORB Sombrero® (Resorba Wundversorgung GmbH, Nürnberg, Germany)

PARASORB Sombrero® is a combination of an absorbable collagen membrane and an absorbable collagen cone in a single product. Both components – membrane and cone – are firmly connected together for easy and reliable handling. They consist of an equine type 1 collagen (31.2 mg) without chemical additives or cross-linking agents, manufactured according to a very special procedure (complete reconstitution of collagen). The dense nature of the membrane component prevents ingrowth of connective tissue and thus guarantees a reliable barrier function, as well as closure sealed against saliva. The special surface microstructure allows growth coverage with bone-forming cells, as well as rapid epithelization above the membrane (Resorba Medical GmbH 2014).

2.1.2. Bio-Oss® (Geistlich Pharma AG, Wolhusen, Switzerland)

Geistlich Bio-Oss® is a natural bone mineral originated from cattle. The granules of spongious bone are produced in a multi-stage purification process. This material is chemically and structurally (macro- and microporous) comparable to mineralized human bone (Geistlich Pharma AG 2011).

2.1.3. Bio-Gide® (Geistlich Pharma AG, Wolhusen, Switzerland)

Geistlich Bio-Gide® is a resorbable collagen membrane with a bilayer structure. The membrane consists of natural collagen obtained from pigs without further cross-linking or chemical additives. The porous surface of the membrane allows for the ingrowth of bone-forming cells. The dense surface prevents the ingrowth of fibrous tissue into the bone defect (Geistlich Pharma AG 2011).

2.2. Animal model

The protocol of the study was approved by the Commission for Animal Studies of Western Pomerania, Germany (LALLF M-V/TSD/7221.3-1.1-012/11). The study was performed on 20 15 month-old domestic pigs (female, about 160 kg). The pigs were randomly distributed into 6 groups according to the different healing periods and inserted materials (Table 1).

2.3. Anesthesia

The extractions, implantations, and euthanasia were performed under general anesthesia under the surveillance of a veterinarian. Anesthesia was induced by intravenous injection of 2 mg/kg body weight azaperon (Stresnil®, Janssen-Cilag, Germany) and 15 mg/kg ketamine (Ursotamin, Serumwerk Bernburg, Bernburg, Germany). To reduce salivation, 0.02 mg/kg atropine (B. Braun Melsungen AG, Melsungen, Germany) was administered. For infection prophylaxis, 3 ml/kg of Veracin®-compositum (Albrecht GmbH, Aulendorf, Germany) were injected intramuscularly. The analgesia and antiinflammation were performed by administration of Flunidol RP (0.08 mg/kg i.m., CP-Pharma, Burgdorf, Germany) intramuscularly.

2.4. Surgical interventions

In a split-mouth design, both mandibles of each pig were treated exactly the same way. For that both permanental P3 premolars have been extracted in all animals. Care was taken to avoid the fracture of bone walls (Fig. 1A). After removal of the premolars extraction alveoli were treated with a collagen cone (CC; PARASORB Sombrero®, Resorba Wundversorgung GmbH, Nürnberg, Germany; Fig. 1B) or demineralized bovine bone mineral (DBBM) concomitant with the placement of a collagen membrane as positive control (PC; Bio-Oss® + Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland; Fig. 1C) as described in Table 1. Untreated alveoli served as a negative control (NC). After insertion of

Table 1
Study protocol for the insertion of bone grafting materials (split mouth).

Group number	Mandibula right side	Mandibula left side	Treatment time	Amount of animals
1	Untreated (negative control; NC)	PARASORB Sombrero® (CC)	4 weeks	3
			12 weeks	3
2	PARASORB Sombrero® (CC)	Bio-Oss® + Bio-Gide® (positive control; PC)	4 weeks	4
			12 weeks	4
3	Bio-Oss® + Bio-Gide® (positive control; PC)	Untreated (negative control; NC)	4 weeks	3
			12 weeks	3

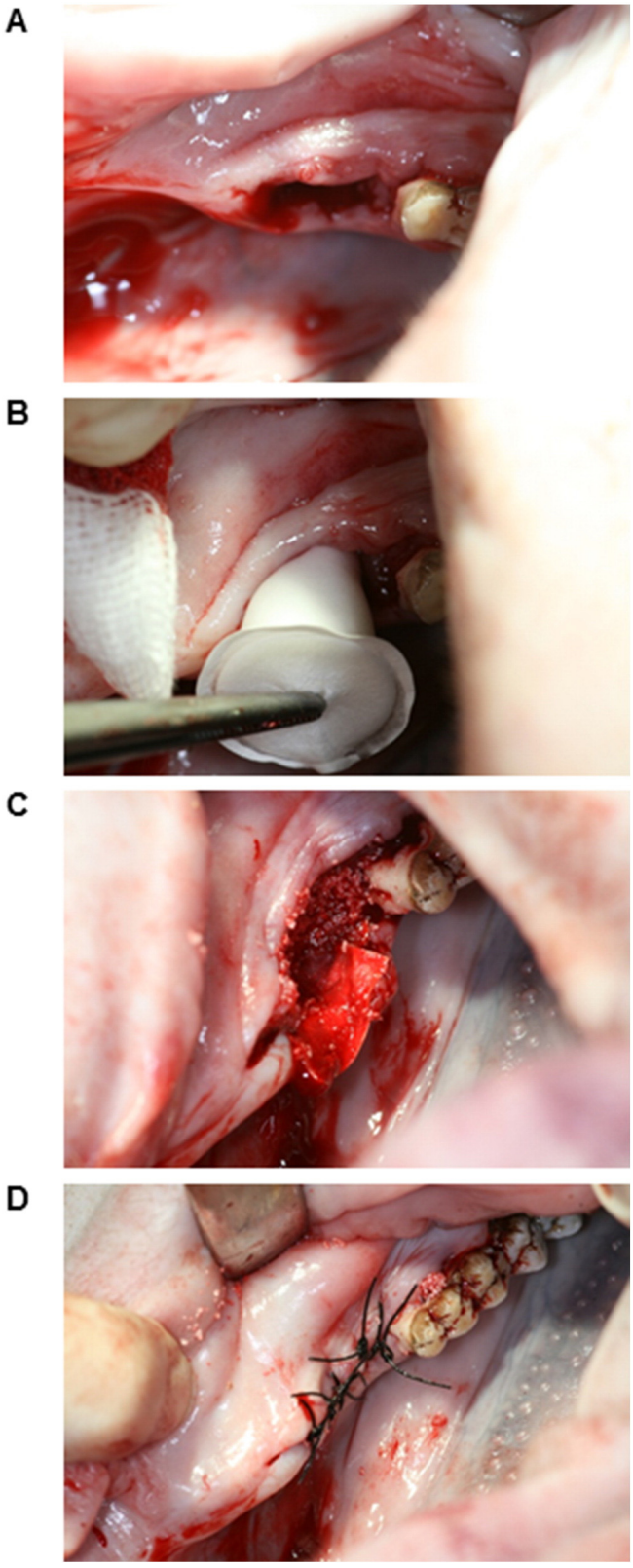

Fig. 1. Surgical procedure. (A) State after tooth extraction of P2. (B) Insertion of the collagen cone. (C) Cutting and insertion of the positive control. (D) Wound closure.

the different bone substitution materials the flap was repositioned using resorbable sutures (PGA Resorba 4x0VR; Fig. 1D). During the healing period the animals were kept without diet confinement or any additional oral hygiene procedures.

The animals were euthanized 4 or 12 weeks after insertion of the bone grafting material using an embutramid/mebezonium iodide/tetracain cocktail (T61VC, Intervet Deutschland GmbH, Unterschleissheim, Germany) and block biopsies of the support zones (premolars P2–P4) were collected.

2.5. X-ray analysis

Lateral radiographs were taken from all block biopsies of the support zone using a digital XIOS^{PLUS} (Sirona, Bensheim, Germany) at 70 kV for 0.06 ms with a distance of 5.5 cm. For semi-quantitative analysis of bone atrophy after tooth extraction and treatment with different bone substitution materials the initial height was calculated on the basis of bone height of the flanking teeth (Fig. 2A). The reduction of the bone amount in the alveolus was then measured at different positions with respect to the initial height of the bone and then averaged for each object.

2.6. Histology and histormorphometry

As described previously, each block biopsy of the support zone was placed in 4% buffered formalin, dehydrated in a graded series of alcohol, and embedded in methylmethacrylate (Technovit 9100 neu, Kulzer, Germany) for histological examination [17,18]. For staining the bone specimens were cut using a diamond saw and successively ground to a thickness of approximately 100 μm with a grinding system (Exakt Apparatebau, Norderstedt, Germany). For differentiation between osteoid, bone marrow and bone tissue the biologics were stained with Masson-Goldner trichrome. Masson's trichrome is a three-color staining protocol used in histology. Histological structures were stained as followed: keratin and muscle fibers in red, collagen and bone in blue or green, cytoplasm in orange, and cell nuclei in dark brown or black. A blind test was conducted at the same time using identical staff, equipment, and chemicals. The data acquisition for the individual bone specimens was performed using the light microscope BX61 with the integrated camera Color View II (Soft Imaging System, Olympus Optical GmbH, Hamburg, Germany). For this purpose, the multiple image alignment method and the motorized measuring stage were used. In a fourfold magnification 3×3 or 4×3 pictures were combined to form a global map of the different bone specimens. The program cellF Ink (analySIS Image Processing Olympus, Münster) was used to subclassify the original recordings in three phases (phase 1: bone tissue, phase 2: osteoid, and phase 3: bone marrow). The percentage of bone tissue (blue or green), bone marrow (white or gray) and osteoid (orange) was analyzed and calculated using the phase color coding system from the computer program. For this purpose, first the evaluated area was determined (frame set). Then the thresholds of the colors for each phase have been defined (see new Fig. 2). This is followed by automatic calculation of the percentage of each phase in the evaluation frame.

2.7. Statistical analysis

All obtained values were compared using Student's unpaired *t*-test. If the normality test failed, the Mann–Whitney U rank sum test was used. Statistical analysis was performed using the SigmaStat Software (Version 3.5, Systat Software, Inc. 1735, Technology Drive, San Jose, CA 95110, USA). Data are given as means ± standard deviation. $P < 0.05$ was considered statistically significant.

3. Results

All animals have recovered without problems from the surgery. No complications such as allergic reactions, abscesses or infections were observed throughout the study periods.

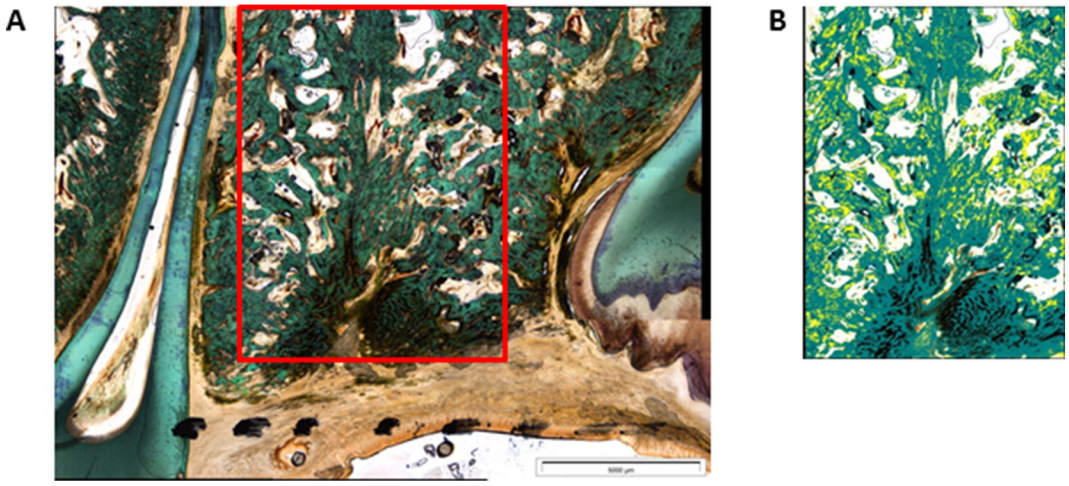

Fig. 2. Histomorphometric analysis using the phase color coding system from the computer program cellF Ink. (A) Evaluation of the analyzed area (red box); (B) color coding based on the thresholds of the colors for each phase, green = trabecular bone, yellow = osteoid, white = bone marrow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1. Quantification of bone atrophy

Fig. 2B illustrates bone atrophy 4 and 12 weeks after insertion of the collagen cone (CC) or demineralized bovine bone mineral (DBBM). After 4 weeks reduction of the bone height was found between 2.5 and 3.7 mm, respectively. At this time point, no significant differences of bone atrophy in the various treatment groups could be detected (Fig. 2B). In contrast, 12 weeks after material insertion significant differences of bone atrophy were detected. The bone atrophy was reduced by approximately 32% after insertion of PC (mean ± S.D.; NC versus PC: 3.67 ± 0.58 versus 2.5 ± 0.58; $P = 0.046$). Using CC bone atrophy reached 37.6% of those from untreated alveoli ($P = 0.002$). The comparison of bone height between CC and PC showed a significant less strong reduction of bone atrophy after treatment with CC (mean ± S.D.; CC versus PC: 1.38 ± 0.48 versus 2.5 ± 0.58; $P = 0.024$; Fig. 2B).

3.2. Histology and histomorphometry

The pattern of wound healing in different groups at 4 and 12 weeks is illustrated in Figs. 3 and 4.

After 4 weeks, bone formation was noticeable in most sites. The mandibulary bone texture showed typical spongious bone structures. Osteoid and collagen fiber formation starting from the host bone could be detected for all treatment groups (Fig. 3A–C). For the evaluation of bone regeneration after application of the materials, the proportion of bone trabeculae and osteoid was determined. In order to make a statement about the bone density, the amount of bone marrow in the spongy bone was analyzed.

Histomorphometric analysis revealed significant lower levels of bone marrow in DBBM treated animals compared to untreated as well as CC treated pigs (mean ± S.D.; PC versus NC: 14.8 ± 9.8 versus 40.9 ± 15.2; $P = 0.016$; PC versus CC: 14.8 ± 9.8 versus 26.5 ± 1.5; $P = 0.05$; Fig. 3D). Quantification of bone tissue and osteoid does not show any significant difference.

After 12 weeks of healing, specimens of all groups exhibited nearly complete osseous organization of the former defect area (Fig. 4A–C). In the histomorphometric analysis, similar results as shown after 4 weeks were found after 12 weeks. The amount of bone marrow was significantly reduced after treatment with PC compared to CC ($P = 0.042$) and NC ($P = 0.018$), whereas the amount of bone tissue and osteoid was unchanged (Fig. 4D).

4. Discussion

Adequate volumes of alveolar bone are necessary to provide favorable esthetic and successful long-term outcomes for dental implants. Socket preservation is necessary immediately after tooth extraction as grafting of extraction sockets does not prevent but reduces alveolar bone atrophy [1,2]. As so the examination of properties, resorption course and resulting bone consistence of augmentation materials is most important as there are different needs in different indications. Buccal augmentation for example should provide long-term stability no matter if residual augmentation material will be in place. Socket preservation on the other hand should stabilize only temporarily until the implant is placed but should provide natural bone as reaction of natural cells for osseointegration and later bone remodeling around the loaded implant is needed [19,20]. A disadvantage of many augmentation materials is that they are not very biodegradable and histological outcome of augmented areas has to be classified into stages of bone regeneration even in exact time controls [14,15,21]. Due to this fact, a number of natural and synthetic biodegradable polymers were established. Ideally, degradable biomaterials to be used as scaffolds for bone tissue repair should be able to support the growth of new blood vessels. It is well known that vascular endothelial cells influence bone cell recruitment, formation and activity, these endothelial cells are critically involved in bone development and remodeling [16]. Collagen materials have shown proangiogenic qualities [22] and acceleration of ingrowth, proliferation and maturation of endothelial cells [23].

The present study identified properties of collagen for augmentation techniques and compared it to a proven xenogen material and natural bone regeneration. One purpose of the insertion of a graft material in a hard-tissue defect is to offer stability for the coagulum and hence avoid volume reduction and surface invaginations. For that, the first aim of this study was to evaluate the decrease in bone atrophy when using a collagen cone in fresh extraction sockets and compare these findings with results from extraction sockets treated with DBBM and a collagen membrane and untreated alveoli. In this animal study, the highest decrease in bone atrophy was found 12 weeks after insertion of the collagen cone followed by the positive control consisting of DBBM and a collagen membrane. The bone loss was only between 1.4 and 2.5 mm after grafting procedure and 3.7 mm after spontaneous bone regeneration. This is in agreement with earlier studies. Changes in horizontal dimension showed an average resorption of 3.6 ± 0.7 mm in extraction sockets after spontaneous healing in humans, where horizontal bone resorption in test sites grafted with corticocancellous porcine bone and a collagen membrane

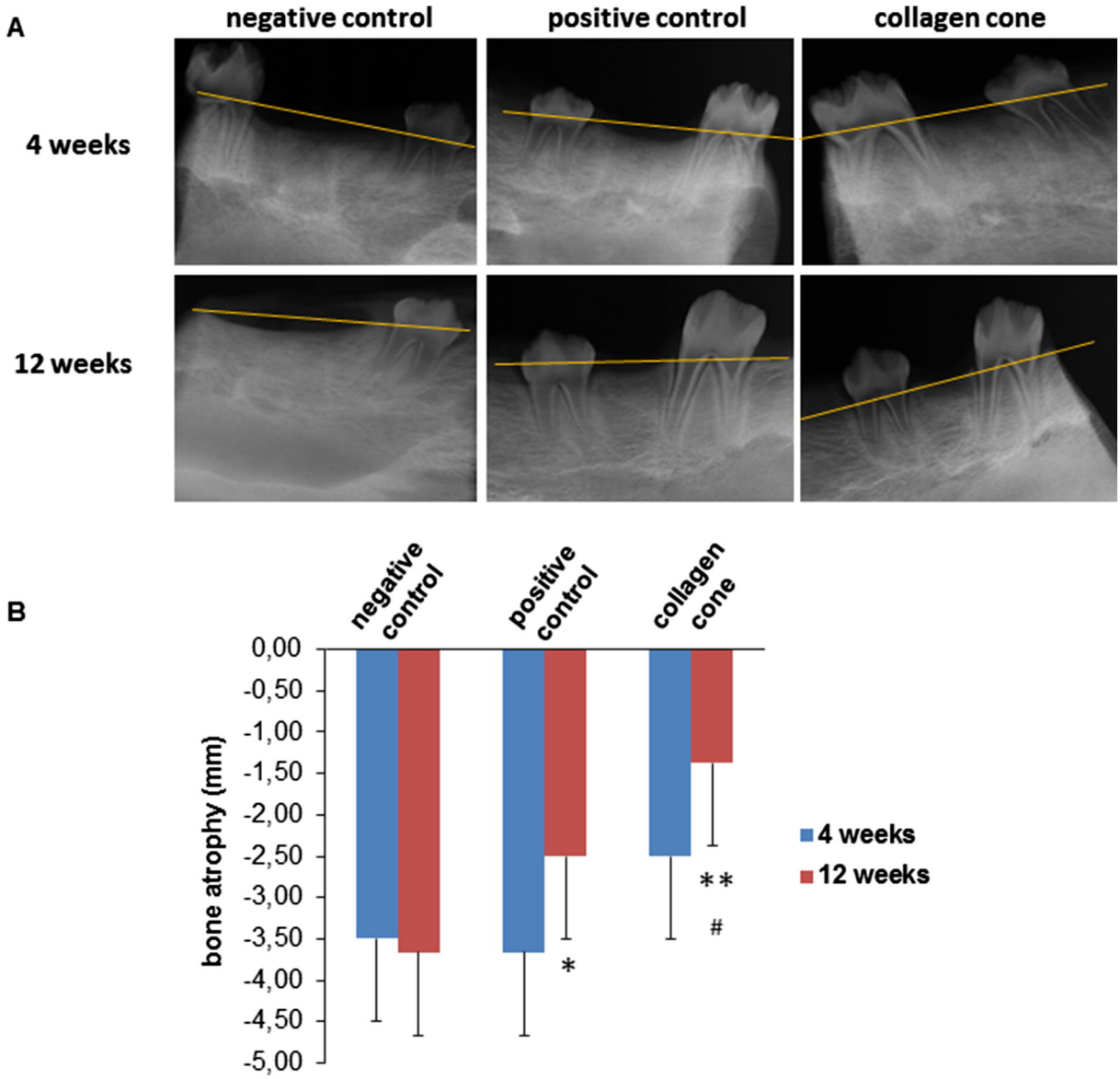

Fig. 3. X-ray analysis of bone resorption 4 and 12 weeks after ridge augmentation. (A) Representative X-ray pictures of each group after 4 and 12 weeks. (B) Quantification of the bone atrophy. mean ± S.D.; Student's *t*-test; *P < 0.05 and **P < 0.01 = negative control versus grafted sockets; #P < 0.05 = collagen cone group versus positive control. The yellow line demonstrates the bone height before tooth extraction and was determined from the adjacent teeth. The black area below the yellow line represents the decline of the alveolar bone (bone atrophy) after tooth extraction and bone healing. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was 1.6 ± 0.6 mm [24]. In a further randomized controlled clinical trial it could be demonstrated that deproteinized bovine bone significantly reduced alveolar ridge resorption after teeth extraction in comparison with untreated sockets [25]. Sockets treated with BioOss demonstrated a loss of less than 20% of the buccal plate in 79% of all test sites, whereas in untreated sockets 71% of all control sites showed a loss of more than 20% of the buccal plate [25]. Using collagen sponges in dogs it was found that the invagination of the surface of the crestal bone was 0.6 mm, whereas the corresponding invagination of non-augmented defects was 0.8 mm. This corresponds to a bone atrophy of 25% [26]. Furthermore, buccal alveolar bone resorption between 1.8 and 2.1 mm in both, DBBM treated and untreated extraction sockets, was shown in dogs 4 months after tooth extraction [27].

Another reason for the usage of a bone grafting material is to provide a scaffold for new bone formation. For this reason, the second aim of this in vivo study was to analyze the bone quality after bone regeneration induced by the collagen cone. For that, comparisons were made regarding the tissue composition after 4 and 12 weeks of healing at grafted extraction sites and non-grafted sockets. It was found that after 4 and 12 weeks of wound healing, the bone formation was noticeable in collagen cone grafted and non-grafted sockets. The regenerated mandibulary bone showed typical spongious structures with no significant difference in the amounts of bone tissue, osteoid and bone marrow between collagen cone grafted alveoli compared to untreated extraction sockets. Thus, the used collagen cone leads to the formation of a natural bone. However, DBBM leads to a compression of the bone, seen in a significant decrease of the amount of bone marrow. Carmagnola and coworkers could also demonstrate that collagen membrane treated and untreated control sockets in humans showed higher amounts of lamellar and woven bone as well as bone marrow after 12 weeks of healing compared to DBBM treated sockets [28]. Furthermore, it was shown that human extraction sockets filled with DBBM and covered with a collagen membrane were comprised of connective tissue and small amounts of newly formed bone surrounding the graft particles [28]. An increase in the amount of new mineralized bone was also found in canine bone defects treated with a collagen sponge as well as Bio-Oss Collagen compared to untreated defects, respectively [26].

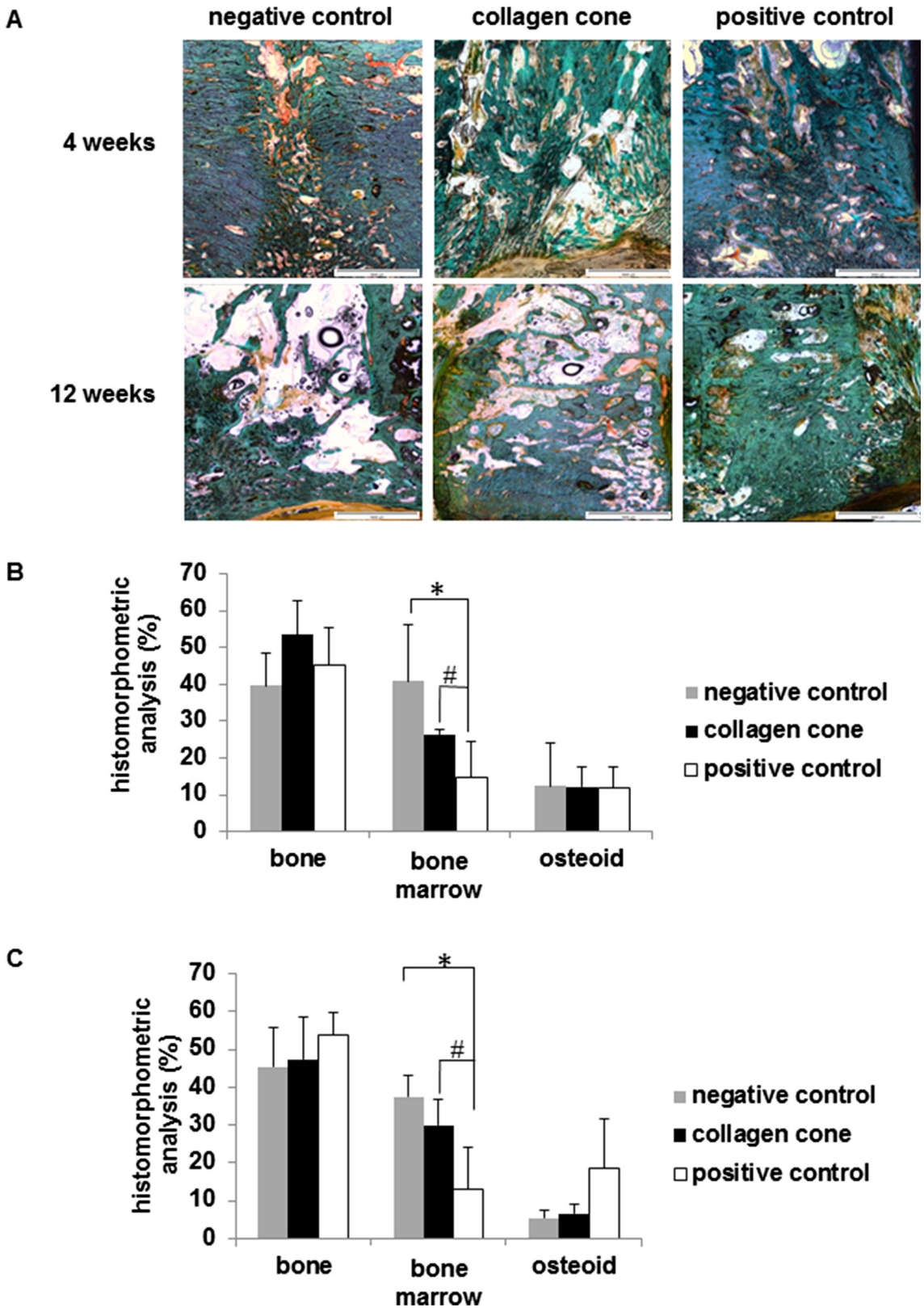

Fig. 4. Masson Goldner trichrome staining of bone specimens after 4 and 12 weeks of healing. (A) Sample images, magnification ×20; (B) histomorphometric analysis after 4 weeks; mean ± S.D.; Student's *t*-test; *P < 0.05 = negative control versus positive control; #P < 0.05 = collagen cone group versus positive control; (C) histomorphometric analysis after 12 weeks; mean ± S.D.; Student's *t*-test; *P < 0.05 = negative control versus positive control; #P < 0.05 = collagen cone group versus positive control.

5. Conclusion

From this animal study, it can be concluded that the collagen membrane cone is suited as a material with good quality for socket preservation. Compared to other xenogenic materials this collagen-based product has several advantages: it can be easily handled, it is completely resorbable, bone resorption after tooth removal is significantly reduced and the bone structure is similar to the natural

structure without bone compression, as seen in DBBM treated extraction sockets.

Acknowledgments

We would like to thank Diana Jünger and Michaela Krause for the excellent technical assistance and Resorba Wundversorgung GmbH (Nürnberg, Germany) for providing the research materials.

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