J Clin Periodontol 2012; 39: 448–456 doi: 10.1111/j.1600-051X.2012.01861.x

Journal of Clinical Periodontology

Regenerative therapy of infrabony defects with or without systemic doxycycline. A randomized placebo-controlled trial

Lasse Röllke[1], Beate Schacher[1], Martin Wohlfeil[1], Ti-Sun Kim[2], Jens Kaltschmitt[2], Jörg Krieger[2], Diana M. Krigar[2], Peter Reitmeir[3] and Peter Eickholz[1]

[1]Department of Periodontology, Center for Dental, Oral, and Maxillofacial Medicine (Carolinum), Johann Wolfgang Goethe-University Frankfurt/Main, Frankfurt/Main, Germany; [2]Section of Periodontology, Department of Conservative Dentistry, Clinic for Oral, Dental and Maxillofacial Diseases, University Hospital Heidelberg, Heidelberg, Germany; [3]Institute of Health Economics and Health Care Management, Helmholtz Zentrum München, Neuherberg, Germany

Röllke L, Schacher B, Wohlfeil M, Kim TS, Kaltschmitt J, Krieger J, Krigar DM, Reitmeir P, Eickholz P. Regenerative therapy of infrabony defects with and without postsurgical doxycycline. A randomized placebo-controlled trial. J Clin Periodontol 2012; 39: 448–456. doi: 10.1111/j.1600-051X.2012.01861.x.

Abstract

Aim: Comparison of regenerative therapy of infrabony defects with and without administration of postsurgical systemic doxycycline (DOXY).

Methods: In each of 61 patients one infrabony defect was treated with enamel matrix derivative (EMD), EMD plus filler or membrane at two centres. By random assignment patients received either 200 mg DOXY per day or placebo (PLAC) for 7 days after surgery. Prior to and 6 months after surgery probing pocket depths (PPD) and vertical attachment level (PAL-V) were obtained.

Results: Fifty-four patients (DOXY: 27; PLAC: 27) were re-examined after 6 months and had been treated exclusively with EMD. Seven to 8 days after surgery 81% of defects in both groups showed complete flap closure. In both groups significant ($p < 0.001$) PPD reduction (DOXY: 3.87 ± 1.44 mm; PLAC: 3.67 ± 1.30 mm) and PAL-V gain (DOXY: 3.11 ± 1.50 mm; PLAC: 3.32 ± 1.83 mm) were observed. However, the differences failed to be statistically significant (PPD: 0.20; $p = 0.588$; PAL-V: 0.21; $p = 0.657$).

Conclusions: Two hundred milligram systemic DOXY administered for 7 days after therapy of infrabony defects with EMD failed to result in better PPD reduction and PAL-V gain compared with PLAC which may be due to low power (50%) and, thus, random chance.

Key words: doxycycline; early wound-healing; infrabony defects; periodontal disease; regenerative therapy: enamel matrix derivative

Accepted for publication 21 January 2012

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests. Six months data management and radiographic measurements were performed as part of a thesis for a Master of Science in Periodontology and Implant Therapy [German Society of Periodontology (DGP) and Dresden International University (DIU)]. This study was in part funded by the authors and their institutions. Study medication, patients' insurance, and monitoring were provided by August Wolff GmbH & Co, Bielefeld, Germany. Microbiological analysis was funded by GABA International, Münchenstein, Switzerland. Furthermore, funding was provided by the DGP GABA research fund and the Medical Faculty of the Johann Wolfgang Goethe-University Frankfurt, Germany. L. Röllke was granted a master scholarship by the DGP which was funded by Straumann GmbH, Freiburg, Germany.

In many clinical studies, regenerative therapy using non-resorbable expanded polytetrafluoroethylene (ePTFE), bioabsorbable barriers as well as enamel matrix derivative (EMD) were shown to be more effective than open flap debridement (OFD) in infrabony defects (Cortellini et al. 1995b, 1998, 2001, Eickholz et al. 1998, Tonetti et al. 1998, Tonetti et al. 2002, Pontoriero et al. 1999, Needleman et al. 2001, Esposito et al. 2009, Wachtel et al. 2003, Francetti et al. 2005).

Periodontal wound-healing is disturbed by bacterial plaque after periodontal surgery in general (Nyman et al. 1977) and after regenerative procedures using membranes in particular (Nowzari et al. 1996). Systemic antibiotics may reduce the number of bacterial pathogens in the wound and enhance healing. Thus, many clinical studies on regenerative therapy using membranes or EMD report post or perisurgical antibiotic regimens: e.g. 250 mg tetracycline four times daily for 1 week (Cortellini et al. 1995a, Cortellini et al. 1995b), 1.5 g amoxicillin once a day for 1 week (Cortellini et al. 1998), 200 mg doxycycline (DOXY) once a day for 1 week (Tonetti et al. 1998, Tonetti et al. 2004, Cortellini et al. 2001, Cortellini & Tonetti 2001), 1 g amoxicillin plus clavulanic acid once a day for 8 days (De Sanctis & Zucchelli 2000), 3 g amoxicillin once 30–60 min. before surgery (Eickholz et al. 2000). Several studies failed to observe better clinical results after use of antibiotics additionally to use of membranes: propicilline (Eickholz et al. 1998), metronidazole & ciprofloxacin (Vest et al. 1999), amoxicillin & metronidazole (Sculean et al. 2001). It seems that additional use of those antibiotics evaluated with regenerative therapy does improve neither clinical results nor prognosis. However, up to now tetracycline derivatives that are used most frequently after regenerative periodontal therapy have not been evaluated for their additional benefit.

Therefore, the purpose of this randomized placebo-controlled clinical trial was to compare the clinical outcomes of regenerative periodontal therapy of infrabony defects with or without postsurgical administration of 200 mg DOXY once a day for 7 days.

Material and Methods

Patients

From April 2007 until February 2009 all patients undergoing periodontal treatment at the Department of Periodontology, Center of Dental, Oral, and Maxillofacial Medicine (Carolinum), Johann Wolfgang Goethe-University Frankfurt/Main and the Section of Periodontology, Department of Conservative Dentistry, Clinic for Oral, Dental and Maxillofacial Diseases, University Hospital Heidelberg were screened for this study (Fig. 1).

All patients were asked about current and past smoking. They were categorized as former smokers if they had quit smoking for at least 5 years, individuals who had quit smoking for <5 years were categorized as current smokers (Lang & Tonetti 2003).

Inclusion criteria

Patient-related

- Adults ($\geq$18 years of age) with moderate to severe periodontitis
- Completed initial periodontal treatment consisting of oral hygiene instruction, scaling and root planing under local anaesthesia according to the concept of full-mouth disinfection and re-evaluation of the tissue response and the patients' plaque control 3 months later. Sites with infrabony defects and persisting pockets [probing pocket depth (PPD) $>$5 mm and bleeding on probing (BOP)] that occurred at re-evaluation or supportive periodontal

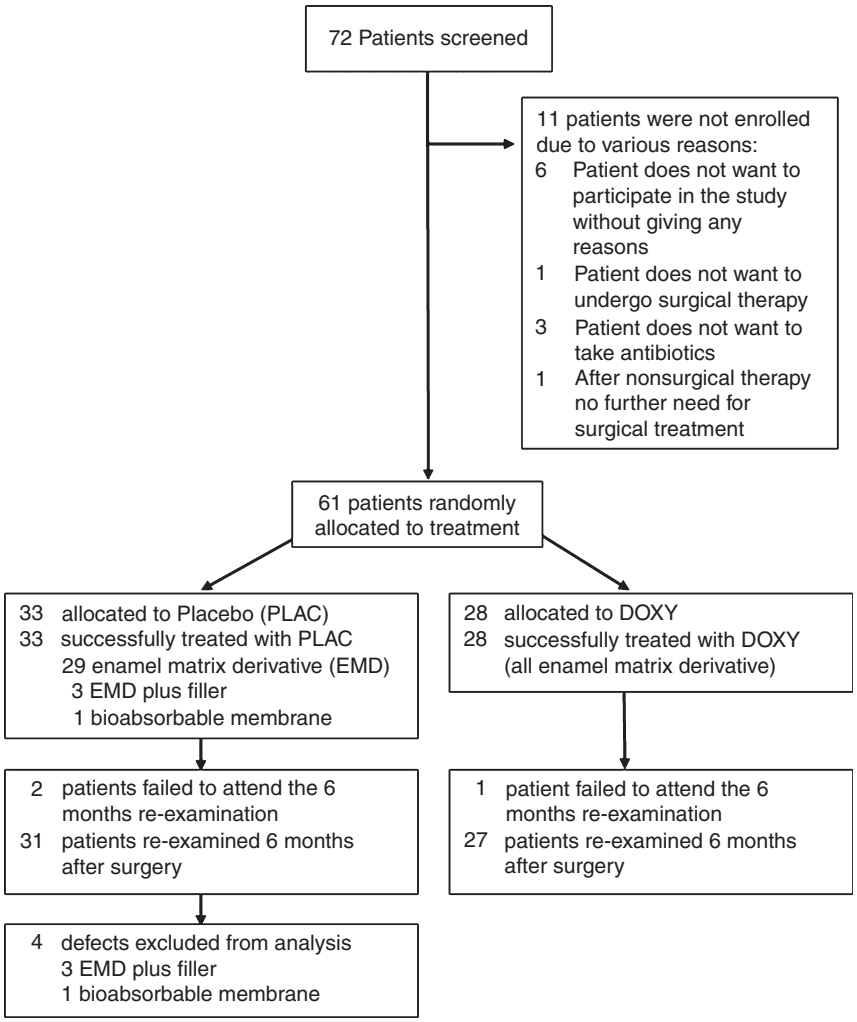

Fig. 1. Fow-chart.

treatment (SPT) underwent surgery.
- At least one radiographically detectable infrabony lesion (Weinberg & Estrow 2000)
- Good physical health and effective individual plaque control [Full-mouth-plaque score PCR $\leq 30\%$ (O'Leary et al. 1972)]
- Only women in childbearing age [< 45 years (Bundeszentrale für politische Bildung 2006)] who provide contraception from screening to at least 1 week after surgery
- Informed written consent.

Site-specific

• Interproximal angular defects on single-rooted teeth or multi-rooted teeth without furcation involvement, radiographic infrabony component (INFRA) ≥ 4 mm, vertical probing attachment loss (PAL-V) > 6 mm and PPD ≥ 6 mm.

Exclusion criteria

- Known allergies to tetracycline or any components of the active drug or PLAC
- Severe liver dysfunction
- Local or systemic antibiotic treatment during the last 3 months before surgery
- kidney dysfunction
- Being medicated with barbiturate, carbamazepin, diphenyhydantoine, sulfonyl-urea, methoxyfluran, cyclosporin A, theophylline, isotretionin
- Chronic alcohol abuse
- Anticoagulative therapy
- Need for antibiotic endocarditis prophylaxis
- Pregnancy (women in childbearing age were tested; Keul-o-test®; BGT BioGenTechnologies GmbH, Steinfurt, Germany)
- Lactation

The trial was approved by the Institutional Review Boards for Human Studies of the Medical Faculties of the Johann Wolfgang Goethe-University Frankfurt/Main (159/06) and the University of Heidelberg (ABmu-179/2006) as well as the German Federal Drug Authority (Bundesamt für Arzneimittel und Medizinprodukte). All participating individuals were informed on risks, benefit and the procedures of the study. All gave written informed consent. The study is registered under the EudraCT-Nr. 2006-001367-36 and under the number NCT01030666 at "http://www.clinicaltrials.gov".

Randomization and examiner blinding

The manufacturer packed and labelled the study medication before handing it over to the sponsor (PE) who distributed it to the study centres. Randomization was performed also by the manufacturer of the DOXY (August Wolff GmbH & Co. Arzneimittel, Bielefeld, Germany). For each patient, treatment assignment was made according to a table of random digits using a block randomization regarding smoking (current smoking: smokers/former and never smoking: non-smokers) stratified for centre. This assignment was stored in sealed envelopes. Medication (DOXY/PLAC) was assigned according to smoking status and schedule of recruitment. Thus, blinding of therapists and examiners was provided.

Clinical measurements

During the entire study all clinical measurements were performed by a maximum of two trained and experienced examiners at a time at each participating centre, who were blinded for the assignment of therapy modes (DOXY/PLAC). Due to changes in staff between April 2007 until February 2009 a total of six examiners performed clinical measurements (Frankfurt: BS, MW, PE; Heidelberg: JK, DK, JK). The following clinical parameters were assessed at six sites per tooth (mesiobuccal, midbuccal, distobuccal, distolingual, midlingual, mesiolingual) at baseline and 6 months after surgery: Gingival Index (GI) (Löe 1967), Plaque Index (PlI) (Löe 1967), PPD and PAL-V to the nearest 0.5 mm using a straight manual periodontal probe (PCPUNC 15; Hu Friedy, Chicago, IL, USA). As reference for the PAL-V measurements, the cemento-enamel junction (CEJ) was used. If the CEJ was destroyed by a restoration the restoration margin (RM) served as reference.

For examiner calibration repeated PPD and PAL-V measurements were performed at one tooth of each of five patients unrelated to this study by each examiner.

Radiographic examination

Standardized radiographs were obtained of test teeth by modified film holders (VIP 2 Positioning; UpRad Corp., Fort Lauderdale,FL, USA) (Duckworth et al. 1983, Eickholz et al. 1996). These were done routinely in the clinical setting, after anti-infective and immediately before surgical treatment. Intra-oral size 0 and 2 dental films (Insight; Eastman Kodak Co., Rochester, MN, USA) were exposed using an x-ray source with 7 mA and 60 kVp (Heliodent DS; Sirona, Bensheim, Germany) and developed under standardized conditions (XR24pro; Dürr Dental GmbH, Bietigheim-Bissingen, Germany).

Radiographic evaluation

All radiographs were evaluated by one examiner who was blinded to the clinical results and to the time point the particular radiographs had been taken (Eickholz et al. 2004).

All radiographs were digitalized using a computer program (SIDEXIS nextGeneration 1.51; Sirona, Bensheim, Germany) and a flatbed scanner (Microtek ScanMaker 4; Microtek, Hsinchu, Taiwan) with 600 dpi resolution and 8 bit grey values. The image files were stored as TIFF files and analysed using the computer program SIDEXIS and a 19' flat screen (Totoku CCL 192 plus; Totoku Electric, Ueda, Japan) in a particular room under exclusion of natural or artificial light except the screen.

For evaluation the analysing tool of the program SIDEXIS was used. The image files were opened and magnified by using the function "zoom" once. Then the distances CEJ/RM to alveolar crest (AC), CEJ/RM to bony defect (BD), and the depth of the INFRA were measured. The definition of these radiographic landmarks has been published in detail before (Eickholz et al. 2004, Pretzl et al. 2009, Meyle et al. 2011).

Microbiological examination

Immediately before and 14 ± 2 days after surgery subgingival plaque samples were taken from the same site of each tooth selected for regenerative therapy. After insulation with cotton rolls a paper point was inserted at the site with the deepest pocket at baseline for 20 s and after that transferred to a transport vial. Samples were analysed by a commercially available real-time PCR test (Meridol Paro Diagnostik; GABA GmbH, Lörrach, Germany) for *Aggregatibacter actinomycetemcomitans (A.a.)*, *Porphyromonas gingivalis (P.g.)*, *Tannerella forsythia (T.f.)*, *Treponema denticola (T.d.)*, *Fusobacterium nucleatum (F.n.)* and *Prevotella intermedia (P.i.)*. The detection limit of this test is 10^2.

Periodontal surgery

Infrabony defects were treated with the following modalities of regenerative therapy: bioabsorbable barrier membranes (Vicryl Membran; Ethicon GmbH & Co. KG, Norderstedt, Germany) or application of enamel matrix derivative (EMD: Emdogain; Institut Straumann AG, Basel, Switzerland) that both could be combined with the use of a filler (Bio-Oss® ø 0.25–1 mm or Bio-Oss Collagen®; Geistlich AG, Wolhusen, Switzerland). The decision for membrane type or EMD with or without filler was up to the clinician. For each defect surgery consisted of an identical procedure. Following an intra-crevicular incision a mucoperiosteal flap was reflected to a height of 5 mm exposing the bony margin of the defect and allowing complete visualization of the infrabony lesion (Zappa 1991). The flap was designed according to the modified or simplified papilla preservation technique to obtain primary closure of the wound and the membrane respectively (Cortellini et al. 1995a, 1999). The flap was extended at least one tooth mesially and distally of the defect side. After complete removal of inflammatory granulation tissue, the root surfaces were thoroughly scaled and root planed. Before closure of the defect the following intra-surgical parameters were measured to the nearest 0.5 mm using the above mentioned probe:

- At six sites per tooth the distances CEJ/RM to AC and CEJ to the most apical extension of the bony defect (BD).
- Infrabony component (INFRA) as well as the 3-, 2- and 1-wall component of the defect (Cortellini et al. 1995b).

When using EMD the root surfaces facing the infrabony defect were conditioned with EDTA (prefgel; Institut Straumann AG, Basel, Switzerland) for 2 min. first. Then the EDTA was washed out with plenty of saline. After drying the surfaces EMD was applied starting at the bottom of the defect and proceeding coronally. If membranes were used they were adapted to the root surface if necessary by a suture around the root trunks. The mucoperiosteal flaps were repositioned tension-free to cover the defect completely. The flaps were fixed with synthetic non-resorbable (Gore Tex 5-0, 6-0; W. L. Gore & Associates, Flagstaff, AZ, USA) sutures. Surgery was performed by six authors.

Postsurgical care

All patients took a postsurgical medication once a day for 7 days after regenerative periodontal therapy:

- Test: 200 mg DOXY.
- Control: 200 mg PLAC.

Doxycycline and PLAC were packaged identically and stored in sealed envelopes that were marked with the randomization code. Patients received DOXY or PLAC after regenerative surgery was accomplished. Patient and clinician were blinded. For safety reasons (e.g. in case of adverse events) a sealed envelope was stored at each participating centre that contained the randomization list.

Furthermore, all patients were advised to rinse with a 0.12% chlorhexidine gluconate solution (ParoEx; Butler, Kriftel, Germany) for 2 min. twice daily for 5–7 weeks after surgery. During this time, all patients had to refrain from individual mechanical plaque control and thus were seen every other week for control and gentle cleaning of the teeth. In addition, 400 mg ibuprofen qd was prescribed for patient's comfort if necessary. If soft tissue dehiscence was noted the patient was advised to use a 1% chlorhexidine gluconate gel (Chlorhexamed 1% Gel; GlaxoSmithKline, Bühl, Germany) twice daily. Sutures were retained as long as they maintained closure.

Patient-centred parameters

Seven to 8 days after surgery wound-healing was classified according to the Early Wound-Healing Index (EHI, Wachtel et al. 2003). Additionally patients were asked if they had experienced postsurgical pain (yes/no). If so, patients were asked (i) about the intensity (10 cm Visual Analogue Scale/VAS) and (ii) duration (h) of pain. (iii) If patients had taken pain killers for pain relief the number was documented. If the patients had investigational medication left, the tablets were taken back and their number documented.

Fourteen ± 2 days and 21 ± 3 days after surgical therapy patients were asked about pain (yes/no, VAS) again. Correspondingly the inter-dental papilla's covering the defects was examined and dehiscence recorded if present.

Supportive periodontal therapy

Postoperative visits were scheduled weekly for the first month. Wound-healing and complications were documented. Thereafter, patients were placed on a maintenance schedule including oral hygiene instruction and professional tooth cleaning once every 3 months.

Statistical analysis

Sample size calculation was based on an observed standard deviation for PAL-V gain (1.6 mm) in a study comparing regenerative therapy with and without antibiotics (Sculean et al. 2001). To detect a mean difference of 1.0 mm with a test power of 80% and a type 1 error $\alpha < 0.05$ a minimal sample size of 84 patients had to be recruited, 42 for each group. Thus, it was decided to recruit a total of 90 patients.

Table 1. Plaque Index (PlI), Gingival Index (GI) and probing pocket depth (PPD) at baseline and 6 months after surgery

	PlI			GI			PPD (mm)		
	DOXY $n = 27$	PLAC $n = 27$	*p-Value*	DOXY $n = 27$	PLAC $n = 27$	*p-Value*	DOXY $n = 27$	PLAC $n = 27$	*p-Value*
Baseline	0.74 ± 0.98	0.37 ± 0.57	0.243	1.52 ± 0.75	1.26 ± 0.94	0.358	8.06 ± 1.82	7.85 ± 1.53	0.658
6 Months	0.93 ± 0.92	0.74 ± 0.90	0.443	0.67 ± 0.88	0.89 ± 0.93	0.372	4.19 ± 1.29	4.19 ± 1.40	1.000
Change	0.19 ± 1.00	0.37 ± 0.93	0.599	−0.85 ± 1.03	−0.37 ± 0.93	0.074	−3.87 ± 1.44	−3.67 ± 1.30	0.588
	0.374	0.045		0.001	0.040		<0.001	<0.001	

The patient was defined as statistical unit. PAL-V gain and early wound-healing were used as primary and PPD reduction, bony fill as well as microbiology as secondary outcome variables. All other parameters (PlI, GI, pain) were control variables. Baseline patient characteristics were described as means ± SD (age, PCR) or frequencies/percentages (gender, smoking). All clinical parameters were calculated as means ± SD (PlI, GI, PPD, PAL-V, INFRA). Patient-centred parameters were provided as means ± SD (EHI, VAS, pain duration, number of pain killers taken) or frequencies/percentages (frequency of pain).

After controlling for normal distribution PAL-V gain and PPD reduction for the two test groups (DOXY/PLAC) were compared using an independent *t*-test. Intra-group comparisons (baseline/6 months) were calculated using the paired *t*-test. Inter- and intra-group comparisons for PlI, GI, radiographic bone and microbiological parameters were calculated using Mann–Whitney *U*- and Wilcoxon-test respectively. All bacterial counts were log-transformed. If the respective bacterium was not detected the lower detection limit of the real time PCR (i.e. 10^2) was entered into analysis.

A multiple regression model was calculated for the dependent variable PAL-V gain with the independent variables baseline clinical parameters (PAL-V, PPD, GI, PlI), postsurgical medication (DOXY/PLAC), defect parameters (INFRA,% 1, 2, 3 wall component), baseline *A. actinomycetemcomitans*, age, gender, centre, smoking, EHI. Gender, centre and smoking habits were defined by indicator variables.

All patients who were recruited, treated according to the study protocol, and attended the 6 month re-examination were considered for analysis (per protocol analysis). Statistical analysis was performed using a PC program (Systat™ for Windows Version 10, Systat Inc. Evanston, IL, USA). Including all studies comparing EMD treatment with or without antibiotics a meta-analysis was calculated (Comprehensive Meta-Analysis; Biostat, Englewood, NJ, USA).

Results

Patients

Due to the fact that the investigational drug expired before the estimated sample size was reached, only 61 patients were included in the study (Frankfurt: 43; Heidelberg: 18). Three patients failed to attend the 6 months re-examination. The majority of infrabony defects (54) were treated exclusively with EMD (Emdogain®; Straumann, Basel, Switzerland). In three defects (Frankfurt) EMD was combined with fillers. In one patient (Heidelberg) a bioabsorbable barrier was used. The later four defects were all in the PLAC group and excluded from analysis to avoid confounding.

Patients in the PLAC group (50.3 ± 10.2 years) were on average 3.5 years younger than in the DOXY group (53.8 ± 10.3 years) ($p = 0.218$). Current smoking (DOXY: 26%; PLAC 19%; $p = 0.745$) and baseline PCR (DOXY: 18.3 ± 7.7; PLAC: 19.0 ± 7.7; $p = 0.737$) were well balanced in both groups. However, there were significantly more females in the PLAC (70%) than in the DOXY group (41%) ($p = 0.028$).

Clinical and radiographic parameters

Plaque Index, GI and PPD at baseline and 6 months after surgery are given in Table 1. Intra-surgical defect characteristics are given in Table 2. In both groups statistically significant ($p < 0.001$) PAL-V gain (DOXY: 3.11 ± 1.50 mm; PLAC: 3.32 ± 1.83 mm) (Table 3) was

Table 2. Baseline defect characteristics

	DOXY $n = 27$	PLAC $n = 27$	*p-Value*
Cemento-enamel junction to bony defect (mm)	10.30 ± 2.41	10.48 ± 2.56	0.721
Intra-bony component (mm)	6.59 ± 2.72	6.70 ± 3.08	0.986
3 wall component (mm)	3.41 ± 1.74	3.00 ± 2.35	0.379
2 wall component (mm)	2.11 ± 2.02	2.15 ± 3.12	0.425
1 wall component (mm)	1.07 ± 1.06	1.56 ± 1.81	0.504

Table 3. Vertical attachment level (mm) at baseline, 6 months after surgery and change 6 months after surgery

	DOXY $n = 27$	PLAC $n = 27$	*p-Value*
Baseline	9.13 ± 1.97	9.17 ± 1.80	0.943
6 Months	5.98 ± 1.76	5.85 ± 1.76	0.788
Change	3.11 ± 1.50	3.32 ± 1.83	0.657
p-Value	<0.001	<0.001	

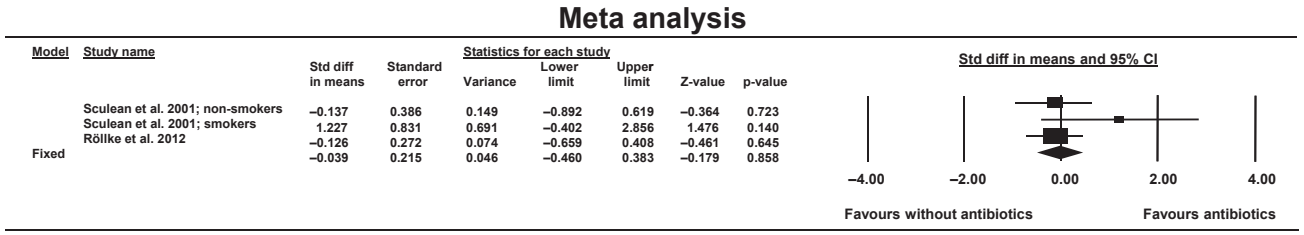

Fig. 2. Forest plot of meta-analysis including 88 subjects (Sculean et al. 2001: smokers: 7 smokers/3 with antibiotics; 27 non-smokers/14 with antibiotics); test power 72%.

Table 4. Backward stepwise multiple regression analysis: vertical attachment gain 6 months after surgery in relation to patient and defect parameters

Dependent variable: vertical attachment gain (mm); $n = 54$; $R^2 = 0.577$; R^2-adjusted = 0.543; standard error of estimate = 1.123				
	b	SE(*b*)	T	*p-Value*
Constant	−1.449	0.834	−1.738	0.088
Baseline probing depth	0.662	0.093	7.090	<0.001
Current smoker	−0.850	0.378	−2.246	0.029
Early Wound-Healing Index	−0.486	0.171	−2.838	0.007
Female gender	0.693	0.310	2.231	0.030

SE, standard error.
Analysis of variance: $p < 0.001$.

observed. However, the difference in PAL-V gain between both groups failed to be statistically significant. Post-hoc analysis revealed a test power of 50%. Meta-analysis including Sculean et al. 2001 and this study also failed to detect better PAL-V gain in the antibiotic group with 72% power (Fig. 2). For Eickholz et al. (1998) group means were not accessible any more. Baseline PPD and female gender influenced PAL-V gain positively, current smoking and EHI negatively (Table 4). Intra-examiner variability was within 0.5 mm in 95% of PPD and 88% of PAL-V measurements. Radiographic parameters are given in Table 5. Two radiographs in the DOXY group and one in the PLAC group could not be evaluated.

Patient-centred parameters

Seven to 8 days after surgery 81% of defects in both groups showed complete flap closure. None of the defects exhibited incomplete flap closure with complete necrosis of the inter-proximal tissue. Analysis failed to find statistically significant differences between the groups regarding EHI (Table 6).

Table 5. Linear distances between CEJ/RM-BD, CEJ/RM-AC and INFRA (mm) at baseline, 6 months after surgery, and change 6 months after surgery

	DOXY $n = 25$	PLAC $n = 26$	*p-Value*
CEJ/RM-BD			
Baseline	8.84 ± 2.62	9.00 ± 3.00	0.828
6Months	7.38 ± 1.63	7.77 ± 1.78	0.486
Change	1.46 ± 2.62	1.23 ± 2.76	0.977
p-Value	0.005	0.026	
CEJ/RM-AC			
Baseline	5.06 ± 1.49	5.12 ± 1.69	0.992
6Months	4.99 ± 1.34	5.35 ± 1.82	0.386
Change	0.07 ± 1.11	−0.23 ± 0.98	0.585
p-Value	0.647	0.304	
INFRA			
Baseline	4.82 ± 2.69	5.09 ± 3.18	0.962
6Months	3.36 ± 1.60	3.57 ± 1.98	0.720
Change	1.47 ± 2.37	1.51 ± 2.60	1.000
p-Value	0.004	0.001	

CEJ, cemento-enamel junction; RM, restoration margin; BD, bone defect; AC, alveolar crest.

Microbiology

The baseline subgingival plaque sample of one patient in the PLAC group was missing at the time of analysis. Fourteen ± 2 days after surgery her sample was above the detection limit of the test (positive) for *A.a.* At baseline bacterial numbers for *A.a.* were positive only in three individuals per group. Fourteen ± 2 days after surgery still three individuals were positive in the PLAC and two in the DOXY group: in three patients *A.a.* had persisted, in two it had disappeared and in one it had risen above the detection limit (Table 7).

Discussion

Systemic antibiotics are commonly used after regenerative therapy with membranes (Cortellini et al. 1995a, b, Cortellini et al. 1998, Tonetti et al. 1998, Tonetti et al. 2004, Cortellini et al. 2001, Cortellini & Tonetti 2001, De Sanctis & Zucchelli 2000, Eickholz et al. 2000) and EMD (Sculean et al. 1999, 2001, Gurinsky et al. 2004, Cortellini & Tonetti 2009). Evidence, however, for added benefit of systemic antibiotics to the results of regenerative therapy is scarce (Mombelli et al. 1996). Most studies failed to observe better clinical results after use of additional antibiotics (Eickholz et al. 1998, Vest et al. 1999, Sculean et al. 2001). Mombelli et al. (1996) and Vest et al. (1999) studied furcation defects, Eickholz et al. (1998) and Sculean et al. (2001) infrabony lesions. To date and to the best of our knowledge this is the first study to evaluate the effect of systemic

Table 6. Early Wound-Healing Index and patient-centred parameters

Early Wound-Healing Index	DOXY $n = 27$	PLAC $n = 27$	*p-Value*
1	17	15	
2	5	7	
3	4	3	
4	1	2	0.817
EHI (mean)	1.6 ± 0.9	1.7 ± 1.0	0.631
Pain 7 days (*n*/%)	12/44	18/67	0.239*
VAS at 7 days	1.3 ± 1.7	2.4 ± 2.2	0.170*
Pain duration (h)	10.5 ± 24.8	26.7 ± 46.5	0.243*
Pain killers taken (*n*)	0.2 ± 0.4	1.4 ± 2.8	0.093*
Pain 14 days (*n*/%)	1/4	1/4	1.000
Pain 21 days (*n*/%)	0/0	1/4	1.000

EHI, Early Wound-Healing Index; DOXY, doxycycline; PLAC, placebo; VAS, Visual Analogue Scale.
*adjusted for gender.

DOXY after regenerative periodontal therapy in a randomized placebo-controlled trial.

All infrabony defects in this trial were treated exclusively with EMD. Thus, conclusions can only be drawn for therapy using EMD. Consequently, to benchmark the results at 6 months of the present study, comparisons with other studies on EMD for treatment of infrabony defects are appropriate. This clinical trial revealed similar or better PAL-V gain in infrabony defects 6 months after therapy using EMD as compared with results reported by other authors 6–8 months after EMD therapy in infrabony defects with unknown and less favourable baseline INFRA. In some of these studies, systemic antibiotics were prescribed (200 mg DOXY at the first postsurgical day and then 100 mg per day for 3 weeks [Heijl et al. 1997,] and for 10 days [Gurinsky et al. 2004,] or 375 mg amoxicillin and 275 mg metronidazole three per day for 7 days [Sculean et al. 1999,] respectively) or none were used (Wachtel et al. 2003, Chambrone et al. 2007, Jepsen et al. 2008). Those studies reporting the smallest PAL-V gain 6 months after use of EMD included exclusively 1- and 2-wall as well as circumferential defects (Heijl et al. 1997, Jepsen et al. 2008). Characteristics of infrabony defects have been shown to influence periodontal healing after regenerative therapy before (Cortellini et al. 1998, Eickholz et al. 2004).

Table 7. Microbiological parameters at baseline and 14 ± 2 days after surgery

	DOXY $n = 27$	PLAC $n = 27$	*p*
Aggregatibacter actinomycetemcomitans			
Baseline	2.25 ± 0.95	2.27 ± 0.88	0.948
2 weeks	2.12 ± 0.51	2.33 ± 0.96	0.594
Porphyromonas gingivalis			
Baseline	4.09 ± 2.02	4.13 ± 2.32	0.913
2 weeks	2.43 ± 0.64*	2.87 ± 1.45	0.749
Tannerella forsythia			
Baseline	4.54 ± 1.99	4.75 ± 2.11	0.659
2 weeks	2.57 ± 0.93*	2.88 ± 1.23*	0.388
Treponema denticola			
Baseline	4.64 ± 1.92	4.57 ± 2.19	0.835
2 weeks	2.28 ± 0.44*	2.72 ± 1.47	0.648
Fusobacterium nucleatum			
Baseline	3.95 ± 1.86	4.46 ± 1.97	0.280
2 weeks	2.52 ± 0.95*	3.67 ± 1.69	0.007
Prevotella intermedia			
Baseline	3.57 ± 2.05	3.49 ± 1.91	0.969
2 weeks	2.76 ± 1.25	3.23 ± 1.77	0.503
Total bacterial load			
Baseline	7.16 ± 0.62	7.12 ± 0.99	0.715
2 weeks	6.73 ± 0.52	6.83 ± 0.94	0.378

*Change to baseline $p \leq 0.001$

Six months after surgery systemic DOXY did not affect the primary outcome PAL-V gain. In the PLAC group 0.21 mm more PAL-V gain was observed than in the DOXY group. This small difference may be statistically insignificant due to the low test power of 50%. However, the difference is clearly clinically irrelevant and may be due to the fact that by chance there were more females in the PLAC group. Multiple regression revealed female gender and baseline PPD to have a positive effect on PAL-V gain, but not systemic doxycycline. Furthermore, meta-analysis including data of another study (Sculean et al. 2001) also failed to detect better PAL-V gain after use of systemic antibiotics. However, postsurgical systemic antibiotics may be beneficial for other regenerative approaches incorporating materials temporarily (membranes) or permanently (filler) into the wound/defect. At least, this provides data to calculate sample sizes for future studies on this issue. Further studies are needed to address the issue of systemic antibiotics after use of membranes and fillers for regenerative therapy.

Interestingly multiple regression revealed also EHI to influence PAL-V gain: the better early wound-healing the more PAL-V gain. This result highlights the significance of perfect defect closure for periodontal regeneration.

Within the limits of the present study the following conclusion may be drawn: 200 mg systemic DOXY for 7 days after regenerative therapy of infra-bony defects using EMD failed to result in better PPD reduction and PAL-V gain compared to PLAC in this study.

Acknowledgements

We thank Dr. Almut von Schwedler, MBBS (Tas), Matcham, NSW, Australia for her valuable and competent support in the preparation of this manuscript.

References

Bundeszentrale für politische Bildung. (2006) Glossar: Demographische Begriffe. www.bpb.de.

Chambrone, D., Pasin, I. M., Conde, M. C., Panutti, C., Carneiro, S. & Lima, L. A. (2007) Effect of enamel matrix proteins on the treatment of intrabony defects: a split-mouth randomized controlled trial study. *Brazilian Oral Research* **21**, 241–246.

Cortellini, P., Carnevale, G., Sanz, M. & Tonetti, M. S. (1998) Treatment of deep and shallow intrabony defects. A multicenter randomized controlled clinical trial. *Journal of Clinical Periodontology* **25**, 981–987.

Cortellini, P., Pini Prato, G. & Tonetti, M. (1995a) The modified papilla preservation technique. A new surgical approach for interproximal regenerative procedure. *Journal of Periodontology* **66**, 261–266.

Cortellini, P., Pini Prato, G. & Tonetti, M. S. (1995b) Periodontal regeneration of human infrabony defects with titanium reinforced membranes. A controlled clinical study. *Journal of Periodontology* **66**, 797–803.

Cortellini, P., Pini Prato, G. & Tonetti, M. S. (1999) The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. *International Journal for Periodontics and Restorative Dentistry* **19**, 589–599.

Cortellini, P. & Tonetti, M. S. (2001) Evaluation of the effect of tooth vitality on regenerative outcomes in infrabony defects. *Journal of Clinical Periodontology* **28**, 672–679.

Cortellini, P. & Tonetti, M. S. (2009) Improved wound stability with a modified minimally invasive surgical technique in the regenerative treatment of isolated interdental intrabony defects. *Journal of Clinical Periodontology* **36**, 157–163.

Cortellini, P., Tonetti, M. S., Lang, N. P., Suvan, J. E., Zucchelli, G., Vangsted, T., Silvestri, M., Rossi, R., McClain, P., Fonzar, A., Dubravec, D. & Adriaens, P. (2001) The simplified papilla preservation flap in the regenerative treatment of deep intrabony defects: clinical outcomes and postoperative morbidity. *Journal of Periodontology* **72**, 1702–1712.

De Sanctis, M. & Zucchelli, G. (2000) Interleukin-1 gene polymorphisms and long-term stability following guided tissue regeneration therapy. *Journal of Periodontology* **71**, 606–613.

Duckworth, J. E., Judy, P. F., Goodson, J. M. & Socransky, S. S. (1983) A method for the geometric and densitometric standardisation of intraoral radiographs. *Journal of Periodontology* **54**, 435–440.

Eickholz, P., Benn, D. K. & Staehle, H. J. (1996) Radiographic evaluation of bone regeneration following periodontal surgery with or without expanded polytetrafluoroethylene barriers. *Journal of Periodontology* **67**, 379–385.

Eickholz, P., Hörr, T., Klein, F., Hassfeld, S. & Kim, T.-S. (2004) Radiographic parameters for prognosis of periodontal healing of infrabony defects: two different definitions of defect depth. *Journal of Periodontology* **75**, 399–407.

Eickholz, P., Kim, T.-S., Steinbrenner, H., Dörfer, C. & Holle, R. (2000) Guided tissue regeneration with bioabsorbable barriers: intrabony defects and class II furcations. *Journal of Periodontology* **71**, 999–1008.

Eickholz, P., Lenhard, M., Benn, D. K. & Staehle, H. J. (1998) Periodontal surgery of vertical bony defects with or without synthetical bioabsorbable barriers. 12 months' results. *Journal of Periodontology* **69**, 1210–1217.

Esposito, M., Grusovin, M. G., Papanikolaou, N., Coulthard, P. & Worthington, H. V. (2009) Enamel matrix derivative (Emdogain®) for periodontal tissue regeneration in intrabony defects. *Cochrane Database of Systematic Reviews* Issue 4. Art. No.: CD003875.

Francetti, L., Trombelli, L., Lombardo, G., Guida, L., Cafiero, C., Rocuzzo, M., Carusi, G. & DelFabro, M. (2005) Evaluation of efficacy of enamel matrix derivative in the treatment of intrabony defects: a 24-month multicenter study. *International Journal for Periodontics and Restorative Dentistry* **25**, 461–473.

Gurinsky, B. S., Mills, M. P. & Mellonig, J. T. (2004) Clinical evaluation of demineralized freeze-dried bone allograft and enamel matrix derivative versus enamel matrix derivative alone for the treatment of periodontal osseous defects in humans. *Journal of Periodontology* **75**, 1309–1318.

Heijl, L., Heden, G., Svärdström, G. & Ostgren, A. (1997) Enamel matrix derivative (EMDOGAIN) in the treatment of intrabony periodontal defects. *Journal of Clinical Periodontology* **24**, 705–714.

Jepsen, S., Topoll, H., Rengers, H., Heinz, B., Teich, M., Hoffmann, T., Al-Machot, E., Meyle, J. & Jervøe-Storm, P. M. (2008) Clinical outcomes after treatment of intra-bony defects with an EMD/synthetic bone graft or EMD alone: a multicentre randomized-controlled clinical trial. *Journal of Clinical Periodontology* **35**, 420–328.

Lang, N. P. & Tonetti, M. S. (2003) Periodontal risk assessment (PRA) for patients in supportive therapy (SPT). *Oral Health Preventive Dentistry* **1**, 7–16.

Löe, H. (1967) The gingival index, the plaque index and the retention index system. *Journal of Periodontology* **38**, 610–616.

Meyle, J., Hoffmann, T., Topoll, H., Heinz, B., Al-Machot, E., Jervøe-Storm, P. M., Meiß, C., Eickholz, P. & Jepsen, S. (2011) A multi-centre randomized controlled clinical trial on the treatment of intra-bony defects with enamel matrix derivatives/synthetic bone graft or enamel matrix derivatives alone: results after 12 months. *Journal of Clinical Periodontology* **38**, 652–660.

Mombelli, A., Zappa, U., Brägger, U. & Lang, N. P. (1996) Systemic antimicrobial treatment and guided tissue regeneration. Clinical and microbiological effects in furcation defects. *Journal of Clinical Periodontology* **23**, 386–396.

Needleman, I. G., Giedrys-Leeper, E., Tucker, R. J. & Worthington, H. V. (2001) Guided tissue regeneration for periodontal infra-bony defects. *Cochrane Database of Systematic Reviews*, Issue 2. Art. No.: CD001724.

Nowzari, H., MacDonald, E. S., Flynn, J., London, R. M., Morrison, J. L. & Slots, J. (1996) The dynamics of microbial colonization of barrier membranes for guided tissue regeneration. *Journal of Periodontology* **67**, 694–702.

Nyman, S., Lindhe, J. & Rosling, B. (1977) Periodontal surgery in plaque-infected dentitions. *Journal of Clinical Periodontology* **4**, 240–249.

O'Leary, T. J., Drake, R. B. & Naylor, J. E. (1972) The plaque control record. *Journal of Periodontology* **43**, 38.

Pontoriero, R., Wennström, J. & Lindhe, J. (1999) The use of barrier membranes and enamel matrix proteins in the treatment of angular bone defects. A prospective controlled clinical study. *Journal of Clinical Periodontology* **26**, 833–840.

Pretzl, B., Kim, T.-S., Steinbrenner, H., Dörfer, C., Himmer, K. & Eickholz, P. (2009) Guided tissue regeneration with bioabsorbable barriers III 10 years results in infrabony defects. *Journal of Clinical Periodontology* **36**, 349–356.

Sculean, A., Blaes, A., Arweiler, N., Reich, E., Donos, N. & Brecx, M. (2001) The effect of postsurgical antibiotics on the healing of intrabony defects following treatment with enamel matrix proteins. *Journal of Periodontology* **72**, 190–195.

Sculean, A., Donos, N., Blaes, A., Lauermann, M., Reich, E. & Brecx, M. (1999) Comparison of enamel matrix proteins and bioabsorbable membranes in the treatment of intrabony periodontal defects. A split-mouth study. *Journal of Periodontology* **70**, 255–262.

Tonetti, M. S., Cortellini, P., Suvan, J. E., Adrians, P., Baldi, C., Dubravec, D., Fonzar, A., Fourmousis, I., Magnani, C., Muller-Campanile, V., Patroni, S., Sanz, M., Vangsted, T., Zabalegui, I., Pini Prato, G. & Lang, N. P. (1998) Generalizability of the added bebefits of guided tissue regeneration in the treatment of deep infrabony defects. Evaluation in a multicenter randomized controlled clinical trial. *Journal of Periodontology* **69**, 1183–1192.

Tonetti, M. S., Cortellini, P., Lang, N. P., Suvan, J. E., Adrians, P., Dubravec, D., Fonzar, A., Fourmousis, I., Rasperini, G., Rossi, R., Silvestri, M., Topoll, H., Wallkamm, B. & Zybutz, M. (2004) Clinical outcomes following treatment of human intrabony defects with GTR/bone replacement material or access flap alone. A multicenter randomised controlled clinical trial. *Journal of Clinical Periodontology* **31**, 770–776.

Tonetti, M. S., Lang, N. P., Cortellini, P., Suvan, J. E., Adians, P., Dubravec, D., Fonzar, A., Fourmosis, I., Mayfield, L., Rossi, R., Silvestri, M., Tiedemann, C., Topoll, H., Vangsted, T. & Wallkamm, B. (2002) Enamel matrix proteins in the regenerative therapy of deep intrabony defects. *Journal of Clinical Periodontology* **29**, 317–325.

Vest, T. M., Greenwell, H., Drisko, C., Wittwer, J. W., Bichara, J., Yancey, J., Goldsmith, J. & Rebitski, G. (1999) The effect of postsurgical antibiotics and a bioabsorbable membrane on regenerative healing in Class II furcation defects. *Journal of Periodontology* **70**, 878–887.

Wachtel, H., Schenk, G., Bohm, S., Weng, D., Zuhr, O. & Hürzeler, M. B. (2003) Microsurgical access flap and enamel matrix derivative for the treatment of periodontal intrabony defects: a controlled clinical study. *Journal of Clinical Periodontology* **30**, 496–504.

Weinberg, M. A. & Estrow, R. N. (2000) Osseous defects: proper terminology revisited. *Journal of Periodontology* **71**, 1928.

Zappa, U. (1991) Resorbierbare Membranen (I). Parodontale Geweberegeneration unter Verwendung von resorbierbaren Membranen - klinische Aspekte. *Schweizerische Monatsschrift für Zahnmedizin* **101**, 1147–1151.

Address:
P. Eickholz
Department of Periodontology
Center for Dental, Oral, and Maxillofacial Medicine (Carolinum)
Johann Wolfgang Goethe-University
Frankfurt am Main
Theodor-Stern-Kai 7
60596 Frankfurt am Main
Germany
E-mail: eickholz@med.uni-frankfurt.de

Clinical Relevance

Scientific rationale for the study: Many clinical studies on regenerative therapy report post or perisurgical antibiotic regimes. However, only a few clinical trials have investigated the additional effect of antibiotics given additionally to regenerative therapy. Several studies failed to observe better clinical results after use of antibiotics. However, up to now tetracycline derivatives have not been evaluated for their additional benefit.
Principal findings: Postsurgical systemic DOXY failed to improve clinical results after regenerative therapy of infrabony defects using EMD. Due to lack of test power in this study this finding is possibly not generalizable.
Practical implication: Wound dehiscence deteriorates clinical results after regenerative therapy of intrabony defects using EMD. The benefit of postsurgical systemic antibiotics may be questioned.