J Periodontal Implant Sci. 2020 Dec;50(6):406-417
https://doi.org/10.5051/jpis.2000400020
pISSN 2093-2278·eISSN 2093-2286

JPIS Journal of Periodontal & Implant Science

Research Article
Implant Science

Check for updates

Open healing of contained and non-contained extraction sockets covered with a ribose cross-linked collagen membrane: a pilot study

Anton Friedmann [1,*], **Viktorija Meskeleviciene** [1], **Mehmet Selim Yildiz** [1,2], **Werner Götz** [3], **Jung-Chul Park** [4], **Kai R. Fischer** [1,5]

[1]Department of Periodontology, Witten/Herdecke University Faculty of Health, Witten, Germany
[2]Department of Periodontology, Altınbaş University Faculty of Dentistry, Istanbul, Turkey
[3]Department of Orthodontics, Oral Biology Laboratory, University of Bonn, Bonn, Germany
[4]Department of Periodontology, Dankook University College of Dentistry, Seoul, Korea
[5]Clinic of Conservative and Preventive Dentistry, Division of Periodontology and Peri-implant Diseases, University of Zurich, Zürich, Switzerland

Received: Jan 14, 2020
Revised: Jul 21, 2020
Accepted: Aug 27, 2020

***Correspondence:**
Anton Friedmann
Department of Periodontology, Witten/Herdecke University Faculty of Health, Alfred-Herrhausen-Straße 44, 58455 Witten, Germany.
E-mail: anton.friedmann@uni-wh.de
Tel: +49-2302/926-655
Fax: +49-2302/926-651

ORCID iDs
Anton Friedmann
https://orcid.org/0000-0002-8708-829X
Viktorija Meskeleviciene
https://orcid.org/0000-0002-4901-7730
Mehmet Selim Yildiz
https://orcid.org/0000-0001-6949-158X
Werner Götz
https://orcid.org/0000-0001-9975-5793
Jung-Chul Park
https://orcid.org/0000-0002-2041-8047
Kai R. Fischer
https://orcid.org/0000-0001-8632-3879

Author Contributions
Conceptualization: Anton Friedmann, Kai R. Fischer; Formal analysis: Viktorija Meskeleviciene, Mehmet Selim Yildiz; Werner Götz; Investigation: Anton Friedmann, Viktorija Meskeleviciene, Kai R. Fischer; Methodology: Anton Friedmann, Kai R. Fischer; Project

ABSTRACT

Purpose: This study investigated whether the placement of ribose cross-linked collagen (RCLC) membranes without primary soft tissue closure predictably resulted in sufficient alveolar ridge preservation in contained and non-contained extraction sockets.
Methods: Membranes were positioned across extraction sockets, undermining full-thickness flaps, and the gingival margins were fixed by double-interrupted sutures without crossed horizontal mattress sutures for 1 week. In non-contained sockets, a bone substitute was used to support the membrane within the bony envelope. Radiographs and clinical images obtained 4 months later were analyzed by ImageJ software using non-parametric tests.
Results: In 18 patients, 20 extraction sockets healed uneventfully and all sites received standard-diameter implants (4.1, 4.8, or 5.0 mm) without additional bone augmentation. Soft tissues and the muco-gingival border were well maintained. A retrospective analysis of X-rays and clinical photographs showed non-significant shrinkage in the vertical and horizontal dimensions (P=0.575 and P=0.444, respectively). The new bone contained vital bone cells embedded in mineralized tissues.
Conclusions: Within the limitations of this pilot study, open healing of RCLC membranes may result in sufficient bone volume for implant placement without additional bone augmentation in contained and non-contained extraction sockets.

Keywords: Bone regeneration; Epithelial tissue; Guided tissue regeneration; Tooth extraction; Wound healing

INTRODUCTION

Tooth extraction is regularly followed by significant changes in soft and hard tissue volume [1-4]. These contour changes might impair implant placement and necessitate bone augmentation procedures [5]. Therefore, various techniques for ridge preservation have been proposed [6-9]. Most studies on this topic have investigated socket grafting using bone substitutes (with or without placement of a collagen membrane and with or without

Administration: Anton Friedmann; Writing - original draft: Anton Friedmann; Writing - review & editing: Werner Götz, Jung-Chul Park, Kai R. Fischer.

Conflict of Interest
No potential conflict of interests relevant to this article was reported.

primary closure) or soft tissue grafts [6,10-13]. Recent studies have also investigated the influence of open healing of single- versus double-layer membrane application, different graft compression forces, and different suturing techniques to overcome the problem of incomplete ridge preservation [14-16]. Collagen membranes treated with the sugar ribose to achieve cross-linking were shown to undergo slow degradation when exposed to collagenases *in vitro* and *in vivo* [17,18]. Previous observations have documented secondary epithelization of exposed membrane surfaces if soft tissue dehiscence occurs during the healing phase, without loss of barrier function [19]. These observations, together with the capacity of ribose cross-linked collagen (RCLC) to withstand proteolytic activity, support the non-submerged application of this material to cover extraction sites. The hypothesis of this study was that placement of an RCLC membrane between the bony crest of the extraction socket and the soft tissue would result in predictable ridge preservation for later implant placement without additional bone augmentation. Core biopsies and samples of tissue attached to membrane residues were evaluated histologically. The time until complete soft tissue closure and alterations at the level of muco-gingival border were monitored.

MATERIALS AND METHODS

Study population

The study protocol was approved by the Ethics Committee of the Witten/Herdecke University (188/2015 from 12/17/2015) and patient consent was obtained. A total of 8 female and 10 male patients, with a mean age of 59.6 ± 8.2 years (range, 42–76 years) were recruited from the patient pool of the dental school, and the sample predominantly contained participants from the maintenance program of the Department of Periodontology. All participants were in a good general condition; patients with diabetes mellitus or a history of heart disease and smokers consuming >10 cigarettes per day were excluded. Contained and non-contained defects were included regardless of their position in the anterior or posterior maxilla or mandible (Table 1). Patients were consecutively enrolled and group allocation was determined

Table 1. Patient and socket characteristics for 18 enrolled participants and 20 sites

Patient	Age	Sex	Tooth number (ADA)	Reason for extraction	Bone defect (Y/N)	Contained (1) or non-contained (2)
1	58	M	4	Caries	N	1
			5	Caries	N	1
2	52	F	9	Internal resorption	N	1
3	61	M	13	Periodontitis	N	1
4	48	F	5	Caries	N	1
5	55	F	19	Endodontics	N	1
6	60	M	4	Endodontics	N	1
7	68	M	13	Periodontitis	N	1
			14	Periodontitis	N	1
8	42	M	13	Endodontics	N	1
9	55	M	14	Endodontics	Y	2
10	56	M	5	Periodontitis	Y	2
11	59	F	5	Periodontitis	Y	2
12	65	M	3	Root fracture	Y	2
13	76	F	7	Endodontics	Y	2
14	64	F	14	Root fracture	Y	2
15	59	F	12	Periodontitis	Y	2
16	72	F	20	Periodontitis	Y	2
17	66	M	2	Endodontics	Y	2
18	57	M	12	Periodontitis	Y	2

ADA: American Dental Association.

based on a clinical examination of the buccal or lingual bone wall after tooth extraction. However, preoperative periodontal probing and intraoperative bone sounding served as indicators for the preliminary allocation.

Surgical interventions

Following tooth extraction and thorough degranulation of the sockets, a porcine RCLC (OSSIX®Plus, Datum Dental, Lod, Israel) was placed to cover at least 2 mm of the bony socket borders in all directions (Figure 1A-F). The membranes were hydrated by sterile saline solution before placement and pre-shaped to the extension required. To allow stable seating of the membranes, soft tissues were slightly elevated on the buccal and lingual aspect to interpose the membrane between the full-thickness flap and alveolar crest on both sides of the ridge. Sockets were considered to be non-contained in the presence of vertical bone resorption exceeding 30% of the buccal or lingual bone wall, as confirmed by a clinical examination after flap elevation. Coronal advancement of the flap was omitted in accordance with the protocol. Double-interrupted sutures without crossed horizontal mattress sutures were used to fix the gingival margins in their original position for 1 week (4-0 Cytoplast PTFE; Osteogenics, Lubbock, TX, USA). Defects missing buccal and/or lingual bony walls were considered to be non-contained and required additional support to prevent the membrane from collapsing (Figure 2A-E). In these sites, the ridge dimensions were re-contoured according to the level of the adjacent crestal margins by applying either a synthetic calcium sulfate (3D Bond™; Regedent, Zurich, Switzerland) or a bovine solvent-preserved (CopiOs™; Zimmer Biomet, Palm Beach Gardens, FL, USA) bone substitute. Patients were instructed to rinse with 0.2% chlorhexidine digluconate (CHX) 3 times a day during the first week and recommended to continue biofilm control using CHX gel locally until soft tissue closure. At weekly follow-up visits during the first 4 weeks of healing, the area was disinfected with cotton pellets

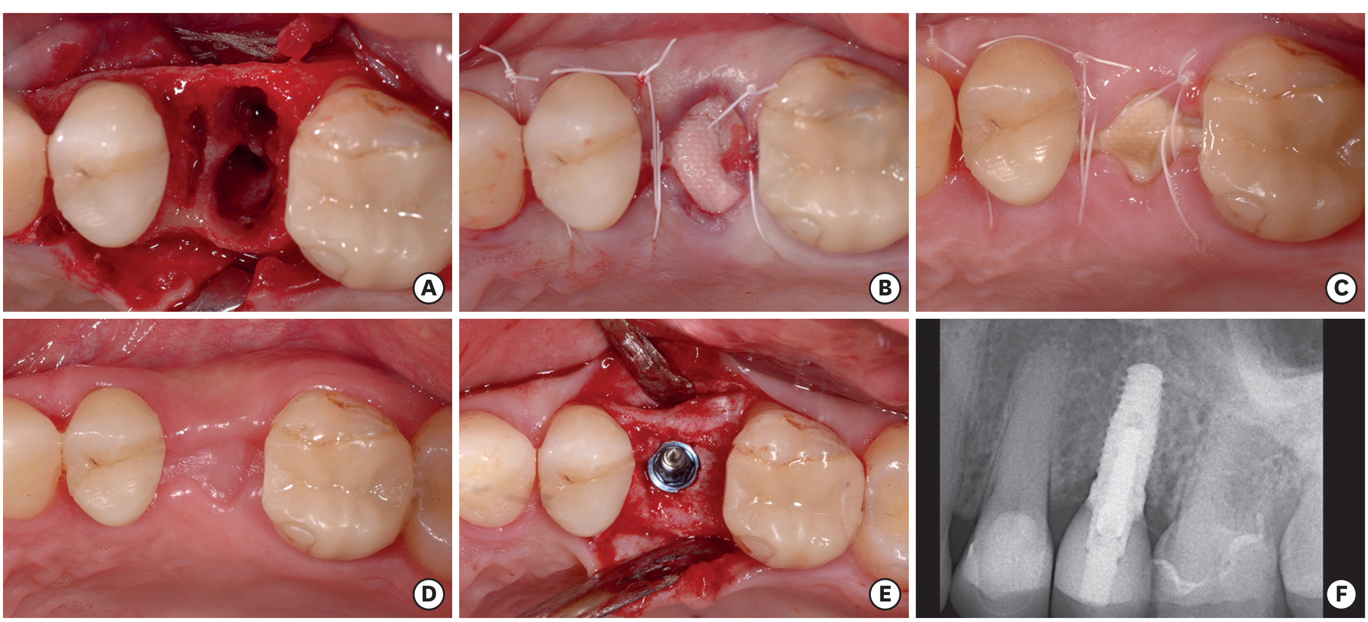

Figure 1. (A) Tooth #13 had to be extracted due to an endodontic complication and surgical crown lengthening was performed simultaneously at tooth #12; intact bony housing was found, and no bone filler was therefore used (contained sockets). (B) After placement of ribose cross-linked collagen (OSSIX®Plus; Datum Dental) to cover the extraction socket, flaps were fixed with double-interrupted sutures, but no primary closure was attempted. (C) Uneventful healing after 7 days: no swelling or signs of inflammation were observed in the surrounding tissues, and the membrane was clearly visible and intact. (D) After 3 weeks, secondary healing was completed over the previously exposed membrane. (E) Implant surgery 4 months post-extraction; sufficient regeneration had been achieved for implant placement (4×11.5 mm, T3; ZimmerBiomet, Warsaw, IN, USA). (F) Periapical radiograph 7 months after ridge preservation and 3 months after implant placement.

https://doi.org/10.5051/jpis.2000400020

JPIS Journal of Periodontal & Implant Science

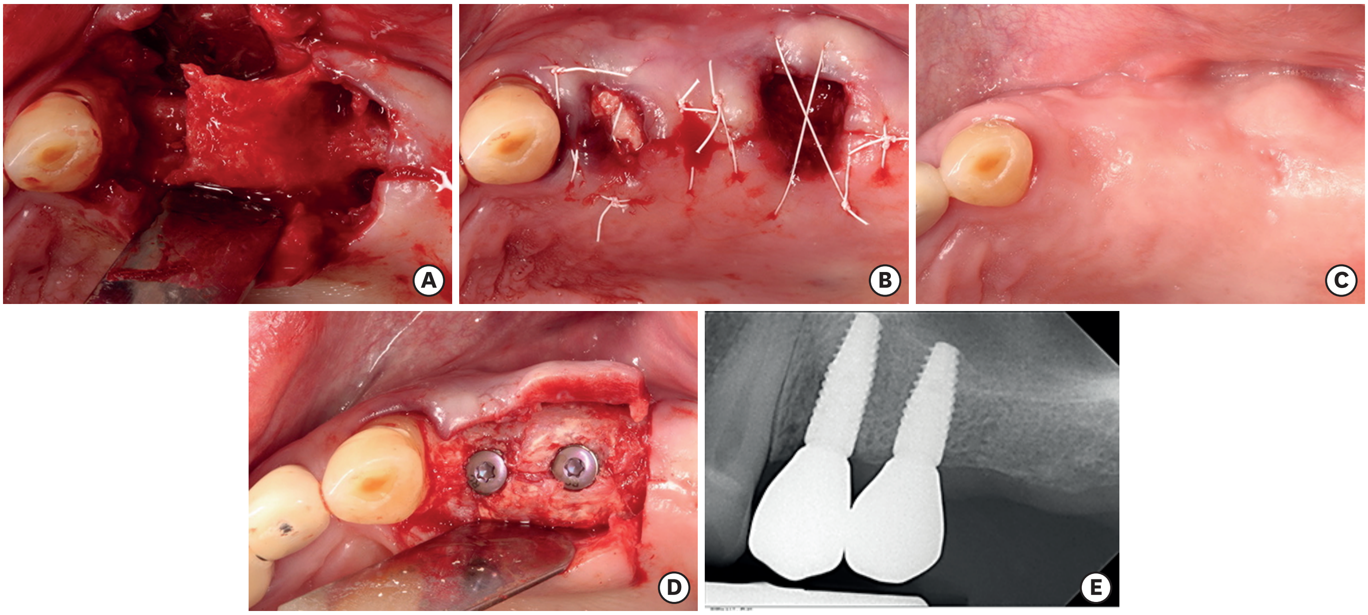

Figure 2. (A) Teeth #12 and #14 had to be removed because of periodontal disease; implant placement was scheduled for positions #12 and #13 (non-contained socket #12; loss of buccal and lingual walls). (B) After placement of a bovine xenograft (CopiOs; ZimmerBiomet) to recreate the ridge outline, a ribose cross-linked collagen membrane was placed and the flaps were fixed with crossed horizontal mattress and double-interrupted sutures; socket #14 was left to heal spontaneously. (C) Primary wound closure was noted 4 weeks after alveolar ridge preservation and after spontaneous healing. (D) Four months after ridge preservation, 2 implants were placed without additional augmentation (#12: 4.1×12 mm BLT; #13: 4.1×10 mm BLT; Straumann GmbH). (E) Intraoral X-ray after prosthetic restoration; both implants were well surrounded by bone, and there was no visible difference between the augmented and pristine area.

hydrated in 3% hydrogen peroxide. Administration of systemic antibiotics was considered on an individual basis. Specifically, patients with periapical lesions or who presented other acute inflammatory signs received 1,000 mg of amoxicillin 3 times daily for 6 days after tooth removal. Ibuprofen (600 mg) was prescribed for anti-inflammatory and analgesic purposes.

Implant placement was scheduled for 4 months after ridge preservation, an interval that was considered to be appropriate based on studies by Chappuis et al. [10]. If the shape of a trephine bur matched the design and diameter of the implant that was planned, a core biopsy was retrieved through the mucosal layer. In other cases, a biopsy was retrieved from the central part of the former socket by using a chisel on the crest of the ridge after reflecting a full-thickness flap before starting the osteotomy.

After tissue sampling, the implant osteotomy was continued according to the manufacturer's protocol (Bone Level Tapered & Tissue Level implants, Straumann GmbH, Basel, Switzerland; T3, Zimmer Biomet, Palm Beach Gardens, FL, USA) in a prosthetically driven position defined by the fissures of the neighboring teeth or the possibility of a screw-retained crown (posterior implant: central access hole; anterior teeth: access hole behind the incisal edge). After implant installation, the dimensions of the buccal/oral bone and the need for further bone augmentation were assessed.

Data collection

Clinical analysis

Clinical images were repeatedly obtained from the buccal and occlusal side in a 1:1 format to compare the soft tissue conditions and the dimensions of the alveolar crest at baseline and at the final exam.

Patients were monitored weekly until secondary epithelization of the socket orifice occurred. Healing of the underlying bony ridge was monitored by occlusal clinical photographs taken immediately before tooth extraction, after tooth extraction/flap elevation, and 4 months later during implant placement. ImageJ2 software was used for 2-dimensional area calculations in the clinical photographs [20]. The images were calibrated using constant landmarks, such as fillings, to match the photographs taken before and after the healing period (month 4). The cross-sectional area of the crestal bone was determined at the stage of flap elevation. The surgical site was photographed in the occlusal direction at an angle of 90° and bony socket borders were defined for bone area and bony socket extension measurements (Figure 3A and B). The areas of bone contact with the teeth adjacent to the extraction socket formed the mesial and distal borders, and the buccal and palatal alveolar bone formed the buccal and palatal borders.

Radiographic analysis

The vertical dimension of crestal bone was measured radiographically by calculating the crestal bone level change between baseline (tooth *in situ*) and after implant placement. Radiographs were standardized by matching the size of certain landmarks such as radiopaque fillings and teeth crown lengths on paired periapical X-rays by using the scaling tool in ImageJ2 software. The alveolar crest level was assessed for each tooth/implant pair by

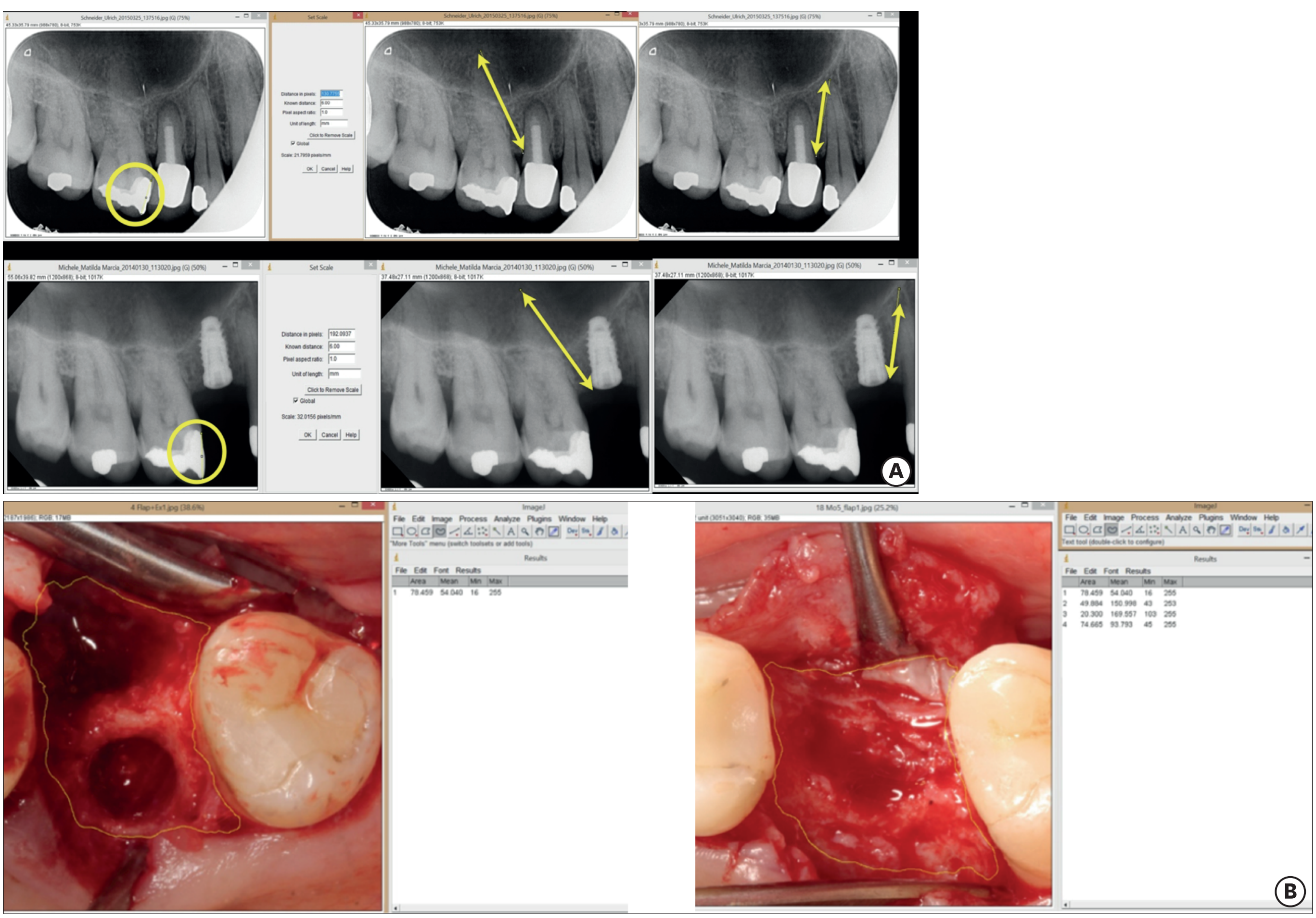

Figure 3. (A) Non-standardized periapical radiographs of tooth #4 obtained before treatment and after replacement by an implant. The landmarks used to align the 2 images and the reference points used to estimate the vertical dimension are highlighted yellow. (B) The clinical occlusal images taken after tooth extraction and at re-entry show the area for 2-dimensional calculations highlighted in yellow, for an extraction socket at tooth #13.

JPIS Journal of Periodontal & Implant Science

calculating the mesial and distal distance from the alveolar crest to the adjacent tooth apex (deepest point), which was assumed to represent a constant reference point. The mean values of the mesial and distal measurements were calculated for the analysis (Figure 3A).

Histological analysis

Tissue samples were fixed in 4% formalin, decalcified in ethylenediaminetetraacetic acid, and embedded for paraffin histology following standard protocols. After vertical serial sectioning, selected sections were deparaffinized, stained with hematoxylin and eosin and Masson-Goldner trichrome according to routine protocols, and evaluated using light microscopy.

Outcomes

The primary outcome was the possibility of placing a standard-diameter implant after placement of an RCLC membrane for ridge preservation and the need for additional augmentation during implant installation. The secondary outcomes were a retrospective analysis of 2-dimensional bone changes (clinical photographs, and peri-apical radiographs), and time to soft tissue closure over the exposed RCLC membrane.

The statistical analysis was carried out using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Intergroup comparisons were made using the Wilcoxon ranked test and intragroup analyses were carried out using the Mann-Whitney *U* Test.

RESULTS

Demographic information

The 18 patients had 20 extraction sockets, of which 10 were contained and 10 were non-contained. Table 1 presents the characteristics of the patients and sockets.

Clinical analysis

All extraction sites healed by secondary epithelization without complications. All 20 implants were inserted in accordance with the original protocol, and none of the sites required additional bone augmentation for compensation of the ridge contour (Figures 2-4). The height and width of the edentulous gaps allowed placement of implants with dimensions between 6 to 12 mm in length and a standard diameter using either tissue or bone level implants (4.1 & 4.8 Straumann BLT & TL; 4.1 & 5.0 Zimmer Biomet T3 tapered). All inserted implants showed successful osseointegration and were loaded according to the standard protocol after a healing period of 3 months. New bone formation within the boundaries of the former socket volume was completed in all 20 sites within 4 months. The bone quality at all sites varied between D2 and D3 as assessed clinically and radiographically and confirmed microscopically.

The muco-gingival junction was maintained in its original position in all 20 defect sites. Complete soft tissue closure and epithelialization was achieved in 75% of the cases after 3 weeks and in all cases after 4 weeks.

Dimensional analysis

The radiographically calculated mean change (ΔT0-T1) in crestal bone height between baseline (bone level at the tooth) and the bone level after implant placement revealed a reduction of 0.62±2.02 (standard deviation) mm. The median difference, which was 0.45 mm (range, −3.60 to 4.00 mm), was statistically non-significant at all sites (P=0.450). In

JPIS Journal of Periodontal & Implant Science

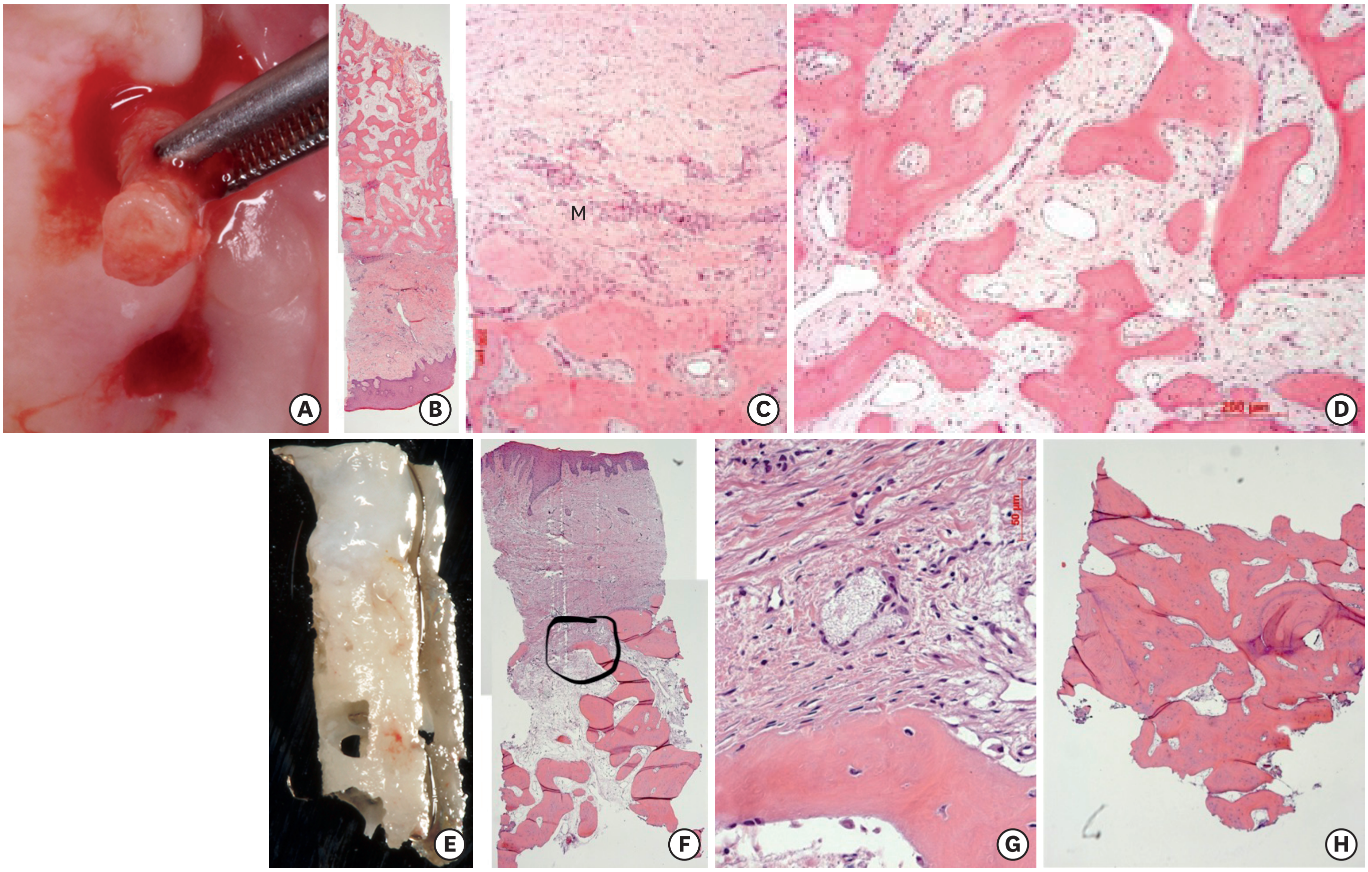

Figure 4. (A) A core biopsy sample was retrieved after using a hollow cylinder bur through the layer of newly formed keratinized tissue firmly attached to the newly formed mineralized tissue underneath. The position represents a formerly contained defect, which was treated by a RCLC membrane without using any substitute to graft the socket. (B) The reconstructed core biopsy reveals regular keratinized epithelium on top of the lamina propria, which was densely attached to the newly formed trabecular bone underneath (magnification ×5; H&E staining). (C) The transition zone from the lamina propria to the newly mineralized trabecular bone, M indicates an area of membrane residue embedded in newly formed tissue. The interface of the spongious trabeculae was seamed by vital (nuclei) osteoblasts, and the connective tissue within the bony structures appeared well vascularized (magnification ×10; H&E staining). (D) Magnification of the bone structures, displaying the woven character of the newly formed trabecular bone, a high level of angiogenesis, complete absence of any grafting material (not used), non-activity of osteoclasts or other multi-nuclear cells, and no infiltrate (magnification ×10; H&E staining). (E) The retrieved core biopsy after reconstructing a non-contained defect with CaS+HA graft (Augma Bond, Regedent, Germany) and an RCLC membrane following a 4-month period. (F) The reconstruction of the core, displaying a regular layer of keratinized epithelium with a well-organized lamina propria underneath. Properly attached mineralized trabecular bone structure underneath, representing newly formed bone. Focally, seams of vital osteoblasts are shown to cover the new bone surfaces. The encircled area (black line) represents the magnification shown by the next figure (magnification ×5; H&E staining). (G) The transition area from the lamina propria to the newly formed bone demonstrates an area of lamellar bone closely attached to the well-vascularized connective tissue. No signs of the residual portion of the RCLC membrane material can be detected (magnification ×10; H&E staining). (H) Magnification showing the center part of the biopsy and residual substitute particulates seamed by some attached multinuclear cells. Appositional bone formation with enclosed vital osteocytes dominates the area, but intertrabecular well-vascularized connective tissue is also shown (magnification ×10; H&E staining).
RCLC: ribose cross-linked collagen, H&E: hematoxylin and eosin.

the subgroups, vertical reduction was more prominent in the non- contained sockets, with a median of 1.11 mm (range, −2.59 to 4.00 mm) versus 0.35 mm (range, −3.60 to 2.75 mm) in the contained sockets. However, neither intragroup nor intergroup comparisons revealed statistically significant changes between T0 and T1 (P=0.575 vs. P=0.232; and P=0.450, respectively) (Table 2).

The reduction of the cross-sectional area of the bone ridge showed a non-significant change (P=0.444), and the area was reduced by 9.60 mm^2 (range, −23.53 to 29.84 mm^2). Subgroup analyses revealed a statistically significant change (P=0.007) in the contained sockets (reduction: 9.74 mm^2 [range, −23.53 to 28.70 mm^2]), but a non-significant difference in

https://doi.org/10.5051/jpis.2000400020

JPIS Journal of Periodontal & Implant Science

Table 2. Assessment medians (range) and changes (Δ) of vertical dimension and cross-sectional area of the bone ridge

Variables	All sockets (n=20)	Contained (n=10)	Non-contained (n=10)	P[a]
Vertical dimension (mm)				
T0 (Baseline)	12.93 (7.68, 21.65)	12.12 (7.68, 19.74)	16.01 (9.20, 21.65)	n.s. (0.226)
T1 (3.5 months)	12.36 (7.39, 23.34)	12.24 (7.39, 23.34)	13.80 (7.45, 22.90)	n.s. (0.450)
Δ T0–T1	0.45 (−3.60, 4.00)	0.35 (−3.60, 2.75)	1.11 (−2.59, 4.00)	n.s. (0.450)
P[b]	n.s. (0.575)	n.s. (0.575)	n.s. (0.232)	
Cross-sectional area of the bone ridge				
T0 (Baseline)	72.62 (22.48, 189.59)	72.62 (43.53, 189.59)	82.32 (22.48, 146.44)	n.s. (0.821)
T1 (3.5 months)	72.00 (20.85, 159.75)	65.56 (33.85, 159.75)	73.03 (20.85, 136.93)	n.s. (0.821)
Δ T0–T1	9.60 (−23.53, 29.84)	9.74 (−23.53, 28.70)	9.38 (−1.97, 29.84)	n.s. (0.650)
P[b]	n.s. (0.444)	(0.007)	n.s. (0.074)	
Δ T0–T1 (%)	100.14 (11.40, 189.28)	115.93 (43.78, 189.28)	46.97 (11.40, 143.33)	n.s. (0.049)

Data are presented as medians (range; min, max) and changes (Δ) (% based on mm^2; min, max; %).
n.s.: not significant.
[a]Mann-Whitney *U* test; [b]Wilcoxon signed rank test.

the non-contained sockets (reduction: 9.38 mm^2 [range, −1.97 to 29.84 mm^2]; $P=0.074$). However, the 2 changes did not show a statistically significant difference ($P=0.650$) (Table 2).

Histologic analysis

The 5 core biopsy samples and 5 crestal non-standardized biopsy samples were microscopically examined (Figure 4A-H). The biopsy samples showed normal keratinized gingival epithelium with fibrous vascularized lamina propria. Formation of cellular spongy woven bone within a loose connective tissue was obvious. The bone surfaces were locally covered by osteoblasts and osteoclasts. Remodeling into lamellar mature bone could be observed.

DISCUSSION

In this pilot study, alveolar ridge preservation was performed with an RCLC membrane with the goal of predictably achieving sufficient preservation of the bone ridge dimension for standard-diameter implant placement without further augmentation. Bone formation was assessed clinically, radiographically, and histologically. The open-healing approach was intended to emphasize the ease of handling and to facilitate the formation of new gingiva with keratinized epithelium by preserving the muco-gingival junction at its initial level.

Clinical and histological observations confirmed osteogenesis and new bone formation within the extraction sockets in the area preserved by the RCLC membranes. These findings were consistent in all 18 patients and sockets. The position of implants was in full agreement with the treatment plan made before extraction, regardless of the subgroup allocation of the sockets. The vertical change in the crestal height of the ridge was non-significant between baseline and control periapical radiographs after implant placement in the area of interest. Clinically, the reduction of the alveolar ridge slightly exceeded 0.5 mm (mean, 0.62 mm), which was almost irrelevant regarding implant placement and estimation of the appropriate implant size. The horizontal reduction of the alveolar ridge, with an overall value of <10% (mean, 9.75%), was non-restrictive to the choice of implant diameter or its prosthetically driven position. The contained sockets treated by the proposed method tended to show somewhat more horizontal bone loss than the non-contained sockets, which showed non-significant changes after the healing period. The classification of contained versus non-contained sockets was made clinically, as full-thickness flap elevation was required in all cases for membrane placement, thereby clarifying the extent of bone loss.

These observations are in agreement with the outcomes reported by Avila-Ortiz et al. [12]. In their study, dense polytetrafluoroethylene membranes in combination with freeze-dried bone allograft and demineralized freeze-dried bone allograft grafts were used in 3 experimental groups, while the controls were grafted with a collagen sponge without a membrane cover. The authors used the open-healing approach, removing the non-resorbable membranes at a separate visit after 4 weeks. After 16 weeks of healing and before implant installation, the amount of keratinized mucosa was unchanged. The volume change, as assessed by cone-beam computed tomography (CBCT) scans, was between 3% and 16% among the groups. Although similarly designed, in their pilot study, only well-maintained contained sockets were enrolled; teeth with a history of periodontitis and molar teeth were excluded according to the protocol [12].

In a recent randomized controlled trial conducted in 30 periodontitis patients, all 30 extraction sites revealed a vertical defect in the buccal bone [13]. The open-healing approach was used for secondary healing above a double layer of native collagen membrane stabilized by titanium pins to the recipient bone with a demineralized bovine bone matrix-collagen graft underneath. Using CBCT scans, the intervention group and non-treated controls were compared regarding changes in vertical and horizontal dimensions and the defect volume after a 12-month period. Although at the most crestal level, the horizontal shrinkage was significantly less pronounced in the intervention group than in controls (−2.60±1.24 mm vs. −4.92±2.45 mm; P=0.0001), the reduction in the intervention group from baseline to 12 months was statistically significant (P=0.0001) and clinically relevant [13].

The results reported by this study contradict the outcomes presented in most studies on alveolar ridge preservation. In an experimental study, CBCT scans were taken 6 months after ridge preservation for 3-dimensional top-down planning of the ideal position for implant-supported restorations [21]. It was found that 22% to 44% of the 87 included patients revealed insufficient dimensions for proper housing of the implants planned at an ideal position, demonstrating the necessity for additional augmentation regardless of baseline allocation to non-compromised or compromised sockets [21]. Sites treated with ridge preservation revealed similar dimensional changes as non-augmented sites in a comparison of ridge alterations in 2 dimensions in a group of 38 adults 12 weeks after tooth extraction [22].

The quality of newly formed bone was sufficient for implant site preparation by drills at re-entry, clinically resembling cancellous bone. Radiography confirmed the presence of D2 to D3 bone [23]. These observations were corroborated by microscopic findings. The core biopsies showed newly formed woven bone in direct contact with the re-grown lamina propria, which presented with varying thickness, likely reflecting the individual background of each patient (Figure 4A-D and 4E-H). In specimens obtained from the crestal surface of the alveolar ridge, the newly formed bone consistently occupied the most crestal portion of the former defect, showing intimate contact between newly mineralized tissues and the membrane residues (Figure 4C). The collagen residues revealed no signs of fibrotic encapsulation or an inflammatory infiltrate in the surrounding tissues. In these specimens, perimembranous and intramembranous osteogenesis was a consistent finding. The position of mineralized tissue areas at the collagen interface indicated that the membrane compartment facing the former socket defect was involved in the ossification process. The bone formation process within the defects was clearly not impaired by the fact that the membranes were incompletely covered by the flap tissue initially, thereby underscoring the osteopromotive character of RCLC.

Similar observations in collagen residues have been reported by Zubery et al. [18]. Their group used RCLC membranes for guided bone regeneration or guided tissue regeneration, along with additional fixation and coronal advancement of the soft tissues for complete primary soft tissue closure [18]. In contrast to their study, neither additional fixation of membranes nor complete membrane cover was included in our protocol. Nevertheless, the clinical and histological results were consistent in all sites treated in the present series.

Soft tissue closure by secondary epithelization occurred consistently, and uneventful healing was completed within 4 weeks after tooth removal in 100% of the treated sites. The muco-gingival junction was maintained consistently in the pre-surgical position; newly formed soft tissues regularly showed keratinized gingiva, irrespective of the allocation of the sockets. The proliferation of keratinized mucosa in edentulous areas was enhanced above the previously exposed membranes, probably due to the non-advanced treatment of the buccal flap. These observations corresponded with the findings reported by Engler-Hamm et al. [6]. The mean coronal displacement of the muco-gingival junction was 3.83 mm in the control sockets, which were primary closed by coronal advancement of the flap. The sockets in both groups were grafted with a bone substitute and a co-polymer membrane, but remained uncovered for open healing in the test group, resulting in significantly less displacement of the muco-gingival junction, with a mean of 1.21.mm (P=0.002) [6]. The capacity of RCLC to facilitate secondary healing was demonstrated by continuous closure of the soft tissue above exposed membrane areas in sites with complications during primary healing following lateral augmentation in a previous case series [19].

As this project was a pilot study, some limitations need to be acknowledged. One weakness might be the lack of a CBCT analysis and a comparison of the outcomes using 3-dimensional. Although CBCT scans would have enabled a more detailed evaluation of the cases, using CBCT scans would have dramatically increased the required radiation exposure. The intraoral radiographs were not standardized and comparison was only possible by extrapolating different landmarks. The primary aim of this ridge preservation evaluation, however, was focused on a patient-centered outcome (i.e., the possibility of placing an implant without further bone augmentation) and it was not aimed to compare 2 different treatment modalities. Based on our preliminary results, a randomized controlled study might include a dimensional comparison of different treatment approaches based on CBCT scans directly before implant placement, as well as radiation-free options like intraoral scanning and digital cast superimposition at multiple time points. Furthermore, only a limited number of core biopsy samples were retrieved during implant placement; hence, the influence of the 2 applied bone substitutes was not assessed, although differences in the healing pattern were seen histologically (but not clinically).

Within the limitations of the case series, the proposed method made additional augmentation predictably redundant, reducing thereby patients' morbidity and simplifying implant placement. The open healing of RCLC membranes was feasible as a way to allow secondary epithelization above exposed membrane areas, while being non-detrimental to bone formation. Thus, the method was a satisfactory alveolar ridge preservation technique in all dimensions.

ACKNOWLEDGEMENTS

The authors deeply appreciate the help of Ms. van Dyke and Ms. Bay for assistance in histological processing. The authors are grateful to Dr. Özgün Pehlivan, Bolu Abant İzzet Baysal University, School of Medicine, Department of Public Health for proofreading the methodology, verifying the results by double-checking the calculations, and revising the conclusions.

REFERENCES

1. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent 2003;23:313-23.
PUBMED
2. Misawa M, Lindhe J, Araújo MG. The alveolar process following single-tooth extraction: a study of maxillary incisor and premolar sites in man. Clin Oral Implants Res 2016;27:884-9.
PUBMED | CROSSREF
3. Chappuis V, Engel O, Reyes M, Shahim K, Nolte LP, Buser D. Ridge alterations post-extraction in the esthetic zone: a 3D analysis with CBCT. J Dent Res 2013;92:195S-201S.
PUBMED | CROSSREF
4. Chappuis V, Engel O, Shahim K, Reyes M, Katsaros C, Buser D. Soft tissue alterations in esthetic postextraction sites: a 3-dimensional analysis. J Dent Res 2015;94:187S-193S.
PUBMED | CROSSREF
5. Chappuis V, Rahman L, Buser R, Janner SF, Belser UC, Buser D. Effectiveness of contour augmentation with guided bone regeneration: 10-year results. J Dent Res 2018;97:266-74.
PUBMED | CROSSREF
6. Engler-Hamm D, Cheung WS, Yen A, Stark PC, Griffin T. Ridge preservation using a composite bone graft and a bioabsorbable membrane with and without primary wound closure: a comparative clinical trial. J Periodontol 2011;82:377-87.
PUBMED | CROSSREF
7. Avila-Ortiz G, Elangovan S, Kramer KW, Blanchette D, Dawson DV. Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. J Dent Res 2014;93:950-8.
PUBMED | CROSSREF
8. Barone A, Borgia V, Covani U, Ricci M, Piattelli A, Iezzi G. Flap versus flapless procedure for ridge preservation in alveolar extraction sockets: a histological evaluation in a randomized clinical trial. Clin Oral Implants Res 2015;26:806-13.
PUBMED | CROSSREF
9. Barone A, Toti P, Quaranta A, Alfonsi F, Cucchi A, Calvo-Guirado JL, et al. Volumetric analysis of remodelling pattern after ridge preservation comparing use of two types of xenografts. A multicentre randomized clinical trial. Clin Oral Implants Res 2016;27:e105-15.
PUBMED | CROSSREF
10. Chappuis V, Araújo MG, Buser D. Clinical relevance of dimensional bone and soft tissue alterations post-extraction in esthetic sites. Periodontol 2000 2017;73:73-83.
PUBMED | CROSSREF
11. Mardas N, Chadha V, Donos N. Alveolar ridge preservation with guided bone regeneration and a synthetic bone substitute or a bovine-derived xenograft: a randomized, controlled clinical trial. Clin Oral Implants Res 2010;21:688-98.
PUBMED | CROSSREF
12. Avila-Ortiz G, Rodriguez JC, Rudek I, Benavides E, Rios H, Wang HL. Effectiveness of three different alveolar ridge preservation techniques: a pilot randomized controlled trial. Int J Periodontics Restorative Dent 2014;34:509-21.
PUBMED | CROSSREF
13. Aimetti M, Manavella V, Corano L, Ercoli E, Bignardi C, Romano F. Three-dimensional analysis of bone remodeling following ridge augmentation of compromised extraction sockets in periodontitis patients: a randomized controlled study. Clin Oral Implants Res 2018;29:202-14.
PUBMED | CROSSREF

14. Cho IW, Park JC, Shin HS. A comparison of different compressive forces on graft materials during alveolar ridge preservation. J Periodontal Implant Sci 2017;47:51-63.
PUBMED | CROSSREF

15. Choi HK, Cho HY, Lee SJ, Cho IW, Shin HS, Koo KT, et al. Alveolar ridge preservation with an open-healing approach using single-layer or double-layer coverage with collagen membranes. J Periodontal Implant Sci 2017;47:372-80.
PUBMED | CROSSREF

16. Park JC, Koo KT, Lim HC. The hidden X suture: a technical note on a novel suture technique for alveolar ridge preservation. J Periodontal Implant Sci 2016;46:415-25.
PUBMED | CROSSREF

17. Klinger A, Asad R, Shapira L, Zubery Y. *In vivo* degradation of collagen barrier membranes exposed to the oral cavity. Clin Oral Implants Res 2010;21:873-6.
PUBMED | CROSSREF

18. Zubery Y, Nir E, Goldlust A. Ossification of a collagen membrane cross-linked by sugar: a human case series. J Periodontol 2008;79:1101-7.
PUBMED | CROSSREF

19. Friedmann A, Strietzel FP, Maretzki B, Pitaru S, Bernimoulin JP. Observations on a new collagen barrier membrane in 16 consecutively treated patients. Clinical and histological findings. J Periodontol 2001;72:1616-23.
PUBMED | CROSSREF

20. Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, et al. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 2017;18:529.
PUBMED | CROSSREF

21. Koutouzis T, Lipton D. Regenerative needs following alveolar ridge preservation procedures in compromised and noncompromised extraction sockets: a cone beam computed tomography study. Int J Oral Maxillofac Implants 2016;31:849-54.
PUBMED | CROSSREF

22. Flügge T, Nelson K, Nack C, Stricker A, Nahles S. 2-Dimensional changes of the soft tissue profile of augmented and non-augmented human extraction sockets: a randomized pilot study. J Clin Periodontol 2015;42:390-7.
PUBMED | CROSSREF

23. Lekholm U, Zarb GA. Patient selection. In: Brånemark PI, Zarb GA, Albrektsson T, editors. Tissue-integrated prosthesis: osseointegration in clinical dentistry. Chicago (IL): Quintessence; 1985. p.195-205.