ON APPROACHES TO

PERIODONTAL INFECTION CONTROL

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ABSTRACT

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The purpose of the project was to gain understanding of clinical possibilities and applicability of non-surgical periodontal therapy.

A clinical study was designed to compare a full-mouth ultrasonic debridement approach with the traditional approach of consecutive sessions of quadrant-wise scaling/root planing with respect to the clinical outcome and long term stability. A second study evaluated the outcome of locally delivered doxycycline as an adjunct to initial subgingival instrumentation in smokers and non-smokers. A third study was designed to evaluate the clinical outcome of mechanical re-treatment of non-responding pockets, with or without the use of adjunctive locally delivered doxycycline. Furthermore, a multilevel analysis was performed to investigate factors affecting the clinical outcome of pocket debridement at initial as well at re-treatment phase.

In patients with moderate to advanced periodontitis an initial, 1-hour session of full-mouth ultrasonic debridement resulted in clinical improvements that were not significantly different from those following the traditional treatment approach. No significant difference with regard to the risk for recurrence of diseased periodontal pockets between the two treatment approaches was found, which lends support to the concept that the full-mouth ultrasonic approach to pocket/root debridement is as effective as quadrant-wise SRP in the initial treatment phase.

Locally applied, controlled-release doxycycline gel partly counteracted the negative effect of smoking on periodontal healing following initial non-surgical therapy. However, when used as an adjunct to mechanical debridement in the re-treatment of periodontal pockets, locally delivered doxycycline did not significantly improve the treatment outcome compared to mechanical debridement alone.

The multilevel analysis demonstrated that smoking habits, presence of supra-gingival plaque at the tooth site and location of the pocket at a molar were significant factors for an inferior outcome of *initial* non-surgical periodontal treatment.

Molars, furcation sites, presence of plaque and presence of angular bony defects were associated with an inferior clinical result after *re-treatment*.

The findings show that a full-mouth debridement approach is justified as an initial treatment modality. Furthermore, the results point to the importance of considering factors associated with the individual tooth site in the decision-making process regarding the selection of treatment procedures, particularly for sites showing poor healing response following initial pocket/root debridement. Locally applied controlled-release doxycycline gel may partly counteract the negative effect of smoking on periodontal healing following initial non-surgical therapy, but showed no significant benefit when applied in conjunction with re-treatment of remaining diseased sites.

Keywords: periodontitis, scaling and root planing, ultrasonic, randomized controlled trial, doxycycline, local drug delivery, smoking, plaque, multilevel analysis

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Contents

Preface

This thesis is based on the following papers which are referred to in the text by their Roman numerals:

I. Wennström, J.L., Tomasi, C., Bertelle, A. & Dellasega, E. (2005) Full-mouth ultrasonic debridement versus quadrant scaling and root planing as an initial approach in the treatment of chronic periodontitis. *Journal of Clinical Periodontology* **32**: 851-859.

II. Tomasi, C., Bertelle, A., Dellasega, E. & Wennström, J.L. (2006) Full-mouth ultrasonic debridement and risk of disease recurrence: a 1-year follow-up. *Journal of Clinical Periodontology* **33**: 626-631.

III. Tomasi, C., Leyland, A.H. & Wennström, J.L. (2007) Factors influencing the outcome of non-surgical periodontal treatment: a multilevel approach. *Journal of Clinical Periodontology* **34**: 682-690

IV. Tomasi, C. & Wennström, J.L. (2004) Locally delivered doxycycline improves the healing following non-surgical periodontal therapy in smokers. *Journal of Clinical Periodontology* **31**: 589-595.

V. Tomasi, C., Koutouzis, T. & Wennström, J.L. (2007) Locally delivered doxycycline as an adjunct to mechanical instrumentation at re-treatment of periodontal pockets. *Journal of Periodontology* (Submitted)

To my father, that would be proud of this.

INTRODUCTION

Periodontal disease is characterized by tissue inflammation and destruction of the tooth supporting structures that eventually leads to the loss of affected teeth (Kinane 2001, Page & Kornman 1997, Pihlstrom et al. 2005). Lesions in the periodontal tissues are clinically identified and diagnosed based on the signs (i) presence of bleeding following periodontal pocket probing and (ii) reduced tissue resistance to pocket probing (i.e. probing depth of > 4 mm). These signs develop as a result of the tissue response to the presence of a subgingival biofilm, resulting in an inflammatory lesion, rich in leukocytes and poor in collagen, in the gingival connective tissue adjacent to the tooth surface (Nanci & Bosshardt 2006, Page et al. 1997). Hence, the main goal of the treatment of patients with periodontitis is to establish proper infection control, i.e. to reduce the bacterial load below the individual threshold level for disease. The achievement of this goal involves various treatment phases:

- Establishing an optimal self-performed plaque control by means of oral hygiene instructions, motivation and elimination of retentive factors (Axelsson & Lindhe 1981, Dahlen et al. 1992, Hellström et al. 1996, Katsanoulas et al. 1992, Magnusson et al. 1984, Westfelt et al. 1998)
- Suppressing the subgingival bacterial load around teeth by the use of non-surgical means
- Accessing the site of infection by a surgical approach that allows the correction of anatomical unfavourable features (DeSanctis & Murphy 2000, Heitz-Mayfield et al. 2002)
- Preventing recurrences of periodontal disease by regular monitoring and supportive periodontal treatment (Axelsson & Lindhe 1981, Axelsson et al. 2004)

The current thesis focused on non-surgical treatment approaches for the establishment of periodontal infection control.

Non-surgical periodontal therapy

A number of systematic reviews on the efficacy of mechanical non-surgical periodontal therapy have been published during the last decade (Table 1). There is a consensus among these reviews that subgingival debridement combined with proper supra-gingival plaque control is an effective treatment modality in reducing probing pocket depth and improving clinical attachment levels. However, the heterogeneity of the studies did not allow a meta-

analysis of the data. Information concerning methods and randomization, masking of examiners and completeness of follow up was seldom reported.

In Table 2 original studies that have been published after the time period covered in the systematic reviews are summarized. Five of these publications are randomized clinical trials comparing scaling and root planing (SRP) performed with hand and various machine-driven instruments. Another 3 RCTs compared SRP performed as a quadrant-wise or a full-mouth approach. Even though these studies are well described, heterogeneity of the data is still an issue of concern: for example probing assessments are divided in categories based on initial pocket depth but the thresholds chosen may differ from one study to another. Overall, it can be stated that non-surgical periodontal treatment will lead to a significant improvement in terms of reduction of inflammation, which is accompanied by a probing pocket depth reduction varying between 1.0-1.6 mm for medium deep and 1.6-2.3 mm for deep pockets. The magnitude of CAL gain may correspond to 70-90% of the pocket depth reduction.

Table 1: Systematic reviews on sub-gingival mechanical instrumentation

Author/year	**Type**	**Aim**	**Inclusion/exclusion criteria**	**Clinical Variab.**	**Number of studies**	**Clinical outcome** (compared with no treatment or baseline)	**Author's Conclusions**	**Comments**
van der Weijden & Timmerman (2002)	Systematic review	Effect of subgingival debridement (SGD) on chronic periodontitis patients.	Randomized Clinical Trials and uncontrolled studies of minimum 3 month duration. Adult patients No antibiotic Patient level analysis	ΔPPD ΔCAL ΔBOP	114 screened **26 selected**: 8 controlled 18 single arm	ΔPPD ΔCAL Weighted mean RCT 1.18 0.64 W. mean no control 0.74 W. m. SGD as control 0.22 6 papers reported a benefit for SGD, 2 showed no effect (no hygiene instructions), 2 had unclear description.	In patients with chronic periodontitis, SGD (in conjunction with supragingival plaque control) is an effective treatment in reducing PPD and improving CAL	- Big variation of time for treatment and number of sessions. - Instruments used seldom reported
Tunkel et al. (2002)	Systematic review	Compare effect of machine driven instruments with hand instruments.	RCT Min. 6 month duration	iCAL-L iAB-L mCAL-G mPPD-R mBOP-R mGI-R iPA, GR, mRH	27 screened **13 selected**	No meta-analysis could be performed on clinical outcome variables. Mean PPD changes Hand Machine Badersten et al. 1981 1.00 1.20 Badersten et al. 1984 1.40 1.20 Kocher et al. 2001 0.77 1.10 Copulos et al. 1993 0.72 0.75 Mean Cal changes Badersten et al. 1981 0.30 0.50 Badersten et al. 1984 0.50 0.20 Kocher et al. 2001 0.53 0.71 Copulos et al. 1993 0.10 0.20 The debridement with ultrasonic/sonic instruments took on average 36.7% less time than the treatment with hand instruments. (2 studies)	No apparent difference in the efficacy of subgingival debridement using ultrasonic/sonic and hand instruments in the treatment of chronic periodontitis in single-rooted teeth. Subgingival debridement may be completed in less time with ultrasonic/ sonic	The methodological quality assessment of the 13 included studies revealed that none of the trials provided sufficient information concerning methods of randomization, allocation concealment, blindness of examiners and completeness of follow-up.
Hallmon & Rees (2003)	Systematic review	To assess and compare the efficacy of mechanical and physical non-surgical therapy with manual instrumentation	RCT or CT or Case-control Min. 3 month duration Patient age ≥ 10 years Sonic ultrasonic and subgingival irrigation as test treatment alone or in combination No antibiotic local or systemic	ΔPPD ΔCAL ΔBOP Recess.	99 screened **9 selected**	3 studies comparing manual and ultrasonic: no difference 1 study comparing SRP and sonic: no difference 1 study comparing manual and motorized curette: no difference 3 studies comparing manual and manual + subgingival irrigation: no difference 1 study comparing manual and manual + subgingival citric acid: no difference No difference in terms of time except for one study.	Based on clinical outcomes, there was comparable efficacy between manual and machine driven instrumentation. The use of subgingival irrigation as an adjunct to MI offered no additional benefit to MI alone.	Meta-analysis not possible due to heterogeneity of the studies

Table 2: Original papers on mechanical sub-gingival instrumentation from 2003.

Author/year	Design	Aim	Inclusion/Excl. criteria	Variable	Patients, treat. and follow-up	Results	Author's conclusion	Comments
Kahl et al. (2007)	RCT Split Mouth	To assess the clinical effects of subgingival polishing with Vector ultrasonic compared with supragingival polishing or with subgingival root debridement	Moderate to advanced chronical periodontal disease Molars excluded At least 2 teeth with 5-8 mm pocket Healthy No Ab No scaling before	ΔPPD ΔCAL ΔBOP	20 patients mean age 47.9 4 treatments: VU-H: Vector ultrasonic HI-H: s/rp with curettes HI-D: s/rp. with Gracey-curettes PO-H: supragingival polishing alone Re-evaluation at 3 and 6 month	Results at 6 month examination Mean PPD ch. VU-H HI-H HI-D PO-H PPD ini <6 1.2 1.3 1.6 0.5 PPD ini ≥6 2.5 2.6 2.5 1.5 Mean Cal ch. PPD ini <6 0.6 0.7 0.7 0.2 PPD ini ≥6 0.7 1.2 0.9 0.1	VU subgingival debridement leads to BOP and PD reduction, in and CAL gain similar to those achieved by hand instrumentation. A tendency towards a smaller reduction in BOP and CAL gain in deep pockets was noted for VU treatment.	Study testing Vector ultrasonic system. No significant difference between treatment groups, all significantly better than polishing. Time for instrumentation limited to 6 minutes /tooth
Christgau et al. (2007)	RCT Split-mouth Single masked	To compare the clinical and microbiological healing outcomes after non-surgical periodontal therapy using the Vector ultrasonic scaling system versus subgingival debridement with hand curettes.	Moderate to severe chronical periodontal disease At least 4 teeth/quadrant with ≥4 mm pocket Healthy No Ab	Plaque Bleeding PPD CAL BoP Micro (DNA probe) Side eff.	20 patient Age 40 median Test:ultrasonic Control:SRP Re-evaluation at 4 weeks and 6 month.	Results at 6 month examination Mean BoP reduct. Test Control PPD ini 4-6 69% 73% PPD ini ≥7 70% * 88% Mean PPD ch. PPD ini 4-6 1.0 1.1 PPD ini ≥7 1.6 2.1 Mean Cal ch. PPD ini 4-6 0.7 0.8 PPD ini ≥7 0.8 1.5 Total bact. load 19 11 Time needed 4.7 4.3	Both Vector system and S/RP provided favourable periodontal healing results. In deep pockets, S/RP achieved a better BoP reduction and CAL gains. Vector system required similar amount of time as hand instrumentation.	Treatment in 24 hours Significant difference between treatment groups only for BoP reduction. A tendency towards better clinical effect in deep pockets for SRP was present. The time was measured but not restricted
Faveri et al. (2006)	RCT Parallel Single-masked	To test the null hypothesis that there was ''no difference in the effect on treatment with the adjunctive use of CHX rinsing during non-surgical periodontal treatment compared with SRP alone'', in subjects with chronic periodontitis	Healthy subjects >30 years At least 15 teeth Minimum of six teeth with at least one site with PD 5-7 and CAL 5-10 Exclusion: Previous periodontal therapy, pregnancy, smokers; antibiotic coverage and allergy to CHX	Plaque GI BoP PD CAL Micro (Bana test)	29 patients Age 45 mean Test: SRP + CHX Control: SRP Re-evaluation at 42 and 63 days	Only graphs reported Plaque, GI, BoP, PPD and CAL reduction significantly higher in test group for medium and deep pockets.	The combination of CHX rinses and SRP leads to clinical benefits and to a better reduction in BANA-positive species.	Recall every week Plaque score remained over 60% in control group. Since numbers not reported, it is difficult to compare with other studies.

Author/year	Design	Aim	Inclusion/Excl. criteria	Variable	Patients, treat. and follow-up	Results	Author's conclusion	Comments
Christgau et al. (2006)	RCT Split mouth Single-masked	To investigate the clinical and microbiological outcomes following non-surgical periodontal treatment using the modified sonic scaler system SonicFlex 2003L in comparison with scaling and rp (S/RP) with hand curettes.	Moderate to severe chronical periodontal disease At least 4 teeth/quadrant with ≥4 mm pocket Healthy No Ab	Plaque Bleeding PPD CAL BoP Micro (DNA probe) Side eff	20 patient Age 46 median Test:sonic Control:SRP Re-evaluation at 4 weeks and 6 month.	Results at 6 month examination Mean BoP reduct. Test Control PPD ini 4-6 66% 63% PPD ini ≥7 57% * 76% Mean PPD ch. PPD ini 4-6 0.9 1.1 PPD ini ≥7 2.0 2.4 Mean Cal ch. PPD ini 4-6 0.8 0.9 PPD ini ≥7 1.3 1.8 Total bact. load 12 7 Time needed 4.3 * 6.1	- The modified sonic scaler system and S/RP by hand curettes provided similarly favourable periodontal healing results. - In deep pockets, S/RP appeared to achieve better resolution of inflammation. - Less time employed with sonic	Treatment in 24 hours The time was measured but not restricted Statistical testing with non-parametric technique, which implies lower power than parametric testing
Quirynen et al. (2006a)	RCT Parallel Single-masked	To evaluate the relative role of antiseptics and of the timing in the full mouth disinfection protocol by comparing different clinical protocols: with versus without antiseptics and short versus long time gap between debridement of 4 quadrants.	Age 30 to 75 years, - minimum of 18 teeth, at least 2 teeth with at least 6 sites having a probing depth ≥6mm, - rx evidence of moderate bone loss no perio treat within 12 months before no use of antimicrobial agents	Staining Plaque Bleeding PPD REC BoP	71 patients Age 48 mean 5 treat. groups: 1) NC (15 pat): quadrant S/RP 2) FRp (14pat): full mouth S/RP 3) FMCHX (14 pat): fm S/RP + CHX 0.2% 2 months 4) FMF(14 pat):fm S/RP+AmF/SnF_2 2m. 5) FMCHX+F (14pat) fm S/RP + CHX 0.2% for 2 m. and AmF/SnF_2 6m.	Results at 8 month re-evaluation ΔPPD(mm) 1 2 3 4 5 Single-r. PPDini 4-5 1.3 1.4 1.8 1.4 1.7 PPDini ≥6 2.3 2.5 2.6 2.4 2.8 Multi-r. PPDini 4-5 1.0 1.5 1.6 1.2 1.7 PPDini ≥6 2.3 2.6 2.7 2.3 3.1 Overall BoP from 85% to 45% 14 drop out patients	The use of antiseptics, as well as the completion of the scaling and root planing sessions within a short time frame, seem to have a beneficial effect in the treatment of moderate and severe periodontitis.	The SRP group was instructed not to use interdental cleaning devices during study to favour cross-infection. 2 weeks interval between each session. No significant difference between full-mouth groups.
Jervøe-Storm et al. (2006)	RCT Parallel Single-masked	To determine the clinical effects after 3 and 6 months of FMRP compared with conventional quadrant wise root planing.	More than 20 teeth, with at least 2 teeth per quadrant with a PPD 5mm or more and bleeding on probing. Good general health, no pregnant females No periodontal or antibiotic treatment during the last 6 m.	PPD RAL BoP	20 patients Age 53 mean 2 groups: Control: Quadrant S/RP Test: Full Mouth SRP Re-examination at 3 and 6 month	Results at 6 month examination Mean BoP reduct. FMRP QSRP PPD ini 5-6 75% 66% PPD ini ≥7 28% 38% Mean PPD ch. PPD ini 5-6 1.6 1.8 PPD ini ≥7 1.7 2.1 Mean Cal ch. PPD ini 5-6 1.1 0.9 PPD ini ≥7 0.7 1.4 1 hour scaling each quadrant for all patients	Both treatment modalities, quadrant wise and full mouth root planing, have been able to show comparable beneficial changes in the periodontal status, and should both be considered as valid treatment approaches in the treatment of patients with chronic p.	Full mouth done with 2 session within 24 hours Quadrant scaling with 1 session each week Plaque score <20% for all patients

Author/year	Design	Aim	Inclusion/Excl. criteria	Variable	Patients, treat. and follow-up	Results	Author's conclusion	Comments
D'Aiuto et al. (2005)	Prospective Longitudinal Masked examiner	To assess, using a multilevel analysis, the relative contribution of patient-, tooth-, and site-associated factors in determining the clinical outcomes of machine-driven subgingival debr.	Severe generalized periodontitis (PD ≥5mm BoP+ and bone loss >30% in at least 50% of the dentition). Exclusion: known systemic diseases; systemic antibiotic or periodontal treatment in the preceding 3 months; pregnant or lactating	PI PPD BoP REC	94 patients Age 46 mean Treatment: Ultrasonic debridement Re-examination at 2 and 6 month	6 month — Mean ± SE 95% ΔFMPS 37.7 ± 1.9 ΔFMBS 45.5 ± 2.5 ΔNPD 57.5 ± 2.4 ΔPD 1.2 ± 0.5 ΔCAL 0.1 ± 0.5 Variance at different levels for ΔPD Patient 0.177 ± 0.029 (8.0%) Tooth 0.262 ± 0.017 (11.6%) Site 1.806 ± 0.024 (80.4%)	These data provided an estimation of the relative contribution of site-, tooth-, and patient-associated variables in terms of PD reductions following a standard course of machine-driven subgingival debridement.	Multilevel analysis allowed to model variance at different levels and to investigate influence of factors related to different levels on the outcome. The clinical changes were calculated on full mouth basis.
Koshy et al. (2005)	RCT Parallel Single-masked	To compare the clinical and microbiological effects of single-visit full-mouth ultrasonic debridement with or without additional anti-microbial agents to those of conventional quadrant-wise therapy.	Patients moderate- to-advanced chronic periodontitis. <u>No smoker included</u> The subjects had at least 5 teeth and 2 pocket sites with PPD ≥ 5mm in each quadrant and rx bone loss. No periodontal treatment and/or antibiotic therapy 6 months before. Patients who were pregnant or lactating, or who were allergic to iodine were excluded	PI PPD BoP PAL Micro (DNA probe)	36 patients Age 50 mean 3 groups: Control: Quadrant debridement Test1: Full Mouth Deb. Test2: Full Mouth Deb. with PVP iodine Re-examination at 3 and 6 month	Results at 6 month examination **Mean PPD ch.** FMD-I FMD QD Single-r. PPD ini 5-6 3.0 3.0 2.8 PPD ini ≥7 4.0 4.2 3.8 Multi-r. PPD ini 5-6 2.3 2.6 2.5 PPD ini ≥7 3.4 3.8 3.9 **Mean Cal ch.** Single-r. PPD ini 5-6 2.0 2.1 1.9 PPD ini ≥7 2.7 3.3 2.8 Multi-r. PPD ini 5-6 1.5 1.7 1.6 PPD ini ≥7 2.3 3.0 2.6 Total Time (min) 139 128 * 178	Fullmouth ultrasonic debridement with or without adjunctive anti-microbial agents may have limited additional benefits over conventional quadrant-wise mechanical therapy, in terms of reduction of bleeding and number of pocket sites, and a shorter treatment time.	The fact that only non-smokers were included may partly explain the relevant clinical results. Ultrasonic instrumentation only was used for all groups. PVP iodine did not improve the outcome.
Darby et al. (2005)	Prospective	To compare the effect of smoking on SRP in CP and GAgP patients, both clinically and microbiologically.	Each patient had at least 2 non-adjacent sites per quadrant with pocket depth of at least 5 mm, with no history of systemic disease or antibiotic therapy within the last 3 months. Chronic and aggressive periodontitis cases both included. Four sites PPD ≥5mm selected.	MGI PI PPD BoP PAL Micro (PCR)	57 patients 12 drop-out 28 Chronic P. Age 47 mean 17 Gen. agr. P. Age 33 mean Divided in Smokers and Non-smokers 8 weeks follow-up	Results at 8 week examination Mean PPD ch. Smokers Non-smokers CP 1.0 * 1.7 GaP 1.3 * 2.4 Mean Cal ch. CP 0.3 0.7 GaP 1.2 1.4 Microb Red. *Pg* CP 9.4 10 GaP 41.7 * 18.8 Microb Red. *Pi* CP 18.8 * 23.8 GaP 25 * 46.9 Microb Red. *Tf* CP -25 * 36.3 GaP 63.9 * 21.3	SRP was effective in reducing clinical parameters in both groups. The inferior improvement in PD following therapy for smokers may reflect the systemic effects of smoking on the host response and the healing process. These detrimental consequences for smokers appear consistent in both aggressive and CP.	Smoking status assessed by interview. Big change in terms of recession between CP and GaP (almost 95% of PPD reduction due to PAL gain in smokers for GaP pat)

Author/year	Design	Aim	Inclusion/Excl. criteria	Variable	Patients, treat. and follow-up	Results	Author's conclusion	Comments
Colombo et al. (2005)	Prospective Longitudinal	The aim of the present investigation was to evaluate the microbiological changes resulting from scaling and root planing therapy in Brazilian patients with untreated chronic periodontitis.	≥35 years of age, had at least 20 teeth, and at least seven sites with PPD >4 mm and CAL >3 mm No history of periodontal therapy. Exclusion criteria pregnancy, and use of antibiotics 6 months prior the study.	PPD CAL BoP	25 patients Age 43 mean Treatment: hand instruments SRP in 4 to 6 session Re-examination at 3, 6 and 9 month	Clinical Parameters Baseline / 9 Months Mean CAL (mm) 3.7 / 2.8 Mean PD (mm) 3.4 / 2.5 BoP 55 / 24	In Brazilians with untreated chronic periodontitis, SRP led to clinical improvement with a decrease of periodontal pathogens for up to 9 months after therapy.	Full mouth clinical measurements. Microbial charge reduced more in mean count than in frequency.
Sculean et al. (2004)	RCT Parallel Single-masked	To assess the clinical effectiveness of Vector US when compared to scaling and root planing with hand instruments.	(a) no treatment of periodontitis for the last 2 years, (b) no use of antibiotics for the 12 months prior to treatment, (c) no systemic diseases, (d) good level of oral hygiene. As criterion for a good level of oral hygiene a mean plaque index (PII) score <1 was chosen.	FMPS PPD REC BoP PAL	38 patients Age 54 mean 2 groups: Test: vector ultrasonic Control: S/RP with hand instruments Re-examination at 6 month	Results at 6 month examination **Mean PPD ch.** Test / Control Single-r. PPD ini 4-5 0.8 / 1.1 PPD ini >6 0.6 / 1.2 Multi-r. PPD ini 4-5 0.8 / 0.8 PPD ini >6 0.9 / 1.1 **Mean Cal ch.** Single-r. PPD ini 4-5 0.6 / 0.8 PPD ini >6 0.5 / 0.7 Multi-r. PPD ini 4-5 0.6 / 0.5 PPD ini >6 0.7 / 0.7 Total Time (min) 6-10 / 8-12	It may be concluded that non-surgical periodontal therapy with the tested ultrasonic device may lead to clinical improvements comparable to those obtained with conventional hand instruments.	Patients selected based also on level of self-performed oral hygiene. As groups based on initial PPD included 4-5 and >6, look like 6mm pockets were excluded. Changes in SRP group quite reduced for deep pockets compared to other studies.
Obeid et al. (2004)	RCT Split-mouth	To evaluate the clinical effectiveness of the mechanical root planing system: Perioplaners & Periopolishers alone or combined with other usual root planing methods (hand and ultrasonic), for periodontal debridement	Generalized moderate-to-severe adult periodontitis systemically healthy. At least 3 sites with probing depth >4mm per quadrant. Exclusion criteria: - antibiotic therapy in the last 2 months - previous and recent periodontal treatment.	Pli PBi PPD PAL	20 patients Age 50 mean 4 treatment: MAN: hand instrument SRP US: ultrasonic debridement US-P: ultrasonic+perio polisher P-P: perioplaners+periopolisher Re-examination at 3 and 6 month	Results at 6 month examination MAN / US / US-P / P-P Mean PPD ch. 1.5 / 1.6 / 1.7 / 1.7 Mean PAL ch. 1.5 / 1.6 / 1.2 / 1.5 Time min/tooth 3 / 2 / 2+1 / 2+1	Mechanized root planing with the Perioplaners/ Periopolishers system, as effective as the common procedures, represents a satisfactory and alternative means of nonsurgical root therapy.	No recession reported in MAN and US groups.

Author/year	Design	Aim	Inclusion/Excl. criteria	Variable	Patients, treat. and follow-up	Results	Author's conclusion	Comments
Apatzidou & Kinane (2004)	RCT Parallel Single-masked	To determine whether same-day full-mouth scaling and root planing (FM-SRP) would show greater improvements in clinical indices than Q-SRP in moderate to advanced chronic periodontitis patients.	At least two non-adjacent sites per quadrant with PD of 5mm or over and radiographic evidence of bone loss. Exclusion: - history of systemic disease - antibiotic therapy within the last 3 months or during the course of the study	PI PPD BoP RAL	40 patients Age 44 mean 2 treatment groups: Q-SRP quadrant scaling and rp FM-SRP full mouth scaling and rp Re-examination at 13 and 25 weeks	Results at 25 weeks examination . FM-SRP Q-SRP Mean BoP reduct 57% 58% Mean PPD ch. 1.7 1.8 Mean Cal ch. 1.1 1.1 Total time approximately 4 hours in both treatments.	No significant differences found in the clinical outcome between Q-SRP at 2-weekly intervals and same-day FM-SRP at 6 months.	Interval of 1 week between treatment of quadrants. Full-mouth in 24 hours.
Kerdvongbundit & Wikesjo (2003)	RCT Parallel	To evaluate the effect of a triclosan/ copolymer/ fluoride dentifrice on healing following non-surgical periodontal therapy in smokers.	A minimum of 20 natural permanent teeth. Smokers with chronic periodontitis. Unremarkable medical history. Exclusion: - antibiotics during the 6 months preceding the study - oral appliances.	PI GI PPD CAL BoP REC	60 smokers Age 47 mean 2 groups: Test: SRP and use of triclosan dentifrice Control: SRP and fluoride dentifrice Re-examination at 6, 12, 18, 24 moth	Test Control PPD Baseline 4.4 4.5 PPD 24 month 2.7 4.0 ΔPPD 1.7 0.5 CAL Baseline 4.6 4.6 CAL 24 month 3.0 4.1 ΔCAL 1.6 0.5	An oral hygiene regimen including a triclosan/ copolymer/ fluoride dentifrice may sustain the short-term effect of non-surgical periodontal therapy in smokers.	Alterations of clinical parameters quite limited in control group compared to other studies. All alterations due to attachment gain, with no recession.

Approaches to pocket/root debridement

Root/pocket instrumentation (scaling and root planing; SRP), combined with effective self-performed supragingival plaque control measures, serves the purpose of infection control by altering the subgingival ecological environment through disruption of the microbial biofilm and suppression of the inflammation.

The traditional modality as an initial periodontal treatment phase is to perform scaling and root planing by jaw quadrant (Q-SRP) at a series of appointments (Badersten et al. 1984). More recently, Quirynen et al. (1995) advocated the benefit of performing full-mouth SRP within 24 hours in order to prevent re-infection of the treated sites from remaining untreated periodontal pockets. The authors also considered the risk of re-infection from other intra-oral niches such as the tongue and tonsils, and therefore included tongue cleaning and extensive anti-microbial regimens with chlorhexidine (full-mouth disinfection). In a series of studies (Bollen et al. 1996, Mongardini et al. 1999, Quirynen et al. 1995), it was documented that this combined approach resulted in improved healing, as assessed by clinical and microbiological means, compared to Q-SRP with 2-week intervals.

Although it was shown in a subsequent study by the same research group (Quirynen et al. 2000) that the major part of the improved treatment outcome of the full-mouth disinfection approach was attributed to the SRP of all four quadrants within 24 hours, rather than to the adjunctive chlorhexidine regimen, a recently published RCT (Quirynen et al. 2006) supported the previous conclusion of an improved outcome with respect to probing depth reduction with the use of the chlorhexidine regimen. Other research groups (Apatzidou & Kinane 2004, Jervøe-Storm et al. 2006, Koshy et al. 2005, Pihlstrom et al. 2005), however, failed to confirm that the full-mouth SRP approach results in a superior healing outcome compared to the traditional approach with quadrant-wise SRP.

A consideration in relation to non-surgically performed SRP is the extent of root instrumentation required for periodontal healing. The original intention with SRP was not only to remove microbial biofilm and calculus but also "contaminated" root cementum or dentin in order to prepare a root surface biocompatible for soft tissue healing. The rationale for performing root planing was based on the concept that bacterial endotoxins penetrate into the cementum (Aleo et al. 1974, Hatfield & Baumhammers 1971), a concept that later was disproved by data from experimental studies showing that the endotoxins were loosely adhering to the surface of the root cementum and not penetrating into it (Cadosch et al. 2003, Hughes et al. 1988, Hughes & Smales 1986, Moore et al. 1986). Hence, intentional removal of

tooth structures by root planing during pocket/root instrumentation may not be considered as a prerequisite for periodontal healing (Nyman et al. 1986, Nyman et al. 1988). Consequently, pocket/root instrumentation should preferably be carried out with instruments that cause minimal root substance removal, but are effective in disrupting the biofilm and removing calculus. In this respect, data reported in studies that evaluated root substance removal following the use of various manual and power-driven instruments (Busslinger et al. 2001, Kawashima et al. 2007, Ritz et al. 1991, Schmidlin et al. 2001) favour the use of ultrasonic devices.

According to the systematic reviews reported in Table 1 there is no major difference between using hand or power-driven instruments in the efficacy of debridement techniques in terms of pocket reduction and gain in clinical attachment. However, there is no consensus regarding a potential difference in treatment time between the two techniques. While Tunkel et al. (2002) concluded in their systematic review that the use of ultrasonic/sonic devices requires less treatment time than manual instrumentation, Hallmon and Rees (2003) in a comparable review considered that there is insufficient evidence to make any conclusion regarding differences in treatment time.

Contradicting reports are available on a potential correlation between the amount of removal of subgingival deposits and the time employed for instrumentation (Braun et al. 2005, Busslinger et al. 2001). In this context, however, one also has to consider that the experience of the operator may be an important factor influencing the efficacy of subgingival debridement (Brayer et al. 1989, Fleischer et al. 1989, Kocher et al. 1997). Furthermore, a number of *in vitro* (Breininger et al. 1987, Rateitschak-Pluss et al. 1992) and *in vivo* studies (e.g. Brayer et al. 1989, Caffesse et al. 1986, Eaton et al. 1985, Sherman et al. 1990b, Waerhaug 1978, Wylam et al. 1993) have shown that a complete removal of hard and soft deposits is a non-feasible objective of closed pocket/root instrumentation.

Hence, a question to be addressed is what level of instrumentation is required for resolution of periodontal lesions. An interesting observation in this respect was that piezoelectric ultrasonic debridement performed as a single-visit full-mouth procedure resulted in a healing outcome comparable to traditionally performed scaling and root planing in the control groups of a study aimed at testing locally delivered doxycycline (Wennström et al. 2001). This finding indicates that sufficient removal of subgingival deposits for resolution of signs and symptoms of periodontal disease may be attainable using markedly less treatment time than that traditionally allocated to non-surgical pocket/root debridement.

The **first aim** of the present thesis was to evaluate the clinical efficacy of a single 1-hour session of full-mouth ultrasonic debridement as an initial periodontal treatment approach in comparison with the traditional treatment modality of consecutive sessions of quadrant scaling/root planing.

The **second aim** was to evaluate the incidence of disease recurrence following a full-mouth pocket/root debridement approach with ultrasonic instrumentation versus that following a traditional approach of quadrant-wise scaling and root planing performed with hand-instrumentation.

Analysis of factors determining the outcome of non-surgical periodontal therapy

A common experience by clinicians is that the treatment outcome of non-surgical periodontal therapy varies not only between patients but also between various tooth sites in the individual subject (Badersten et al. 1984, Serino et al. 2001, van der Weijden & Timmerman 2002). Hence, the gain of knowledge about factors that may be responsible for such variation in treatment response would be beneficial for the selection of treatment approaches aiming at the establishment of infection control. Such factors may be related to the patient, the tooth, or the single tooth site (Axtelius et al. 1999, D'Aiuto et al. 2005, Hughes et al. 2006).

The inherent hierarchical structure of periodontal data poses difficulties for data analysis (McDonald & Pack 1990, Sterne et al. 1990). The outcome variable may be related to teeth or tooth sites, which are clustered in patients, who in turn may be clustered in centres. The key feature of this correlated (or clustered) data is that items under study are bound together in sets (or clusters) that are known to the data analyst (Begg & Parides 2003). The correlation invalidates classical assumptions of independence that are assumed to exist when applying common regression techniques such as ordinary least square (OLS).

A common approach to analyse hierarchical data is to perform an aggregate level analysis. This often involves computing mean values and combining these in a simple regression model to relate an outcome of interest (e.g. mean PPD for each patient) to a set of explanatory variables also computed at patient level (e.g. mean plaque score). However, aggregating site data within patients using mean values runs the risk of loosing information and overestimating the standard error due to collinearity among explanatory variables. Furthermore, a risk of this approach is also the so called “ecological fallacy”, which arises because association between two variables at group level (or ecological level, which in our example could be the patient) may differ from associations between analogous variables measured at the tooth site level

(Diez Roux 2002). On the contrary, an analysis at tooth or site level, without taking in account the dependence (or correlation) between teeth/sites in the same patient, may result in an underestimation of the standard error (Rice & Leyland 1996) and run the opposite risk called "atomistic fallacy", which shares the same origin of ecological fallacy (Fig. 1).

Two common regression approaches for analysing clustered data are the generalized estimating equations (GEE), also called marginal models, and multilevel analysis. A thorough discussion about differences between these two approaches may be found in a publication by Begg et al. (2003). Theoretical and software development have facilitated the analysis of nested structures within a generalized linear model framework, with introduction of multilevel models such as random coefficient models, variance component models and hierarchical linear models (Rice & Leyland 1996).

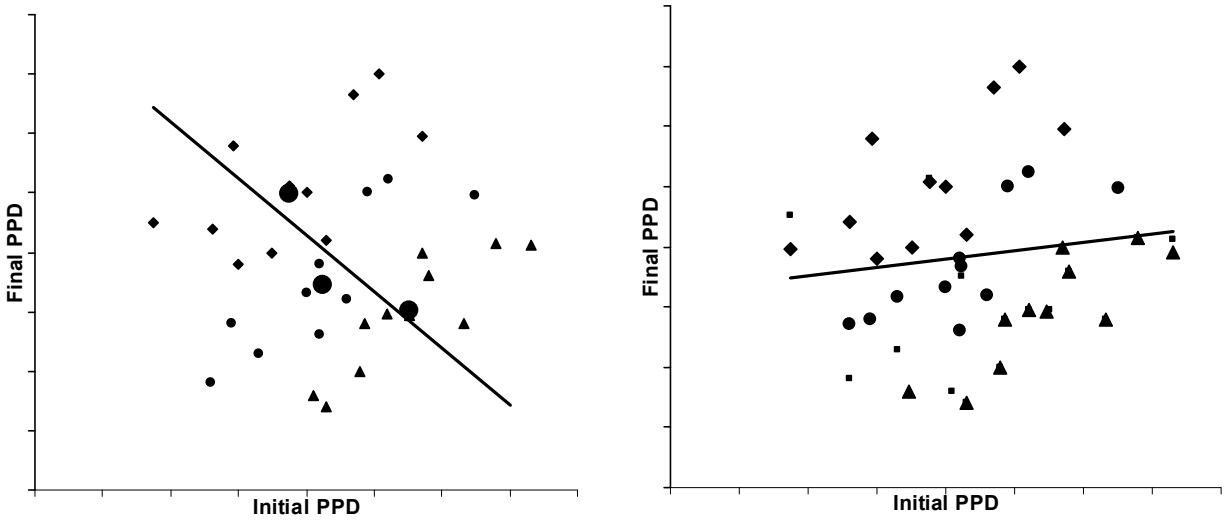

Fig. 1 Model based on ordinary least square aggregate level regression on the left and site level regression on the right.

Multilevel models

As defined by Snijders & Bosker (1999), multilevel analysis is a methodology for the analysis of data with complex patterns of variability, with a focus on nested sources of variability.

Multilevel analysis, which originally was developed in the fields of education, sociology and demography, has received increasing attention in public health, epidemiological and medical research (Goldstein 1987, Goldstein et al. 2002, Leyland & Goldstein 2001, Leyland & Groenewegen 2003, Rice & Leyland 1996, Snijders & Bosker 1999). Multilevel modelling is a generalization of regression methods, and as such can be used for a variety of purposes, including prediction, data reduction, and causal inference from experiments and observational studies (Gelman 2006).

The most powerful feature of MLM is the facility to investigate the underlying complexity of hierarchical systems, simultaneously modelling fixed effects and complex variation. Methods that accommodate hierarchy but fail to model variation explicitly (e.g., Generalized Estimating Equations) are not as efficacious when analysing hierarchical data.

Multilevel analysis is an extension of ordinary least square (OLS) analysis under which, for example, the mean relationship between initial PPD and final PPD after treatment can be estimated.

The algebraic notation of an OLS regression equation is:

$$y_i = \beta_0 + \beta_1 x_i + e_i$$

where β_0 is the intercept of the regression line (the value of y when x=0), β_1 is the slope associated with the independent variable x and e_i is the residual for the i^{th} site.

The multilevel model comes in at two points (Leyland & Groenewegen 2003). First, as the average outcome (mean final PPD) for each patient may differ, the mean is modelled as a random sample from a hypothetical distribution of all possible patients. The relationship between initial and final PPD is assumed to be the same for all patients: what we are really fitting is a set of parallel lines indicating that the mean final PPD differs between patients for pockets sharing the same characteristic (in this case initial PPD) as illustrated in the left graph of Fig. 2. This is called the random intercept model, which equation is:

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + u_j + e_{ji}$$

where u_j is the residual of the higher level unit (the patient in this case) and e_{ij} the residual associated with a site within each patient. The higher level residual u_j is an effect of the j^{th} patient shared by all sites of that patient.

Second, in a more complex model, the relationship between site characteristic and outcome variable may differ between patients. For some subjects the response of deep sites may be more pronounced than in other patients, as illustrated in the right graph of Fig. 2. To take account of such differential relationships, the regression slopes are allowed to differ between patients and again these slopes are modelled as a random sample. The equation will therefore be:

$$y_{ij} = \beta_0 + \beta_1 x_{ij} + u_{0j} + u_{1j} x_{ij} + e_{ji}$$

where u_{1j} is the slope residual in patient j just as u_{0j} is the intercept residual.

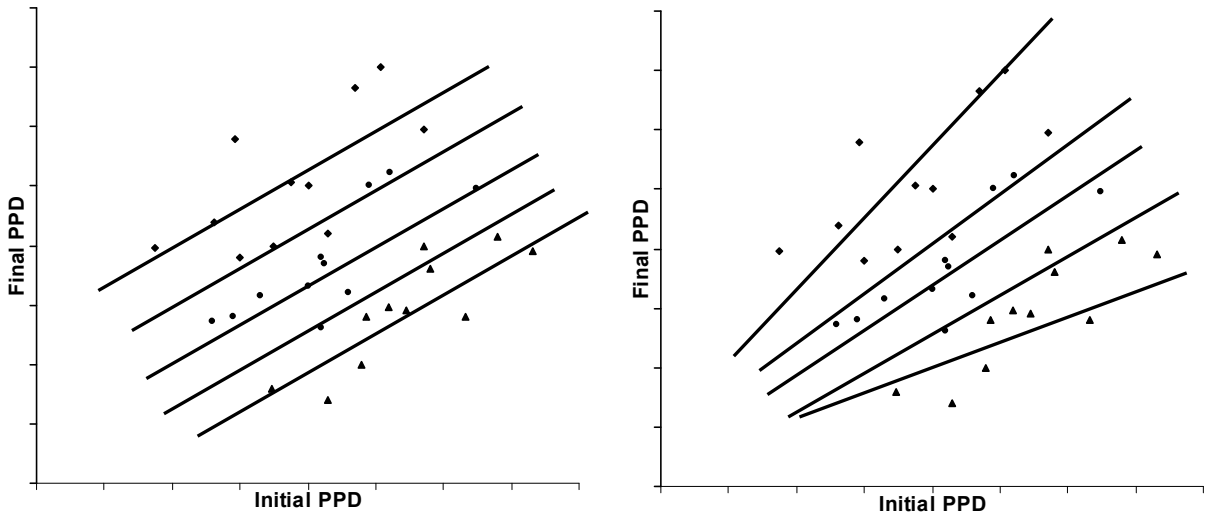

Fig. 2 Multilevel model with random intercept on the left and random slope and intercept on the right

The multilevel models will then include continuous and dichotomous explanatory variables and may be further developed to complex variance, multivariate models, discrete response models, repeated measures models etc.

One of the first studies that applied multilevel analysis to dental research (Albandar & Goldstein 1992) explored in the same model explanatory factors at the subject and at the tooth-site level for periodontal disease progression. The statistical method has subsequently been used for analysis of longitudinal data on gingivitis (Müller & Stadermann 2006), disease characteristics and progression (Gilthorpe et al. 2003, Nieri et al. 2002, Tu et al. 2004a, b), and factors affecting treatment outcome (Axtelius et al. 1999, D'Aiuto et al. 2005).

The **third aim** of the thesis was to investigate, by means of multilevel analysis, factors that may affect the clinical outcome of non-surgical periodontal treatment.

Effects of tobacco smoking

Patients that are smokers show consistently a poorer clinical outcome, as demonstrated in 2 recent reviews on the topic (Heasman et al. 2006, Labriola et al. 2005).

Contradicting results have been reported on the effect of tobacco smoking on the vascular circulation in gingival tissue. Nicotine has been reported to induce localised vasoconstriction in rabbits inoculated with nicotine solution (Clarke et al. 1981). However, in humans, no difference could be detected in gingival blood flow during the act of smoking, while a vasoconstriction could be detected in skin vessels of light smokers (Meekin et al. 2000, Palmer et al. 1999b). On the contrary, an acute effect of smoking with a transitory increase of gingival blood flow and gingival crevicular fluid flow was also reported (Baab & Oberg 1987,

McLaughlin et al. 1993, Morozumi et al. 2004). Smokers was reported to have a reduced vascular response to plaque accumulation (Bergström et al. 1988) and to present a lower BoP compared to non smokers (Shimazaki et al. 2006). On the contrary, the transition from a status of non-inflamed to inflamed gingival margin in response to plaque seems to be more prevalent in smokers compared to non-smokers (Muller et al. 2002). However a re-analysis of the data with a more appropriate statistical technique did not reveal any effect of smoking on the response of gingival tissues to a steady plaque state (Muller & Stadermann 2006).

Pockets oxygen tension was found to be lower in smokers and uncorrelated with oxygen saturation of hemoglobin (Hanioka et al. 2000). The reduction in pO2 was also related to pocket depth, confirming an environmental shift toward anaerobic species in deep sites that seems enhanced in smokers. An influence of smoking habits on subgingival microbial environment was reported in papers considering this variable as a possible explanation of poorer treatment outcome. A high prevalence of *Bacteroides forsythus* (now called *Tannerella forsythia*) and *Prevotella intermedia* in association or not with *Campylobacter rectus* was detected in smokers (Haffajee & Socransky 2001, van Winkelhoff et al. 2001, Zambon et al. 1996). A more limited reduction of these species after treatment of periodontal patients who were smokers compared to non-smokers has also been reported (Darby et al. 2005, Grossi et al. 1997). However other authors did not find a difference in terms of microbial pocket population comparing smokers and non-smokers before (Boström et al. 2001, Darby et al. 2000) or after treatment (Apatzidou et al. 2005).

An influence of smoking on host response could partly explain its effect on periodontal healing after treatment. Different response mechanisms seem to be affected from smoking. Granulocyte activity like enzyme release (Söder 1999, Söder et al. 2002) or mobility (Guntsch et al. 2006, Ryder et al. 2002b) seems to be impaired in smokers. Despite some methodological issues that may have influenced the results (Gustafsson 1996), a lower elastase level in the gingival crevicolar fluid of smokers was reported by some authors (Alavi et al. 1995, Murray et al. 1995, Pauletto et al. 2000). This seems to be in contrast with the association between deep pockets and high elastase levels (Gustafsson et al. 1994). However, this result could reflect an impaired function of neutrophils, with an early release of elastase in the gingival tissues. In contrast, a higher level of elastase-α2-MG complex was found in smoking periodontal patients (Söder 1999), but the fact that MMP-8 and elastase levels were not correlated in smokers as they were in non-smokers still reflects an altered neutrophils function. Considering other aspects of immune response, smokers exhibited reduced

cytotoxicity, increased pro-inflammatory cytokines production and an increased T-cell proliferative response in collected peripheral blood (Zeidel et al. 2002), confirming a systemic effect of smoking. An altered cytokines release from mononuclear blood cells exposed to in-vitro smoking has also been reported (Ryder et al. 2002a). Also plasminogen activator system is affected from smoking (Buduneli et al. 2005). A final effect on the healing mechanism may be related to an impaired fibroblasts adhesion to root surfaces in an in-vitro model (Gamal & Bayomy 2002).

Adjunctive antimicrobial therapy

Since periodontitis is an infectious disease, the use of antimicrobials has rendered attention as a means in periodontal treatment. Considering that antimicrobials show low potential to penetrate subgingival biofilms, it is suggested that the use of antimicrobials in the treatment of periodontitis should be as an adjunct to mechanical debridement and not as an alternative therapy (Cosyn & Wyn 2006, Hanes & Purvis 2003).

In a recent systematic review by Haffajee et al. (2003) on the effect of systemic antibiotic therapy in conjunction with mechanical instrumentation, meta-analysis revealed a statistically significant positive effect, particularly in aggressive periodontitis patients. However, the clinical relevance of a mean effect size of 0.2 mm in CAL gain for chronic periodontitis patients may be questioned. Herrera et al. (2002) published a systematic review on the use of systemic antimicrobials as adjunct to SRP, including 25 studies, and concluded that the overall additive effects of antibiotics on clinical parameters were limited. By the meta-analysis an improvement in terms of CAL and PPD for deep pockets and a reduction of the risk of further attachment loss could be demonstrated. However 3 problems related to the systemic administration were highlighted:

- Adverse effects, particularly related to the gastrointestinal tract
- Risk for development of bacterial resistance
- Compliance.

An alternative approach to the administration of antimicrobials is to apply the drug directly into the diseased pocket. Hence, locally delivered antimicrobials can provide effective concentration of the drug at the site of infection with minimal systemic load (Goodson & Tanner 1992) and a low risk for the emergence of bacterial resistance (Walker et al. 2000). In Table 3 the systematic reviews regarding clinical outcomes of adjunctive use of locally delivered antimicrobials compared to mechanical instrumentation alone are summarized.

Original studies that have been published after the time period covered in the systematic reviews are summarized in Table 4.

Despite a statistically significant adjunctive effect could be demonstrated, two of the systematic reviews (Bonito et al. 2005, Hanes & Purvis 2003) questioned the clinical significance of locally applied antibiotics based on cost/benefit considerations. A similar concern was expressed also in a previous consensus report on the topic (Greenstein & Tonetti 2000), suggesting that the use of locally delivered antibiotics should be restricted to sites or patients not responding adequately to mechanical instrumentation.

Sustained and controlled release devices

Various methods have been utilized to deliver antimicrobial agents into periodontal pockets (Greenstein & Polson 1998, Quirynen et al. 2002). Delivery devices like gels and fibres have been developed to provide an effective concentration of the drug subgingivally for an extended period of time. Metronidazole gel and minocycline gel, categorized as sustained local drug delivery devices, provided increased drug concentration for up to 24 hours, but subsequently decreased rapidly. Other drug devices such as tetracycline fibers, referred to as controlled delivery systems, maintained an effective drug concentration for a period 7 days or more. However insertion of the fibers is time consuming and they have to be removed from the pocket at a recall appointment. These disadvantages led to the development of re-absorbable controlled delivery devices, like microspheres and polymers that maintain an effective drug concentration until they are re-absorbed after a time varying from 7 to 10 days.

Tetracyclines

The most commonly used antibiotics incorporated in controlled delivery devices are tetracyclines. This group of antibiotics have a broad spectrum activity and inhibit bacterial protein synthesis, hence requiring a long exposure time to exert antimicrobial effects at the concentration found in the crevicular fluid after systemic administration (3-6 μg/ml), but bactericidal at the high concentrations reached with sustained release devices (Stoller et al. 1998). Tetracyclines show also substantivity (retained on root surfaces) and can penetrate the epithelial tissue for 1 to 20 μm after local delivery.

Tetracyclines present also non-antimicrobial properties that could be important in the healing process, as they can modulate the inflammatory host response. These properties have been extensively evaluated by the use of chemically modified tetracyclines (CMTs) that are depleted from antimicrobial function.

In in-vitro experiments doxycycline and CMTs are reported to inhibit proteases by means of 3 mechanisms: blocking the conversion of latent proteases in active mature forms, preventing MMPs activation by chelating metal ions and preventing the inactivation of proteinase inhibitors both for proteinases of bacterial and of host tissues origin (Acharya et al. 2004, Grenier et al. 2002), (Golub et al. 1995, Korostoff et al. 2000). CMT-5, which lacks the structural elements required for cation chelation, did not show the same properties in those *in-vitro* tests.

Table 3 Systematic reviews on locally delivered antibiotics in non-surgical therapy

Author/year	Type	Aim	Inclusion/exclusion criteria	Clinical Variab.	Number of studies	Clinical outcome	Author's Conclusions	Observations
Hung & Douglass (2002)	Systematic review and Meta-analysis	To report a meta-analysis of studies that have investigated the effect of scaling and root planing on PPD and attachment loss Report on evidence related to the effect of SRP when compared to or combined with local antibiotic	RCT with SRP as primary treatment arm 80% of patients included in 1 year follow up PPD and CAL reported Sample size reported Stratification based on initial PPD	ΔPPD ΔCAL	**38 papers selected** 9 selected on SRP 12 selected on tetracycline 11 selected on 25% metronidazole 6 selected on 2% minocycline	Effect of SRP Initial pocket ΔPPD ΔCAL Shallow PPD 0.2 -0.3 Medium PPD 1.2 0.5 Deep PPD 2.2 1.2 Difference between Tetrac.+SRP and SRP ΔPPD ΔCAL TC/RP -RP 0.35 0.18 Difference between Metro..+SRP and SRP ΔPPD ΔCAL Met/RP -RP 0.23 0.15 Difference between Mino.+SRP and SRP ΔPPD ΔCAL Min/RP -RP 0.61 0.24	Patient preferences for one type of periodontal therapy over another, and/or the self-perceived skill or style of a provider within a particular dental practice may be factors that currently determine which therapies among reasonable alternatives are chosen.	The effect of scaling and root planing is not significant for shallow pockets but it is significant for medium and deep pockets. The three local antibiotic therapies alone did not show any benefit. Combination with SRP showed a tendency for better outcome.
Greenstein (2006)	Systematic review and Meta-analysis	Controlled clinical trials were selected that assessed the capability of local drug delivery to improve periodontal health.	RCT	ΔPPD ΔCAL	**19 papers selected** 4 on chlorhexidine chip 2 on doxycycline gel 2 on metronidazole gel 4 on minocycline gel 3 on minocycline microspheres 4 on tetracycline fibers	SRP plus local anti-infective versus SRP alone ΔPPD ΔCAL Chlorhexidine chip 0.553 0.269 Doxycyline gel 0.360 0.231 Metronidazole gel 0.020 0.041 Minocycline gel 0.306 0.131 Minocycline microspheres 0.538 -1.286 Tetracycline fiber 0.180 -0.129 Combined result 0.338 0.058	The clinician's decision to use local drug delivery as an adjunct to SRP needs to be determined on an individual case basis, and factors that should be considered include data reported in the literature, clinical findings, desired clinical outcomes, and the patient's medical and dental history.	Use of local antimicrobials alone was found not significant. Systemic antibiotics were also compared but with a non-systematic approach.

Authro/year	Type	Aim	Inclusion/exclusion criteria	Clinical Variab.	Number of studies	Clinical outcome	Author's Conclusions	Observations
Bonito et al. (2005)	Systematic review and Meta-analysis	To report on treatment of chronic periodontitis in adults focused on the use of locally applied adjunctive antimicrobials.	Clinical trials published in English 1) involved adults with chronic periodontitis 2) tested one or more antimicrobial agents as an adjunct to SRP 3) had a concurrent control group 4) fixed time periods; 5) if multiple antimicrobials were tested, reported outcomes separately	ΔPPD ΔCAL	**50 paper (52 arms) selected** 17 on chlorhexidine 11 on metronidazole 8 on minocycline 16 on tetracycline	SRP plus local anti-infective versus SRP alone ΔPPD ΔCAL Chlorhexidine 0.24 0.16 Metronidazole 0.32 0.12 Minocycline 0.49 0.46 Tetracycline 0.47 0.24	SRP alone seems to produce significant improvements in mean PD reductions or CAL gains in the range of 1.5 mm or more. Improvements produced by adjunctive antimicrobials pose two difficult questions: - whether such improvements are clinically meaningful over time. - whether these improvements justify the likely added costs	All different delivery devices were pooled together according to antimicrobial used. Only one paper with doxycycline included, therefore no meta-analysis was done.
Hanes & Purvis (2003)	Systematic review	To evaluate literature-based evidence in an effort to determine the efficacy of currently available anti-infective agents with and without concurrent SRP in controlling chronic periodontitis	Types of studies included were RCT, case-controlled studies and cohort studies of at least 3 months duration in patients with chronic periodontitis. Both parallel patient groups and split-mouth designs were acceptable. Required therapeutic interventions were 1) scaling and root planing (SRP) alone 2) local anti-infective drug therapy combined with SRP or 3) local anti-infective drug therapy alone	ΔPPD ΔCAL BoP	80 screened **32 selected**	Effect of SRP (adjusted mean) ΔPPD ΔCAL 1.45 0.89 BoP red.48.7% GI red.41% Pl. red.40.6% SRP plus local anti-infective versus SRP alone ΔPPD ΔCAL Chlorhexidine 0.35 0.16 Doxycycline 0.51 0.34 Metronidazole 0.06 0.07 Minocycline gel 0.36 0.39 Minocycline microspheres 0.26 -0.40 Tetracycline 0.21 -0.17 SRP alone versus local anti-infective ΔPPD ΔCAL -0.03 0.08 SRP alone versus SRP and CHX irrigation ΔPPD ΔCAL No difference	In patients with chronic periodontitis scaling and root planing alone results in statistically significant reductions in PPD, gains in CAL and improvements in measures of gingival inflammation. Specific agents and sustained-release systems with significant summary effects on PD reduction were MINO gel and microencaps. MINO. Significant effects on CAL gain were observed in studies of CHX chip and DOXY gel.	Quite considerable variation between studies and devices. Reports on adverse events very infrequent. No differentiation based on initial PPD or smoking habits.

Table 4 Original papers on locally delivered antibiotics in non-surgical therapy from 2005 on

Author/year	Design	Aim	Inclusion/Excl. criteria	Variable	Patients, treat. and follow-up	Results	Author's conclusion	Comments
Goodson et al. (2007)	RCT Parallel Single-masked	To measure the antimicrobial effects of a minocycline HCl microsphere (MM) local drug-delivery system when used as an adjunct to scaling and root planing (SRP).	Good general health; Age 30 to 65 years; ≥16 teeth and 5 sites with PDs ≥5 mm in 5 non-adjacent sites Exclusion: pregnant, lactating, no perio or antibiotic therapy within 3 m; allergic to tetracyclines.	PPD BoP CAL Micro (DNA probe for 40 species)	127 patients Age 50 mean 2 groups: MM: SRP + Arestin SRP: SRP alone Re-examination at	MM SRP PPD reduction 1.38 * 1.01 CAL reduction 1.16 * 0.80 BOP reduction 25.2% * 13.8% *P.g* proportion 2.5% * 1.7% *T.f* proportions 2.7% * 2.5% *T.d* proportions 1.4% * 0.8% *P.g* numbers X10^5 3.71 * 1.54 *T.f* number X10^5 4.32 3.57 *T.d* numbers X10^5 1.40 * -0.003 ·	Locally delivered MM inhibited periodontal pathogens. Ajunctive MM significantly reduced RCB, PD, CAL, and BOP to a greater extent than treat by SRP alone.	The clinical relevance of reported microbial changes may be questioned. Differential between clinical parameters is in line with previous reviews.
Cortelli et al. (2006)	RCT Parallel Double-masked	To evaluate the clinical response to S/RP combined with the use of locally delivered minocycline microsp. in individuals with advanced chronic periodontitis	Non-treated chronic periodontitis; non-smoker; good general health. Exclusion: diabetes;immunoc. subjects; pregnancy; periodontal treatment 12 months before.	PI GI PPD	59 enrolled, 33 drop-out Age 47 mean Test: SRP + M. Control: SRP + Vehicle Re-examination at 90, 180, 270, 360, 720 days	Time Test Control Baseline 7.47 7.73 90 days 4.33 5.07 180 days 4.07 4.93 270 days 3.93 * 5.07 360 days 3.89 * 5.20 720 days 5.20 5.93	Both therapies reduced mean PD from 90 to 360 days; SRP combined with the use of subgingival minocycline showed a higher reduction at 270 and 360 days	11 patients left and other 22 excluded due to maintenance protocol not followed. A patient selection bias cannot be excluded. Smokers not included
Machion et al. (2006)	RCT Parallel Single-masked	To evaluate the long term effects of the association of locally delivered doxycycline to scaling and root planing compared to conventional mechanical therapy in the treatment of chronic periodontitis in smokers.	1) chronic periodontitis patients, showing a minimum of 4 pockets (≥5 mm BOP+) on anterior teeth 2) patients who smoked ≥10 cigarettes/day for a minimum of 5 years; Exclusion criteria: SRP 6 months prior to the study; periapical lesion; allergy; antimicrobials last 6 months	PI GI PPD RAL	48 patients included, 18 drop-out Age 41 mean 2 groups. Test: SRP + Locally delivered Doxycycline Control: SRP Treatment repeated after 12 month in PPD ≥5 mm BoP	Periods Test Control **PD Reduction** 3 months 2.02 1.62 6 months 2.08 1.76 12 months 1.63 1.61 15 months (Retr.) 1.84 2.15 24 months 2.29 2.19 **RAL Gain** 3 months 1.40 1.11 6 months 1.64 * 1.04 12 months 1.31 0.99 15 months (Retr.) 1.20 1.07 24 months 1.58 * 0.70	The use of locally delivered doxycycline may constitute an important adjunct for the active and supportive treatments of severe periodontal disease in smokers.	Significance as reported in the paper. Recession more limited in Test group at 2 years. Results at 6 month reported previously (Machion et al. 2004) for 43 patients.
Lu & Chei (2005)	RCT Split-mouth	To compare the clinical effect of subgingivally applied 2% minocycline hydrochloride plus S/RP vs. S/RP alone on clinical for the treatment of chronic periodontitis	- good general health age> 20 years - more than 16 teeth; - 6-mm deep residual pockets and BoP+. Exclusion criteria: - pregnant / nursing; allergic; antibiotic treatment; perio treatment previous 3 months.	PPD PAL GI BoP	15 patients Age 43 mean 2 groups: Test: SRP + Mynocycline Control: SRP Re-examination at 6, 10, 14, 18 weeks	Results at 3 month re-examination Test Control PPD 5.1 * 3.9	S/RP combined with subgingival administration of minocycline ointment have a significantly better and prolonged effect compared to S/RP alone on the PPD, CAL, GI, but not on BoP	Site used as statistical unit without considering clustering. Data reported in graphs

In the rat model, where periodontitis was induced inoculating bacterial LPS, CMT-8 reduced the activity of enzymes like collagenase, gelatinase, MMP8 and elastase which cause tissue degradation (Golub et al. 1999, Llavaneras et al. 1999). Doxycycline showed the same effect on inflammation in a similar rat model, with in addition an increased collagen I and collagen XII mRNA expression and a consequent increased secretion from fibroblast (Karimbux et al. 1994). In this animal model doxycycline was found to inhibit matrix metalloproteinase's activity (Buduneli et al. 2007, Llavaneras et al. 2001, Ramamurthy et al. 2002) and down-regulate bone resorption (Bezerra et al. 2002).

The **fourth aim** of the present thesis was to evaluate if adjunctive, locally delivered controlled-release doxycycline might counteract the negative effect of smoking on periodontal wound healing following non-surgical pocket instrumentation.

Taking into consideration the general concern of avoiding un-necessary use of antibiotics due to the risk for emergence of resistant bacterial strains (Aracil et al. 2001, Perez-Trallero et al. 2001), as well as the fact that a majority of periodontal pockets will respond favourable to initial, mechanical pocket/root debridement, the utilization of antibiotics in the treatment of chronic periodontitis may preferably be in the phase of re-treatment of sites with persisting pathology at time of re-evaluation after initially performed SRP. However, most studies in the literature involved the use of antibiotic therapy in conjunction with initial SRP and information on the effect of locally delivered antibiotics as an adjunct to repeated instrumentation of initially poorly responding periodontal pockets is inconclusive (Kinane & Radvar 1999, Tonetti et al. 1998, Wennström et al. 2001).

The **fifth aim** of this thesis was to evaluate if adjunctive, locally delivered controlled-release doxycycline might improve the outcome of re-instrumentation of pathological pockets persisting after initial periodontal therapy.

AIMS

The specific objectives of the studies included in this thesis were:

- To evaluate the clinical efficacy of a single session of full-mouth ultrasonic debridement as an initial periodontal treatment approach in comparison with the traditional treatment modality of consecutive sessions of quadrant scaling/root planing (Study I).
- To analyze the effect of re-instrumentation of periodontal pockets not properly responding to initial subgingival instrumentation (Study I).
- To evaluate the incidence of disease recurrence following a full-mouth pocket/root debridement approach with ultrasonic instrumentation versus that following a traditional approach of quadrant-wise scaling and root planing performed with hand-instrumentation (Study II).
- To investigate, by means of multilevel analysis, factors that may affect the clinical outcome of non-surgical periodontal treatment (Study III).
- To evaluate if adjunctive, locally delivered controlled-release doxycycline might counteract the negative effect of smoking on periodontal wound healing following non-surgical pocket instrumentation (Study IV).
- To evaluate if adjunctive, locally delivered controlled-release doxycycline might improve the outcome of re-instrumentation of pathological pockets persisting after initial periodontal therapy (Study V).

MATERIAL AND METHODS

Study samples

Subjects were recruited among patients referred for treatment of chronic periodontitis at the Clinic of Periodontics, Department of Periodontology, Sahlgrenska Academy at Göteborg University, Sweden in all the studies. For studies I-III, around half of patients were recruited from a private practice in Trento, Italy. In study IV, patients were also recruited in the periodontal clinic of Eastman Dental Institute, London, UK and of University of Missouri, Kansas City, USA.

Inclusion and exclusion criteria for patients

For all studies, the age of the patients had to be between 25 and 75 years. Furthermore, patients had to be in good general health and were excluded if subjected to subgingival instrumentation or to the use of antibiotics during the year prior to the start of the study.

The inclusion criteria regarding periodontal status were the following:

Study I-III: a minimum of 18 teeth of which at least 8 teeth had to s3how probing pocket depths (PPD) of ≥ 5 mm and bleeding on probing. At least 2 of these teeth had to have a PPD of ≥ 7 mm and at additional 2 teeth the pockets must measure ≥ 6 mm.

Study IV: at least 8 periodontal sites with PPD ≥5 mm located in 2 jaw quadrants.

Study V: a minimum of 20 teeth of which at least 10 teeth must show probing pocket depths (PPD) of ≥ 5 mm and bleeding on probing. At least 2 of these teeth must have a PPD of ≥ 7 mm and at additional 2 teeth the pockets must measure ≥ 6 mm.

Power calculation and ethical approval

Power calculation was performed before the start of each study based on the detection of a difference in mean PPD reduction of 0.5 mm between treatment groups, assuming that the common standard deviation was 0.6 mm (or 0.5 mm in study V), and with an alpha error defined at 0.05 and beta error at 0.20.

Approval of the study protocols by the Ethics Committee at Göteborg University (and at Eastman Dental Institute, London, UK and at University of Missouri, Kansas City, USA for study IV) was obtained and all participating subjects provided informed consent before the start of each study.

Study designs

All study protocols included repeated instructions in self-performed oral hygiene measures.

Study I to III

Fig. 3 illustrates the flowchart of the study. 42 chronic periodontitis patients were randomly assigned to 2 treatment groups:

Full-mouth ultrasonic debridement (Fm-UD) – Test. The patients assigned to this treatment group received at baseline (Day 0) a one-hour session of full-mouth subgingival debridement using a piezoceramic ultrasonic instrument (EMS Piezon Master 400 with A+PerioSlim tips, water coolant and power setting to 75%; EMS, Nyon, Switzerland). After re-examination at 3 months, re-instrumentation (no time restriction) with the use of the ultrasonic device was performed in all sites with a remaining PPD of ≥ 5 mm.

Quadrant scaling/root planing (Q-SRP) – Control. The patients in the this group were subjected to quadrant-wise scaling and root planing at 4 sessions with an interval of one week. An assortment of manual periodontal curettes was used (LM-dental, Turku, Finland). Following a re-examination 3 months after completion of the baseline treatment, all sites with a remaining PPD of ≥ 5 mm were carefully re-scaled and root planed (no time restriction).

One month following the completion of the baseline treatment all patients were recalled for professional *supragingival* plaque control and reinforcement of oral hygiene.

Clinical re-examinations were performed 3 and 6 months after the completion of baseline treatment. The data collected at the 3 month re-examination were used with regard to analysis of factors affecting the treatment outcome in study III. 1 year after the last re-examination (18 month from baseline), the patients were recalled for a follow-up examination (study II).

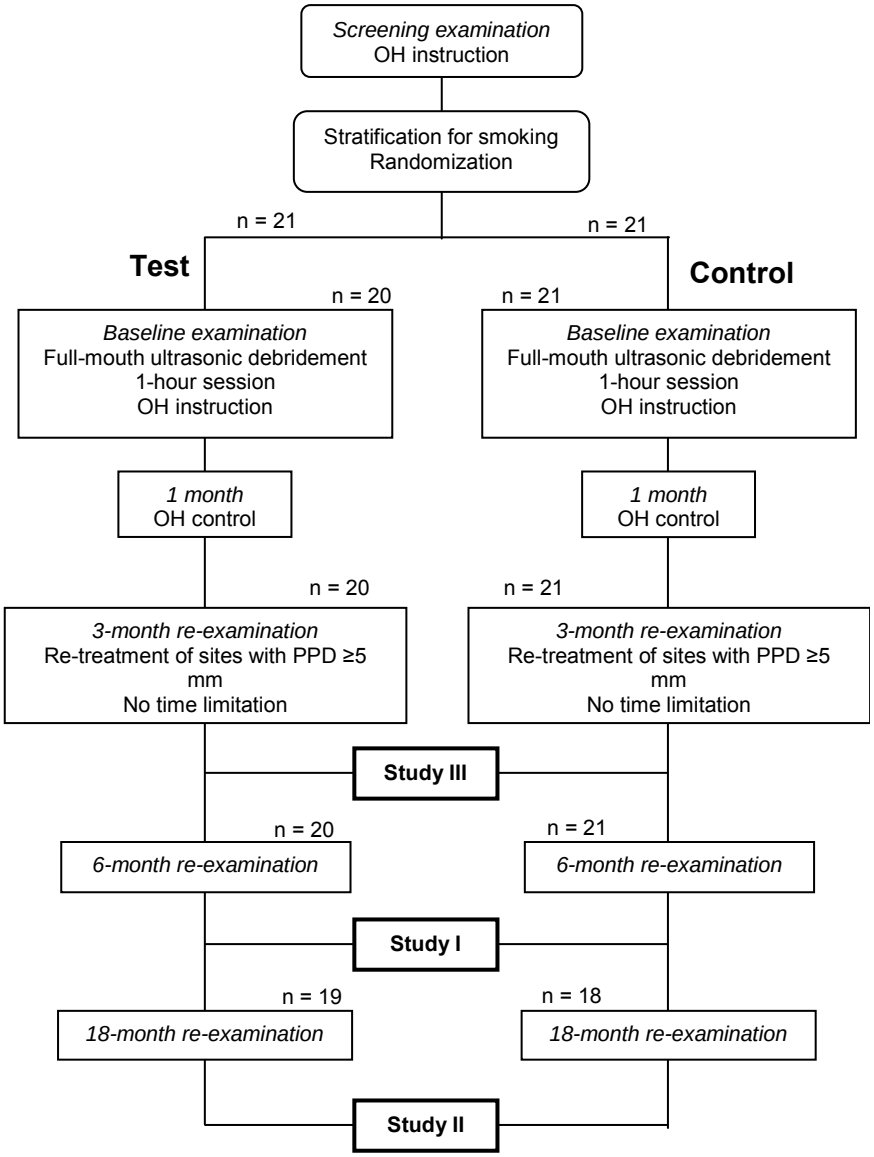

Fig. 3 Flow chart of study design (I-III)

Study IV

Fig. 4 illustrates the flowchart of study IV. 103 patients (42 smokers, 61 non-smokers) with chronic periodontitis were, following stratification for smoking, randomly assigned to 2 different treatment protocols (Test and Control). The subjects of the Test group received at baseline a single session of full-mouth supra-/subgingival debridement by ultrasonic instrumentation. Immediately following the pocket instrumentation, a 8.5% w/w doxycycline gel (Atridox™; Block Drug Corporation, Inc., Jersey City, NJ, USA) was applied in all sites with probing depth ≥ 5 mm in 2 experimental jaw quadrants. The patients randomized to the Control group were subjected to full-mouth supra- and subgingival scaling/root planing using ultrasonic and hand instruments but no application of antibiotic gel.

Clinical examinations were performed before treatment (baseline) and after 3 months.

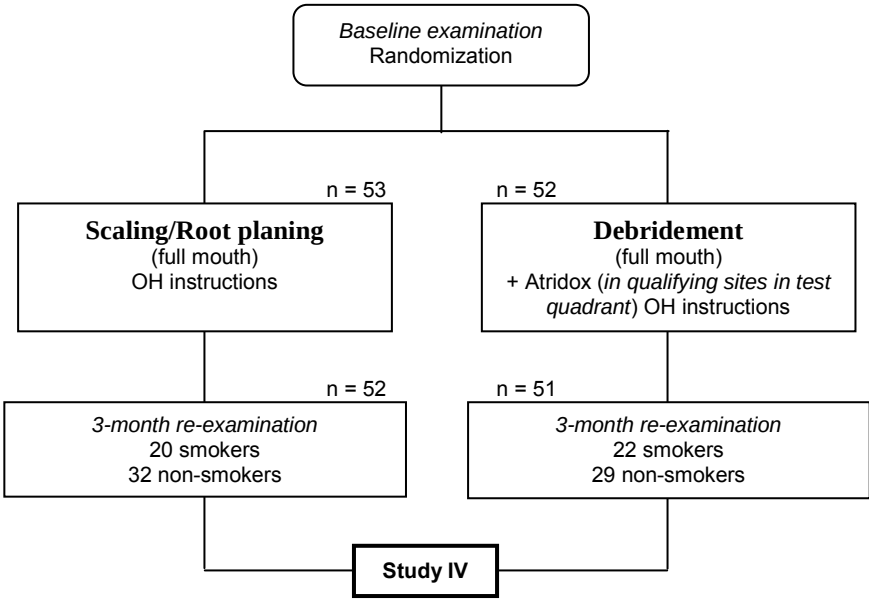

Fig. 4 Flowchart of study design (IV)

Study V

Fig. 5 illustrates the flowchart of study V. Following an initial examination, 32 chronic periodontitis patients were given a 1-hour session of full-mouth supra-/subgingival debridement using a piezoceramic ultrasonic instrument with A tip and PS tip, water coolant and power setting to 75% (Piezon Master 400, EMS, Nyon, Switzerland). The patients were recalled after 1 month for professional supragingival plaque control and reinforcement of oral hygiene and after 3 months for the baseline examination. The selected subjects were then stratified according to smoking habits, i.e. current smokers and non-smokers and randomly assignment to two treatment protocols for re-treatment of all sites with a PPD of ≥ 5 mm (experimental sites).

The patients assigned to the Test group received re-instrumentation (no time restriction) with the use of the ultrasonic device. Immediately following pocket instrumentation, an 8.8% w/w doxycycline gel was applied in all re-treated pockets. The patients randomized to the Control group were subjected to ultrasonic re-instrumentation only. One and 3 months following the completion of the re-treatment all patients were recalled for professional supra-gingival plaque control and reinforcement of oral hygiene. Re-examinations were performed 3 and 9 months after the re-treatment.

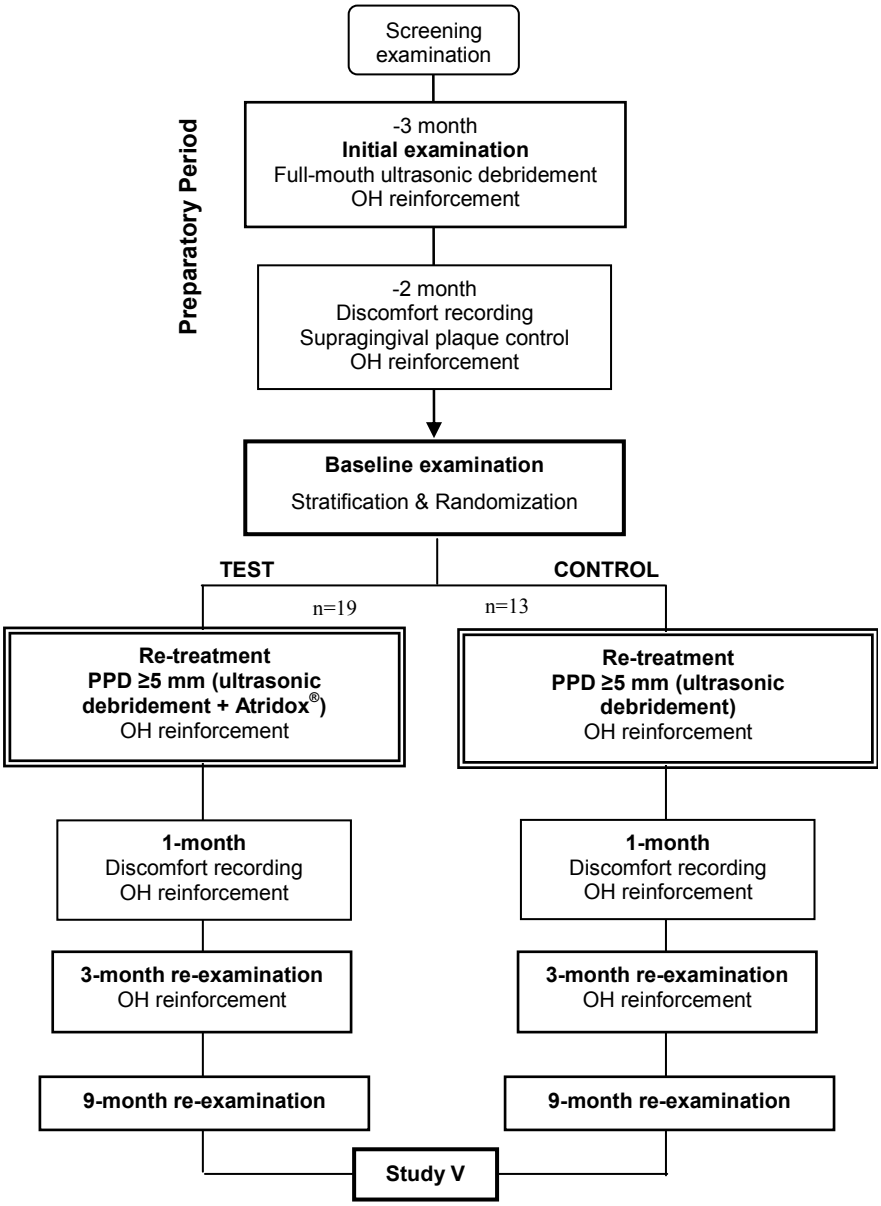

Fig. 5 Flowchart of study V design

Clinical examinations

The following variables were recorded at the mesial, buccal, distal and lingual surfaces of each tooth (study I - III) or at 6 points around each tooth (study IV) or at 6 points around monoradiculated teeth, 9 points around upper molars and 12 points around lower molars (study V):

- *Plaque* (PI) - presence/absence of plaque at the cervical area of the tooth detected by running a probe along the surface.

- *Probing pocket depth* (PPD) - the distance in mm from the gingival margin to the bottom of the probeable pocket.
- *Bleeding on probing* (BoP) - presence/absence of bleeding within 15 sec following pocket probing.
- *Location of gingival margin* (GM): the distance between the gingival margin and a fixed reference point on the tooth (CEJ or the margin of a restoration). A negative value was given when the gingival margin was located coronal to the CEJ.
- *Relative attachment level* (RAL) was calculated as PPD + GM.
- *Furcation involvement* (FI) was assessed according to Hamp et al. 1975.

Radiographic examination

A full-mouth set of intraoral radiographs was obtained at time of screening. Presence of infrabony defects ≥ 3 mm was recorded for each tooth (Studies I - III and V).

Other recordings

Information regarding smoking habits was obtained through a questionnaire. As smokers were considered all patients who reported that they currently were regular smokers.

Adverse events (abscess, pain, swelling) and use of drugs for post-treatment pain control were recorded by the use of a questionnaire. The patients were also asked to judge the overall degree of treatment discomfort on a 100 mm Visual Analogue Scale (VAS). Time spent for the various phases of the treatments was also recorded.

Quality control of assessments

For all studies, each patient was assigned to one examiner that was masked with respect to the treatment assignments. Before the start of the studies, the examiners were trained to adequate levels of accuracy and reproducibility for the various clinical parameters and indices to be used (Polson 1997). Repeated assessments were performed during the course of the study in randomly selected subjects in order to determine the intra- and inter-examiner reproducibility.

Data handling and analysis

All data handling and statistical testing was performed with the use of the SPSS 12.0 or 13.0 software package (SPSS inc., Chicago, Illinois, USA). A statistical package specifically designed for multilevel modeling (MLwiN 2.02, © Centre for Multilevel Modelling at

University of Bristol, UK) was used to investigate the influence of covariates at different levels on selected outcome variables.

Patient mean values were calculated as a basis for the statistical analysis. Mean values, standard deviations and proportions of sites within various categories of scoring units were calculated for data description using the patient as statistical unit. The distribution of continuous variables was initially analyzed with the Kolmogorov-Smirnov test to verify the normality of the data.

Study I: Primary efficacy variables were considered to be percentage of "closed pockets", i.e. PPD $\leq$ 4 mm, and changes in BoP, PPD and RAL. Difference in PPD between the groups at baseline was tested by the use of the Student t-test for independent samples. Changes in PPD and RAL were statistically analyzed by the use of repeated measures analysis of variance and differences in proportions with the use of 2x2 tables and the Fisher's Exact test. Differences in mean proportions of "closed pockets" were analyzed using the Mann-Whitney U-test. As a descriptor of the efficiency of the two treatment protocols, the mean treatment time taken to achieve closure (i.e. PPD $\leq$ 4 mm) of one pocket was determined (time used for instrumentation /number of pockets closed) and differences were analyzed using the Mann-Whitney U-test. All statistical tests were 2-tailed and conducted at a significance level of $p < 0.05$.

Study II: The primary outcome variable was the incidence of recurrent sites (i.e. sites showing PPD $\geq$ 5 mm and BoP+) between the post-treatment and 1-year follow-up examinations. Differences between mean values were statistically analyzed by the use of repeated measurements analysis of variance and differences in proportions with the use of 2x2 tables and the Fisher's Exact test. The Chi-square test was used to determine the differences in dichotomous variables. A p-value of < 0.05 was considered as statistically significant.

Study III: The primary outcome variable was "pocket closure" (PPD $\leq$ 4mm) at the 3-month re-examination. A secondary outcome variable tested was the PPD at 3 months. The levels that were identified for the hierarchical analysis were the patient, the tooth and the tooth site. The database consisted of 1,447 tooth sites at 771 teeth in 41 patients. The various factors associated with the 3 levels that were tested are given in Table 5.

Table 5 Variables included at patient-, tooth-, and site levels

Patient-related variables (level 3)	Tooth-related variables (level 2)	Site-related variables (level 1)
Gender	Tooth type	Initial PPD (mm)
Smoker		3 month PPD (mm)
Treatment		Intrabony defect
Treatment Time		Tooth site (m/b/d/l)
Age		Plaque presence
Plaque score		BoP positive
BoP score		
% of qualified sites		
% of closed pockets		

As the main outcome variable (pocket closure) was dichotomous, with a value of 1 indicating treatment success and 0 otherwise, a logistic regression model was created to evaluate factors affecting the probability of closing a pocket.

Let y_{ij} denote a binary response (0 or 1) for the i^{th} site in the j^{th} patient, and let π_{ij} be the probability of success (i.e. $y_{ij}=1$) such that $y_{ij} \sim Bin(1,\pi_{ij})$. The generalized linear model approach transforms the binary response using the logit function in order to estimate the effect of covariates on the probability of success. The linking function for a 2 levels model will be:

$$\text{logit}(\pi_{ij}) = \log\left\{ \pi_{ij} \Big/ 1-\pi_{ij} \right\} = \beta_0 + \beta_1 x_1 + u_j$$

where β_0 represents the intercept, β_1 the parameter for the tested covariate and u_{0j} the random part of the equation, namely the patient effects or residuals.

The logit function was used to link the linear model with the probability of the binary event so that, if ß is the intercept, the antilogit function of the parameter ß was calculated with the formula: [(1+exp(-β))-1] to obtain the probability of "pocket closure" (Snijders & Bosker 1999).

The model was applied to the data and the parameters estimated with a 2nd order PQL (penalised quasi-likelihood) procedure implemented in the software and the significance of each covariate was tested using a Wald test. The covariates were estimated individually by adding them to the null model and testing the significance. The final model included all factors that were found significant. The intra-class correlation (ICC), i.e. the proportion of the

total variance attributed to the patient level, was approximated using the formula:

$ICC = \frac{\sigma_u^2}{\sigma_u^2 + \frac{\pi^2}{3}}$ according to Snijders & Bosker (1999), where σ_u^2 is the variance of u_{0j}.

For the secondary outcome variable, PPD at 3 months, a multilevel model for a continuous variable was formulated including tests for the normality of the residuals at the different levels. With y_{ijk} denoting a continuous response for the i^{th} site in the j^{th} tooth in the k^{th} patient, the model is:

$$y_{ijk} = \beta_0 + \beta_1 x_{ijk} + v_{0k} + u_{0jk} + e_{0ijk}$$

where v_{0k} are the residuals at patient level, u_{0jk} at tooth level and e_{0ijk} the residuals at site level. Regression coefficients were estimated using IGLS (iterative generalized least squares). Nested models were tested for significant improvements in model fit by comparing the reduction in -2LL (-2 log likelihood) with a Chi-squared distribution. As the interpretation of the intercept with the value 0 mm as initial PPD has no clinical meaning, a new "centered" initial PPD (PPD-5) was introduced in the models.

Study IV: The primary efficacy endpoints were changes in probing pocket depth and clinical attachment level. The individual mean PPD and PAL changes at 3 months were plotted against initial mean PPD and regression lines were calculated for illustration of a potential relation between the variables.

Stepwise regression analysis was used to identify factors predicting the primary outcome variables (PPD and PAL). A simple correlation analysis was first carried out and the variables found to be significantly correlated with any of the outcome variables were included as dependent variables in the stepwise regression models. The variable "smoking" was included in all analyses.

Study V: Primary efficacy variables were considered to be percentage of "closed pockets", i.e. a PPD ≤4 mm, and changes in BoP, PPD and RAL. Difference in PPD between the groups at baseline was tested by the use of the Student t-test for independent samples. Changes in PPD and RAL were statistically analyzed by the use of repeated measures analysis of variance and differences in proportions with the use of 2x2 tables and the Fisher's Exact test.

The probability of the binary events of "pocket closure" and a change in PPD or RAL of ≥2 mm was analyzed with the use of logistic multilevel bi-variate analyses. The influence of

different factors on the outcome was investigated by the use of multilevel regression analyses.

RESULTS

Study I

Aim: to evaluate the clinical efficacy of a single session of full-mouth ultrasonic debridement as an initial periodontal treatment approach in comparison with the traditional treatment modality of consecutive sessions of quadrant scaling/root planing and analyze the effect of re-instrumentation of periodontal pockets not properly responding to initial subgingival instrumentation.

Treatment outcome

Following the baseline treatment, a marked reduction of the full-mouth BoP scores was observed in both treatment groups. Hence, at the 3-month re-examination the BoP score was reduced from 74% to 29% in Fm-UD group and from 80% to 32% in the Q-SRP group. The re-treatment at 3 months resulted in a further reduction of the BoP scores. No statistically significant difference between the 2 treatment groups was observed at any of the examination intervals.

At the 3-month re-examination, the probing assessments revealed a mean PPD reduction of 1.8 mm and a mean RAL gain of 1.2-1.3 mm in the two treatment groups (Table 6). The re-treatment of remaining pathological pockets resulted in a further overall mean PPD reduction of 0.4 mm and a mean RAL gain of 0.3 mm at the 6-month re-examination. Analyzing the data only for sites subjected to re-treatment, the mean PPD reduction amounted to 1.0 mm (ultrasonic instrumentation) and 0.8 mm (hand instrumentation), with a RAL gain of 0.7 and 0.6 mm, respectively. No significant differences were found between the treatment groups at any of the time intervals in terms of overall mean alterations or when the probing data were analyzed according to baseline PPD categories (5-6 mm and ≥ 7 mm).

Table 6: PPD and RAL change at the various examination intervals and according to initial PPD category. Mean values in mm (S.D.). Subject level.

Initial PPD	all		5-6 mm	≥ 7 mm	5-6 mm	≥ 7 mm
	Q-SRP	Fm-UD	Q-SRP		Fm-UD	
Baseline PPD	6.1 (0.5)	6.2 (0.5)	5.4 (0.2)	7.8 (0.5)	5.4 (0.2)	7.8 (0.4)
PPD change						
3 months	1.8 (0.6)	1.8 (0.5)	1.6 (0.5)	2.3 (0.9)	1.6 (0.4)	2.2 (0.8)
6 months	2.2 (0.6)	2.2 (0.5)	1.8 (0.5)	2.9 (0.7)	1.8 (0.4)	2.9 (0.7)
RAL gain						
3 months	1.2 (0.4)	1.3 (0.5)	1.1 (0.4)	1.6 (0.8)	1.1 (0.5)	1.7 (0.7)
6 months	1.5 (0.5)	1.6 (0.4)	1.3 (0.5)	2.1 (0.7)	1.3 (0.5)	2.2 (0.7)

The proportion of sites reaching the successful treatment endpoint of "pocket closure", i.e. a PPD of ≤ 4 mm, after the initial treatment phase is presented in Table 7. The Q-SRP showed at 3 months a tendency to have a more favourable outcome in sites with PPD ≥ 7 mm compared to the Fm-UD approach (36% vs. 25%). Following re-treatment of remaining pockets, the mean percentage of closed pockets increased to 74% for Fm-UD and to 77% for Q-SRP. For sites with an initial PPD of ≥ 7 mm, the corresponding figure was 47% and 50%, respectively. No statistical significant differences were observed between the treatment groups at the various examination intervals.

Table 7: Proportion (%) of pockets closed (PPD ≤ 4 mm)at all time points and according to initial PPD. Mean values and standard deviation.

Initial PPD	all		5-6 mm	≥ 7 mm	5-6 mm	≥ 7 mm
	Q-SRP	Fm-UD	Q-SRP		Fm-UD	
% Closed pockets						
3 months	66% (21)	58% (16)	77% (20)	36% (28)	73% (13)	25% (24)
6 months	77% (18)	74% (15)	86% (17)	50% (28)	86% (12)	47% (23)

Treatment efficiency

The efficiency of the treatment approaches was expressed as average number of minutes of instrumentation used to achieve "pocket closure" at 1 site. For the initial treatment phase, the Fm-UD approach showed significantly higher efficiency than Q-SRP; 3.3 versus 8.8 min per closed pocket ($p<0.01$). Compared to the initial treatment phase, the efficiency of the re-treatment session at 3 months was markedly lower in both treatment groups (11.5 - 12.6 min) and without significant difference between hand and ultrasonic instrumentation.

Treatment discomfort

The subjective rating of the degree of treatment discomfort following the initial treatment phase revealed no difference between the two treatment approaches; median VAS scores 2.0 (range 0-5). One (5%) of the patients subjected to the Fm-UD approach reported increased root sensitivity for a duration of ≥ 5 days post-treatment, whereas the corresponding figure for the Q-SRP approach was 7 (33%).

Study II

Aim: to evaluate the incidence of disease recurrence following a full-mouth pocket/root debridement approach with ultrasonic instrumentation (Fm-UD) versus that following a traditional approach of quadrant-wise scaling and root planing performed with hand-instrumentation (Q-SRP).

Recurrence of diseased periodontal pockets

At the 1-year follow-up examination 12 patients (63%) in the Fm-UD group presented recurrent diseased pockets (i.e. PPD ≥5 mm and BoP+), compared to 14 patients (78%) in the Q-SRP group. Of these patients, 9 patients in the Fm-UD treatment group presented 2 or more sites with recurrent pockets versus 11 in the Q-SRP group.

29 pockets (7%) in the Fm-UD group and 47 pockets (11%) in the Q-SRP group showed recurrence of clinical signs of disease; 15 sites (52%) in the Fm-UD group and 31 (66%) in the Q-SRP group revealed an increase in probing depth of ≥2 mm. A PPD of ≥6 mm was observed at 8 sites (2%) in the Fm-UD group and 10 (2%) in the Q-SRP group. The difference in terms of number of patients or sites with recurrence of disease between the 2 treatment groups was not found to be statistically significant.

Table 8 describes characteristics of the patient sample according to absence or presence of recurrent sites. All but one of the 16 smokers included in the study belonged to the group of patients that showed recurrent sites at the 1-year follow-up examination. While no significant differences in clinical parameters were detected at the pre-treatment examination, patients with recurrent sites showed a significantly higher bleeding score at the post-treatment examination than patients with no recurrent site (28% versus 16%; $p<0.05$). At the 1-year follow-up examination, the patients with recurrent sites presented also a somewhat higher plaque score than the patients without recurrent sites (40% versus 21%; $p=0.066$) and a significantly higher bleeding score (46% versus 17%; $p<0.05$).

In Table 9 the baseline characteristics of recurrent and "stable" sites are compared. The proportions of pockets located at molars were higher for recurrent sites than for the "stable" sites ($p<0.01$). Furthermore, recurrent sites showed a tendency for higher prevalence of sites with an initial PPD of ≥7 mm compared to "stable" sites ($p=0.053$).

Table 8: Characteristics of patients with and without recurrent sites (PPD ≥5 mm and BoP+); mean values (95% CI).

	No recurrent sites		≥1 recurrent site
Number of subjects	11		26
Mean age	49 (41-57)		50 (47-54)
Gender (F/M)	5/6		13/13
Smokers	1	*p<0.05*	15
Mean number recall visits	2.4 (1.5-3.2)		2 (1.5-2.5)
Plaque score			
Pre-treatment	28% (9-47)		27% (20-32)
Post-treatment	17% (7-27)		22% (14-30)
1-year follow-up	21% (3-39)		40% (28-52)
BoP score			
Pre-treatment	91% (78-104)		94% (89-98)
Post-treatment	16% (12-20)	*p<0.05*	28% (20-34)
1-year follow-up	17% (9-26)	*p<0.05*	46% (35-53)
Mean PPD (mm)			
Pre-treatment	5.9 (5.6-6.3)		5.7 (5.5-5.8)
Post-treatment	3.0 (2.8-3.1)		3.2 (3.0-3.3)
1-year follow-up	2.7 (2.5-2.9)	*p<0.05*	3.4 (3.3-3.6)

Table 9: Characteristics of recurrent and stable sites.

	Recurrent		Stable
Number of sites	76		794
Molar location	40 %	p<0.01	21 %
Pre-treatment PPD ≥ 7 mm	26 %		18 %
Presence of angular bone defect	1 (1%)		9 (1%)

Study III

Aim: to investigate, by means of multilevel analysis, factors that may affect the short-term clinical outcome of non-surgical periodontal treatment.

Logistic model with "pocket closure" at 3 months as the outcome variable

The logistic multilevel model without covariates revealed a probability of 0.63 (i.e. 63%) of obtaining "pocket closure" (0.63 = exp(0.55)/(1+exp(0.55)) for a site following initial pocket debridement in the average patient, with a 95%CI of 0.26-0.89. The intra-class correlation (ICC) showed that 17% of the variation in whether pockets were closed or not was due to variation between the patients and 83% due to variations between tooth sites within the patients. As the variance at the tooth level was estimated to be zero, this level was dropped from subsequent analyses.

The final model, including all the significant covariates, explained 44% of the total variability. Treatment type did not have a significant effect (p=0.31), nor did age or gender.

The predicted probabilities of "pocket closure" in relation to different patient and tooth site characteristics are given in Table 10. The probability of achieving "pocket closure" 3 months after subgingival debridement at a site with initial PPD of 6 mm was at best 84% (single-rooted tooth without plaque at baseline in a non-smoker), and decreased markedly for greater initial PPD, presence of plaque at baseline, location at a multi-rooted tooth and/or if the patient was a smoker.

Table 10: Predicted probability of "pocket closure" following treatment in the average patient

Initial PPD			5mm	6mm	7mm	8mm	9mm
Non-moking	Plaque-	Single-Rooted	94%(91-96)	84%(77-90)	63%(52-73)	36%(25-48)	15%(9-25)
		Multi-Rooted	88%(81-92)	70%(59-79)	43%(31-55)	19%(12-29)	7%(4-13)
	Plaque+	Single-Rooted	91%(85-94)	76%(66-84)	50%(38-63)	24%(16-37)	9%(5-17)
		Multi-Rooted	81%(71-87)	57%(45-69)	30%(21-42)	12%(7-20)	4%(2-9)
Smoking	Plaque-	Single-Rooted	85%(78-90)	64%(53-73)	36%(26-48)	16%(10-24)	6%(3-10)
		Multi-Rooted	70%(58-80)	43%(31-56)	20%(12-29)	7%(4-12)	2%(1-5)
	Plaque+	Single-Rooted	76%(65-85)	51%(38-64)	25%(16-37)	10%(5-16)	3%(2-7)
		Multi-Rooted	58%(44-70)	31%(20-43)	12%(7-20)	4%(2-8)	1%(1-2)

Continuous model with PPD at 3 months as the outcome

First, a model with fixed intercept and random slope was built (Table 11). Although not found to be significant, "Treatment" was maintained in the model as a factor because it was the main objective of the study comparison. Plaque at the site level was also included since the interaction of this factor with initial PPD and tooth type was significant. The model represented a significant improvement in terms of fit compared to the null model, and explained 50% of the variability of the outcome variable (R2=0.50). The ICC of 0.14 suggests that 14% of the unexplained variance was attributable to differences between patients.

The variance components at patient and site levels were then explored with the use of random slope models. First, the slope related to initial PPD was allowed to vary randomly at the patient level, as shown in Table 11. A Wald test of the random terms (compared to a Chi squared distribution with 2 degree of freedom) confirmed their significance ($p<0.01$).

The correlation between the intercept and slope was 0.19 (=0.01/sqrt[0.10*0.03]), indicating that the greatest pocket reduction for deep sites was achieved in patients with the best response for 5 mm pockets.

The final step consisted in modelling heterogeneity at the site level. The -2*log(likelihood) decreased significantly, confirming that the variance in final PPD was not constant but differed according to the initial PPD of the tooth site. The correlation between the intercept and the slope at the patient level was 0.83.

Table 11: The final continuous model (dependent variable: PPD at 3 months) with random intercepts and random slopes at different levels

		Fixed slope			Random slope p. lev			Random slopes		
Predictors		**Value**	**SE**	**p**	**Value**	**SE**	**p**	**Value**	**SE**	**p**
Initial PPD		0.52	0.04	<0.000	0.46	0.06	<0.000	0.44	0.05	<0.000
Treatment		0.10	0.14	ns	0.06	0.12	ns	-0.05	0.11	ns
Smoking		0.42	0.15	<0.001	0.37	0.13	<0.001	0.39	0.11	<0.001
Smok.*PPD		0.20	0.04	<0.000	0.21	0.07	<0.000	0.19	0.07	<0.000
Plaque (site)		-0.04	0.11	ns	-0.03	0.10	ns	0.00	0.09	ns*
Plaque*PPD		0.12	0.05	<0.01	0.14	0.05	<0.01	0.11	0.06	ns*
Multi-rooted		0.20	0.10	<0.05	0.20	0.10	<0.05	0.18	0.08	<0.05
Multi-r*PPD		0.18	0.05	<0.000	0.18	0.05	<0.000	0.22	0.06	<0.000
Multi-r*Plaque		0.29	0.14	<0.05	0.27	0.14	<0.05	0.21	0.12	ns*
Intercept (β_0)		3.22	0.13		3.28	0.11		3.37	0.10	
Random part										
Pat.	var (u_{0j})	0.17	0.05		0.10	0.03		0.09	0.03	
	var (u_{1j})				0.03	0.01		0.02	0.01	
	cov (u_{0j},u_{1j})				0.01	0.01		0.03	0.01	
Site	var (e_{0i})	1.10	0.04		1.04	0.04		0.55	0.03	
	var (e_{1i})							0.05	0.03	
	cov (e_{0i},e_{1i})							0.15	0.04	
-2*loglikelihood		4283.75		p<0.000	4230.72		p<0.000	4025.13		

* the joint test was significant p<0.01

In the final model, the outcome "PPD at 3 months" was determined from predictors that relate to the patient (smoking - negative impact more evident in deep pockets) and the tooth site (plaque - negative impact with interaction with PPD and tooth type; location of the site - single rooted teeth respond better than multi-rooted teeth). 86% of the unexplained variance was attributable to site level and 14% to patient level. The graph in Fig. 7 shows the regression lines for combinations of presence/absence of the factors determined as significant.

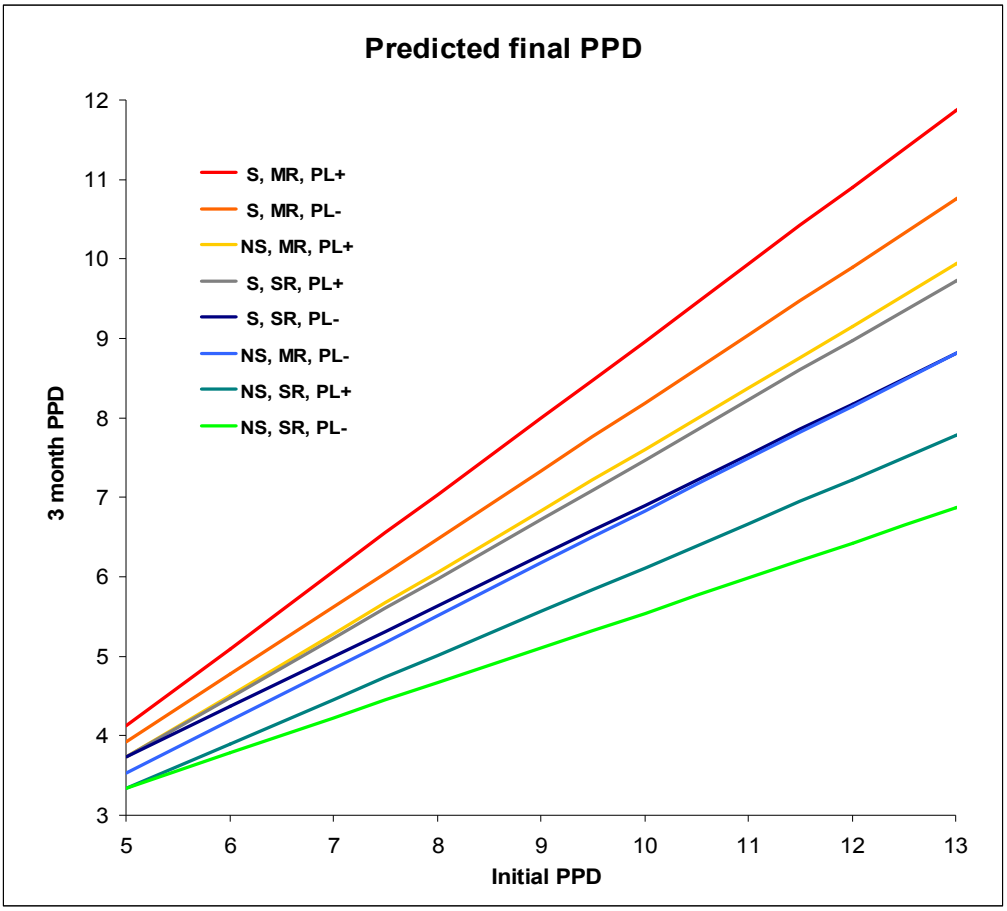

Fig. 6 Final PPD on the initial PPD for different patient and site categories (S: smoker; NS: non-smoker; SR: single-rooted teeth; MR: multi-rooted teeth; PL: presence of plaque at the tooth site).

Study IV

Aim: to evaluate if adjunctive, locally delivered controlled-release doxycycline might counteract the negative effect of smoking on periodontal wound healing following non-surgical pocket instrumentation.

Treatment outcome in smokers and non-smokers

The mean PPD reduction in the control treatment group (scaling and root planing) amounted to 1.1 mm (S.D. 0.45) for smokers and 1.5 mm (0.67) for non-smokers, while in the doxycycline treatment group the PPD reduction was 1.4 mm (0.60) and 1.6 mm (0.45), respectively (Fig. 8). 55% of the smokers in the control group showed a mean PPD reduction of at least 1 mm. The corresponding figure for smokers in the doxycycline group was 68%. A mean PPD reduction of ≥1.5 mm was observed in 20% of the smokers following scaling and root planing and 32% of the smokers treated with adjunctive doxycycline.

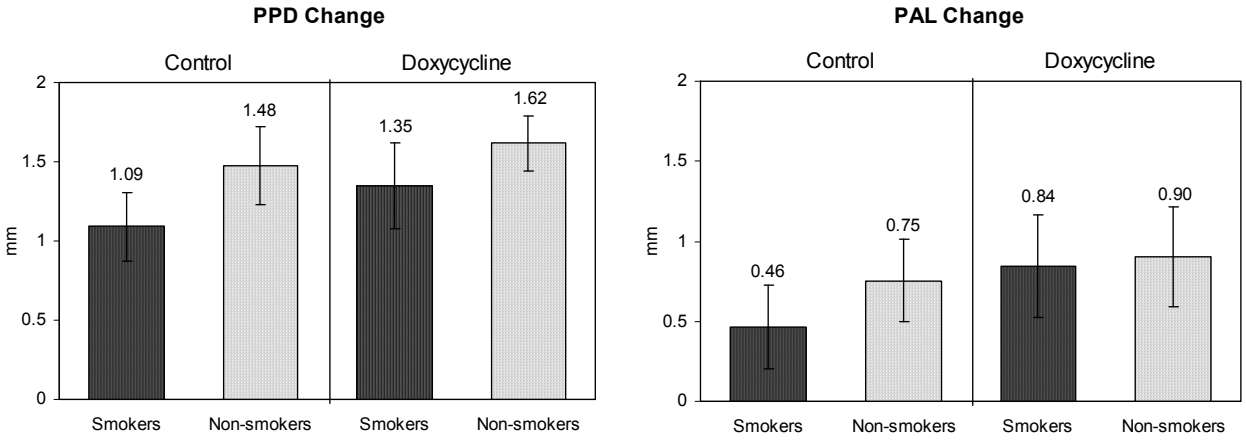

Fig. 7. PPD and PAL alterations at 3-month examination (bars represent 95% confidence interval).

The mean PAL gain for smokers and non-smokers in the control group amounted to 0.5 mm (0.56) and 0.8 mm (0.71), respectively, and to 0.8 mm (0.72) and 0.9 mm (0.82), respectively in the doxycycline group. A mean PAL gain of ≥1 mm was found for 41% of the smokers in the doxycycline group compared to 10% in the control group.

A stepwise regression model was formulated to statistically analyze the relative influence of various factors on the treatment outcome, expressed as mean PPD (Table 12). The independent variables included in the models were those showing significant correlation based on an initial correlation analysis. The regression model with individual mean PPD at 3 months as the dependent variable could explain 55% of the variability in the mean PPD ($p<0.000$). The explanatory variables that entered into the model and showed a negative influence on the 3-

month PPD were mean baseline PPD, smoking and 3-month plaque score, while treatment modality positively influenced the outcome variable.

Table 12*:* Multiple regression analysis with mean PPD at 3 months as dependent variable (Mean 4.4mm, SD 0.80). Adjusted $R^2 = 0.55$

	Coefficient	S.E.	p-value
Constant	-3.332	0.697	0.000
Mean baseline PPD	1.291	0.120	0.000
Smoking (0=NS, 1=S)	0.368	0.108	0.001
3-month plaque score	0.243	0,093	0.011
Treatment (0=Control, 1=Doxycycline)	-0.258	0.109	0.019

Study V

Aim: to evaluate if adjunctive, locally delivered controlled-release doxycycline might improve the outcome of re-instrumentation of pathological pockets persisting after initial periodontal therapy.

Treatment outcome

The re-treatment resulted in about 1.1 mm of mean PPD reduction at 9 months for both the Test and the Control group (Table 13). The mean RAL gain was similar for the two treatment groups and amounted to about 0.9 mm at final examination.

Table 13: PPD and RAL change at final examination. Mean values in mm (95% C.I.). Experimental sites - Subject level

	Test	Control
Baseline PPD	6.0 (5.8-6.2)	5.8 (5.6-6.1)
PPD reduction	1.1 (0.9-1.3)	1.1 (0.8-1.4)
RAL gain	0.8 (0.5-1.0)	0.9 (0.5-1.3)

The probability of achieving the defined endpoint of "pocket closure", i.e. a PPD of ≤ 4 mm, in an average patient at final examination was 46% for the Test versus 53% for the Control treatment (Table 14). Considering only deep pockets (baseline PPD of >6 mm), the probability was markedly lower (Test 15%; Control 17%). There was no significant difference between the treatment groups at any of the time intervals.

Table 14: Probability (%) of "pocket closure" (PPD ≤4 mm) after re-treatment (95%C.I.). - Site level (multilevel analysis)

	Test	Control
All exp. sites	46% (37-53)	53% (43-63)
Baseline PPD 5-6 mm	55% (47-64)	60% (50-70)
Baseline PPD > 6mm	15% (10-21)	17% (11-26)

Multilevel regression model

In order to identify factors affecting the outcome of the treatment, a multilevel regression model was formulated with final PPD as the dependent variable. The baseline PPD was introduced as a covariate and the factors tested included treatment approach, age, gender,

smoking status, plaque presence at site level, tooth type, furcation involvement and presence of intrabony defect. The analyses revealed that none of the patient-related variables had significant impact on the outcome. The full-mouth plaque score was not significant, whereas the presence of plaque at the single site (initial and baseline examination) had a significant negative impact on the outcome. In addition, molar sites, furcation sites (involvement degree 2 or 3), and tooth sites associated with the presence an angular bone defect showed significantly poorer treatment result. The final model, including all the significant factors, and with treatment as the main variable, explained 41% of the variability of the outcome. Of the unexplained variance 12% was attributable to inter-patient variability. The intercept of the model indicated a mean final PPD of 3.8 mm for an initially 5 mm deep pocket; for each millimeter of increment the final PPD increased by 0.7 mm.

MAIN FINDINGS

- In patients with moderately advanced periodontitis an initial, single 1-hour session of "full-mouth ultrasonic debridement" resulted in clinical improvements that were not significantly different from those following the traditional approach of consecutive sessions of quadrant scaling/root planing.

- Comparable healing results were obtained following re-treatment of remaining pathological pockets with ultrasonic instrumentation and root planing using hand instruments. Compared to the outcome of initial instrumentation, the efficiency of the re-treatment was low.

- No significant difference was found with regard to the risk for recurrence of diseased periodontal pockets between the full-mouth ultrasonic debridement approach and the traditional approach of quadrant-wise scaling and root planing.

- Smoking habits, presence of supra-gingival plaque at the tooth site and location of the pocket at a molar had a negative effect on the outcome of non-surgical periodontal treatment. More than 85% of the unexplained variability in outcome parameters was associated with tooth-site factors.

- Locally applied controlled-release doxycycline gel partly counteracted the negative effect of smoking on periodontal healing following non-surgical therapy.

- Locally delivered doxycycline as an adjunct to mechanical instrumentation at re-treatment of periodontal pockets poorly responding to initial debridement did not significantly improve the treatment outcome compared to mechanical debridement alone. Location of the pocket at a molar or a furcation involved site, presence of an angular bony defect and presence of plaque showed a significant negative impact on the clinical outcome of pocket re-treatment.

DISCUSSION

Pocket closure as an outcome variable

The use of surrogate variables such as probing pocket depth and relative attachment level to evaluate the clinical outcome of various treatment procedures is a common approach, since the true outcome variable to be assessed - tooth loss - is not a feasible variable in short-term clinical trials (Greenstein 2005). Since clinical signs of resolution of the inflammatory lesion (increased resistance of the tissues and absence of bleeding) would indicate sufficient removal of biofilm/calculus, "pocket closure" (PPD $\leq$ 4 mm) was defined as a clinical endpoint of treatment success in the current studies. The clinical value of "pocket closure" as an outcome variable is validated by data demonstrating (i) lower risk for disease progression in patients with non-bleeding shallow pockets (Badersten et al. 1990, Claffey 1991, Claffey & Egelberg 1995, Lang & Tonetti 2003), (ii) the effectiveness of pocket reduction in changing subgingival environmental conditions and microbial composition (Mombelli et al. 1995), and (iii) the risk of attachment loss in sites with PPD $\geq$6 mm (Westfelt et al. 1998).

Efficiency of the full-mouth ultrasonic debridement approach

The ultimate goal with instrumentation of a pathological periodontal pocket is to render the root free from microbial deposits and calculus. However, numerous studies have demonstrated that this goal is frequently not attainable by SRP (e.g. Brayer et al. 1989, Caffesse et al. 1986, Eaton et al. 1985, Sherman et al. 1990, Waerhaug 1978, Wylam et al. 1993).

In an attempt to test what level of instrumentation might be required for periodontal healing (study I), the initial Fm-UD approach was restricted to one hour of instrumentation (i.e. about 2 min per tooth). The efficiency of this treatment approach, i.e. the time used for instrumentation during the initial phase of therapy in relation to number of pockets reaching the endpoint of PPD $\leq$ 4 mm, was found to be significantly more favourable than that for the traditional Q-SRP approach. Moreover, the lack of a significant difference in the incidence of disease recurrences during the follow-up period (study II) indicates that the ultrasonic debridement approach was not inferior to the traditional quadrant-wise scaling and root planing approach in terms of removal of subgingival soft and hard deposits. Hence, sufficient removal of subgingival deposits and biofilm seems to be attainable with ultrasonic instrumentation in a markedly shorter treatment time than is traditionally employed for non-surgical pocket/root debridement. This interpretation is further supported by data from a recent

clinical trial by Koshy et al. (2005) demonstrating that ultrasonic debridement performed as a single-visit full-mouth procedure results in a comparable healing outcome 6 months post-treatment as that of a quadrant-wise approach at weekly intervals, even though the time spent to complete the treatment was significantly shorter.

The positive outcome of the Fm-UD approach may partly be explained by observations made in an in vitro study by Busslinger et al. (2001) showing that markedly less treatment time is required for root debridement with the use of a piezoelectric ultrasonic instrument compared to hand instruments. Moreover, the use of a thin periodontal probe-like insert for ultrasonic instrumentation may improve the efficacy of ultrasonic subgingival debridement in terms of accessibility to deep periodontal pockets and removing subgingival plaque/calculus compared to conventional ultrasonic tips and hand instruments (Clifford et al. 1999, Dragoo 1992).

Whether a beneficial effect could be attributed to the fact that the entire dentition was instrumented at a single session may be argued. Quirynen and co-workers (Bollen et al. 1996, Mongardini et al. 1999, Quirynen et al. 1995, Quirynen et al. 2006a) demonstrated the benefit of performing full-mouth SRP within 24 hours in order to prevent re-infection of the treated sites by remaining untreated periodontal pockets. Other research groups (Apatzidou & Kinane 2004, Jervøe-Storm et al. 2006, Koshy et al. 2005, Pihlstrom et al. 2005), however, failed to confirm that the full-mouth SRP approach results in a superior healing outcome compared to the traditional approach with quadrant-wise SRP.

Taken together the observations of the current studies suggest that a threshold level of bacterial load following pocket/root instrumentation may exist below which the host can cope with the remaining infection. Besides the quantity and quality of the remaining subgingival microbiota, the individual threshold level might be influenced by various host-related and modifying factors, e.g. smoking, as shown in study III. Furthermore, the predicted probability of "pocket closure" following the initial phase of pocket/root debridement is markedly influenced by site characteristics. Although the chance of achieving "pocket closure" was low for deep sites, mechanical instrumentation resulted in a significant improvement in terms of reduction of inflammation and pocket depth.

Efficacy of re-treatment

Mechanical re-instrumentation of sites poorly responding to initial mechanical debridement had a limited effect, independent of the use of ultrasonic or hand instruments, as only additionally 11-16% of the total number of target sites were brought to a successful treatment

endpoint at the 6-month examination, and about 50% of the pockets with an initial PPD ≥ 7 mm still remained as non-successful sites (study I). Moreover, the results of study V showed that the overall probability of achieving "pocket closure" 3 months after re-treatment was about 45%, while at sites with PPD > 6 mm the probability was only 12%. Also other investigators have reported that the outcome of pocket re-treatment by non-surgical scaling and root planing is limited compared to that following the initial phase of subgingival instrumentation (Badersten et al. 1984b, Anderson et al. 1996, Wennström et al. 2001).

Smokers versus non-smokers

In interpreting the current results with regard to the treatment effects in smokers and non-smokers, one has to consider the potential risk of misclassification bias of the subjects since the information on smoking habits was obtained through interview. By assessing cotinine levels in self-reported non-smokers, Wells et al. (1998) calculated the misclassification bias to be about 1% and 5.5% for regular and occasional smokers respectively, as defined by the level of the marker.

Tobacco smoking showed a negative impact, both on the probability of "pocket closure" and on the magnitude of pocket reduction (studies III and IV). Additionally, the multilevel analysis performed in study III revealed an interaction between smoking and initial PPD, i.e. the negative effect of smoking was more evident in initially deep pockets. Also stability of the treatment outcome seemed to be affected by smoking, as all but one of the smokers presented recurrent pockets (study II). These findings corroborates results of previous studies comparing the outcome of various periodontal treatment modalities in smokers and non-smokers (Ah et al. 1994, Grossi et al. 1997, Kinane & Radvar 1997, Palmer et al. 1999a, Preber & Bergstrom 1986, Ryder et al. 1999, Scabbia et al. 2001) (for review see Heasman et al. 2006, Labriola et al. 2005). However, in study V smoking was not identified as a negative factor for the healing of re-treated sites. The fact that the patient material in study V did not include any heavy smokers (≥20 cig/day), and that about 50% of the smokers were classified as "light smokers" (<10 cig/day), may have limited the possibility to detect a potentially negative effect of smoking.

Possible explanations for the inferior outcome of initial therapy in smokers may be that the ecological environment of deep periodontal pockets in the smoker is more difficult to alter by mechanical instrumentation. Such an interpretation is supported by the observations that smokers show a lower reduction of the subgingival microbial load following pocket

instrumentation (Van der Velden et al. 2003, van Winkelhoff et al. 2001), and that periodontally untreated as well as treated smokers harbour a subgingival microflora that shows a higher prevalence of e.g. *Tannerella forsythia* than non-smokers (Bostrom et al. 2001, Darby et al. 2000, Haffajee & Socransky 2001, van Winkelhoff et al. 2001, Zambon et al. 1996), which in part also may be related to an impaired host response (Labriola et al. 2005, Palmer et al. 2005).

It has also been suggested (Biddle et al. 2001) that the poorer response to non-surgical treatment observed in smokers may in part be explained by less probe tip penetration of the tissue in smokers compared to non-smokers, particularly in sites measuring 5 mm or more. The authors based their conclusion on a comparison of clinical probing measurements at human molar tooth sites and microscopic assessments of the connective tissue level at the same sites following extraction of the tooth. This in turn would entail less potential for reduction in probing assessments as a result of successful resolution of the inflammation.

The higher incidence of recurrent diseased sites following non-surgical periodontal therapy observed in smokers compared to non-smokers (study II) corroborate findings by e.g. Kamma & Baehni (2003), Loesche et al. (2002) and MacFarlane et al. (1992). MacFarlane et al. (1992) found in their study that 90% of the patients poorly responding to repeated periodontal treatment were smokers. One explanation could be that smokers usually have a higher number of remaining pathological pockets following active treatment, with a higher possibility for re-infection of healed sites (Quirynen et al. 2006b), and in our patient sample smokers at the post-treatment examination presented a prevalence of 13% of diseased pockets compared to 5% in non-smokers.

Effect of locally delivered doxycycline

The results of study V failed to demonstrate an additive effect of doxycycline on the outcome of mechanical re-treatment of teeth with persisting deep pockets after initial subgingival debridement.

Other investigators have reported that the beneficial effect of repeated episodes of scaling combined with locally delivered antibiotics (Kinane & Radvar 1999, Tonetti et al. 1998, van Steenberghe et al. 1999, Wennström et al. 2001) is comparatively limited. A significant improvement in PPD reduction was however reported by Kinane and Radvar (1999) with the use of tetracycline fibres as an adjunct to repeated mechanical debridement (Kinane & Radvar 1999). A closer analysis of their data reveals that the greater PPD reduction was due to

recession of the soft tissue rather than improved gain in clinical attachment level. Wilson et al. (1997) also evaluated the effect of the application of tetracycline fibres and reported that gingival recession accounted for 2/3 of the amount of reduction in PPD, compared to 1/3 for the control group treated by SRP alone (Wilson et al. 1997). In study V, where the antibiotic was delivered by means of a gel vehicle, recession accounted for about 30% of the PPD reduction and was similar for test and control groups. Taken together these findings indicate that the type of device selected for the local delivery of antibiotics most likely accounts for the difference between the studies with regard to the magnitude of PPD reduction.

When used during the *initial treatment phase* (study IV), locally delivered doxycycline was found to counteract the negative effects of tobacco smoking, whereas no such potential could be detected when used at the *re-treatment* of sites with remaining signs of pathology after initial pocket/root debridement (study V). As discussed above, the fact that a majority of the patients included in the latter study were only "light smokers" may be one explanation for the observed difference between the two studies. It cannot be ruled out however that the sites in need of re-treatment after the initial phase of debridement showed local environmental conditions that compromised the potential for a positive treatment effect.

Since the doxycycline gel used in this project provides gingival crevicular fluid concentrations ranging from over 1900 μg/ml at placement to about 300 μg/ml at 7 days (Stoller et al. 1998), it is likely that the enhanced treatment outcome is attributable to a change in the subgingival ecology as a result of antimicrobial effects. Furthermore, doxycycline as well as other tetracyclines possesses non-antimicrobial properties that may positively contribute to pocket healing, as discussed in the introduction. Thus, since an increased protease activity is associated with smoking, these non-antimicrobial properties may offer an additional explanation to the observed improved treatment outcome in smokers. A recent study addressing the impact of systemic administration of low dose doxycycline on non-surgical periodontal treatment of smokers, however, failed to demonstrate any impact of potential host modulating properties of doxycycline on the clinical outcome variables (Needleman et al. 2007).

Subject and site level variables

The use of multilevel models in study III and V explored, besides the effect of the treatment modalities, the impact of different factors on the treatment outcome. Presence of plaque at the tooth site was found to have a significant impact on the outcome both at initial phase as well as at re-treatment. Presence of plaque at the tooth site level has rarely been considered in

studies on the outcome of non-surgical periodontal therapy. In the present studies, the aggregated variable of plaque score on the subject level was not a significant factor, but the presence of plaque at the site level was identified as significant. Hughes et al. (2006) used the plaque score on the subject level, pre- as well as post-treatment, as prognostic factors for treatment outcome, and found plaque not to be associated with the pocket depth reduction after initial cause-related therapy in patients with generalized aggressive periodontitis. Furthermore, in a multilevel analysis of factors influencing the 6-month clinical outcome of subgingival debridement, the full-mouth plaque score on both the final PPD and the change in PPD were not significant (D'Aiuto et al. 2005). On the other hand, in a study by Axtelius et al. (1999) in which the influence of plaque at the tooth-site level on the treatment outcome was evaluated, a significant negative effect was demonstrated.

The multilevel analysis further revealed a poorer outcome of non-surgical therapy at sites located at molars and at furcations, which is in accord with findings reported by other authors who utilized multilevel analysis for evaluations of the treatment outcome (D'Aiuto et al. 2005, Axtelius et al. 1999). Another factor on the site level found to negatively influence the outcome of pocket re-treatment was the presence of an angular bone defect. Furthermore, the significant interaction with plaque shows that the cleaning efficiency of the patient is a crucial factor for pocket reduction.

Factors added into the regression models explained about 50% of the total variance in the outcome variables in study III and about 40% in study V. It is noteworthy that more than 85% of the unexplained variance was attributable to intra-patient variation (between sites) in both studies. Interestingly, these figures are fairly similar to those described in a recent publication by D'Aiuto et al. (2005) where a multilevel analysis was used to evaluate the clinical outcome of subgingival debridement.

CONCLUSION AND FUTURE CONSIDERATIONS

In the treatment of patients affected by chronic periodontitis, a one stage “full-mouth ultrasonic debridement” approach, preceded and followed by careful instructions in self-performed plaque control means, can be an efficient initial step toward infection control. The outcome of the initial phase of treatment may be improved if a smoking cessation program is included in the treatment protocol.

After re-evaluation of the clinical parameters, the decisions on the approach to re-treatment of remaining diseased pockets have to be based on the local characteristics of the tooth sites. Mechanical re-instrumentation is effective at single-rooted teeth, while a surgical approach may be preferable when anatomical corrections are needed. Adjunctive antimicrobial therapy may be considered in selected cases.

The decision regarding the approach to re-treatment of periodontal pockets showing poor response to initial pocket/root debridement has to be based on the local characteristics at the tooth/sites level. Mechanical re-instrumentation is effective at single-rooted teeth, while a surgical approach may be considered when anatomical corrections of soft and hard tissues are indicated. Adjunctive antimicrobial therapy may only be considered in selected cases.

Reasons for the variability in treatment response between different sites of the same patient need to be further studied. In order to provide the clinician with tools to improve the efficacy of periodontal therapy, choosing the most appropriate treatment for a given clinical situation, future research should aim at identifying factors that affect the healing response. With the gain of such knowledge, the cost/benefit ratio of periodontal treatment may be optimized.

REFERENCES

Acharya, M. R., Venitz, J., Figg, W. D. & Sparreboom, A. (2004) Chemically modified tetracyclines as inhibitors of matrix metalloproteinases. *Drug Resistance Updates* **7**, 195-208.

Ah, M. K., Johnson, G. K., Kaldahl, W. B., Patil, K. D. & Kalkwarf, K. L. (1994) The effect of smoking on the response to periodontal therapy. *Journal of Clinical Periodontology* **21**, 91-97.

Alavi, A. L., Palmer, R. M., Odell, E. W., Coward, P. Y. & Wilson, R. F. (1995) Elastase in gingival crevicular fluid from smokers and non-smokers with chronic inflammatory periodontal disease. *Oral Diseases* **1**, 110-114.

Albandar, J. M. & Goldstein, H. (1992) Multi-level statistical models in studies of periodontal diseases. *Journal of Periodontology* **63**, 690-695.

Aleo, J. J., De Renzis, F. A., Farber, P. A. & Varboncoeur, A. P. (1974) The presence and biologic activity of cementum-bound endotoxin. *Journal of Periodontology* **45**, 672-675.

Apatzidou, D. A. & Kinane, D. F. (2004) Quadrant root planing versus same-day full-mouth root planing. I. Clinical findings. *Journal of Clinical Periodontology* **31**, 132-140.

Apatzidou, D. A., Riggio, M. P. & Kinane, D. F. (2005) Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis. *Journal of Clinical Periodontology* **32**, 973-983.

Aracil, B., Minambres, M., Oteo, J., Torres, C., Gomez-Garces, J. L. & Alos, J. I. (2001) High prevalence of erythromycin-resistant and clindamycin-susceptible (M phenotype) viridans group streptococci from pharyngeal samples: a reservoir of mef genes in commensal bacteria. *Journal of Antimicrobial Chemotherapy* **48**, 592-594.

Axelsson, P. & Lindhe, J. (1981) The significance of maintenance care in the treatment of periodontal disease. *Journal of Clinical Periodontology* **8**, pp. 281-294.

Axelsson, P., Nyström, B. & Lindhe, J. (2004) The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. *Journal of Clinical Periodontology* **31**, 749-757.

Axtelius, B., Söderfeldt, B. & Attström, R. (1999) A multilevel analysis of factors affecting pocket probing depth in patients responding differently to periodontal treatment. *Journal of Clinical Periodontology* **26**, 67-76.

Baab, D. A. & Oberg, P. A. (1987) The effect of cigarette smoking on gingival blood flow in humans. *Journal of Clinical Periodontology* **14**, 418-424.

Badersten, A., Nilveus, R. & Egelberg, J. (1984) Effect of nonsurgical periodontal therapy. II. Severely advanced periodontitis. *Journal of Clinical Periodontology* **11**, 63-76.

Badersten, A., Nilveus, R. & Egelberg, J. (1990) Scores of plaque, bleeding, suppuration and probing depth to predict probing attachment loss. 5 years of observation following nonsurgical periodontal therapy. *Journal of Clinical Periodontology* **17**, 102-107.

Begg, M. D. & Parides, M. K. (2003) Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. *Statistics in Medicine* **22**, 2591-2602.

Bergström, J., Persson, L. & Preber, H. (1988) Influence of cigarette smoking on vascular reaction during experimental gingivitis. *Scandinavian Journal of Dental Research* **96**, 34-39.

Bezerra, M. M., Brito, G. A., Ribeiro, R. A. & Rocha, F. A. (2002) Low-dose doxycycline prevents inflammatory bone resorption in rats. *Brazilian Journal of Medical and Biological Research* **35**, 613-616.

Biddle, A. J., Palmer, R. M., Wilson, R. F. & Watts, T. L. (2001) Comparison of the validity of periodontal probing measurements in smokers and non-smokers. *Journal of Clinical Periodontology* **28**, 806-812.

Bollen, C. M., Vandekerckhove, B. N., Papaioannou, W., Van Eldere, J. & Quirynen, M. (1996) Full- versus partial-mouth disinfection in the treatment of periodontal infections. A pilot study: long-term microbiological observations. *Journal of Clinical Periodontology* **23**, 960-970.

Bonito, A. J., Lux, L. & Lohr, K. N. (2005) Impact of Local Adjuncts to Scaling and Root Planing in Periodontal Disease Therapy: A Systematic Review. *Journal of Periodontology* **76**, 1227-1236.

Boström, L., Bergström, J., Dahlen, G. & Linder, L. E. (2001) Smoking and subgingival microflora in periodontal disease. *Journal of Clinical Periodontology* **28**, 212-219.

Braun, A., Krause, F., Frentzen, M. & Jepsen, S. (2005) Efficiency of subgingival calculus removal with the Vector-system compared to ultrasonic scaling and hand instrumentation *Journal of Periodontal Research* **40**, 48-52.

Brayer, W. K., Mellonig, J. T., Dunlap, R. M., Marinak, K. W. & Carson, R. E. (1989) Scaling and root planing effectiveness: the effect of root surface access and operator experience. *Journal of Periodontology* **60**, 67-72.

Breininger, D. R., O'Leary, T. J. & Blumenshine, R. V. (1987) Comparative effectiveness of ultrasonic and hand scaling for the removal of subgingival plaque and calculus. *Journal of Periodontology* **58**, 9-18.

Brochut, P. F., Marin, I., Baehni, P. & Mombelli, A. (2005) Predictive value of clinical and microbiological parameters for the treatment outcome of scaling and root planing. *Journal of Clinical Periodontology* **32**, 695-701.

Buduneli, E., Vardar-Sengul, S., Buduneli, N., Atilla, G., Wahlgren, J. & Sorsa, T. (2007) Matrix metalloproteinases, tissue inhibitor of matrix metalloproteinase-1, and laminin-5 gamma2 chain immunolocalization in gingival tissue of endotoxin-induced periodontitis in rats: effects of low-dose doxycycline and alendronate. *Journal of Periodontology* **78**, 127-134.

Buduneli, N., Buduneli, E., Kardesler, L., Lappin, D. & Kinane, D. F. (2005) Plasminogen activator system in smokers and non-smokers with and without periodontal disease. *Journal of Clinical Periodontology* **32**, 417-424.

Busslinger, A., Lampe, K., Beuchat, M. & Lehmann, B. (2001) A comparative in vitro study of a magnetostrictive and a piezoelectric ultrasonic scaling instrument. *Journal of Clinical Periodontology* **28**, 642-649.

Cadosch, J., Zimmermann, U., Ruppert, M., Guindy, J., Case, D. & Zappa, U. (2003) Root surface debridement and endotoxin removal. *Journal of Periodontal Research* **38**, 229-236.

Caffesse, R. G., Sweeney, P. L. & Smith, B. A. (1986) Scaling and root planing with and without periodontal flap surgery. *Journal of Clinical Periodontology* **13**, 205-210.

Christgau, M., Manner, T., Beuer, S., Hiller, K. A. & Schmalz, G. (2006) Periodontal healing after non-surgical therapy with a modified sonic scaler: a controlled clinical trial. *Journal of Clinical Periodontology* **33**, 749-758.

Christgau, M., Manner, T., Beuer, S., Hiller, K. A. & Schmalz, G. (2007) Periodontal healing after non-surgical therapy with a new ultrasonic device: a randomized controlled clinical trial. *Journal of Clinical Periodontology* **34**, 137-147.

Claffey, N. (1991) Decision making in periodontal therapy. The re-evaluation. *Journal of Clinical Periodontology* **18**, 384-389.

Claffey, N. & Egelberg, J. (1995) Clinical indicators of probing attachment loss following initial periodontal treatment in advanced periodontitis patients. *Journal of Clinical Periodontology* **22**, 690-696.

Clarke, N. G., Shephard, B. C. & Hirsch, R. S. (1981) The effects of intra-arterial epinephrine and nicotine on gingival circulation. *Oral Surgery, Oral Medicine, and Oral Pathology* **52**, 577-582.

Clifford, L. R., Needleman, I. G. & Chan, Y. K. (1999) Comparison of periodontal pocket penetration by conventional and microultrasonic inserts. *Journal of Clinical Periodontology* **26**, 124-130.

Colombo, A. P., Teles, R. P., Torres, M. C., Rosalem, W., Mendes, M. C., Souto, R. M. & Uzeda, M. (2005) Effects of non-surgical mechanical therapy on the subgingival microbiota of Brazilians with untreated chronic periodontitis: 9-month results. *Journal of Periodontology* **76**, 778-784.

Cortelli, J. R., Querido, S. M., Aquino, D. R., Ricardo, L. H. & Pallos, D. (2006) Longitudinal clinical evaluation of adjunct minocycline in the treatment of chronic periodontitis. *Journal of Periodontology* **77**, 161-166.

Cosyn, J. & Wyn, I. (2006) A systematic review on the effects of the chlorhexidine chip when used as an adjunct to scaling and root planing in the treatment of chronic periodontitis. *Journal of Periodontology* **77**, 257-264.

D'Aiuto, F., Ready, D., Parkar, M. & Tonetti, M. S. (2005) Relative contribution of patient-, tooth-, and site-associated variability on the clinical outcomes of subgingival debridement. I. Probing depths. *Journal of Periodontology* **76**, 398-405.

Dahlen, G., Lindhe, J., Sato, K., Hanamura, H. & Okamoto, H. (1992) The effect of supragingival plaque control on the subgingival microbiota in subjects with periodontal disease. *Journal of Clinical Periodontology* **19**, 802-809.

Darby, I. B., Hodge, P. J., Riggio, M. P. & Kinane, D. F. (2000) Microbial comparison of smoker and non-smoker adult and early-onset periodontitis patients by polymerase chain reaction. *Journal of Clinical Periodontology* **27**, 417-424.

Darby, I. B., Hodge, P. J., Riggio, M. P. & Kinane, D. F. (2005) Clinical and microbiological effect of scaling and root planing in smoker and non-smoker chronic and aggressive periodontitis patients. *Journal of Clinical Periodontology* **32**, 200-206.

DeSanctis, M. & Murphy, K. G. (2000) The role of resective periodontal surgery in the treatment of furcation defects. *Periodontology 2000* **22**, 154-168.

Diez Roux, A. V. (2002) A glossary for multilevel analysis. *Journal of Epidemiology and Community Health* **56**, 588-594.

Dragoo, M. R. (1992) A clinical evaluation of hand and ultrasonic instruments on subgingival debridement. 1. With unmodified and modified ultrasonic inserts. *International Journal Periodontics Restorative Dentistry* **12**, 310-323.

Eaton, K. A., Kieser, J. B. & Davies, R. M. (1985) The removal of root surface deposits. *Journal of Clinical Periodontology* **12**, 141-152.

Faveri, M., Gursky, L. C., Feres, M., Shibli, J. A., Salvador, S. L. & de Figueiredo, L. C. (2006) Scaling and root planing and chlorhexidine mouthrinses in the treatment of chronic periodontitis: a randomized, placebo-controlled clinical trial. *Journal of Clinical Periodontology* **33**, 819-828.

Fleischer, H. C., Mellonig, J. T., Brayer, W. K., Gray, J. L. & Barnett, J. D. (1989) Scaling and root planing efficacy in multirooted teeth. *Journal of Periodontology* **60**, 402-409.

Gamal, A. Y. & Bayomy, M. M. (2002) Effect of cigarette smoking on human PDL fibroblasts attachment to periodontally involved root surfaces in vitro. *Journal of Clinical Periodontology* **29**, 763-770.

Gelman, A. (2006) Multilevel (hierarchical) modeling: What it can and cannot do. *Technometrics* **48**, 432-435.

Gilthorpe, M. S., Zamzuri, A. T., Griffiths, G. S., Maddick, I. H., Eaton, K. A. & Johnson, N. W. (2003) Unification of the "burst" and "linear" theories of periodontal disease progression: a multilevel manifestation of the same phenomenon. *Journal of Dental Research* **82**, 200-205.

Goldstein, H. (1987) Developing the use of multilevel models. London: Economic and Social Research Council (ESRC).

Goldstein, H., Browne, W. & Rasbash, J. (2002) Multilevel modelling of medical data. *Statistics in Medicine* **21**, 3291-3315.

Golub, L. M., Ramamurthy, N. S., Llavaneras, A., Ryan, M. E., Lee, H. M., Liu, Y., Bain, S. & Sorsa, T. (1999) A chemically modified nonantimicrobial tetracycline (CMT-8) inhibits gingival matrix metalloproteinases, periodontal breakdown, and extra-oral bone loss in ovariectomized rats. *Annals of the N Y Academy of Sciences* **878**, 290-310.

Golub, L. M., Sorsa, T., Lee, H. M., Ciancio, S., Sorbi, D., Ramamurthy, N. S., Gruber, B., Salo, T. & Konttinen, Y. T. (1995) Doxycycline inhibits neutrophil (PMN)-type matrix metalloproteinases in human adult periodontitis gingiva. *Journal of Clinical Periodontology* **22**, 100-109.

Goodson, J. M., Gunsolley, J. C., Grossi, S. G., Bland, P. S., Otomo-Corgel, J., Doherty, F. & Comiskey, J. (2007) Minocycline HCl Microspheres Reduce Red-Complex Bacteria in Periodontal Disease Therapy. *Journal of Periodontology* **78**, 1568-1579.

Goodson, J. M. & Tanner, A. (1992) Antibiotic resistance of the subgingival microbiota following local tetracycline therapy. *Oral Microbiological Immunology* **7**, 113-117.

Greenstein, G. (2005) The Use of Surrogate Variables to Reflect Long-Term Tooth Survivability. *Journal of Periodontology* **76**, 1398-1402.

Greenstein, G. (2006) Local drug delivery in the treatment of periodontal diseases: assessing the clinical significance of the results. *Journal of Periodontology* **77**, 565-578.

Greenstein, G. & Polson, A. (1998) The role of local drug delivery in the management of periodontal diseases: a comprehensive review. *Journal of Periodontology* **69**, 507-520.

Greenstein, G. & Tonetti, M. (2000) The role of controlled drug delivery for periodontitis. The Research, Science and Therapy Committee of the American Academy of Periodontology. *Journal of Periodontology* **71**, 125-140.

Grenier, D., Plamondon, P., Sorsa, T., Lee, H. M., McNamara, T., Ramamurthy, N. S., Golub, L. M., Teronen, O. & Mayrand, D. (2002) Inhibition of proteolytic, serpinolytic, and progelatinase-b activation activities of periodontopathogens by doxycycline and the non-antimicrobial chemically modified tetracycline derivatives. *Journal of Periodontology* **73**, 79-85.

Grossi, S. G., Zambon, J., Machtei, E. E., Schifferle, R., Andreana, S., Genco, R. J., Cummins, D. & Harrap, G. (1997) Effects of smoking and smoking cessation on healing after mechanical periodontal therapy. *Journal of American Dental Association* **128**, 599-607.

Guntsch, A., Erler, M., Preshaw, P. M., Sigusch, B. W., Klinger, G. & Glockmann, E. (2006) Effect of smoking on crevicular polymorphonuclear neutrophil function in periodontally healthy subjects. *Journal of Periodontal Research* **41**, 184-188.

Gustafsson, A. (1996) Methodological considerations in GCF sampling with paper strips: poor recovery of uncomplexed elastase. *Journal of Clinical Periodontology* **23**, 432-436.

Gustafsson, A., Asman, B. & Bergstrom, K. (1994) Altered relation between granulocyte elastase and alpha-2-macroglobulin in gingival crevicular fluid from sites with periodontal destruction. *Journal of Clinical Periodontology* **21**, 17-21.

Haffajee, A. D. & Socransky, S. S. (2001) Relationship of cigarette smoking to the subgingival microbiota. *Journal of Clinical Periodontology* **28**, 377-388.

Haffajee, A. D., Socransky, S. S. & Gunsolley, J. C. (2003) Systemic anti-infective periodontal therapy. A systematic review. *Annals of Periodontology* **8**, 115-181.

Hallmon, W. W. & Rees, T. D. (2003) Local anti-infective therapy: mechanical and physical approaches. A systematic review. *Annals of Periodontology* **8**, 99-114.

Hanes, P. J. & Purvis, J. P. (2003) Local anti-infective therapy: pharmacological agents. A systematic review. *Annals of Periodontology* **8**, 79-98.

Hanioka, T., Tanaka, M., Takaya, K., Matsumori, Y. & Shizukuishi, S. (2000) Pocket oxygen tension in smokers and non-smokers with periodontal disease. *Journal of Periodontology* **71**, 550-554.

Hatfield, C. G. & Baumhammers, A. (1971) Cytotoxic effects of periodontally involved surfaces of human teeth. *Archives of Oral Biology* **16**, 465-468.

Heasman, L., Stacey, F., Preshaw, P. M., McCracken, G. I., Hepburn, S. & Heasman, P. A. (2006) The effect of smoking on periodontal treatment response: a review of clinical evidence. *Journal of Clinical Periodontology* **33**, 241-253.

Heitz-Mayfield, L. J. A., Trombelli, L., Heitz, F., Needleman, I. & Moles, D. (2002) A systematic review of the effect of surgical debridement vs. non-surgical debridement for the treatment of chronic periodontitis. *Journal of Clinical Periodontology* **29**, 92-102.

Hellström, M. K., Ramberg, P., Krok, L. & Lindhe, J. (1996) The effect of supragingival plaque control on the subgingival microflora in human periodontitis. *Journal of Clinical Periodontology* **23**, 934-940.

Herrera, D., Sanz, M., Jepsen, S., Needleman, I. & Roldan, S. (2002) A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. *Journal of Clinical Periodontology* **29 Suppl 3**, 136-159; discussion 160-132.

Hughes, F. J., Auger, D. W. & Smales, F. C. (1988) Investigation of the distribution of cementum-associated lipopolysaccharides in periodontal disease by scanning electron microscope immunohistochemistry. *Journal of Periodontal Research* **23**, 100-106.

Hughes, F. J. & Smales, F. C. (1986) Immunohistochemical investigation of the presence and distribution of cementum-associated lipopolysaccharides in periodontal disease. *Journal of Periodontal Research* **21**, 660-667.

Hughes, F. J., Syed, M., Koshy, B., Marinho, V., Bostanci, N., McKay, I. J., Curtis, M. A., Croucher, R. E. & Marcenes, W. (2006) Prognostic factors in the treatment of generalized aggressive periodontitis: I. Clinical features and initial outcome. *Journal of Clinical Periodontology* **33**, 663-670.

Hung, H. C. & Douglass, C. W. (2002) Meta-analysis of the effect of scaling and root planing, surgical treatment and antibiotic therapies on periodontal probing depth and attachment loss. *Journal of Clinical Periodontology* **29**, 975-986.

Jervøe-Storm, P. M., Semaan, E., AlAhdab, H., Engel, S., Fimmers, R. & Jepsen, S. (2006) Clinical outcomes of quadrant root planing versus full-mouth root planing. *Journal of Clinical Periodontology* **33**, 209-215.

Kahl, M., Haase, E., Kocher, T. & Ruhling, A. (2007) Clinical effects after subgingival polishing with a non-aggressive ultrasonic device in initial therapy. *Journal of Clinical Periodontology* **34**, 318-324.

Kamma, J. J. & Baehni, P. C. (2003) Five-year maintenance follow-up of early-onset periodontitis patients. *Journal of Clinical Periodontology* **30**, 562-572.

Karimbux, N. Y., Ramamurthy, N. S., Golub, L. M. & Nishimura, I. (1994) Tissue healing with doxycycline and chemically modified tetracycline treatments in rats with Porphyromonas gingivalis-induced periodontitis. *Annals of the N Y Academy of Sciences* **732**, 433-435.

Katsanoulas, T., Renee, I. & Attstrom, R. (1992) The effect of supragingival plaque control on the composition of the subgingival flora in periodontal pockets. *Journal of Clinical Periodontology* **19**, 760-765.

Kawashima, H., Sato, S., Kishida, M. & Ito, K. (2007) A comparison of root surface instrumentation using two piezoelectric ultrasonic scalers and a hand scaler in vivo. *Journal of Periodontal Research* **42**, 90-95.

Kerdvongbundit, V. & Wikesjo, U. M. (2003) Effect of triclosan on healing following non-surgical periodontal therapy in smokers. *Journal of Clinical Periodontology* **30**, 1024-1030.

Kinane, D. F. (2001) Causation and pathogenesis of periodontal disease. *Periodontology 2000* **25**, 8-20.

Kinane, D. F. & Radvar, M. (1997) The effect of smoking on mechanical and antimicrobial periodontal therapy. *Journal of Periodontology* **68**, 467-472.

Kinane, D. F. & Radvar, M. (1999) A six-month comparison of three periodontal local antimicrobial therapies in persistent periodontal pockets. *Journal of Periodontology* **70**, 1-7.

Kocher, T., Riedel, D. & Plagmann, H. C. (1997) Debridement by operators with varying degrees of experience: a comparative study on manikins. *Quintessence International* **28**, 191-196.

Korostoff, J. M., Wang, J. F., Sarment, D. P., Stewart, J. C., Feldman, R. S. & Billings, P. C. (2000) Analysis of in situ protease activity in chronic adult periodontitis patients: expression of activated MMP-2 and a 40 kDa serine protease. *Journal of Periodontology* **71**, 353-360.

Koshy, G., Kawashima, Y., Kiji, M., Nitta, H., Umeda, M., Nagasawa, T. & Ishikawa, I. (2005) Effects of single-visit full-mouth ultrasonic debridement versus quadrant-wise ultrasonic debridement. *Journal of Clinical Periodontology* **32**, 734-743.

Labriola, A., Needleman, I. & Moles, D. R. (2005) Systematic review of the effect of smoking on nonsurgical periodontal therapy. *Periodontology 2000* **37**, 124-137.

Lachin, J. M. (2004) The role of measurement reliability in clinical trials. *Clin Trials* **1**, 553-566.

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

Leyland, A. H. & Goldstein, H. (2001) *Multilevel modelling of health statistics*. Chichester: Wiley.

Leyland, A. H. & Groenewegen, P. P. (2003) Multilevel modelling and public health policy. *Scandinavian Journal of Public Health* **31**, 267-274.

Llavaneras, A., Golub, L. M., Rifkin, B. R., Heikkila, P., Sorsa, T., Teronen, O., Salo, T., Liu, Y., Ryan, M. E. & Ramamurthy, N. S. (1999) CMT-8/clodronate combination therapy synergistically inhibits alveolar bone loss in LPS-induced periodontitis. *Annals of the N Y Academy of Sciences* **878**, 671-674.

Llavaneras, A., Ramamurthy, N. S., Heikkila, P., Teronen, O., Salo, T., Rifkin, B. R., Ryan, M. E., Golub, L. M. & Sorsa, T. (2001) A combination of a chemically modified doxycycline and a bisphosphonate synergistically inhibits endotoxin-induced periodontal breakdown in rats. *Journal of Periodontology* **72**, 1069-1077.

Loesche, W. J., Giordano, J. R., Soehren, S. & Kaciroti, N. (2002) The nonsurgical treatment of patients with periodontal disease: results after five years. *Journal American Dental Association* **133**, 311-320.

Lu, H. K. & Chei, C. J. (2005) Efficacy of subgingivally applied minocycline in the treatment of chronic periodontitis. *Journal of Periodontal Research* **40**, 20-27.

MacFarlane, G. D., Herzberg, M. C., Wolff, L. F. & Hardie, N. A. (1992) Refractory periodontitis associated with abnormal polymorphonuclear leukocyte phagocytosis and cigarette smoking. *Journal of Periodontology* **63**, 908-913.

Machion, L., Andia, D. C., Benatti, B. B., Carvalho, M. D., Nogueira-Filho, G. R., Casati, M. Z., Nociti, F. H., Jr. & Sallum, E. A. (2004) Locally delivered doxycycline as an adjunctive therapy to scaling and root planing in the treatment of smokers: a clinical study. *Journal of Periodontology* **75**, 464-469.

Machion, L., Andia, D. C., Lecio, G., Nociti, F. H., Jr., Casati, M. Z., Sallum, A. W. & Sallum, E. A. (2006) Locally delivered doxycycline as an adjunctive therapy to scaling and root planing in the treatment of smokers: a 2-year follow-up. *Journal of Periodontology* **77**, 606-613.

Magnusson, I., Lindhe, J., Yoneyama, T. & Liljenberg, B. (1984) Recolonization of a subgingival microbiota following scaling in deep pockets. *Journal of Clinical Periodontology* **11**, 193-207.

McDonald, B. W. & Pack, A. R. (1990) Concepts determining statistical analysis of dental data. *Journal of Clinical Periodontology* **17**, 153-158.

McLaughlin, W. S., Lovat, F. M., Macgregor, I. D. & Kelly, P. J. (1993) The immediate effects of smoking on gingival fluid flow. *Journal of Clinical Periodontology* **20**, 448-451.

Meekin, T. N., Wilson, R. F., Scott, D. A., Ide, M. & Palmer, R. M. (2000) Laser Doppler flowmeter measurement of relative gingival and forehead skin blood flow in light and heavy smokers during and after smoking. *Journal of Clinical Periodontology* **27**, 236-242.

Mombelli, A., Nyman, S., Bragger, U., Wennstrom, J. & Lang, N. P. (1995) Clinical and microbiological changes associated with an altered subgingival environment induced by periodontal pocket reduction. *Journal of Clinical Periodontology* **22**, 780-787.

Mongardini, C., van Steenberghe, D., Dekeyser, C. & Quirynen, M. (1999) One stage full- versus partial-mouth disinfection in the treatment of chronic adult or generalized early-onset periodontitis. I. Long-term clinical observations In *Journal of Periodontology* (Vol. 70), pp. 632-645.

Moore, J., Wilson, M. & Kieser, J. B. (1986) The distribution of bacterial lipopolysaccharide (endotoxin) in relation to periodontally involved root surfaces. *Journal of Clinical Periodontology* **13**, 748-751.

Morozumi, T., Kubota, T., Sato, T., Okuda, K. & Yoshie, H. (2004) Smoking cessation increases gingival blood flow and gingival crevicular fluid. *Journal of Clinical Periodontology* **31**, 267-272.

Müller, H. P. & Stadermann, S. (2006) Multivariate multilevel models for repeated measures in the study of smoking effects on the association between plaque and gingival bleeding. *Clinical Oral Investigations* **10**, 311-316.

Müller, H. P., Stadermann, S. & Heinecke, A. (2002) Longitudinal association between plaque and gingival bleeding in smokers and non-smokers. *Journal of Clinical Periodontology* **29**, 287-294.

Murray, M. C., Mooney, J. & Kinane, D. F. (1995) The relationship between elastase and lactoferrin in healthy, gingivitis and periodontitis sites. *Oral Diseases* **1**, 106-109.

Nanci, A. & Bosshardt, D. D. (2006) Structure of periodontal tissues in health and disease. *Periodontology 2000* **40**, 11-28.

Needleman, I., Suvan, J., Gilthorpe, M. S., Tucker, R., St George, G., Giannobile, W., Tonetti, M. & Jarvis, M. (2007) A randomized-controlled trial of low-dose doxycycline for periodontitis in smokers. *Journal of Clinical Periodontology* **34**, 325-333.

Nieri, M., Muzzi, L., Cattabriga, M., Rotundo, R., Cairo, F. & Prato, G. P. P. (2002) The Prognostic Value of Several Periodontal Factors Measured as Radiographic Bone

Level Variation: A 10-Year Retrospective Multilevel Analysis of Treated and Maintained Periodontal Patients. *Journal of Periodontology* **73**, 1485-1493.

Nyman, S., Sarhed, G., Ericsson, I., Gottlow, J. & Karring, T. (1986) Role of "diseased" root cementum in healing following treatment of periodontal disease. An experimental study in the dog. *Journal of Periodontal Research* **21**, 496-503.

Nyman, S., Westfelt, E., Sarhed, G. & Karring, T. (1988) Role of "diseased" root cementum in healing following treatment of periodontal disease. A clinical study. *Journal of Clinical Periodontology* **15**, 464-468.

Obeid, P. R., D'hoore, W. & Bercy, P. (2004) Comparative clinical responses related to the use of various periodontal instrumentation. *Journal of Clinical Periodontology* **31**, 193-199.

Page, R. C. & Kornman, K. S. (1997) The pathogenesis of human periodontitis: an introduction. *Periodontology 2000* **14**, 9-11.

Page, R. C., Offenbacher, S., Schroeder, H. E., Seymour, G. J. & Kornman, K. S. (1997) Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontology 2000* **14**, 216-248.

Palmer, R. M., Matthews, J. P. & Wilson, R. F. (1999a) Non-surgical periodontal treatment with and without adjunctive metronidazole in smokers and non-smokers. *Journal of Clinical Periodontology* **26**, 158-163.

Palmer, R. M., Scott, D. A., Meekin, T. N., Poston, R. N., Odell, E. W. & Wilson, R. F. (1999b) Potential mechanisms of susceptibility to periodontitis in tobacco smokers. *Journal of Periodontal Research* **34**, 363-369.

Pauletto, N. C., Liede, K., Nieminen, A., Larjava, H. & Uitto, V. J. (2000) Effect of cigarette smoking on oral elastase activity in adult periodontitis patients. *Journal of Periodontology* **71**, 58-62.

Perez-Trallero, E., Vicente, D., Montes, M., Marimon, J. M. & Pineiro, L. (2001) High proportion of pharyngeal carriers of commensal streptococci resistant to erythromycin in Spanish adults. *Journal of Antimicrobial Chemotherapy* **48**, 225-229.

Pihlstrom, B. L., Michalowicz, B. S. & Johnson, N. W. (2005) Periodontal diseases. *Lancet* **366**, 1809-1820.

Polson, A. M. (1997) The research team, calibration, and quality assurance in clinical trials in periodontics. *Annals of Periodontology* **2**, 75-82.

Preber, H. & Bergstrom, J. (1986) The effect of non-surgical treatment on periodontal pockets in smokers and non-smokers. *Journal of Clinical Periodontology* **13**, 319-323.

Quirynen, M., Bollen, C. M., Vandekerckhove, B. N., Dekeyser, C., Papaioannou, W. & Eyssen, H. (1995) Full- vs. partial-mouth disinfection in the treatment of periodontal infections: short-term clinical and microbiological observations. *Journal of Dental Research* **74**, 1459-1467.

Quirynen, M., De Soete, M., Boschmans, G., Pauwels, M., Coucke, W., Teughels, W. & van Steenberghe, D. (2006a) Benefit of "one-stage full-mouth disinfection" is explained by disinfection and root planing within 24 hours: a randomized controlled trial. *Journal of Clinical Periodontology* **33**, 639-647.

Quirynen, M., Mongardini, C., de Soete , M., Pauwels, M., Coucke, W., van Eldere , J. & van Steenberghe , D. (2000) The role of chlorhexidine in the one-stage full-mouth

disinfection treatment of patients with advanced adult periodontitis. Long-term clinical and microbiological observations. *Journal of Clinical Periodontology* **27**, 578-589.

Quirynen, M., Teughels, W., De Soete, M. & van Steenberghe, D. (2002) Topical antiseptics and antibiotics in the initial therapy of chronic adult periodontitis: microbiological aspects. *Periodontology 2000* **28**, 72-90.

Quirynen, M., Teughels, W. & van Steenberghe, D. (2006b) Impact of antiseptics on one-stage, full-mouth disinfection. *Journal of Clinical Periodontology* **33**, 49-52.

Ramamurthy, N. S., Rifkin, B. R., Greenwald, R. A., Xu, J. W., Liu, Y., Turner, G., Golub, L. M. & Vernillo, A. T. (2002) Inhibition of matrix metalloproteinase-mediated periodontal bone loss in rats: a comparison of 6 chemically modified tetracyclines. *Journal of Periodontology* **73**, 726-734.

Rateitschak-Pluss, E. M., Schwarz, J. P., Guggenheim, R., Duggelin, M. & Rateitschak, K. H. (1992) Non-surgical periodontal treatment: where are the limits? An SEM study. *Journal of Clinical Periodontology* **19**, 240-244.

Rice, N. & Leyland, A. (1996) Multilevel models: applications to health data. *Journal of Health Services Research and Policy* **1**, 154-164.

Ritz, L., Hefti, A. F. & Rateitschak, K. H. (1991) An in vitro investigation on the loss of root substance in scaling with various instruments. *Journal of Clinical Periodontology* **18**, 643-647.

Ryder, M. I., Pons, B., Adams, D., Beiswanger, B., Blanco, V., Bogle, G., Donly, K., Hallmon, W., Hancock, E. B., Hanes, P., Hawley, C., Johnson, L., Wang, H. L., Wolinsky, L., Yukna, R., Polson, A., Carron, G. & Garrett, S. (1999) Effects of smoking on local delivery of controlled-release doxycycline as compared to scaling and root planing. *Journal of Clinical Periodontology* **26**, 683-691.

Ryder, M. I., Saghizadeh, M., Ding, Y., Nguyen, N. & Soskolne, A. (2002a) Effects of tobacco smoke on the secretion of interleukin-1beta, tumor necrosis factor-alpha, and transforming growth factor-beta from peripheral blood mononuclear cells. *Oral Microbiological Immunology* **17**, 331-336.

Ryder, M. I., Wu, T. C., Kallaos, S. S. & Hyun, W. (2002b) Alterations of neutrophil f-actin kinetics by tobacco smoke: implications for periodontal diseases. *Journal of Periodontal Research* **37**, 286-292.

Scabbia, A., Cho, K. S., Sigurdsson, T. J., Kim, C. K. & Trombelli, L. (2001) Cigarette smoking negatively affects healing response following flap debridement surgery. *Journal of Periodontology* **72**, 43-49.

Schmidlin, P. R., Beuchat, M., Busslinger, A., Lehmann, B. & Lutz, F. (2001) Tooth substance loss resulting from mechanical, sonic and ultrasonic root instrumentation assessed by liquid scintillation. *Journal of Clinical Periodontology* **28**, 1058-1066.

Sculean, A., Schwarz, F., Berakdar, M., Romanos, G. E., Brecx, M., Willershausen, B. & Becker, J. (2004) Non-surgical periodontal treatment with a new ultrasonic device (Vector-ultrasonic system) or hand instruments. *Journal of Clinical Periodontology* **31**, 428-433.

Serino, G., Rosling, B., Ramberg, P., Socransky, S. S. & Lindhe, J. (2001) Initial outcome and long-term effect of surgical and non-surgical treatment of advanced periodontal disease. *Journal of Clinical Periodontology* **28**, 910-916.

Sherman, P. R., Hutchens, L. H., Jr. & Jewson, L. G. (1990a) The effectiveness of subgingival scaling and root planing. II. Clinical responses related to residual calculus. *Journal of Periodontology* **61**, 9-15.

Sherman, P. R., Hutchens, L. H., Jr., Jewson, L. G., Moriarty, J. M., Greco, G. W. & McFall, W. T., Jr. (1990b) The effectiveness of subgingival scaling and root planning. I. Clinical detection of residual calculus. *Journal of Periodontology* **61**, 3-8.

Shimazaki, Y., Saito, T., Kiyohara, Y., Kato, I., Kubo, M., Iida, M. & Yamashita, Y. (2006) The influence of current and former smoking on gingival bleeding: the Hisayama study. *Journal of Periodontology* **77**, 1430-1435.

Snijders, T. A. B. & Bosker, R. J. (1999) *Multilevel analysis : an introduction to basic and advanced multilevel modeling*. Thousand Oaks, Calif. ; London: SAGE.

Söder, B. (1999) Neutrophil elastase activity, levels of prostaglandin E2, and matrix metalloproteinase-8 in refractory periodontitis sites in smokers and non-smokers. *Acta Odontologica Scandinavica* **57**, 77-82.

Söder, B., Jin, L. J. & Wickholm, S. (2002) Granulocyte elastase, matrix metalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers with persistent periodontitis. *Journal of Clinical Periodontology* **29**, 384-391.

Sterne, J. A., Curtis, M. A., Gillett, I. R., Griffiths, G. S., Maiden, M. F., Wilton, J. M. & Johnson, N. W. (1990) Statistical models for data from periodontal research. *Journal of Clinical Periodontology* **17**, 129-137.

Stoller, N. H., Johnson, L. R., Trapnell, S., Harrold, C. Q. & Garrett, S. (1998) The pharmacokinetic profile of a biodegradable controlled-release delivery system containing doxycycline compared to systemically delivered doxycycline in gingival crevicular fluid, saliva, and serum. *Journal of Periodontology* **69**, 1085-1091.

Tonetti, M. S., Cortellini, P., Carnevale, G., Cattabriga, M., de Sanctis, M. & Pini Prato, G. P. (1998) A controlled multicenter study of adjunctive use of tetracycline periodontal fibers in mandibular class II furcations with persistent bleeding. *Journal of Clinical Periodontology* **25**, 728-736.

Tu, Y.-K., Gilthorpe, M. S., Griffiths, G. S., Maddick, I. H., Eaton, K. A. & Johnson, N. W. (2004a) The Application of Multilevel Modeling in the Analysis of Longitudinal Periodontal Data; Part I: Absolute Levels of Disease. *Journal of Periodontology* **75**, 127-136.

Tu, Y.-K., Gilthorpe, M. S., Griffiths, G. S., Maddick, I. H., Eaton, K. A. & Johnson, N. W. (2004b) The Application of Multilevel Modeling in the Analysis of Longitudinal Periodontal Data;Part II: Changes in Disease Levels over Time. *Journal of Periodontology* **75**, 137-145.

Tunkel, J., Heinecke, A. & Flemmig, T. F. (2002) A systematic review of efficacy of machine-driven and manual subgingival debridement in the treatment of chronic periodontitis. *Journal of Clinical Periodontology* **29 (Suppl. 3)**, 72-81.

Van der Velden, U., Varoufaki, A., Hutter, J. W., Xu, L., Timmerman, M. F., Van Winkelhoff, A. J. & Loos, B. G. (2003) Effect of smoking and periodontal treatment on the subgingival microflora. A retrospective study. *Journal of Clinical Periodontology* **30**, 603-610.

van der Weijden, G. A. & Timmerman, M. F. (2002) A systematic review on the clinical efficacy of subgingival debridement in the treatment of chronic periodontitis. *Journal of Clinical Periodontology* **29 (Suppl. 3)**, 55-71.

van Steenberghe, D., Rosling, B., Soder, P. O., Landry, R. G., van der Velden, U., Timmerman, M. F., McCarthy, E. F., Vandenhoven, G., Wouters, C., Wilson, M., Matthews, J. & Newman, H. N. (1999) A 15-month evaluation of the effects of repeated subgingival minocycline in chronic adult periodontitis. *Journal of Periodontology* **70**, 657-667.

van Winkelhoff, A. J., Bosch-Tijhof, C. J., Winkel, E. G. & van der Reijden, W. A. (2001) Smoking affects the subgingival microflora in periodontitis. *Journal of Periodontology* **72**, 666-671.

van Winkelhoff, A. J., Herrera Gonzales, D., Winkel, E. G., Dellemijn-Kippuw, N., Vandenbroucke-Grauls, C. M. & Sanz, M. (2000) Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. *Journal of Clinical Periodontology* **27**, 79-86.

Waerhaug, J. (1978) Healing of the dento-epithelial junction following subgingival plaque control. II: As observed on extracted teeth. *Journal of Periodontology* **49**, 119-134.

Walker, C. B., Godowski, K. C., Borden, L., Lennon, J., Nango, S., Stone, C. & Garrett, S. (2000) The effects of sustained release doxycycline on the anaerobic flora and antibiotic-resistant patterns in subgingival plaque and saliva. *Journal of Periodontology* **71**, 768-774.

Wells, A. J., English, P. B., Posner, S. F., Wagenknecht, L. E. & Perez-Stable, E. J. (1998) Misclassification rates for current smokers misclassified as nonsmokers. *American Journal of Public Health* **88**, 1503-1509.

Wennström, J. L., Newman, H. N., MacNeill, S. R., Killoy, W. J., Griffiths, G. S., Gillam, D. G., Krok, L., Needleman, I. G., Weiss, G. & Garrett, S. (2001) Utilisation of locally delivered doxycycline in non-surgical treatment of chronic periodontitis. A comparative multi-centre trial of 2 treatment approaches. *Journal of Clinical Periodontology* **28**, 753-761.

Westfelt, E., Rylander, H., Dahlen, G. & Lindhe, J. (1998) The effect of supragingival plaque control on the progression of advanced periodontal disease. *Journal of Clinical Periodontology* **25**, 536-541.

Wilson, T. G., Jr., McGuire, M. K., Greenstein, G. & Nunn, M. (1997) Tetracycline fibers plus scaling and root planing versus scaling and root planing alone: similar results after 5 years. *Journal of Periodontology* **68**, 1029-1032.

Wylam, J. M., Mealey, B. L., Mills, M. P., Waldrop, T. C. & Moskowicz, D. C. (1993) The clinical effectiveness of open versus closed scaling and root planing on multi-rooted teeth. *Journal of Periodontology* **64**, 1023-1028.

Zambon, J. J., Grossi, S. G., Machtei, E. E., Ho, A. W., Dunford, R. & Genco, R. J. (1996) Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. *Journal of Periodontology* **67**, 1050-1054.

Zeidel, A., Beilin, B., Yardeni, I., Mayburd, E., Smirnov, G. & Bessler, H. (2002) Immune response in asymptomatic smokers. *Acta Anaesthesiologica Scandinavica* **46**, 959-964.

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