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PMN responses following use of 2 biodegradable GTR membranes

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Abstract

Objectives: In the present prospective trial, the PMN response following resorbable GTR barrier placement was evaluated in mandibular class II furcation lesions.

Materials and Methods: In 10 patients with treated chronic periodontitis, we randomly selected the 1st molars in the mandible with buccal degree II furcation involvement for either polylactic-citric-acid-ester (PLA) or glycolide-lactic-copolymer (PGL) GTR membrane therapy. We examined contralateral healthy molar sites as untreated controls. We then evaluated the PMN-derived inflammatory tissue response at baseline, weekly up to 6 weeks post-therapy and at 12 and 24 weeks using GCF myeloperoxidase (MPO), beta-glucuronidase (βG) and beta-N-acetyl-hexosaminidase (βNAH).

Results: The enzyme levels increased from baseline to the 6-week examination. After the 6-week reappointment, enzyme levels dropped reaching the baseline scores at both the 12- and 24-week visit. At PGL sites, the enzyme levels decreased earlier. Compared with healthy control sites, the MPO, βNAH and βG tests revealed different maximum levels at week 2 and 3 (PGL) and week 4, 5 and 6 (PLA). For both of the barriers the clinical parameters revealed a sustained improvement following therapy.

Conclusion: The release of PMN enzymes following placement of bioabsorbable membranes reflects the early soft tissue healing process. Our results suggest that the PMN response is barrier-dependent with the maximum response occuring at different times. However, the host response did not measureably affect the course of clinical healing.

Key words: gingival crevicular fluid; inflammatory response; polymorphonuclear enzymes; ester hydrolysis; guided tissue regeneration

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The promotion of tissue formation around periodontally compromised teeth is the principal goal of GTR therapy. The physiology of cell types involved in the regenerative process and their individual response to different barrier materials today is poorly characterized. Proteolytic enzymes have been considered important in the outcome of regenerative processes, especially during the initial remodeling phase when the tissues have been regenerated but may not be fully mature. In this step of regeneration, proteases could degrade the newly formed extracellular matrix and ultimately may be a major factor in determining the final clinical result (Grosso et al. 1997). The insertion of a GTR membrane into the gingival tissues induces an inflammatory cellular response of the adjacent connective tissues. Protease repertoires of cells adherent to membranes recovered after guided tissue regeneration yielded human cell lines that produced additional proteases including low molecular weight proteases that were not routinely seen in gingival fibroblasts, periodontal ligament (PDL) cells or keratinocytes (Wakabayashi et al. 1996).

Resorbable periodontal membranes are subjected to proteolytic degradation (Gottlow 1993, Lundgren et al. 1994). The ester-composed synthetic polymers of polylactic-citric-acid-ester barriers (PLA) and glycolide-lactic-copolymer

membranes (PGL) are degradated in vivo by enzymatic hydrolysis. Proteolytic enzymes released from polymorphonuclear leukocytes, especially those with an esterase activity, participate in the biodegradation process. As a result of membrane placement, an inflammatory process is initiated that is associated with a release of lysosomal enzymes from activated polymorphonuclear leukocytes accumulating at the GTR-treated periodontium.

The liberation of myeloperoxidase as a potent injurious oxidative enzyme from the azurophilic granules into the extracellular space gives rise to highly reactive oxygen species, including O_2^- and HOCl (McCulloch 1994). In the Krebs cycle they accelerate the membrane degradation by oxidizing the barrier endproducts CO_2 and H_2O. The acid hydrolase beta-glucuronidase is an indicator of neutrophil infiltration. Elevated βG levels correlate with the total number of PMNs in the gingival crevice (Nakashima et al. 1994) and represent the inflammatory changes following barrier placement. Beta-N-acetylhexosaminidase is recognized to be both of PMN and microbial origin. Cloning of the beta-NAH gene in *E. coli* revealed that beta-NAH is an outer-membrane-associated lipoprotein of *P. gingivalis* (Lovatt & Roberts 1994). The salivary beta-NAH-glycosidase activities correlate with the degree of periodontal inflammation and follow periodontal healing after treatment (Niemen et al. 1993). β-NAH measurements in gingival crevicular fluid are considered to play a role in monitoring the efficacy of periodontal therapy (Beighton et al. 1992).

The purpose of the present prospective investigation was to evaluate the PMN response following resorbable GTR barrier placement in mandibular class II furcation lesions using an array of 3 different gingival crevicular fluid enzymes.

Material and Methods

Patients

10 medically healthy subjects from the Department of Periodontology who had received comprehensive treatment for chronic periodontal disease for at least six months were admitted to enter into the study. All subjects were enrolled into a three-months scheduled periodontal maintenance program including prophylaxis, motivation and reinstruction in self-performed oral hygiene. The average age of the patients, three women and seven men, was 51.2 ± 10.9 years at the last visit. The health questionaire elicited that all patients were non-smokers or former smokers who did not smoke within the last 6 months. At the outset, each subject revealed a mandibular buccal degree II furcation lesion at the left or right first molar with a buccal probing furcation involvement between 3 and 6 mm and radiographic evidence of interradicular bone loss. The subjects were selected at random to receive either a polylactic-citric-acid-ester membrane (PLA, molar straight large, Guidor AB, Huddinge, Sweden) or a glycolid-lactic-copolymer periodontal barrier (PGL, polyglactin-910 membrane, Vicryl-Netz Parodontal-Zuschnitt VPM 2942, Ethicon, Norderstedt, Germany) to cover the molar defect. Contralateral situated buccal first molar sites served as healthy controls. Informed consent was obtained from all patients after briefing on the study protocol, procedures, risks and benefits. Patients requiring additional antibiotic medications related to other acute infectious diseases during the 6-month period were not admitted to participate further and must leave the trial.

Periodontal examinations

The degree of the buccal furcation involvement at the first mandibular molars assigned for GTR therapy was assessed using a calibrated Nabers probe (P2N, Aesculap AG, Tuttlingen, Germany) according to Hamp et al. (1975). The extent of the furcation defect was determined radiographically with intraoral dental films size W2 (Ektaspeed plus EP-22P, Eastman Kodak Co, Rochester, NY, USA) using the XCP-technique. The probing depths (PD) as distance between the gingival margin and the bottom of the periodontal pocket and the relative vertical clinical attachment levels (CAL) as distance from the cemento-enamel junction (CEJ) to the bottom of the periodontal pocket were assessed at six sites per tooth (buccal: mb/b/db; lingual: dl/l/ml) using a straight rigid periodontal probe (PCP 11, HuFriedy, Chicago, IL, USA) with a 3–3–2–3 mm calibration and a 0.4 mm diameter tip. The PD and CAL measurements (reproducibility ± 1 mm greater than 95%) were performed by the same examiner. The presence of supragingival plaque was assessed at six sites per tooth according to the plaque index [PI] (Silness & Löe 1964), and was scored from 0 to 3 related to the amount of visible plaque on the individual tooth surface area. The gingival conditions were visually examined at six surfaces per tooth using the gingival index [GI] (Löe & Silness 1963) that classifies the redness and swelling of the marginal gingiva into the degrees 0–3.

Treatment

We gained access to the periodontal defects through surgery that followed the principles of guided tissue regeneration. An intrasulcular incision extended to both of the neighbouring teeth including the adjacent interdental papillae was performed under local anesthesia (Xylocain Spezial 2%, Astra Chemicals, Sweden). A mucoperiosteal flap was raised beyond the mucogingival border to get access to the vestibular furcation area. The denuded root surfaces were mechanically scaled and root planed and repeatedly rinsed with 0.1% chlorhexidine-digluconate solution (Chlorhexamed fluid, Procter & Gamble, Schwalbach, Germany). Then, each defect was randomly selected for either a PLA or a PGL barrier treatment. The membranes covered the furcation defect and exceeded the adjacent bone margin for at least 3 mm. They were adapted to the root trunk by circumferential resorbable sutures (Vicryl 4–0) or preplaced ligatures (Guidor). For complete coverage of the barrier a periosteal incision was performed to coronally reposition the mucoperiosteal flap up to the cemento-enamel border. To facilitate a primary closure, the reflected flaps were sutured with modified mattress sutures.

During the first 6 weeks of the post-surgical follow-up, all subjects were asked to refrain from mechanical plaque control at the GTR-treated molars and the healthy molar sites, but to perform oral hygiene in the remaining dentition. In week 1 and 2, an 0.1% chlorhexidine-digluconate solution was administered to the patients who were instructed to rinse 2× daily for 2 min. At each post-therapy visit for up to 6 weeks, a professional cleaning restricted to the supragingival areas of the GTR-treated and healthy molars was carried out without usage of polishing agents to avoid any irritation of the GTR-treated sites. Thereafter, a 0.1% chlorhexidine gel was prescribed for a patient-directed

application in the furcation area. Patients were maintained on a plaque-control program that included prophylaxis, motivation and reinstruction in self-performed oral hygiene. During the six months trial no subgingival instrumentation was allowed neither at the GTR-treated nor at the healthy control sites. No needs for any antibiotic treatment or application of NSAID that might affect the postsurgical healing dynamics and the evaluation of the PMN response did occur.

GCF sampling

Gingival crevicular fluid (GCF) was harvested prior to surgery at week –1, at each posttherapy visit up to week 6 (+1–6), and at week 12 and 24 at the buccal center of the furcation defect and at the healthy control sites. Following removal of supragingival plaque with a sterile Gracey 11/12 curette tip, we isolated the furcation area with cotton rolls, gently air dried the furcation and inserted a Periopaper strip (Harco, Tustin, California, USA) at the entrance of the periodontal pocket for 30 s according to Brill (1962). The gingival crevicular fluid volume was determined using the Periotron 6000 (Harco Periotron 304 600, Siemens, Bensheim, Germany). The Periotron 6000 was calibrated with a quadratic regression equation curve using serum, 0.9% saline and 1:1 aliquots of serum and saline (Preshaw et al. 1996). Before each periodontal examination the GCF samples were collected and the GCF volumes immediately read with the Periotron. The strips were placed into empty 1.5 ml Eppendorf vials and stored at 4°C. The samples were eluted in 160 μl 0.9% sterile saline at pH 7.4 for 10 min and centrifuged at 14,000 *g* over a 2-min period. The supernatant was separated into 30 μl fractions (Safe Twist, Eppendorf, Hamburg, Germany) and frozen in liquid nitrogen at −196 °C.

Biochemical analysis

Recovery and validation process
Into 4×10 μl aliqots of each of the diluted enzymes a periopaper was inserted. The samples were immersed into 160 μl 0.9% sterile saline and vortexed. Strips and liquid were separated by centrifugation (2 min, 14,000 *g*). In the supernatant, the total amount of enzyme activity that was liberated from the periopaper was determined according to the assay procedures described below and calculated as a percentage of the enzyme standard. According to data from Nakashima et al. (1994), the average recovery of the original total enzyme activities from periopaper was 92.4%±17.7% (range for all samples 71–125%). The validation of the enzyme assays was performed with HL-60 promyelocytes (Hasilik 1992). The mean variation coefficient, which was calculated following duplicate measurements of the total enzyme activities, was 10.7% (range 8–18%).

Myeloperoxidase (MPO; EC 1.11.1.7)
2×10 μl of the diluted GCF sample were added to 0.1 M citric acid buffer, pH 5.5, containing 0.125% Triton-X-100 solution and 0.1 M hydrogen peroxide. The oxidation of H_20_2 was performed using 0.8 mM σ-Dianisidin as substrate. After 7 min of incubation at 21°C, the samples turned brown, and the oxidative reaction was stopped by adding Glycin/NaOH, pH 10.4. The myeloperoxidase activity of the GCF eluates was determined spectrophotometrically at a wavelength (wv) of 405 nm (reference wv 650 nm) on uncoated 96-well microtiterplates using a microplate reader (Behring ELISA Prozessor II, Behringwerke AG, Marburg, Germany). The total MPO activities were calculated in duplicate in μU GCF sample.

Beta-N-acetyl-hexosaminidase (βNAH; EC 3.2.1.30)
10 μl of the diluted GCF sample were eluted in 25 μl 0.9% NaCl, and 0.1 M citric acid buffer, pH 4.6, and 25 μl p-nitrophenyl-N-acetyl-beta-D-glusoaminide (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) were added. The reaction was allowed to proceed at 37°C for 30 min, and then stopped with 225 μl 0.4 M Glycin-NaOH stopping buffer, pH 10.4. The hydrolytic β-N-acetyl-glucosaminidase activities were assessed at a wv of 405 nm (reference wv 492 nm). The total βNAH activities were calculated in duplicate in μU GCF sample (Hasilik 1992).

β-glucuronidase (βG; EC 3.2.1.31)
10 μl of the diluted sample were eluted in 25 μl 0.9% NaCl. The total volume of 35 μl GCF eluate was added to 25 μl substrate containing 4-nitrophenyl-β-D-glucuronide (Serva, Heidelberg, Germany), 0.2% bovine serum albumin (BSA), and 0.04% natrium acide NaN_3 and dissolved in 0.1 M citric acid buffer, pH 4.6. The plates were incubated at 37°C for 24 h, and 225 μl 0.4 M Glycin-NaOH stopping buffer, pH 10.4, was added until a final color change to yellow occurred. The hydrolytic β-glucuronidase activities were determined at a wv of 405 nm (reference wv 492 nm). The total βG activities were calculated in duplicate in μU GCF sample.

Study protocol
Before each examination, we determined the gingival crevicular fluid volumes and the GCF samples harvested at the GTR-treated molar test and at the contralateral healthy sites. At the presurgical visit, the clinical setting was evaluated for each surgical and control site. The PD and CAL measurements were assessed at baseline (week –1) and six months following therapy by the

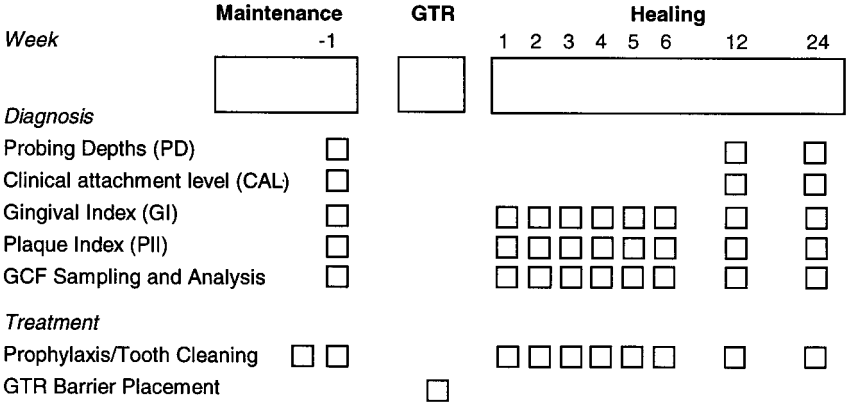

Fig. 1. Experimental protocol of the study displaying the schedule for the clinical and biochemical examinations.

Table 1. Mean±standard deviation of periodontal parameters at healthy sites (H), PLA barrier sites (M1) and PGL barrier sites (M2) pre- and 6 months post-therapy

	Healthy sites (H)	PLA barrier sites (M1)	PGL barrier sites (M2)
PD (mm)			
baseline (−1)*	2.8±1.2	4.4±1.4	4.9±1.6
6 months (24)	2.6±0.8	2.0±1.3	3.1±1.5
change	−0.2±0.9	−2.4±1.4	−1.8±1.3
significance level	NS	<0.05	<0.05
CAL (mm)			
baseline (−1)	3.7±1.1	6.0±1.3	5.8±1.4
6 months (24)	3.6±1.0	4.8±1.1	4.4±1.2
change	−0.1±0.9	−1.2±0.9	−1.4±1.2
significance level	NS	<0.05	<0.05
GCF volume (30 s)			
baseline (−1)	68.6±57.4	127.4±68.5	88.8±33.9
6 months (24)	50.4±41.1	68.5±13.4	106.0±70.5
change	−18.2±43.6	−58.9±31.7	17.2±33.4
significance level	NS	<0.05	NS
GI			
baseline (−1)	0.5±0.5	1.2±0.6	1.0±0.8
6 months (24)	0.3±0.2	0.4±0.3	0.9±0.5
change	−0.2±0.4	−0.8±0.5	−0.1±0.2
significance level	NS	<0.05	NS
PI			
baseline (−1)	0.7±0.7	0.6±0.6	1.0±0.9
6 months (24)	0.8±0.7	0.5±0.4	1.3±0.6
change	0.1±0.8	−0.1±0.6	0.3±0.7
significance level	NS	NS	NS

* Week.
PD=probing depths, CAL=clinical attachment level, GCF volume=gingival crevicular fluid volume, GI=gingival index, PI=plaque index; NS=not significant.

same dentist. The PI, GI indices and the GCF scores were recorded at the pre-therapy visit, weekly during the post-operative care up to 6 weeks post-therapy, and re-examined 3 and 6 months after GTR therapy (Fig. 1).

Statistical analysis

The data analysis was performed using the SAS software (SAS Institute Inc. 1989). The clinical parameters were evaluated using mean values and standard deviations. For both of the grouped patient categories an intra-group comparison of the changes between the pre- and 6-months post-therapy clinical setting at healthy and GTR-treated barrier sites was performed and analyzed by Mann-Whitney at a significance level of $\alpha=0.05$. The biochemical data were calculated with the site values for each visit and plotted as medians with the Q1–Q3 quartiles, minima and maxima with outliers exceeding more than 1.5-fold of the individual boxplot height.

Results

Clinical results

The average probing depths (PD) and clinical attachment levels (CAL) with standard deviations for both of the PLA and PGL membranes before and after GTR therapy are displayed in Table 1. The changes in PD from the outset to the six-month reexamination were −2.4±1.4 mm for PLA (M1) and −1.8±1.3 mm for PGL (M2) barrier sites, $p\leqslant 0.05$. The CAL levels yielded statistically significant changes of −1.2±0.9 mm for PLA and −1.4±1.2 mm for PGL membranes, $p\leqslant 0.05$. In addition, a statistically significant 6-months change of −58.9±31.7 was seen for GCF volumes at PLA barrier sites, but not at PGL sites that yielded a GCF change of 17.2±33.4. The gingival index (GI) at PLA-treated sites displayed a significant change of −0.8±0.5 whereas the change at PGL-treated sites of −0.1±0.2 was not statistically significant. For all sites (H, M1, M2), the plaque scores (PI) did not change statistically. In each of the PLA- and PGL-group 1 subject exhibited a moderate membrane exposure of 1 mm at the gingival margin that occured between week 2 and 4, but did not affect the 6-months results. The 6-months course of the single GCF parameters and GI indices at healthy sites (H), PLA barrier sites (M1) and PGL barrier sites (M2) is represented in Table 2.

Biochemical results

Following PLA therapy (M1), the MPO levels significantly increased from 3.20 μU/30 s at baseline to 8.40 μU/30 s (a 2.6-fold increase) at the 6-week post-therapy visit. They declined at the 12-week re-examination. The increase of the MPO activities at PGL-treated molar sites (M2) was moderate and

Table 2. 6-months course of GCF parameters and GI indices at healthy (H), PLA barrier (M1) and PGL barrier sites (M2)

	Weeks								
	−1	1	2	3	4	5	6	12	24
GCF volume (30 s)									
H	68.6±57.4*	79.7±35.2	56.4±42.5	61.6±45.5	86.8±56.9	52.4±30.28	44.9±27.9	58.0±23.5	50.4±41.1
PLA (M1)	127.4±68.5	172.4±27.3	147.2±53.2	150.8±32.9	163.3±16.9	135.3±58.5	122.6±12.7	167.5±24.8	68.5±13.4
PGL (M2)	88.8±33.9	152.3±55.6	124.3±32.2	118.8±86.3	156.5±60.1	96.5±45.9	133.0±49.5	84.5±40.3	106.0±70.5
GI									
H	0.5±0.5	0.3±0.4	0.6±0.5	0.5±0.5	0.5±0.6	0.3±0.5	0.4±0.6	0.5±0.6	0.3±0.2
PLA (M1)	1.2±0.6	1.6±0.6	1.4±0.7	1.5±0.6	1.5±1.0	1.1±0.5	1.2±0.8	1.3±0.5	0.4±0.3
PGL (M2)	1.0±0.8	1.0±0.8	1.3±1.1	1.3±0.7	1.2±0.8	0.9±0.8	1.0±1.4	1.0±1.4	0.9±0.5

* Means±standard deviation. GCF volume=gingival crevicular fluid volume. GI=gingival index.

occurred earlier. GTR treatment with PGL barriers (M2) resulted in an only 1.8-fold increase of the MPO activities from the outset to week 2, followed by an early rebound at the third post-therapy week. The MPO scores at healthy control sites (H) revealed values between 3.10 and 3.50 μU/30 s (Fig. 2).

After GTR treatment with PLA barriers (M1), the GCF βNAH scores increased from 133.20 at the outset to 185.70 μU/30 s (a 1.3-fold increase) at the 4-week visit. At the 5-week examination, they dropped slightly falling below the initial levels 6 months after membrane placement. 2 weeks following GTR therapy, the GCF βNAH activities (PGL) (M2) increased 1.5-fold from the baseline values. At the 3-week post-therapy re-examination, the values decreased and reached the baseline scores at week 4 with values between 89.10 and 110.2 μU/30 s. At healthy molar sites (H) the GCF βNAH levels ranged from 106.7 to 141.3 μU/30 s (Fig. 3).

PLA membrane placement (M1) resulted in a slight increase of the βG levels from the outset (9.10 μU/s) to the 3-week re-examination. At week 5, a 1.8-fold increase from baseline occurred in βG scores (16.40 μU/s). 12 weeks after PLA treatment, the βG levels started to drop and felt under the baseline scores at the 6-months visit. At PGL-treated molar sites (M2), the βG increase was limited to the week 2 appointment (13.50 μU/30s , 1.3-fold from baseline). The βG levels both at PLA and PGL barriers revealed slightly enhanced values throughout the re-examination. At the healthy molar sites (H) the GCF βG measurements ranged from 5.80 to 7.40 μU/30 s (Fig. 4).

Discussion

Data from longitudinal GTR trials demonstrated improvement of clinical periodontal parameters, especially for mandibular buccal class II furcations (Gottlow et al. 1992, Cortellini et al. 1994, Becker et al. 1996, Machtei et al. 1996, Garrett 1996, Garrett et al. 1997). With regard to the clinical parameters, we did not expect any significant differences between both of the membranes. We observed a vertical attachment gain of 1.20 mm at PLA (M1) and 1.40 mm at PGL barriers (M2) in the class II furcation. Our results agree with recent data from Eickholz & Hausmann (1997, 1999) who reported for class II furcation lesions a CAL-v gain of 1.35 mm at the 6-months visit and 1.06 mm at the 2-year re-examination. Becker et al. (1996) reported a 12-months post-treatment CAL-v gain with bioresorbable membranes (Resolut) of 2.10 mm. Differences in clinical outcomes might be related to individual tooth and defect factors. The extent of the horizontal furcation depths (Eickholz 1995), the intraindividual variability of the defects, i.e., the width and height of the furcation access, the tooth endodontic status, mobility or gingival recessions have impact on the healing pattern and improvement of clinical conditions following regenerative therapy (Garrett 1996). Patient-related factors such as smoking, plaque control, maintenance procedures, early wound stability and infection of the barrier with periodontal pathogens might modify the cellular repopulation of the GTR site and contribute to the potential regeneration (Cortellini et al. 1994, 1996, Tonetti et al. 1995).

Data on tissue healing responses fol-

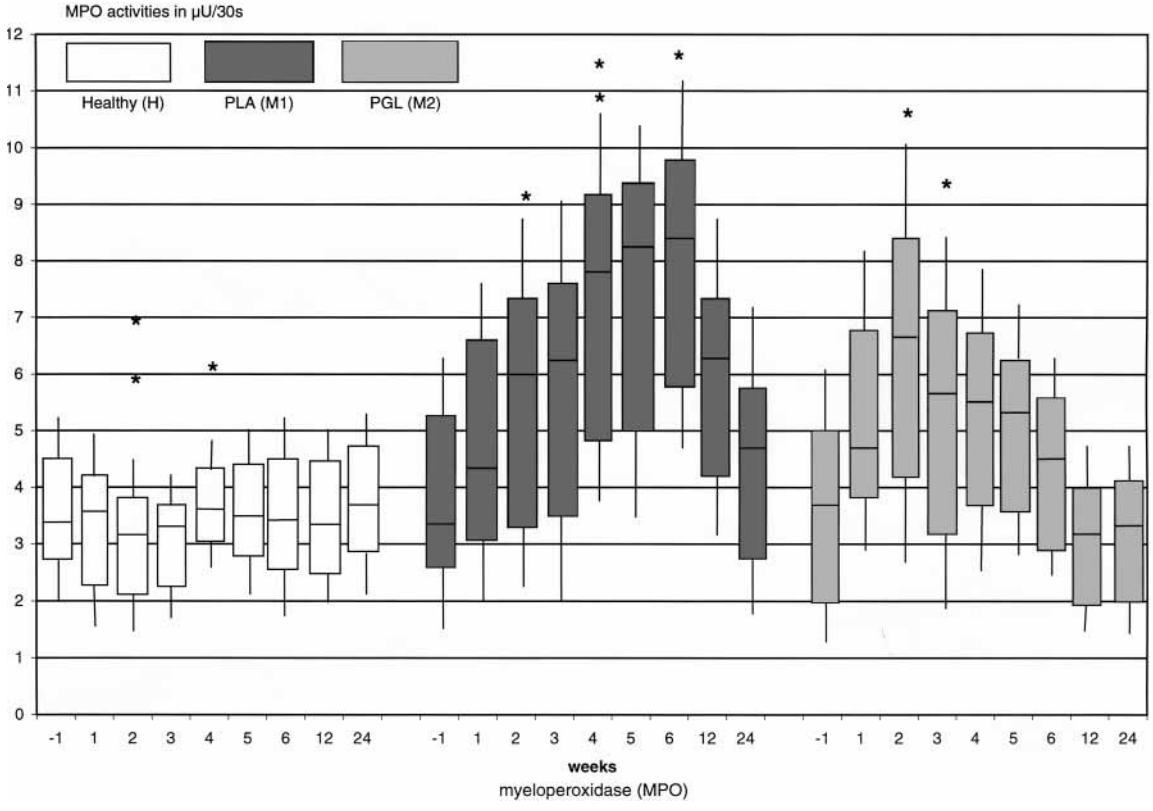

Fig. 2. Boxplots for the medians and Q1–Q3 quartils of GCF myeloperoxidase (MPO) levels at healthy molar (H), PLA barrier (M1) and PGL barrier sites (M2) at baseline and for each of the 6 months re-examination visit. Lines below and above box plots=min, max. * Outliers.

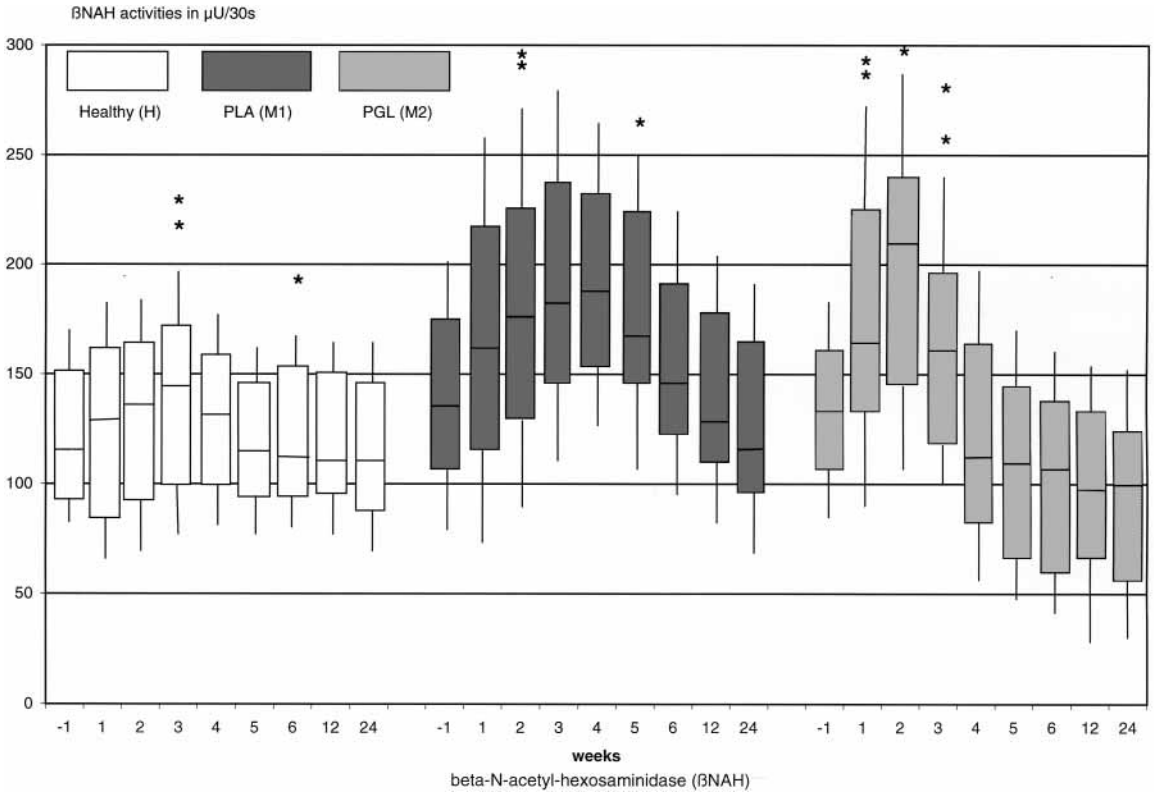

Fig. 3. Boxplots for the medians and Q1–Q3 quartils of GCF beta-N-acetyl-hexosaminidase (ßNAH) levels at healthy molar (H), PLA barrier (M1) and PGL barrier sites (M2) at baseline and for each of the 6 months re-examination visit. Lines below and above box plots=min, max. * Outliers.

lowing regenerative procedures in gingival crevicular fluid from Said et al. (1999) evaluated by matrix-metalloproteinases show that GCF MMP-8 levels increased immediately following surgical and regenerative therapy representing the early wound healing period. They dropped at week 4 to the baseline levels, indicating a collagen and proteoglycan core tissue remodelling on the membranes. We observed a prolonged increase of MPO, βNAH and βG activities at PLA-barrier sites up 6 weeks post-therapy whereas the levels at PGL sites increased only until week 2. This finding might be explained by the material properties of the Guidor barriers (PLA) which represent an enhanced dimensional stability due to the use of the hydrophobe acetyl-tri-N-butyl-citrate-ester (Lundgren et al. 1994). Adverse clinical effects due to foreign body reactions associated with resorbable PLA-barrier defragmentation that include moderate pain, release of caseous-appearing exsudate and soft tissue swelling, as reported by Tatakis & Trombelli 1999, might interfere with the PMN healing responses. However, they are benign and reveal a self-limiting course, and did not occur in our patient population group. It is important to note, that they could be observed more frequently following guided tissue regeneration of recession type defects where the overlying buccal mucosa is of very thin nature. At PGL sites the enzyme activities dropped already at week 3 following membrane placement that suggests an earlier enzymatic hydrolysis of the ester composed matrix of the Vicryl mesh.

Our data on enzyme activities in GTR therapy are preliminary because comparable investigations of GCF factors in GTR therapy are limited. Kennett et al. (1997) reported that proteases from inflammatory cells make up most of the measured enzyme activity in GCF. The biodegradation is always associated with a broad range of cellular responses that regulate the dynamics of membrane resolution (Wakabayashi et al. 1996). The inflammatory mechanisms ought to be moderate and reversible without affecting the regeneration and maturation of the new connective tissues. The extent and quality of the new connective tissue attachment correlate with the inflammatory tissue reactions that occur following GTR therapy (Weigel et al. 1995). Because of the limited number of patients in our study we could not validate our data by further statistical testing.

Within the limitations of the present study, the following conclusions may be drawn: (i) PLA and PGL GTR barriers provide evidence of a barrier-dependent PMN response following membrane placement. (ii) PGL barriers in the treatment of class II furcation defects are associated with an early decrease of the PMN enzymes compared with PLA membranes. (iii) The maximum PMN response occuring at different times does not affect the course of clinical healing.

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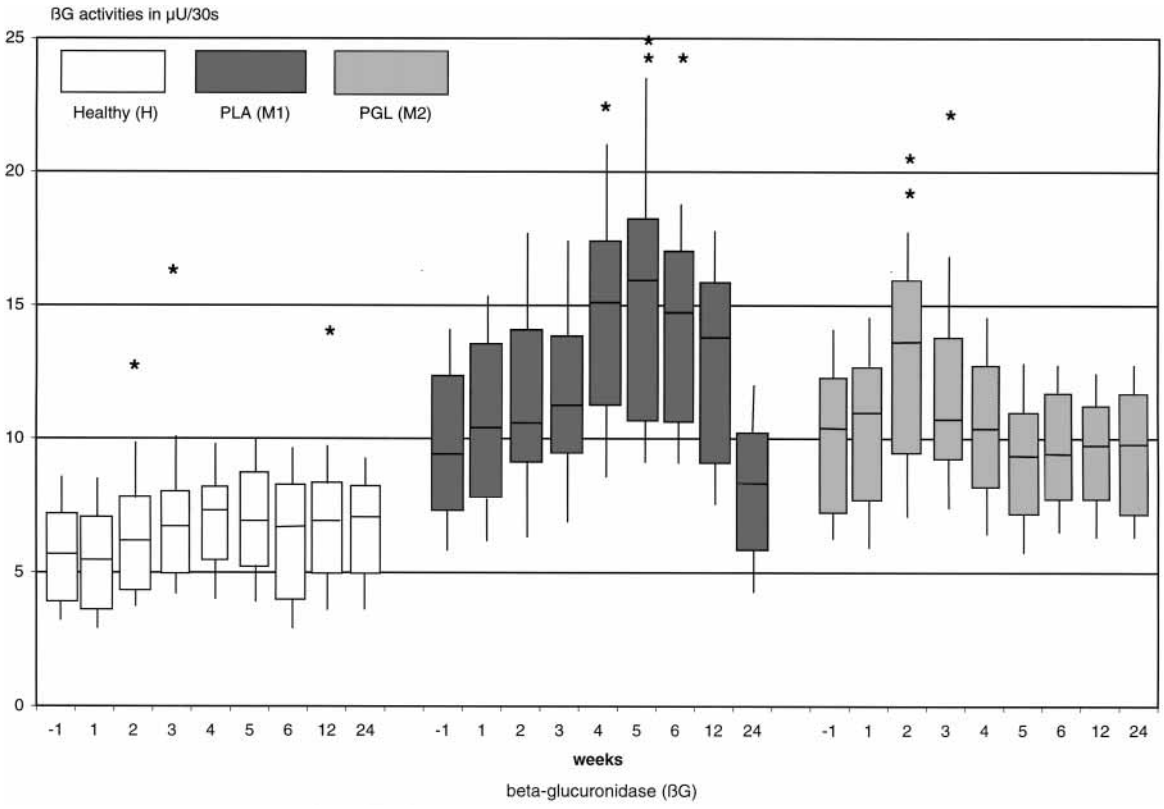

Fig. 4. Boxplots with outliers for the medians and Q1–Q3 quartils of GCF beta-glucuronidase (ßG) levels at healthy molar (H), PLA barrier (M1) and PGL barrier sites (M2) at baseline and for each of the 6 months re-examination visit. Lines below and above box plots=min, max. * Outliers.

assays, and to Professor Thomas E. Van Dyke for his contributions in reviewing the manuscript.

Zusammenfassung

PMN-Freisetzung nach Applikation zweier resorbierbarer GTR-Membranen

Ziel: In vorliegender prospektiver Studie wird die Freisetzung polymorphkerniger neutrophiler Granulozyten (PMN) in der Sulkusflüssigkeit nach Insertion von 2 bioresorbierbaren GTR-Barrieren bei Unterkiefermolaren mit Furkationsbefall Grad II untersucht.
Material und Methoden: Bei 10 Patienten mit behandelter chronischer Parodontitis erfolgte an den 1. Molaren im Unterkiefer mit vestibulärer Furkationsbeteiligung Grad II eine regenerative Therapie mit Polylaktid-Zitronensäureester- (PLA) oder Glykolid-Laktid-Copolymer-Membranen (PGL). Kontralateral gelegene, nicht erkrankte Molaren dienten als gesunde Kontrollparodontien. Die PMN-assoziierte inflammatorische Gewebeantwort wurde zu Therapiebeginn, wöchentlich bis zur 6. postoperativen Kontrollwoche sowie 12 und 24 Wochen nach GTR-Therapie anhand der Myeloperoxidase (MPO)-, β-Glucuronidase (βG)- und β-N-Acetyl-Hexosaminidase (βNAH)-Aktivitäten in der Sulkusflüssigkeit beurteilt.
Ergebnisse: Die Enzymaktivitäten zeigten einen kontinuierlichen Anstieg bis zur 6. Woche. Ab der 6. Kontrollwoche fielen die gemessenen Enzymlevel und erreichten in der 12. und 24. Woche die Basiswerte. An den PGL-behandelten Parodontien veringerten sich die Enzymaktivitäten frühzeitiger. Im Vergleich zu den Kontrollflächen erreichten die MPO-, βG- und βNAH-Aktivitäten unterschiedliche Maximumlevel in der 2. und 3. Woche (PGL) und 4., 5. und 6. Woche (PLA). Die klinischen Parameter wiesen für beiden GTR-Barrieren eine signifikante Verbesserung auf.
Schlußfolgerungen: Die Freisetzung PMN-assozierter Enzyme in der Sulkusflüssigkeit ist Indikator der frühen Weichgewebsheilung nach Insertion bioresorbierbarer Membranen. Die PMN-Antwort ist membranabhängig mit maximaler Freisetzung zu unterschiedlichen Zeitpunkten. Die klinische Heilung wird durch die Wirtsantwort jedoch nicht meßbar beeinflußt.

Résumé

Les réponses des PMN suivant l'utilisation de 2 membranes GTR biodégradables

But: Dans cette essai prospectif, la réponse des PMN suivant le placement d'une membrane barrière GTR résorbable a été évaluée au niveau de lésions de furcation de degré II de la mandibule.
Matériaux: Chez 10 patients ayant une parodontite chronique traitée, les premières molaires inférieures avec des furcations de degré II en vestibulaire ont été sélectionnées et traitées avec soit une membrane GTR ester-acide citrique-polylactique (PLA) ou copolymère-lactique-glycolide (PGL). Les sites molaires sains contralatéraux ont été utilisés comme contrôles non-traités. La réponse tissulaire inflammatoire des PMN a été mesurée lor de l'examen initial et toutes les semaines jusqu'à 6 semaines après la chirurgie, et ensuite à 12 et 24 semaines en utilisant la myéloperoxydase (MPO) du fluide créviculaire gingivale la β-glucuronidase (βG) et la β-N-acétylhexosaminidase (βNAH).
Résultats: Les niveaux d'enzymes augmentaient entre l'examen de départ et l'examen à 6 semaines. Ensuite les niveaux d'enzymes diminuaient jusqu'à atteindre des scores semblables à ceux de l'examen initial tant à 12 qu'à 24 semaines. Au niveau des sites PGL, les niveaux d'enzymes diminuaient plus tôt. Comparés aux sites contrôles sains, les tests MPO, βNAH et βG ont révélé différents niveaux maximum aux semaines 2 et 3 pour PGL, et aux semaines 4, 5 et 6 pour PLA. Pour les deux types de barrières, les paramètres cliniques révélaient une amélioration soutenue après le traitement.
Conclusions: La libération d'enzymes PMN suivant le placement de membranes biorésorbables reflète le processus de guérison préco-

ce des tissus mous. La réponse PMN est dépendante de la membrane-barrière avec une réponse maximale se déroulant à différents moments. Cependant, la réponse de l'hôte n'affectait pas de manière appréciable le déroulemement de la guérison clinique.

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