J Clin Periodontol 2011; 38: 677–685 doi: 10.1111/j.1600-051X.2011.01738.x

Journal of Clinical Periodontology

Randomized controlled trial on lateral augmentation using two collagen membranes: morphometric results on mineralized tissue compound

Anton Friedmann[1], Kirsten Gissel[2], Mouaz Soudan[1], Bernd-Michael Kleber[2], Sandu Pitaru[3] and Thomas Dietrich[4]

[1]Department of Periodontology, School of Dentistry, Faculty of Health, University of Witten, Witten, Germany; [2]Department of Periodontology, Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Oral Biology, School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel; [4]Department of Oral Surgery, School of Dental Medicine, University of Birmingham, Birmingham, UK

Friedmann A, Gissel K, Soudan M, Kleber B-M, Pitaru S, Dietrich T. Randomized controlled trial on lateral augmentation using two collagen membranes: morphometric results on mineralized tissue compound. J Clin Peridontol 2011; 38: 677–685. doi: 10.1111/j.1600-051X.2011.01738.x.

Abstract

Background: Guided bone regeneration is considered an effective tool for gaining mineralized tissue either at exposed implant surface or in deficient alveolar ridge areas before implant placement.

Material and methods: Customized casts obtained following impression taking at surgery and re-entry allowed for morphometric assessment of alveolar ridge alterations 6 months after one-stage augmentation of bone dehiscences. In a randomized pilot study using biphasic calcium phosphate tests ($n = 17$) received treatment with ribose cross-linked collagen membranes (RCLM), whereas controls ($n = 20$) received non-cross-linked membranes. The primary endpoint was to quantify the effect of membrane type on dimensional changes in bone margins at crestal level of endosseous implants.

Results: Soft tissue dehiscencies occurred at 70.5% and 55% frequency for tests and controls, respectively. Gain in clinically hard newly mineralized tissue at the crestal level was significantly higher in test group in lateral (1.8 *versus* 0.7 mm; $p = .046$) and in vertical dimensions (1.1 *versus* 0.2 mm; $p = .035$) compared with controls. Second measurement obtained at the border of reflected flap revealed no significant difference between groups (3.0 *versus* 2.1 mm; $p = 0.57$) for lateral dimension.

Conclusions: Both collagen devices were effective in bone augmentation. RCLMs supported mineralization process and remodelling even in sites showing compromised healing as indicated by morphometric outcome.

Key words: lateral augmentation; morphometry; non-cross-linked collagen; randomized trial; ribose cross-linked collagen

Accepted for publication 11 April 2011

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest.

The study was funded in parts by *Biomet* 3i (Palm Beach; FL, USA) as unrestricted research grant to Department of Periodontology and Synoptic Dentistry, Charité, Berlin, Germany and by material donations from Institute Straumann AG (Basel, Switzerland). Originally, *Biomet 3i* was the study initiator proposing the initial steps for the protocol and providing the membranes as study material. After the company lost the rights of distribution for the OSSIX membrane, it was no longer part of this investigation. The final protocol, as described in the manuscript and approved by the Ethic Committee of the Charité University was designed by the principal investigator (A. F.). The data processing and preparation of the manuscript were completed by the co-authors all named on the cover page. Dr. Pitaru is one of the two inventors of Ossix and holds a patent on this invention together with Dr. M. Noff. Dr. Pitaru is one of the two co-founders of Colbar Life Sciences, held equities in this Company until 2005 and served as Chief Scientist Officer until 2003.

Guided bone regeneration (GBR) is one of several well-documented, evidence-based augmentation techniques (Hammerle & Karring 1998, McAllister & Haghighat 2007). Several animal and clinical studies showed gain in marginal bone, using resorbable membranes in combination with an underlying, osteoconductive membrane-supporting material (Hockers et al. 1999, Hammerle & Lang 2001, Zitzmann et al. 2001, Strietzel et al. 2006). Collagen membranes, as the most frequently used type of degradable membranes, lack enough stiffness for space maintenance and tend to collapse (Strietzel et al. 2006). Therefore, titanium re-inforced non-degradable e-PTFE membranes are still favoured by some clinicians. Early exposure of barrier membranes to the oral environment jeopardizes the outcome due to infection, mostly manifested around non-resorbable membranes, or due to rapid disintegration in case of resorbable membrane (Mayrand & Grenier 1985, Tempro & Nalbandian 1993, Simion et al. 1994, Nowzari & Slots 1995, De Sanctis et al. 1996, Sela et al. 2003).

Collagen cross-linking contributes to prolonged membrane barrier function (von Arx et al. 2005, Bornstein et al. 2007). Barriers with high degradation rates could have a shorter-than-indicated effect (Moses et al. 2008). Membrane degradation starts shortly after implantation (von Arx et al. 2005). The integration pattern of various collagen membranes into soft tissues were analysed in recently published animal studies (Rothamel et al. 2005, Schwarz et al. 2006). A larger membrane porosity (less collagen contents per area) would allow for cell ingrowths within the membrane, resulting in better tissue (Rothamel et al. 2005, Schwarz et al. 2006), but may result in reduced barrier function (Rothamel et al., 2004). Importantly, nutrient diffusion for cell proliferation and differentiation was not affected by collagen cross-linking *versus* non-cross linking in an in vitro study (Friedmann et al. 2008). Significantly more new bone formation in animal defects augmented with biphasic calcium phosphate (BCP) together with either a non-degradable (e-PTFE) or a newly introduced slowly degrading polyethylene glycol membranes *versus* controls grafted without membranes was recently confirmed by Jung et al. (2006). Nevertheless, the discussion upon the benefits and disadvantages of cross-linked collagen material as slowly degradable *versus* membranes from native collagen, a rapidly degrading type is still ongoing. In this respect, there is no data regarding the efficiency of resorbable membranes to support bone gain in cases in which bone augmentation is performed with simultaneous installation of implants with transgingival surgical elements.

Morphometric assessment of outcomes in bone augmentation in terms of volume gain has been the topic for in vitro and clinical studies (Studer et al. 1997, Kohles et al. 2000, Tai et al. 2000, Proussaefs et al. 2002, Proussaefs & Lozada 2005, Llambes et al. 2007, Windisch et al. 2007). Our group introduced a method to perform morphometric measurements on casts obtained from impressions of the residual alveolar ridge taken during surgery and of the post-augmented ridge taken during re-entry (Pitaru et al. 2006).

The aim of the present randomized clinical trial was to test the efficiency of supporting bone gain and promoting soft tissue healing of a ribose cross-linked collagen membrane (RCLM) *versus* a non-cross-linked collagen membrane (NCLM) during augmentation of bone dehiscences and fenestrations with BCP and concomitant implant placement using a new morphometric approach for human clinical trials.

Material and Methods

This study was a randomized controlled, single-blinded pilot clinical trial with an observation period of 6 months. All participants signed a written consent before the beginning of surgical procedures. The study was conducted in accordance with the guidelines of Good Clinical Practice (GCP-ICH) and the principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Universitätsmedizin Charité, Berlin, Germany, under protocol number EA2/054/05 and registered at ClinicalTrials.gov under ID: NCT00835432. Patients were recruited from the pool of recall patients at the Department of Periodontology and Synoptic Dentistry or from referring dentists during the years 2005–2006. Included were partially edentulous patients in good general health with one or more teeth missing and requiring bone augmentation with placement of implants. There were no restrictions with regard to the location of the edentulous area, i.e. anterior and posterior regions of the maxilla and/or mandible were included. The study used a block randomization to randomize patients to either the cross-linked test (OSSIX, 3i, Palm Beach, FL, USA) or the non-cross-linked control (BioGide, Geistlich, Wohlhusen, Switzerland) membranes in a 1:1 ratio (Fig. 1). This was a pilot study and no sample size calculation was performed. At the beginning of the study, treatment allocation for each patient was placed in a sealed envelope to be opened during the surgery. In patients who had two surgical sites that met the inclusion criteria, both sites were treated concomitantly and the first site received the membrane as per treatment allocation while the second site received the alternative treatment, facilitating comparisons within patients with two surgical sites.

Smokers consuming 10 or more cigarettes per day were excluded. All treatments were performed between November 2005 and May 2007. All surgeries were carried out under local anaesthesia (Ultracain DS forte, Aventis, Germany) by a single surgeon (A. F.). All patients received a one-stage procedure including implant placement, concomitant augmentation, and primary wound closure. Defect extensions were recorded using periodontal probe (PCP 15, Hu-Friedy, Leimen, Germany) during surgery. While in vivo measurements were performed by the surgeon, morphometric analyses on the casts obtained were carried out by the clinical investigator (K. G.), who was blinded to the randomized patient assignment. In addition, impressions were taken for ex vivo measurements by a blinded investigator (K. G.) using individualized plastic trays and a sterile A-silicone material (Elite® Implant, Zhermack, Germany). These impressions were taken before and after reflection of a muco-periosteal flap exposing the defect area. Straumann Soft Tissue Level implants with 4.1 mm in diameter and varying from 8 to 12 mm in length (Institute Straumann AG) were used in accordance with established guidelines. In brief, recipient site was prepared for placing implant with it, rough SLA surface at the crestal level of alveolar bone leaving the machined part of the implant neck above the bone level. Standard plus implants with a machined neck of 1.8 mm were used. BCP (BoneCeramic, Institute Straumann AG) was used for grafting dehiscences and fenestrations in alveolar bone in exposed implant areas. No autogenous bone was used. For easier

application, a coagulum was formed by the calcium phosphate grafting material with 1–2 ml of the patients' own blood collected in the wound area after the incisions were carried out (Friedmann et al. 2002, Artzi et al. 2005, Friedmann et al. 2009). The composite grafting material was placed in the defect up to the level of the machined surface of the implant, in the vertical direction and up to the completion of the bony envelop in the lateral direction. After application of the augmentation material, envelopes containing the randomization code were opened and membrane placement was performed as assigned. Each membrane was applied in a monolayer. All flaps were coronally advanced by periosteal release to achieve a tension-free adaptation of soft tissue margins for complete closure of the wound. The post-operative regimen included twice daily mouth rinses with chlorhexidine 0.2% (chlorhexamed, Glaxo Smith Kline, Bühl, Germany). Systemic antibiotics, either amoxicillin 750 mg 3 × daily (Amoxicillin-Ratiopharm, Ratiopharm, Ulm, Germany) or clindamycine 300 mg 4 × daily (Clindamycin-Ratiopharm, Ratiopharm) were administered for 1 week; and ibuprofen (Ibuprofen-Ratiopharm 600 mg, Ratiopharm) as necessary for post-operative analgesia. Sutures were removed after 14 days. Fixed partial dentures or semi-permanent splinting were used as temporary prosthetic devices only. In edentulous posterior regions, no dentures were applied during the study.

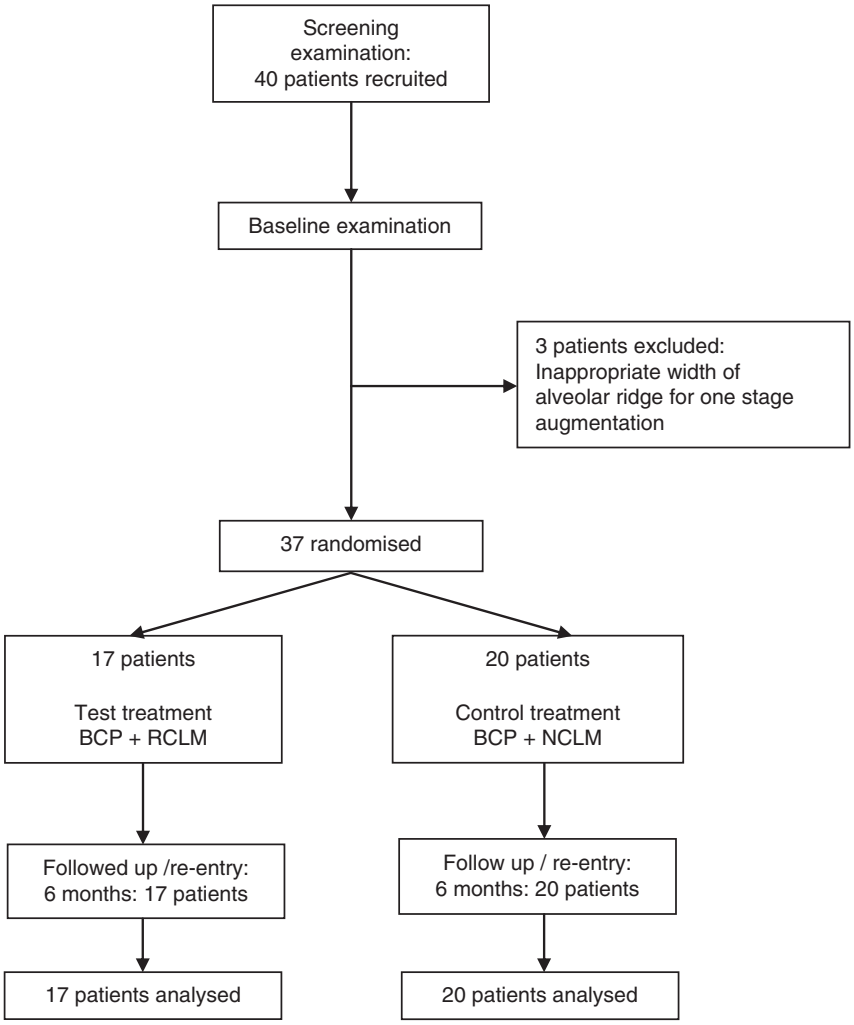

Fig. 1. Flow diagram of study outline.

At re-entry after 6 months, an impression was taken to document tissue dimensions and full thickness flaps were then raised using similar incision extensions as during the first surgery, with the exception for lingual flaps which were not raised if the area augmented was sufficiently exposed (Fig. 2a–f). Following thorough removal of any remaining non-mineralized tissue with sterile curettes another impression was taken to document hard-tissue dimensions. In nine cases requiring additional augmentation, BCP and RCLM were used. All implants received gingival formers (Straumann AG) and the flap was sutured back by single sutures using Monocryl 6.0 suture (Ethicon, Hamburg, Germany).

Morphometric procedure

The primary objective of the study was to quantify the effect of the type of collagen membrane on the level of bone augmentation at the crestal level of endosseous implants. To do this the following morphometric procedure was undertaken. All impressions were cast in stone rock plaster (Octa Stone CN, Hereus – Kulzer, Bensheim, Germany). Casts of impressions obtained at initial surgery and at re-entry (post-augmentation) are designated as casts-0 and casts-1, respectively. Negative templates of casts-1 were obtained by taking silicon (C-silicon, Silaplast®, Detax, Germany) impressions. Using landmarks as teeth adjacent to the augmented site and the implant neck, the negative templates were adjusted upon the casts-1 (Fig. 3a and b). To determine reference points and to ascertain reproducibility in positioning one and the same template on casts obtained at both surgeries, the top aspects of the templates were trimmed parallel with the basis of cast 1; furthermore the side walls of templates were trimmed perpendicular to top and bottom aspect of the templates (Fig. 4a–d). While kept together, the casts-1 and their negative silicon templates were sectioned through bucco-lingual plans that mid-crossed in mesio-distal dimension of implants around which augmentation was performed. A perpendicular was dropped from the edge of template to the middle of the cover screw of a cross-sectioned implant to label central implant axis (Fig. 4a). To assess vertical dimension, a parallel perpendicular was marked on the template at the crossing point of alveolar crest and implant surface and the distance between this point and the top plan of the silicon template was measured with a digitized calliper and termed – *H*1/1 (Figs 3a and 4a). A perpendicular to *H*1/1 passing through the crossing point of alveolar crest and implant surface was drawn. The distance between the implant middle axis and the buccal aspect of the alveolar

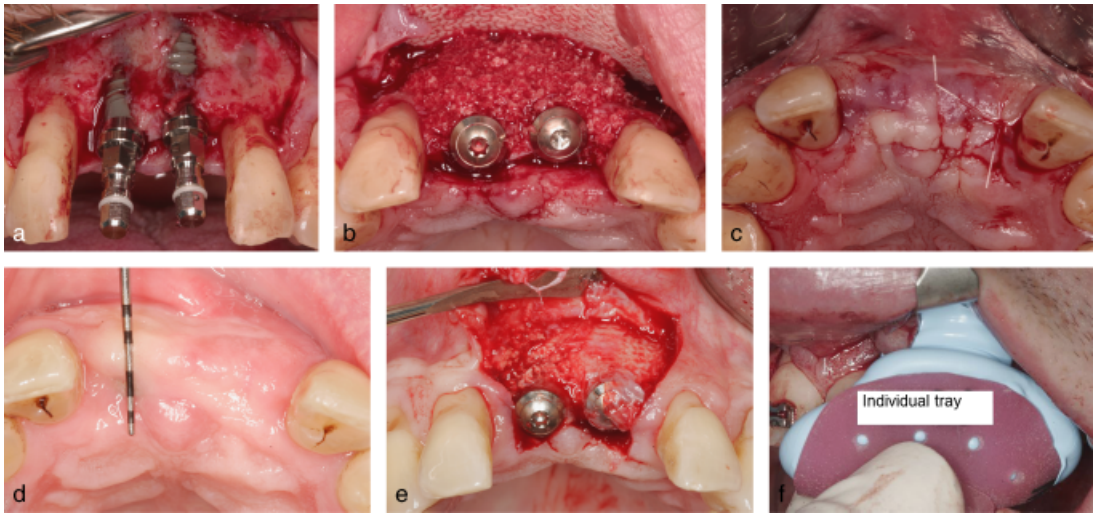

Fig. 2. Clinical images of surgical procedure. (a) Two Straumann Soft Tissue Level implants are placed displaying a dehiscence and a fenestration bone defects on the buccal aspect of the ridge. The necks of both implants are positioned supra-crestally according to the protocol of use. (b) Augmentation with biphasic calcium phosphate is performed; the randomization assigned this site for test group (ribose cross-linked membrane). (c) Primary closure by coronally advancing the flap tissue for submerged healing is achieved. (d) The site 6 months later before re-entry shows sufficient gain in width indicated by a perioprobe. (e) Re-opening demonstrates a complete fill of the defect extension by newly organized hard tissue in both, the area of former dehiscence and fenestration. (f) An example of intra-surgical impression taking with sterile silicon and an individual tray.

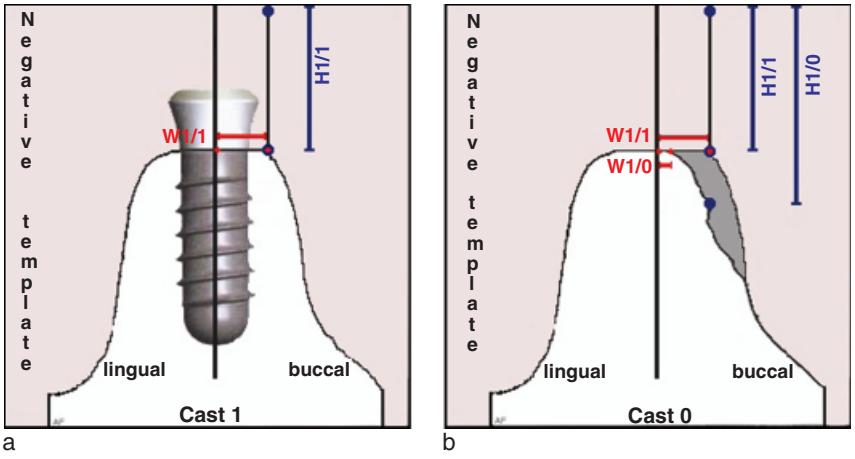

Fig. 3. Schematic drawing of the principle for assessment of the parameters width (*W*) and height (*H*) at most crestal extension of augmented area on casts out of stone. The reflected portions of buccal flaps were reduced in stone for easier adaptation of the silicon template on both, cast-1 and cast-0. (a) Mid-crestal section across alveolar ridge casted in plaster together with negative silicon template mounted on top shows position of implant neck and crestal extension of newly organized bone (cast-1). Perpendicular is marked on the silicon template indicating the middle axis of the implant. A perpendicular to this one at the crestal level indicates width of the ridge in buccal direction termed as *W*1/1. *H*1/1 is the distance from the edge of silicon template down to the crossing point with the buccal proximity of the crest. (b) Mid-crestal section of the cast obtained at baseline surgery before implant positioning (cast-0) with the template mounted according to references determined by teeth adjacent to the gap thus reproducing the position previously achieved on cast-1. The perpendicular indicating the middle axis of the implant is the reference for assessment of *W*1/0 along the second perpendicular at the crestal level again. Assessment of the distance *H*1/0 repeats the measurement *H*1/1, however, the crossing point with alveolar crest is supposed to be more apical.

crest was measured on this perpendicular and termed – *W*1/1 (Figs 3a and 4a). A second perpendicular, parallel to *H*1/1 was drawn at the level of most pronounced buccal aspect of augmented area and the distance from the middle of implant to this aspect was recorded and termed – *W*2/1 (Fig. 4a and b). The distance from this point to the top plan of the silicon template was measured with a digitized caliper and termed – *H*2/1 (Fig. 4a). Then, the cross-sectioned silicon templates were adapted to the casts-0 obtained during the augmentation procedure using the reference marks mentioned above (Fig. 4c and d). Using the templates as landmarks the casts-0 were sectioned in the same plan as described above for the casts-1. Using the landmark lines drawn on the silicon templates, now adapted to casts-0, the distances measured on casts-1 were measured for casts-0 and termed *W*1/0; *H*1/0; *W*2/0; and *H*2/0. This methodology ensured that casts-0 and casts-1 were sectioned in the same plans and exactly at the same site, that is a bucco-lingual plan crossing the central axis of the implants (Fig. 4b). Bone gain in width and height was calculated by:

- Crestal (coronal) width gain: $\Delta W1 = W1/1 - W1/0$
- Crestal (coronal) vertical gain: $\Delta H1 = H1/0 - H1/1$
- Apical width gain: $\Delta W2 = W2/1 - W2/0$
- Apical vertical gain: $\Delta H2 = H2/0 - H2/1$

Statistical analysis

Changes in hard and soft-tissue dimensions between baseline and re-entry were

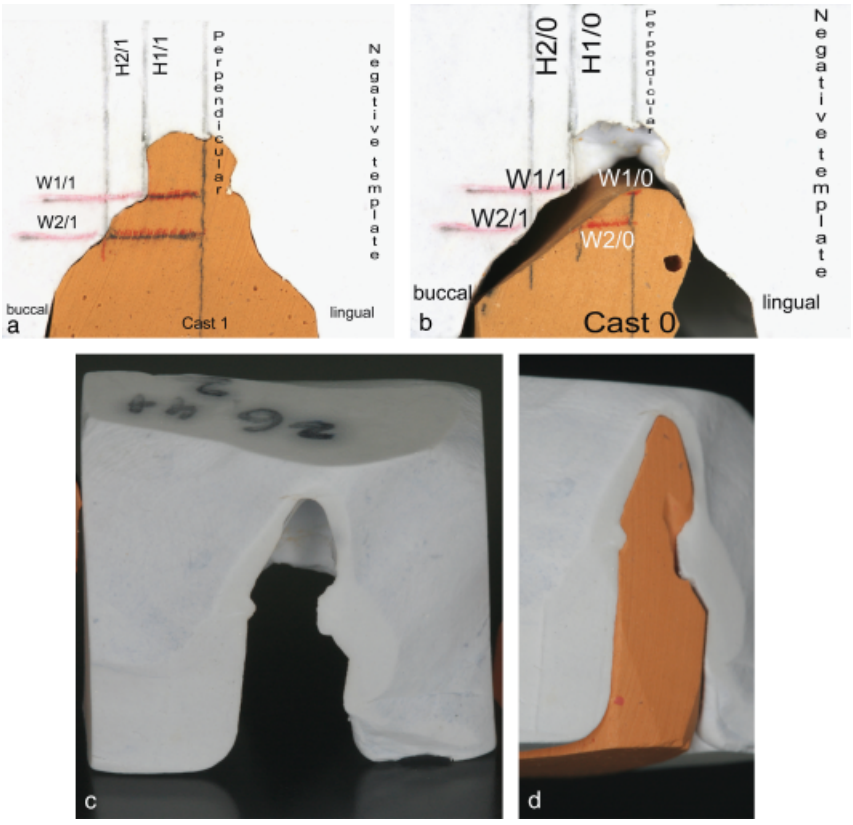

Fig. 4. Illustration of morphometric steps performed on casts under laboratory conditions. (a) Mid-crestal section of cast-1 (obtained after augmentation at re-entry) with mounted silicon template shows the implant neck casted in plaster and the perpendicular dropped from the edge of the template at the middle of cover screw indicating the middle axis of the implant. According to the scheme in the Fig. 3 *H*1/1 marks the distance from the edge of the template to the first crestal contact with the bone; *W*1/1 determines the distance from the mid-implant axis to the same crossing point, respectively. *H*2/1 and *W*2/1 reflect the distances in the same manner more apically. (b) Mid-section of the cast-0 with the template transferred from cast-1 displaying the reference lines obtained from cast-1 post-augmentation for assessment of baseline dimensions of the ridge. The gap between the ridge casted in plaster and the edge of the template indicates bone deficiency in buccal–lingual direction. The labeled landmarks repeat those schematically drawn in Fig. 3. (c) Silicon template shows parallel walls and perpendicularly arranged top plan and bottom for exact positioning transferring the template from cast-1 to cast-0. (d) The image shows the tooth next to the area of interest from the opposite to the edentulous area site with the template being exactly adapted.

outcomes of interest. Data were analysed in two different ways. Firstly, data of all 37 patients were analysed using one randomly selected site of those patients with two surgical sites. Comparisons between groups and between baseline and re-entry within groups were made using *t*-tests and paired *t*-tests as appropriate. Further analyses used multiple linear regression adjusting for maxillary *versus* mandibular defects, anterior and posterior sites, smoking, membrane exposure. Secondly, comparisons between groups were made limited to data of those patients who had two surgical sites (split-mouth comparison) using non-parametric Wilxocon's sign rank tests. All statistical tests were two-sided at $\alpha = 0.05$ and performed using statistical software (STATA 11, Stata Corp, College Station, TX, USA).

Results

Patient recruitment involved 40 patients; three of them were excluded due to the extensive loss of alveolar bone making therefore the implant placement at one stage with augmentation not feasible (Fig. 1). All patients included completed the study, resulting in a total of 37 patients with 46 defect sites. All implants inserted (in total: 73) were well integrated after 6 months of healing. All implants received gingival formers for 4–6 weeks being loaded subsequently by single crowns or fixed partial dentures. There were 37 implants placed in 17 defect sites in the test group and 36 implants in 20 sites in the control group (Table 1). The overall survival rate at time point of loading was 100%.

Albeit patient assignment to the test and control groups was performed randomly, groups matched well in regard to patient's age, proportions between females and males and distribution of sites between maxilla and mandible (Table 1). In both groups no-smoking patients and smoking <10 cigarettes/day were represented in similar frequency. A group of nine patients formed a split-mouth group and were treated at two sites each site with a different membrane. Analysis of the morphometric results within this group indicated no-statistically significant differences between the sites treated with the RCLM and those treated with NCLM. Therefore, only one site per patient has been chosen by secondary randomization assigning one study site and implant per patient, either to tests or to controls. Thus, Table 2 represents 37 patient data sets and the distribution among test and control group for 37 study sites randomly selected for analysis. The age ranged from 24 to 69 years with a median of 53 years (Table 2).

No adverse events were recorded but one participant in the control group showed hypersensitive reaction to amoxicillin, which then was exchanged by zithromax (azithromycine, Pfizer, Berlin, Germany).

Morphometric results

Statistical outcomes for all 37 sites including randomly selected one site per patient out of former split-mouth randomization are given by Table 3. At reference point 1 (*W*1/*H*1), indicating the most crestal bone-to-implant contact the median gain of mineralized tissue in the vertical dimension (vertical gain; $\Delta H1$) was 1.1 mm in the test sites compared with 0.2 mm in the control sites ($p = 0.0463$). The median gain in the horizontal dimension (width gain; $\Delta W1$) was 1.8 mm in the test sites *versus* 0.7 mm in the control sites ($p = 0.0359$). There were no statistically significant associations between the primary outcome and membrane exposure ($p = 0.6845$ for $\Delta H1$ and $p = 0.2809$ for $\Delta W1$, respectively).

Table 1. Comprehensive patient data ($n = 37$)

Patients	Age	Sex	Region*	Defect size Baseline (mm) Depth/width		No. of implants (total)	Test/control 1/ 0	Exposure Yes/no 1/0
1	58	F	2	4	3	3	1	0
2	68	F	2	5	5	2	1	0
3	64	M	3	6	5	2	1	1
4	67	F	2	5	5	2	0	1
5	61	M	3	7	5	2	1	1
6***	47	F	2	4	5	1	1	1
6			2	4	8	1	0	1
7	59	F	2	5	4	1	0	1
7***			2	5	4	2	1	1
8	37	M	2	7	6	1	1	1
9	62	F	2	5	4	2	1	1
10***	52	F	2	8	4	2	0	1
10			2	5	4	2	1	1
11	64	F	3	8	6	1	0	1
12	44	F	2	2	4	1	0	0
13***	49	F	2	4	4	2	0	1
13			2	6	5	2	1	0
14	49	F	2	5	4	1	0	0
14***			2	5	5	1	1	1
15	40	M	2	5	6	1	0	0
16	69	M	3	10	6	1	1	1
17***	65	M	1	7	5	1	1	0
17			1	10	15	1	0	1
18	68	F	2	6	6	2	0	0
19	33	M	3	10	6	1	1	1
20	63	F	4	8	5	2	0	1
21	64	F	2	5	5	2	0	1
22	55	F	2	4	6	2	1	1
23	40	M	2	7	6	1	0	1
24	58	F	3	8	4	1	0	1
25	65	F	2	7	4	2	0	0
26	56	F	2	3	5	2	1	1
27	67	F	2	4	3	2	1	1
28	30	M	3	10	6	1	0	1
29***	67	M	2	4	4	1	0	1
29			2	3	3	2	1	0
30	69	F	2	5	4	2	1	1
31***	64	F	2	4	3	2	0	0
31			2	3	3	2	1	0
32	69	F	2	6	4	2	0	0
33***	24	M	3	7	4	1	1	0
33			3	6	4	1	0	1
34	44	F	1	8	10	1	1	1
35	49	F	2	5	7	2	0	0
36	62	F	2	7	5	2	0	0
37	59	F	1	1	1	2	0	0

*1, maxilla posterior;
2, mandible posterior;
3, maxilla anterior;
4, mandible anterior.
***Indicates sites excluded from statistical analysis by secondary randomization.

Table 2: Baseline characteristics for patients and sites randomly selected for statistical analysis ($n = 37$)

	Test ($N = 17$)	Control ($N = 20$)
Age: median (range)	61 years (33–69)	59 years (24– 9)
Gender: F:M	11:6	15:5
Smoking: −:+	16:1	18:2
Soft tissue dehiscence: −:+ (%)	5:12 (70.5)	9:11 (55.0)

At the second reference point ($\Delta W2$/ $\Delta H2$), the most apically accessible point for measurement, the median vertical gains were 2.5 and 2.7 mm for the test and control sites, respectively ($p = 0.5674$). The median width gains at this reference point were 3.0 and 2.1 mm for test and control sites, respectively ($p = 0.1189$).

Table 3. Median values and ranges of morphometric outcome

	Test ($N = 17$) (mm)		Control ($N = 20$) (mm)
$\Delta H1$ (= *H*1/1–*H*1/0)	1.1 (0.8–1.9)		0.2 (– 0.2–1.8)
$p =$		0.0463*	
$\Delta W1$ (= *W*1/1–*W*1/0)	1.8 (1.0–3.0)		0.7 (– 0.0–2.3)
$p =$	0.0359*		
$\Delta H2$ (= *H*2/1–*H*2/0)	2.5 (1.4–3.7)		2.7 (0.2 ± 4.3)
$p =$		0.5674	
$\Delta W2$ (= *W*2/1–*W*2/0)	3.0 (2.2–3.3)		2.1 (0.8–3.0)
$P =$	0.1189		

*$p < 0.05$.

In both groups of patients, test and control sites which exhibited soft tissue dehiscence required additional augmentation at exposed implant surface. There were four sites in the test group and five sites in the control group (23.5% *versus* 20%, respectively) requiring additional treatment; implants involved received secondary application of BCP plus RCLM at re-entry.

Discussion

The aim of the present randomized clinical trial was to test the effectivity of RCLM (test) *versus* a NCLM (control) in GBR treatment using a new method for morphometric analysis. Generally, the results indicate that both membranes improved the bone volume and predictably supported GBR procedures at dehiscence-type and fenestration-type defects.

The rate of soft tissue dehiscences during healing was almost equally distributed for both groups. Over 50% of sites were exposed and underwent secondary epithelization. The figures are high compared with the data presented for the group of dehisence-type defects within the review by Jensen and Terheyden. They found rates of soft tissue dehiscences up to 14.5% for sites with resorbable and of 26.3% for those with non-resorbable mebranes (Jensen & Terheyden 2009). Similar observations in regard to RCLM samples were reported previously by our group (Friedmann et al. 2001). Moses et al. (2005) found the RCLM demonstrating a higher reduction of the bony defect area in cases of premature membrane exposure. The history of premature membrane exposure may have a negative effect on new bone formation even if soft tissue dehiscence recovers by secondary epithelization. Although RCLM might be associated with a higher incidence rate of soft tissue dehiscences (Moses et al. 2005, Tal et al. 2008), the frequency of concomitant inflammatory reactions reported is almost zero (Friedmann et al. 2001). On the opposite, the histological report by Zubery and colleagues demonstrated nicely the ossification of RCLM remnants facing underlying newly mineralized tissues. These observations support the idea that slow degradation of RCLM is beneficial for mineralization process (Zubery et al. 2007, Zubery et al. 2008).

In our study Soft Tissue Level Straumann implants were inserted in accordance with the ITI surgical protocol resulting in a position of the machined part of implant neck supra-crestally. However, all implants and augmentation sites were planned to heal in the submerged mode according to the study protocol. Therefore, complete flap closure required mobilization and coronal advancing of the flap to achieve tension-free primary wound closure over the implant neck exceeding almost 2 mm coronally to the crest. No grafting material was applied in the supra-crestal area of bone dehiscencies laterally and therefore it is conceivable that flap stabilization was under optimal in this study. This lack of membrane support by grafting material might explain the unexpected high frequency of soft tissue dehiscence and premature membrane exposure. In a recent study by Burkhard and Lang tensions exceeding 0.1 N determined before suturering the flap resulted in a wound dehiscence rate over 40% (Burkhardt & Lang 2010). The high prevalence of dehiscences in our study may be a result of mechanical challenges due to masticatory movements as a complementary factor to membrane properties. According to the implant design secondary epithelialization in this study did not always result in complete soft tissue closure over the cover screw. However, the difference in frequency of dehiscence onset following augmentation and the need of additional augmentation at re-entry was obvious.

The method used to assess bone gain was originally introduced by Pitaru et al. 2006 at Europerio in Madrid, Spain (Pitaru et al. 2006). Customizing each defect site before and after augmentation in plaster of Paris allowed for determining identically positioned reference points on boths. A silicon template transferred the reference points from one cast to the other. Implants inserted concomitantly with augmentation of dehiscence bone defects served as reference points. Albeit the implant necks were positioned supra-crestally the equicrestal level of roughened sand blasted and acid-etched (SLA) surface on casts obtained at re-entry was clearly indicated. Therefore, measurements across alveolar crest perpendicular to the most coronal bony margin performed in this study can be considered standardized. In a previous publication our group demonstrated the degree of mineralization in newly formed tissues exceeding 40% in the area grafted laterally with BCP (Friedmann et al. 2009). As in this study biopsies for the histomorphometric analysis were harvested during re-entry surgeries from sites augmented in the present study, histologic outcome indicates an effective denudation of bony crest (Friedmann et al. 2009). Taking these observations into account and furthermore by removing all non-mineralized tissue from underneath the flap before impression we payed maximum attention to establish accuracy of the impressions. As all surgeries were performed by one co-worker (A. F.) we consider the approach standardized for all sites. Our study presents for first time an accurate method for quantifying vertical and horizontal changes of the alveolar ridge in GBR. Furthermore this is first prospective report on efficacy of collagen membranes in one stage GBR with one stage Soft Tissue Level Straumann implants. Lang et al. (1994) reported successful implementation of Gore Tex membranes placing them around necks of Straumann implants which, however, healed in transmucosal mode. The methods for tracking defect changes introduced in periodontal regeneration as standardized probing from the cemento-enamel junction to the deepest defect extension at baseline and at re-entry surgery (Yukna et al. 2000, Lekovic et al. 2002) are not applicable for purposes outlined in this study. Even in a situation illustrated by

Fig. 2a–f probing across the ridge would result in significant variation in positioning any measuring device at baseline and the re-entry surgeries.

Retrospective analyses presented by Jensen and Terhyeden at ITI consensus conference 2008 revealed an average fill of 85.5% together with 68.5% completely regenerated defects, respectively, calculated for the group of dehiscence type of defects if a resorbable membrane was used. The rate of infectious complications was 13.75%. These data are based on a review of 20 studies (Jensen & Terheyden 2009).

Our results did not evaluate the level of defect fill, but rather the change in the bone dimensions in the defect. Nevertheless, sites requiring additional augmentation can be considered showing incomplete regeneration. Implants exposed in the area to be grafted are generally considered seeking new bone formation. A failure in apposition of new bone results therefore in an unlikely contact of the implant surface with soft tissues bearing odds for onsetting periimplantitis. Therefore, the gain in mineralized tissue at the crestal level of implant roughened surface is the crucial parameter in testing the efficacy of GBR procedure. The statistically significant differences in gain of clinically hard mineralized tissues at the crestal border of previously exposed SLA-surface ($H1/W1$) favours the use of the RCLM. Clinically viable amounts of mineralized tissue was gained in the test group as indicated by without exception positive values for the difference between baseline and 6 months results in height and width parameters ($\Delta H1$; $\Delta W1$) of the bone covering previously exposed implant areas (Table 3). There was none additional gain and even a slight loss of bone occurred obviously in some controls as expressed by negative differences in crestal height and width for this group. Taking into account similar frequencies of dehiscence onset in both groups, we had to assume that cases treated with RCLM (tests) had a greater benefit in regard to primary outcome than those treated with NCLM (controls). This interpretation is supported by results of an animal study, which showed significantly greater membrane stability for the RCLM than for NCLM sutured onto oral mucosa in rats, both materials being exactly same as used in our study (Rothamel et al. 2005). The unpublished in vitro data by Pitaru and colleagues showed greater resistance of RCLM *versus* NCLM against bacterial collagenases (personal communication, 14 October 2010). Evaluating at re-entry the degradation status of both collagen membranes histologically Tal and colleagues found that among 26 initially applied RCLMs 13 were pre-maturely exposed (50%). Five out of them appeared interrupted and four were completely degraded 6 months following placement, whereas all devices from none-exposed sites remained intact. On the contrary, none of 18 NCLM devices initially applied was detectible at re-entry histologically. The authors concluded RCLM being more resistant against tissue degradation and retaining its integrity for a longer period of time compared with NCLM (Tal et al. 2008).

Although none significant differences between both groups existed in regard to measurements at the second level addressed as $\Delta H2/\Delta W2$, we conclude that the results are clearly superior for the use of RCLM material in lateral augmentation if compared with native collagen membranes, especially in regard to alterations in soft tissue healing.

Acknowledgements

We are grateful for initiation of the study by 3i Implant Innovations and their support with study materials. We also highly appreciate the contribution made by Institute Straumann AG supplying this study with additional materials such as BCP, Tissue Level Implants and gingival formers. We are deeply indebted to Asisa Saidowa for providing our group with schematic drawings of the principle used for assessments. Our gratitude goes also to Mr. Bodenstein and Mr. Schnitzer, who both supported the laboratory part of study providing clinical investigator with lab equipment and facilities.

References

Artzi, Z., Kozlovsky, A., Nemcovsky, C. E. & Weinreb, M. (2005) The amount of newly formed bone in sinus grafting procedures depends on tissue depth as well as the type and residual amount of the grafted material. *Journal of Clinical Periodontology* **32**, 193–199.

Bornstein, M. M., Bosshardt, D. & Buser, D. (2007) Effect of two different bioabsorbable collagen membranes on guided bone regeneration: a comparative histomorphometric study in the dog mandible. *Journal of Periodontology* **78**, 1943–1953.

Burkhardt, R. & Lang, N. P. (2010) Role of flap tension in primary wound closure of mucoperiosteal flaps: a prospective cohort study. *Clinical Oral Implants Research* **21**, 50–54.

De Sanctis, M., Zucchelli, G. & Clauser, C. (1996) Bacterial colonization of barrier material and periodontal regeneration. *Journal of Clinical Periodontology* **23**, 1039–1046.

Friedmann, A., Dard, M., Kleber, B. M., Bernimoulin, J. P. & Bosshardt, D. D. (2009) Ridge augmentation and maxillary sinus grafting with a biphasic calcium phosphate: histologic and histomorphometric observations. *Clinical Oral Implants Research* **20**, 708–714.

Friedmann, A., Dehnhardt, J., Kleber, B. M. & Bernimoulin, J. P. (2008) Cytobiocompatibility of collagen and ePTFE membranes on osteoblast-like cells in vitro. *Journal of Biomedical Materials Research A* **86**, 935–941.

Friedmann, A., Strietzel, F. P., Maretzki, B., Pitaru, S. & Bernimoulin, J.-P. (2001) Observations on a new collagen barrier membrane in 16 consecutively treated patients. Clinical and histological findings. *Journal of Periodontology* **72**, 1616–1624.

Friedmann, A., Strietzel, F. P., Maretzki, B., Pitaru, S. & Bernimoulin, J. P. (2002) Histological assessment of augmented jaw bone utilizing a new collagen barrier membrane compared to a standard barrier membrane to protect a granular bone substitute material. *Clinical Oral Implants Research* **13**, 587–594.

Hammerle, C. H. & Karring, T. (1998) Guided bone regeneration at oral implant sites. *Periodontology 2000* **17**, 151–175.

Hammerle, C. H. & Lang, N. P. (2001) Single stage surgery combining transmucosal implant placement with guided bone regeneration and bioresorbable materials. *Clinical Oral Implants Research* **12**, 9–18.

Hockers, T., Abensur, D., Valentini, P., Legrand, R. & Hammerle, C. H. (1999) The combined use of bioresorbable membranes and xenografts or autografts in the treatment of bone defects around implants. A study in beagle dogs. *Clinical Oral Implants Research* **10**, 487–498.

Jensen, S. S. & Terheyden, H. (2009) Bone augmentation procedures in localized defects in the alveolar ridge: clinical results with different bone grafts and bone-substitute materials. *International Journal of Oral Maxillofacial Implants* **24** (Suppl.), 218–236.

Jung, R. E., Zwahlen, R., Weber, F. E., Molenberg, A., van Lenthe, G. H. & Hammerle, C. H. (2006) Evaluation of an in situ formed synthetic hydrogel as a biodegradable membrane for guided bone regeneration. *Clinical Oral Implants Research* **17**, 426–433.

Kohles, S. S., Vernino, A. R., Clagett, J. A., Yang, J. C., Severson, S. & Holt, R. A. (2000) A morphometric evaluation of allograft matrix combinations in the treatment of osseous defects in a baboon model. *Calcified Tissue International* **67**, 156–162.

Lang, N. P., Bragger, U., Hammerle, C. H. & Sutter, F. (1994) Immediate transmucosal implants using the principle of guided tissue regeneration. I. Rationale, clinical procedures and 30-month results. *Clinical Oral Implants Research* **5**, 154–163.

Lekovic, V., Camargo, P. M., Weinlaender, M., Vasilic, N. & Kenney, E. B. (2002) Comparison of platelet-rich plasma, bovine porous bone mineral, and guided tissue regeneration versus platelet-rich plasma and bovine porous bone mineral in the treatment of intrabony defects: a reentry study. *Journal of Periodontology* **73**, 198–205.

Llambes, F., Silvestre, F. J. & Caffesse, R. (2007) Vertical guided bone regeneration with bioabsorb-

able barriers. *Journal of Periodontology* **78**, 2036–2042.

Mayrand, D. & Grenier, D. (1985) Detection of collagenase activity in oral bacteria. *Canadian Journal of Microbiology* **31**, 134–138.

McAllister, B. S. & Haghighat, K. (2007) Bone augmentation techniques. *Journal of Periodontology* **78**, 377–396.

Moses, O., Pitaru, S., Artzi, Z. & Nemcovsky, C. E. (2005) Healing of dehiscence-type defects in implants placed together with different barrier membranes: a comparative clinical study. *Clinical Oral Implants Research* **16**, 210–219.

Moses, O., Vitrial, D., Aboodi, G., Sculean, A., Tal, H., Kozlovsky, A., Artzi, Z., Weinreb, M. & Nemcovsky, C. E. (2008) Biodegradation of three different collagen membranes in the rat calvarium: a comparative study. *Journal of Periodontology* **79**, 905–911.

Nowzari, H. & Slots, J. (1995) Microbiologic and clinical study of polytetrafluoroethylene membranes for guided bone regeneration around implants. *The International Journal of Oral Maxillofacial Implants* **10**, 67–73.

Pitaru, S., Friedmann, A., Meidan, L. & Bernimoulin, J. P. (2006) Ribose-crosslinked collagen membranes are more effective than ePTFE membranes in supporting bone augmentation in humans: a morphometric study. *Journal of Clinical Periodontology* **33**, 53 (abstract).

Proussaefs, P. T., Valencia, G., Lozada, J. & Tatakis, D. N. (2002) A method to assess the clinical outcome of ridge augmentation procedures. *Journal of Periodontology* **73**, 302–306.

Proussaefs, P. & Lozada, J. (2005) The use of intraorally harvested autogenous block grafts for vertical alveolar ridge augmentation: a human study. *The International Journal of Periodontics Restorative Dentistry* **25**, 351–363.

Rothamel, D., Schwarz, F., Sager, M., Herten, M., Sculean, A. & Becker, J. (2005) Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clinical Oral Implants Research* **16**, 369–378.

Rothamel, D., Schwarz, F., Sculean, A., Herten, M., Scherbaum, W. & Becker, J. (2004) Biocompatibility of various collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells. *Clinical Oral Implants Research* **15**, 443–449.

Schwarz, F., Rothamel, D., Herten, M., Sager, M. & Becker, J. (2006) Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. *Clinical Oral Implants Research* **17**, 403–409.

Sela, M. N., Kohavi, D., Krausz, E., Steinberg, D. & Rosen, G. (2003) Enzymatic degradation of collagen-guided tissue regeneration membranes by periodontal bacteria. *Clinical Oral Implants Research* **14**, 263–268.

Simion, M., Baldoni, M., Rossi, P. & Zaffe, D. (1994) A comparative study of the effectiveness of e-PTFE membranes with and without early exposure during the healing period. *The International Journal of Periodontics Restorative Dentistry* **14**, 166–180.

Strietzel, F. P., Khongkhunthian, P., Khattiya, R., Patchanee, P. & Reichart, P. A. (2006) Healing pattern of bone defects covered by different membrane types – a histologic study in the porcine mandible. *Journal of Biomedical Materials Research B Applied Biomaterials* **78**, 35–46.

Studer, S. P., Sourlier, D., Wegmann, U., Scharer, P. & Rees, T. D. (1997) Quantitative measurement of volume changes induced by oral plastic surgery: validation of an optical method using different geometrically-formed specimens. *Journal of Periodontology* **68**, 950–962.

Tai, C. C., Sutherland, I. S. & McFadden, L. (2000) Prospective analysis of secondary alveolar bone grafting using computed tomography. *Journal of Oral Maxillofacial Surgery* **58**, 1241–1249; discussion 1250.

Tal, H., Kozlovsky, A., Artzi, Z., Nemcovsky, C. E. & Moses, O. (2008) Long-term bio-degradation of cross-linked and non-cross-linked collagen barriers in human guided bone regeneration. *Clinical Oral Implants Research* **19**, 295–302.

Tempro, P. J. & Nalbandian, J. (1993) Colonization of retrieved polytetrafluoroethylene membranes: morphological and microbiological observations. *Journal of Periodontology* **64**, 162–168.

von Arx, T., Broggini, N., Jensen, S. S., Bornstein, M. M., Schenk, R. K. & Buser, D. (2005) Membrane durability and tissue response of different bioresorbable barrier membranes: a histologic study in the rabbit calvarium. *The International Journal of Oral Maxillofacial Implants* **20**, 843–853.

Windisch, S. I., Jung, R. E., Sailer, I., Studer, S. P., Ender, A. & Hammerle, C. H. (2007) A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study. *Clinical Oral Implants Research* **18**, 545–551.

Yukna, R. A., Krauser, J. T., Callan, D. P., Evans, G. H., Cruz, R. & Martin, M. (2000) Multi-center clinical comparison of combination anorganic bovine-derived hydroxyapatite matrix (ABM)/cell binding peptide (P-15) and ABM in human periodontal osseous defects. 6-month results. *Journal of Periodontology* **71**, 1671–1679.

Zitzmann, N. U., Scharer, P. & Marinello, C. P. (2001) Long-term results of implants treated with guided bone regeneration: a 5-year prospective study. *The International Journal of Oral Maxillofacial Implants* **16**, 355–366.

Zubery, Y., Goldlust, A., Alves, A. & Nir, E. (2007) Ossification of a novel cross-linked porcine collagen barrier in guided bone regeneration in dogs. *Journal of Periodontology* **78**, 112–121.

Zubery, Y., Nir, E. & Goldlust, A. (2008) Ossification of a collagen membrane cross-linked by sugar: a human case series. *Journal of Periodontology* **79**, 1101–1107.

Address:
Anton Friedmann
Department of Periodontology
School of Dentistry
Faculty of Health
University of Witten
Alfred-Herrhausen-Str. 50
58448 Witten
Germany
E-mail: anton.friedmann@uni-wh.de

Clinical Relevance

Scientific rationale for the study: Limited data are available regarding morphometric outcome in terms of mineralized tissue gain if either cross-linked collagen membranes or NCLM were used in lateral augmentation.

Principal findings: Results indicate both membranes having potential to effectively support GBR in dehiscence type of defect. Dehiscence of the soft tissue occurred in 70.5% of test and in 55% of control sites. However, significantly higher amount of newly mineralized tissue in a most critical zone argues for the use of cross-linked collagen membranes although more exposures occurred in test sites.

Practical implications: Cross-linked membranes combined with BCP are suitable for GBR and likely to support formation of significant amount of newly mineralized tissue under unlikely clinical conditions.