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In vivo degradation of collagen barrier membranes exposed to the oral cavity

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Abstract

Objectives: The purpose of this human clinical trial was to compare the degradation profiles of three different collagen membranes under conditions mimicking exposure to the oral cavity.

Materials and methods: Three collagen membranes, ribose cross-linked (RCL), glutaraldehyde cross-linked (GCL), and non-cross-linked (NCL) were tested. The membranes were placed over the buccal mucosa of 20 human volunteers, apical to the gingival margins in the maxillary premolar and molar region. A periodontal dressing was placed over the membranes and secured in the interproximal spaces. The dressing was removed after 10 days, and membrane integrity was evaluated by two examiners using a Likert-like grading scale (grades 1–5).

Results: Eight subjects withdrew from the study due to discomfort, pain, or dislodging of the pack. Of the three membranes tested, RCL appeared to be the most resistant to degradation (median grade 5), compared with GCL (2.25) and NCL (1.75).

Conclusions: Marked differences in membrane integrity were found between the three tested membranes after 10 days in the oral cavity. These differences may be part of the important factors determining the outcome of the regenerative treatment modality in cases of premature membrane exposure.

Resorbable barrier membranes are being widely used for guided tissue and guided bone regeneration (GTR and GBR, respectively) in periodontal and dental implant procedures. Of these, collagen membranes are the most commonly used, with a proven efficacy in animal models and in humans (Gelb 1993; Majzoub et al. 1999; Saldanha et al. 2004; Cesar-Neto et al. 2005). These membranes exhibit high biocompatibility, low antigenicity (Zitzmann et al. 1997; Zitzmann et al. 1999), low cytotoxicity (Carpio et al. 2000), and physiological degradation over time. Their barrier effect and defect seclusion should ideally be maintained for 2–4 months to enable the generation and maturation of cells from osteoblastic or cementoblastic cell lineage. Many factors can influence the outcome of GBR using collagen membranes, including patient age, smoking, systemic diseases, defect morphology, surgical handling, and the biomaterials used (Gelb 1993; Zitzmann et al. 1997, 1999; Carpio et al. 2000; Saldanha et al. 2004; Vanden 2004; Cesar-Neto et al. 2005). Even under the care of an experienced surgeon, soft tissue healing may be impaired, often resulting in premature membrane exposure. The reported frequency of premature exposure of collagen membranes in GBR procedures ranges from 30% to

50% (Oh et al. 2003; Tal et al. 2008a). Premature exposure may reduce the amount of regenerated bone by up to 81% (Jovanovic et al. 1992; Nowzari & Slots 1995; Zitzmann et al. 1997; Lekholm et al. 2001, Machtei 2001). This is probably due to infection and premature loss of the barrier. Therefore, when premature exposure of a membrane does occur, its resistance to degradation by bacterial collagenolitic enzymes may be beneficial to the regenerative process and may allow soft tissue healing with minimal loss of the grafted bone. Cross-linking of collagen was proposed as a method to reduce membrane degradation by enzymes and to maintain the membrane's barrier effect (Pitaru et al. 1988; Petite et al. 1990; Goissis et al. 1999). Animal and human studies had shown that in cases with primary flap closure, cross-linked membranes are more resistant to degradation (Zubery et al. 2007, 2008). However, the degree of cross-linking and the resultant resistance to degradation may depend on the method used for the cross-linking of the collagen.

When premature exposure of a collagen membrane to the oral environment occurs, oral bacteria adhere to the membrane surface (Sela et al. 1999). Bacteria of the oral flora produce proteinases with the ability to degrade collagen membranes (Bunyaratavej & Wang 2001). In early exposure cases, a membrane more resistant to degradation may allow better soft tissue healing and prevent loss of graft material, and hence a more successful augmentation procedure. The differences in the degradation rates of collagen membranes upon exposure to the oral cavity may be dependent on the cross-linking level of the collagen in a given membrane, as was recently shown in an in-vitro study by Sela et al. (2009).

The purpose of this human clinical trial was to compare the degradation rates of three commercially available collagen membranes – two cross-linked and one non-cross-linked – under conditions mimicking exposure to the oral environment.

Material and methods

The study was designed as a single-blind, parallel group, single-center, three-arm, controlled clinical trial. The study was approved by the IRB for Human Studies of the Hadassah Medical Center, and all participants signed a consent form.

Twenty generally healthy adult volunteers (medical center employees and medical students, aged 18–65) were recruited and selected for the study. Exclusion criteria were pregnancy, uncontrolled systemic disease, and untreated periodontal or gingival pathology. Upon enrollment, all subjects were given instructions regarding oral hygiene, and were administered scaling (supra- and sub-gingival) and dental prophylaxis by a trained hygienist. Four weeks later, each patient was reexamined, and membranes (three membranes in each patient, one from each group) were placed in the mouths of subjects who showed no clinical signs of gingival inflammation. The tested membranes were a ribose cross-linked membrane (RCL, Ossix-plus, OraPharma Inc. Warminster, NJ, USA); a glutaraldehyde cross-linked membrane (GCL, BioMend, Zimmer Dental, Carlsbad, CA, USA); and a non-cross-linked membrane (NCL, Bio-gide, Geistlich, Wolhusen, Switzerland). Membranes were trimmed to a uniform size of 8 × 15 mm and were placed in a randomized mesiodistal order on the buccal mucosa, apical to the gingival margins of maxillary premolar and molar teeth. A light-cured periodontal dressing (Barricaid, Denstply Caulk, Denstply Int. Inc., York, PA, USA) was placed over the membranes and securely attached to the teeth at the interproximal spaces and undercuts. Subjects were instructed to avoid hard food, brushing, and other forms of mechanical trauma, and to avoid rinsing with an antiseptic mouthwash of any kind. On the next visit, on Day 10, the dressing was removed, along with the attached membranes, and photographed. Membrane integrity was evaluated by two independent blinded examiners, using a scale of 1–5 (Table 1). Subjects who complained of significant discomfort or pain, and subjects in whose mouths the pack was dislodged, were excluded from the study.

Table 1. **Membrane integrity in all participants as evaluated by two blinded examiners**

Participant	RCL			NCL			GCL		
	Examiner 1	Examiner 2	Mean	Examiner 1	Examiner 2	Mean	Examiner 1	Examiner 2	Mean
2	4	4	4	1	1	1	2	2	2
3	5	5	5	1	1	1	1	1	1
5	5	5	5	5	5	5	4	3	3.5
6	5	5	5	1	1	1	1	1	1
8	2	2	2	1	1	1	1	1	1
9	5	5	5	5	5	5	5	4	4.5
10	5	5	5	2	2	2	5	5	5
11	5	5	5	5	5	5	4	4	4
12	1	3	2	4	4	4	4	5	4.5
13	5	5	5	1	2	1.5	1	1	1
14	5	5	5	1	2	1.5	1	1	1
17	5	5	5	4	5	4.5	3	2	2.5
Mean			4.42*			2.71			2.58
(± SD)			1.16			1.8			1.62

*Statistically significant difference compared with other tested membranes (Friedman test followed by Wilcoxon's signed-rank test $P = 0.004$).
RCL, ribose cross-linked membrane; GCL, glutaraldehyde cross-linked membrane; NCL, non-cross-linked membrane.

Statistical analysis

The following parameters were used to estimate the required sample size: power of 90%, $\alpha = 0.05$. After the last subject has completed the study, and all readings of the degraded membranes were recorded, the data were analyzed using the SPSS software. For the comparisons of the three membranes, the non-parametric Friedman test was first applied, followed by Wilcoxon's signed rank test for each pair of membranes. Exact *P*-values were calculated instead of the usual asymptotic ones. The Holm's corrected for multiple comparisons *P*-values are reported.

Results

Two subjects complained of pain and discomfort from the pack, and their packs were removed. In five subjects, the dres-

sing was dislodged due to anatomical limitations such as pressure from a high buccal frenum, shallow vestibule, or robust masticatory muscle activity. One participant used antibacterial rinse during the experimental period and was disqualified from the study. Twelve subjects completed the study, and the results are presented in Table 1.

The inter-examiner agreement, as expressed in κ values, was 0.8 for the RCL membrane, 0.65 for the NCL membrane, and 0.55 for the GCL membrane. As shown in Table 1, Fig. 1, and Fig. 2, there were significant differences in membrane integrity between the three tested membranes (Friedman's test: P-value = 0.004). The RCL membrane appeared to be more resistant to degradation (mean score = 4.42; median = 5), compared with the NCL membrane (mean score = 2.71; median = 1.75 P-value = 0.02, Holm's-adjusted P-value = 0.047) and the GCL membrane (mean score = 2.58; median = 2.25; P-value = 0.016, Holm's-adjusted P-value = 0.047). In eight of the 12 samples, the RCL membrane appeared to be more resistant as compared with the NCL membrane, and in 10 of the 12 samples, the RCL membrane also appeared more resistant as compared with the GCL membrane. No statistically significant differences were found between the NCL and GCL membranes (P-value = 0.54, Holm's adjusted P-value = 0.54).

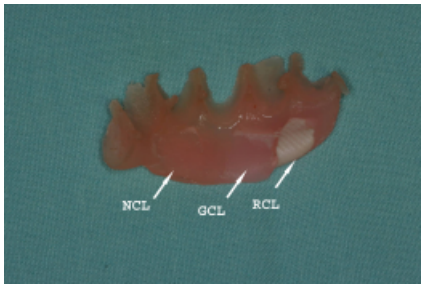

Fig. 1. An example of the periodontal pack with remnants of the three tested membranes upon retrieval from the oral cavity of one of the tested subjects. RCL, ribose cross-linked membrane; GCL, glutaraldehyde cross-linked membrane; NCL, non-cross-linked membrane.

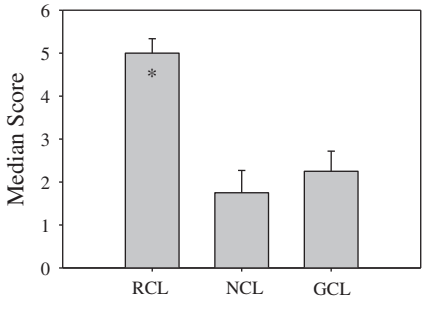

Fig. 2. Membrane integrity, expressed as the median ± standard error. *Statistically significant difference between RCL and the other two tested membranes (Friedman test followed by Wilcoxon's signed-rank test. P = 0.004). RCL, ribose cross-linked membrane; GCL, glutaraldehyde cross-linked membrane; NCL, non-cross-linked membrane.

Discussion

In the present study, we compared the resistance to degradation in the oral cavity of three commercially available collagen membranes, upon exposure to the oral cavity in humans. Several studies had tested this issue using in-vitro and animal models (Tal et al. 2008b; Sela et al 2009), but we tried to mimic the common clinical situation of soft tissue dehiscence immediately following regenerative procedures in a human model. The present model allows the membranes to be exposed to oral fluids and oral bacteria, but protects them from mechanical forces that may also cause membrane degradation. In the present study, all three tested membranes lost their integrity to a certain degree after 10 days of exposure to the oral environment. No differences were found between NCL and GCL, as both membranes disintegrated to an average degree of 1/3–2/3 of their original surface area after 10 days, and the cross-linking of the GCL membrane did not seem to affect the degradation process. This minimal resistance to degradation may be explained by the limited amount of cross-linking possible in GCL membranes, because glutaraldehyde is a toxic material when released into a living tissue. This result is in agreement with Moses et al. (2008), who, using a rat model, showed that 14 days after the implantation of membranes in a completely submerged condition, degradation profiles of the GCL and NCL membranes were similar. Our results also demonstrated that the RCL membrane maintained its integrity to a much higher degree than the GCL and NCL membranes, and in most cases maintained its original structure. Taken together, the results confirmed that a high degree of collagen cross-linking has a significant effect on the degradation rate in submerged and exposed conditions. These results can be explained by the chemical nature of the collagen molecules with and without cross-linking. The collagenolytic proteinases hydrolyze the peptide bond in the collagen molecule at the amino-terminal side of Gly in the –X–Gly–Pro peptide, resulting in the breakdown of collagen molecules into small peptides (Watanabe 2004). When collagen molecules are cross-linked, some of the sites that usually serve as a substrate for bacterial or human collagenase are hidden or modified due to protein folding, and enzymatic digestion may be significantly reduced. In conclusion, it appears that the level of collagen cross-linking may determine the resistance of the collagen membrane to degradation by enzymes.

Maintenance of a barrier effect of exposed membranes even for several weeks may be significant to the results of GBR procedures. Clinically, it appears that cross-linking of the collagen by ribose may prolong its biological effect. Friedmann et al. (2002) had shown that the volume of augmented bone after early exposure of a cross-linked collagen membrane was significantly higher as compared with the premature exposure of an e-PTFE membrane. Using a cat palate model, Tal et al. (2008b) compared the degradation rate of RCL and NCL collagen membranes following experimental exposure to the oral cavity. In contrast to our results, in this animal model, neither of the two types of membranes were resistant to degradation following exposure periods of 7 days, and no differences were found between degradation profiles of the RCL and NCL collagen membranes. The difference between the two studies may be explained by the fact that in an open-wound cat palate model, mechanical rather than enzymatic degradation may have played a more significant role, as the wound is accessible to the grinding forces of the cat tongue.

In two animal studies, the biodegradation of different membranes implanted subcutaneously was compared (Rothamel et al. 2005; Moses et al. 2008). In both studies, the RCL membrane showed the least amount of biodegradation com-

pared with the NCL and GCL membranes. It may be concluded that cross-linking of collagen by ribose is superior to cross-linking by glutaraldehyde, and is associated with prolonged biodegradation. In addition, there is recent evidence that increasing the resistance to degradation of NCL membrane using it in a double-layer form (Kozlovsky et al. 2009), results in improved bone regeneration (Kim et al. 2009). It should be pointed out that the previous results were for unexposed membranes and cannot be compared directly with our study. But the same trend was found in these two different models, suggesting that biodegradation in the tissues as well as after exposure to the oral cavity is caused by similar mechanisms.

The present study demonstrated marked differences in membrane degradation profiles *in vivo* between three commercially available collagen membranes after ten days under a complete exposure to the oral environment. The high resistance of the RCL membrane may be an advantage during regenerative procedures, in cases where a premature membrane exposure is encountered.

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