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Title: Microflora cultivable from minocycline strips placed in persisting periodontal pockets

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Abstract:

Summary *Objective:* The microflora that develops on Minocycline strips, used as an adjunct in non-surgical periodontal therapy was studied. *Design and Methods:* Minocycline (1.4 mg in polycaprolactone vehicle) and control strips were applied into all residual pockets (PD $\geq$ 5mm, $\geq$ 2 pockets/subject) of patients with chronic periodontitis one month after a course of non-surgical periodontal therapy. Strips were inserted and retained for 3 days, changed to new strips for 3 more days and then removed. Strips were recovered from 14 (8 test, 6 control) of the 34 participants at day 0 (strip inserted, left for 30s, removed), days 3 and 6, for i) anaerobic culture, ii) coliforms culture, using MacConkey agar, iii) yeast culture, using Sabouraud's dextrose agar. *Results:* The mean anaerobic c.f.u./strip ($\times 10^5$; control/test) were 2/6, 24/2, 11/2 at days 0, 3 and 6, respectively ($P > 0.05$). The corresponding mean proportion of gram-negative rods and fusiforms were 27%/21%, 27%/15%, 55%/8%. The proportions of gram-negative rods on test strips by day 6 were significantly reduced ($P < 0.05$). A significantly increased prevalence of *Streptococcus mitis* biovar 1 was found on spent test strips (control vs test; 0% vs 38%, Fisher exact test, $P = 0.01$). Coliform prevalence at days 0, 3 and 6 on control/test strips were 0/13%, 50%/38%, 50%/13%. Yeasts were occasionally isolated. *Conclusions:* the findings indicated that the minocycline strips but not the control strip supported a microbial colonization compatible with periodontal health by day 6.

Abbreviations: API, Analytical Profile Index; CBABS, Columbia blood agar base with 5% defibrinated horse blood, 5 mg/l hemin and 500 μg/l menadione supplement; c.f.u., colony-forming unit; GCF, gingival crevicular fluid; RTF, Reduced transport fluid

Microflora on locally delivered minocycline strips

Introduction

The local delivery of antimicrobial agents into periodontal and/or peri-implant pockets is an increasingly used therapy[1]. Clinical studies have shown some agents tested to be as effective as conventional mechanical therapy[2-4], however, in most studies the agents were shown to be adjunctive, that is, providing additional clinical improvements when used in combination with mechanical therapy[5-7]. A number of agents using different vehicles have been developed including tetracycline[8] and chlorhexidine[9] in controlled delivery devices, metronidazole[10], minocycline[3,11,12], doxycycline[13] and silver nitrate[14] in sustained–release devices.

No definitive protocol is available as to when and how these agents should be most appropriately used. This project investigated whether local delivery of minocycline strips could produce added microbiological effects when placed in pockets that persisted one month after non-surgical periodontal therapy.

Materials and Methods

Participants and clinical procedures

Sixteen Chinese individuals with chronic periodontitis who had undergone one course of non-surgical periodontal therapy, including oral hygiene instructions, quadrant-wise scaling and root planing over 4-6 weeks were recruited for this study. The inclusion criteria were: no known allergies to tetracyclines, non-smoker, age > 35 years, no systemic disease, no history of antibiotics taken within the previous 6 months, having at least 2 residual pockets $\geq$ 5 mm in at least 2 individual sextants. The project was approved by the Ethics Committee, Faculty of Dentistry, The University of Hong Kong and written informed consent was obtained from all participants.

The deepest pocket from each patient was selected for study. The control and test strips were provided by Dong Kook Pharmaceutical Co. Ltd.[15] (Choong Cheong Book-Do, Korea) and came in exactly the same packaging format. They were randomly assigned strip numbers 1-10; 5 test and 5 control. The identity of the strips was recorded and sealed in an envelope, and decoding was only performed at the end of the project. All strips were hour-glass shaped measuring 5.5 mm in length, 2.5 mm in width at the wider end portions and 2.2 mm at the narrowest mid-portion dimension and 0.3 mm in thickness[16].

During the study period, minocycline (test: 30% or 1.4 mg active ingredient per strip in polycaprolactone vehicle) and control strips (polycaprolactone vehicle only) were inserted by the same clinician into all residual periodontal pockets $\geq$ 5 mm of the participants and these sites were covered with a periodontal dressing, Coe-Pak (GC America, Alsip, IL, USA) to assist in strip retention. All subjects used 0.2% (wt/vol) chlorhexidine mouth rinses 2 times a day during the week of strip retention as per the manufacturer's instructions. A single strip or multiple strips were placed inside each residual pocket until the pocket was completely filled with the strips. At day 3, all strips placed at day 0 were removed and were then immediately replaced by new strips, which were left for 3 more days until day 6 when these were removed. The numbers of strips inserted in each pocket and their corresponding code number were carefully recorded to make sure that all inserted strips which were eventually removed and sequential strips which were inserted should have the same code number.

Sampling

Before the clinical sampling, the ability of common oral bacteria to colonize the polycaprolactone strip was tested. In brief, control polycarprolactone strips were

suspended into pure culture of common oral bacteria including *Streptococcus mutans* ATCC 35668, *Actinomyces israelii* ATCC 10048, *Lactobacillus acidophilus* ATCC 9224, *Actinobacillus actinomycetemcomitans* ATCC 29523, *Eikenella corrodens* ATCC 23834, and *Prevotella nigrescens* ATCC 33563 for 3 days inside anaerobic chambers. Colonization on strips was also subsequently tested using mixed bacterial cultures: *L. acidophilus* mixed with *P. nigrescens*, *L. acidophilus* with *E. corrodens*, *A. actinomycetemcomitans* with *A. israelii*, *A. israelii* with *P. nigrescans*, *S. mutans* with *P. nigresencs*, *S. mutans* with *E. corrodens*, *S. mutans* with *L. acidophilus*, and *S. mutans* with *A. israelii*.

Each individual tested strip was then transferred to 10 ml sterile saline, rinsed 3× and then put into 1 ml RTF, vortexed, plated and cultured to recover the individual bacterial members. Out of the 6 single and 8 combinations tested, all bacterial strain, singly or in combinations, were found to be able to adhere to the polycaprolactone strips and could be recovered from anaerobic culture.

Samples were taken from the sixteen participants in the following manner. On day 0 the sample site was dried and isolated with cotton wool rolls. All supragingival plaque was removed using a sterile scaler. Then an assigned strip was placed in the pocket using sterile tweezers until mild resistance was felt and the strip was left for 30s (day 0) and removed and processed. Used strips, after three days of insertion, were retrieved using a sterile tweezers from sample sites after carefully removing the Coe-Pak together with any supra-gingival debris. The strips were counted, stored in reduced transport fluid (RTF) and sent to the laboratory. The specimens were processed within 30-60 min by one investigator, who was blind to both the clinical data and the subject grouping. Data from the control strips at day 0 was regarded as baseline data.

Anaerobic culture

The strips in RTF were dispersed by vortexing (Autovortex Mixer SA2, Stuart Scientific, London, UK) at maximum setting for 30s. The dispersed microbial suspension was serially diluted in RTF and innoculated onto Columbia blood agar base (Difco Laboratories, Detroit, MI) supplemented with 5% defibrinated horse blood, 5 mg/l hemin and 500 μg/l menadione (CBABS) using a spiral plater (Spiral system, Cincinnati, OH). The plates were incubated for 5-7 days at 37°C in an anaerobic chamber (Forma Scientific, Marietta, OH) under an atmosphere of 80% N_2, 10% H_2 and 10% CO_2. Anaerobiosis of the chamber was monitored daily using disposable anaerobic indicator strips (BBL, Becton Dickinson, MD).

After incubation, plates with colonies that were well separated and evenly dispersed were selected and a stainless steel template was used to subdivide the agar surface into sectors with a defined area. The relative proportions of all different colony morphotypes were determined. Representative colonies were subcultured as described by McNabb and cowokers[17] on CBABS plates to obtain pure cultures. The c.f.u. of these colonies with different morphotypes and the dilution factor of the inoculum were recorded for later quantification. All dispersion, plating and subculturing were performed on a laboratory bench.

Selective culture of aerobic and facultative anaerobic gram-negative rods and yeasts

The dispersed microbial suspension was inoculated by spiral plating onto duplicated MacConkey agar and Sabouraud's dextrose agar (Oxoid, Hampshire, UK) plates and incubated for 18h at 37°C[18]. Cultures were examined and the c.f.u. of representative

colonies with different morphotypes and the dilution factor of the inoculum was noted. The aerobic and facultative anaerobic gram-negative rod and yeast colonies were subcultured on their respective culture media to obtain pure isolates.

Identification of isolates

Isolates were identified presumptively using colony morphology, hemolysis, pigmentation, cell morphology, catalase and oxidase tests and the ability to grow in air supplemented with 10% CO_2[19]. Additional tests for the facultative anaerobic gram-positive cocci included colony morphology on mitis-salivarius agar (Difco Laboratories) and characterization using API 20 Strep and API Staph Kits (Analytical Profile Index, BioMérieux, Hazelwood, MO). Additional tests for the facultative anaerobic gram-negative rods were glucose fermentation and motility in a semi-solid medium[19]. Facultative anaerobic gram-negative fusiforms were further characterized as per Koneman et al.[20]. Obligate anaeobes were characterized using the Rapid ANA II system (Innovative Diagnostic systems, Norcross, GA) and motility in a semi-solid medium[19]. The corresponding quantitative/percentage proportion data (c.f.u. per strip or percentage proportion of the identified species per total count of the sample) of individual species isolated were then calculated.

Facultative anaerobic gram-negative rods isolates were further characterized biochemically using API 20E Kit. Pure cultures of yeasts were identified based on colony morphology, cell morphology, Gram staining reaction, the germ tube test and the API 20C AUX and API ZYM tests[21].

Statistics

The demographic, clinical, and microbiological data of control and test group were analyzed by Statview 4.5 for Macintosh (SAS Institute, Cary, NC). Differences between groups and time points were tested by analysis of variance (with or without Bonferroni multiple comparison when indicated) or Fisher exact test, as appropriate. Groups were regarded as significantly different from each other if P was < 0.05.

Results

Clinical data

Of an initial 50 non-smoking, systemically healthy subjects with chronic periodontitis who had all undergone a course of non-surgical mechanical periodontal therapy, 34 were found to have residual periodontal pockets one month post-treatment and who fulfilled the inclusion criteria. Sixteen randomly selected subjects accepted invitation to participate in the microbiological arm of the project. Data from two female control subjects, whose strips were not retained due to premature loss of the Coe-Pak and hence loss of the strips from the sample sites, were excluded. Relevant demographic and clinical data are shown in Table 1.

Anaerobic culture

Following purification, a total of 243 isolates were obtained from the 42 samples. The mean numbers of isolates obtained from the two groups at the 3 time points were similar (days 0, 3 and 6; control/test: 7.2/7.5, 6.2/4.5, 5.0/4.6 respectively), as were the mean numbers of identifiable species (days 0, 3 and 6, control/test: 5.8/7.0, 5.2/4.4, 4.5/4.3 respectively). Twenty-five isolates, however, were lost or could not be identified.

Marked sample-to-sample variations were observed in the two groups regarding the total quantity of predominant cultivable facultative and obligate anaerobic bacteria. The mean c.f.u./strip for control and test groups at days 0, 3 and 6 are as shown in Table 2. No significant difference was detectable in total quantity or proportions of microbes isolated between control and test groups at the 3 time points. Up to 36% of the isolates in the groups were obligatory anaerobes. Their quantities are as shown in Table 2. A significantly reduced proportion of gram-negative species was observed in the test strips removed on day 6 compared to the corresponding control (control: 56.0 $\pm$ 41.0%, vs test: 10.3 $\pm$ 13.7%, $P = 0.026$, Bonferroni multiple comparison). The proportion of gram negative rods and fusiforms at day 6 was significantly less on test strips (control: 56.0 $\pm$ 41.0%, vs test: 8.5 $\pm$ 13.6%, $P = 0.016$, Bonferroni multiple comparison, Fig. 1). Up to 13% of the isolates were lost before being tested (Fig. 1).

A total of 66 different organisms were identified, comprising facultative or obligatory anaerobic gram-positive cocci (30 species), gram-positive rods (11 species), gram-negative coccus (1 species), gram-negative rods (15 species), gram-negative fusiforms (8 species), and gram-negative motile rod (1 species). The frequently isolated bacterial species (defined here as species having a frequency isolation $\geq$ 20%, n = 20) are shown in Table 3. The prevalence of *Anaerococcus prevotti* at day 0 was found to be higher in test than control sites while there was no significant differences among the total quantity or proportion of this microbe at day 6 between the groups (Table 3). Considering bacterial prevalence data on the used test strips (days 3 and 6 result pooled together), *Streptococcus mitis* biovar 1 was prevalent only on test strips (0% vs 38%, Fisher exact test, $P = 0.02$). *Streptococcus constellatus* was present only in control group samples but was undetectable by culture in test samples, while

Granulicatella adiacens, *Kingella kingae*, *Peptostreptococcus micros*, *Prevotella melaninogenica* and *Veillonella* spp., were undetectable by culture in control samples. The prevalence of lost/unidentified species was high in both groups however lost species were only a minor proportion of the total cultivable count (Table 3, Fig 1).

The 45 bacterial species that were isolated at lower frequencies included 16 species of facultative anaerobic gram-positive cocci; one species of obligatory anaerobic gram-positive coccus; 10 species of facultative or obligatory anaerobic gram-positive rods; 11 species of facultative or obligatory anaerobic gram-negative rods including one species of *Prevotella intermedia* isolated from a control patient at days 0 and 3; 6 species of facultative or obligatory anaerobic gram-negative fusiforms and one species of an obligatory anaerobic gram-negative motile rod. Of all bacteria species isolated, 24% were isolated only in control samples, and 23% were isolated only in test samples.

Aerobic and facultative anaerobic gram-negative rods and yeasts

Aerobic and facultative anaerobic gram-negative rods species isolation prevalence at days 0, 3 and 6 on control/test strips was 0%/13%, 33%/25%, 33%/13% respectively with corresponding quantities as shown in Table 2. The prevalence of aerobic and facultative anaerobic gram-negative rod species at days 0, 3 and 6 on control/test strips were 0%/13%, 50%/33%, 50%/13% respectively. A total of seven strains by MacConkey agar and/or anaerobic culture were isolated. *Pseudomonas aeruginosa* was isolated from more than one sample of both groups. *Enterobacter cloacae* was isolated from 2 control and one test strips respectively while *Raoultella terrigena* was isolated from one sample of both groups. *Enterobacter aerogenes*, *Enterobacter sakazakii* and *Klebsiella oxytoca* were detected once on control strips. *Enterobacter*

gergoviae was detected once on a test strip. Significantly elevated prevalence of *Enterobacter cloacae* was observed on the used control strips (day 3 and 6 data pooled; 50% vs 13%, Fisher exact test, $P = 0.04$).

Yeast species were isolated at a low prevalence and in low quantities on both control and test strips. Three Candida strains and one non-oral yeast *Trichosporon mucoides* were isolated. *Candida parapsilosis* was found on one control and two test strips while *Candida guilliermondii* and *Candida albicans* were detected only once on a control and a test strip. *T. mucoides* was isolated from one control strip.

Discussion

Topical administration of antimicrobial agents in diseased periodontal pockets reduced the risk of disturbance of the intestinal flora associated with systemic administration. Local delivery, however, is still associated with substantial risk of development of microbial antibiotic resistance at the site of application[23]. The microbial effects of local delivery of tetracycline[24,25], minocycline[26,27], metronidazole[25] and sliver nitrate[14] alongside scaling and root planing in periodontitis patients have been reported. Utilization of local delivery 25% tetracycline in poly (D, L-lactide/glycolide) strips for residual inflamed periodontal pockets of patients undergoing supportive periodontal therapy has also been studied showing that adjunctive tetracycline enhanced reduction of motile rods compared to scaling and root planing, but no significant additional clinical benefit was recorded[28].

The current clinical project was designed in such a way as to study the utility of local delivery of the antimicrobial minocycline as a delayed adjunct for promoting healing of residual periodontal pockets one-month after non-surgical periodontal therapy. The design of the study allowed the minimal possible use of the agent in that

only residual periodontal pockets required local delivery. If the minocycline strips were effective, the need for surgical or further periodontal treatment of these residual pockets could be eliminated. This study investigated the early subgingival microbial composition during local minocycline delivery which might assist the appreciation of the corresponding clinical healing responses. The tetracyclines, including minocycline, are bacteriostatic agents with broad-spectrum anti-microbial activity against gram-positive, gram-negative, aerobic and obligate anaerobic bacteria including spirochetes[29,30]. Minocycline was also shown to be effective against a variety of oral microbes including black pigmenting anaerobic gram negative cods, *Fusobacterium nucleatum*, *Actinobacillus actinomycetemcomitans*, and *Capnocytophaga* species[31].

It is assumed that the microbial biofilm colonizing a strip was reflective of the biofilm colonizing the subgingival environment where it was located and at the time when it was removed. We also observed that the biofilm formed on the polycaprolactone strip could be conveniently released for culturing. The removed strips after having been inserted into the pocket for 3 days, could be effectively broken up into pieces by the vortexing process. However, to confirm that the sample on the strips were a fair reflection of sampled bacteria, a parallel paper point sample for the study site alongside the sampling/used strip would have been needed. That was not carried out in the current investigation. On the other hand, previous data published by this research group regarding subgingival microbiology of paper point samples of renal transplant recipients who had no periodontal disease but gingival hyperplasia showed a mean of 6.5 identifiable species[18]. That observation was similar to the mean 7.2 identifiable species sampled by polycaprolactone control strip from residual pockets in the current study. The commonly identifiable bacterial strains isolated from day 0 control strip also appeared relatively diverse (Table 3)

implying that the control polycaprolactone strip did not appear to selectively sample bacteria. Together with the pre-study observation that laboratory oral microbial isolates in pure or mixed cultures appeared to colonize readily the control strips, we postulate that the strips could function reasonably effectively as a sampling device. The code for the strips was not broken until the conclusion of the study. Hence some test strips were used as the sampling collection device at day 0. The day 0 samples from test strips may not reflect the microbial biofilm at the sampled sites, whereas the control strips acted as efficient sampling devices at day 0. The considerable work load required for the anaerobic culture and characterization in this study meant that a relatively small sample size could be managed and hence only one-half of the subjects taking part in the clinical trial were recruited for the microbiological study.

The rate of minocycline release from the strips was described earlier[32]. *In vivo* experiment showed an initial 2 hour burst of minocycline release into the GCF followed by a steady state kinetics of release (1200 μg/ml GCF) within the first 4-5 days. The *in vitro* release data showed slightly more than 50% of the total minocycline was released from the strips after 3 days[32]. In order to maintain an optimal therapeutic level of minocycline at the treated sites during the 6 days study, it was then decided to have the strips changed once at end of day 3. Therefore, at the moment of strip removal, the test strip would still retain certain level of minocycline. We postulate that if one set of strips were inserted and kept throughout the 6 days period, higher proportions of gram negative species could be recovered on the strip.

Chlorhexidine mouthrinse was used during strip retention. This agent has a wide spectrum antibacterial or anti-plaque effects, possibility due to a variety of mechanisms[33]. Previous studies have shown that twice daily 1 minute rinsing with chlorhexidine for one week could significantly reduce the proportion live bacteria and

the total cultivable c.f.u. on a *in situ* supragingival plaque model[34]. However, the study by Langbaek and Bay[35] had shown that twice daily 0.2% chlorhexidine gluconate mouthrinse for 3 weeks did not redue the amount of plaque under a periodontal dressing compared to placebo, indicating possible reduced availability of the agent under the dressing used, Coe-Pak. In the current study chlorhexidine was used in both test and control groups. We postulate that any effect of this agent on subgingival plaque colonization on the strips, covered by Coe-Pak to assist in their retention, would be minimal.

A few studies have demonstrated reductions in proportions of motile and/or spirochetal species and corresponding increases in coccal species after one to two weeks introduction of tetracycline/minocycline into the untreated subgingival environment[27,28,36]. Some studies on local delivery tetracycline/minocycline have also shown reductions of total facultative or obligate anaerobic counts, with reductions in *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Treponema denticola*, *Eikenella corrodens*, *Actinomyces viscosus*[3,24,27] or the overall *Actinomyces*, *Bacterioides*, *Fusobacterium*, and *Streptococcus* species[25]. Similar short term results regarding reduction of various species and obligate anaerobe quantities were observed using metronidazole and silver nitrate[14,25]. The designs of the previously published studies did not include repeat subgingival debridement to disrupt the biofilm in persistent pockets. The mean proportion of predominantly cultivable gram-positive species at day 0 in the present study was more than 60% reflecting to some extent the effect of the initial mechanical therapy, despite the persistence of pockets. Significant reductions in proportions of total gram-negative species and gram-negative rods and fusiforms after 6 days of minocycline strip insertion demonstrated an additional antimicrobial effect of the

locally delivered minocycline in the absence of any further mechanical disruption of the subgingival microbial biofilm.

A multicentre study investigated the repeated subgingival application of minocycline ointment as an adjunct to non-surgical periodontal therapy[3]. Over the 12 months clinical trial period, a small proportion of the subjects (27% of control and 15% of test subjects) reported minor clinical adverse experiences with the agent, principally in terms of soft tissue reactions at application sites[3]. No superinfection with yeasts or antibiotic sensitivity was reported in that study[3]. In the present study no adverse reactions were reported in response to specific and systematic questioning.

Plaque antimicrobial resistance development as a result of local drug delivery has been studied under *in vitro*[37,38] and *in vivo* conditions[39-41]. Around 10% of the predominantly cultivable periodontitis-associated subgingival microbes in one study were resistant to tetracycline[42] with all resistant *Prevotella* and *Bacteriodes* species carrying the *tetQ* gene. *In vitro*, the tetracycline resistant element, Tn*916* was found to be able to transfer horizontally among *Streptococuss* species in a plaque microcosm format[43]. Later, the effect of tetracycline on a plaque biofilm model was studied and it was found that the drug altered the plaque biofilm composition and allowed for bacteria resistant to tetracycline and other unrelated antimicrobials to fluorish[38]. Preus and Co-workers[39] reported a transient increase in proportions of minocycline resistant plaque microbes in response to local delivery of 2% minocyline ointment. This plaque minocycline resistant profile returned to normal levels by 3 month post-therapy. A later PCR-based study regarding the presence of specific tetracycline resistance genes (*tetM*, *tetO*, and *tetQ*) showed individually stable or highly variable resistance genes carriage patterns regardless of therapy[40]. The same observation was recently reported from a study which followed up the effects of minocycline ointment

on *in vivo* subgingival plaque *tetQ* gene carriage[41]. So it seems, up to the present, that the exact antimicrobial resistance induction effect of locally delivered tetracycline/minocycline, if any, is yet to be elucidated. The present study did not adddress the issue of resistance to the minocycline.

Few studies have investigated the subgingival plaque bacterial counts when under the influence of local delivery minocycline. The anaerobic culture results of the current investigation compliment the observation in a quantitative real-time PCR study[41] that the total bacterial count in periodontal pockets treated locally with minocycline did not consistently reduce. The same study[41] observed marked reductions in the quantities of *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*. While the current study did not specifically select for *A. actinomycetemcomitans*, *P. intermedia* was detected in low quantities as well as at low prevalence per site upon anaerobic culture. *P. gingivalis* was not detectable. One possible explanation might be the fact that the levels of these periodontopathogens in subgingival biofilm in the persistent pockets of the patients studied were dramatically reduced as a result of the non-surgical periodontal therapy delivered one month before minocycline strip insertion. Such per site microbial effect of non-surgical mechanical periodontal therapy has been previously established[44]. The low number of subjects/sites, however, also reduced the chances of the current study to detect accurately the presence of low prevalence microbes.

The current study observed significantly increased prevalence of *S. mitis* biovar 1 in subgingival plaque associated with test strips used (Table 3). *S. mitis* is known to possess the tetracycline resistance gene[43], so this increase in prevalence after exposure to minocycline was probably because the agent had provided a better competitive edge for this microbe. Together with the significant reduction in percentage

proportion of gram-negative rods and fusiforms attributed to the use of the minocycline strips, the subgingival microflora at the test sites on strip removal was shown to resemble the subgingival plaque compatible with periodontal health.

Low levels of coliform bacteria and yeasts associated with the strip placement confirmed the relatively low complication risk associated with use of the agent[3]. However, clinicians should still be aware of the observation that enteric bacteria are possibly associated with refractory periodontitis[45]. It was shown in the present study that the foreign bodies, both in the form of control and test strips, were commonly associated with coliform colonization, especially *Enterobacter cloacae* on strips without any minocycline (control strips, Table 3). So it seems that strip removal at the conclusion of the treatment, at day six of this study, would be a justified procedure.

This small scale study showed that minocycline, as locally delivered, in pockets persisting one-month after non-surgical periodontal therapy, showed the potential to change further the subgingival microflora towards microflora compatible with periodontal health.

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Table(s)

Table 1 Demographic data and clinical parameter of subjects immediately prior to control or test strip insertion[a].

	Control	Test
n	6	8
Age (years)[b]	46.7 ± 8.4	48.0 ± 6.5
% Female	33.3	50.0
Oral condition after initial therapy		
% Plaque[b,c]	21.2 ± 13.1	28.0 ± 17.3
% BOP[a]	43.0 ± 22.0	45.7 ± 20.7
No. of teeth with PD ≥ 5mm[b]	4.7 ± 2.5	3.1 ± 1.6
No. of sites with PD ≥ 5mm[b]	8.8 ± 6.0	4.1 ± 2.4
Sampling site PD (mm)[b]	7.5 ± 1.0	7.3 ± 2.0

[a] Two female control subjects excluded due to premature loss of strips.

[b] mean ± SD

[c] % Plaque: present of plaque detectable by an explorer at 6 sites per tooth (Modified O'Leary Plaque index[22]).

Table 2 Quantity (c.f.u./strip) of cultivable species from control and test minocycline strips[a].

	Control	Test
Total count ($\times 10^5$)		
day 0	1.8 ± 2.7	6.2 ± 5.3
day 3	23.8 ± 56.3	2.4 ± 3.9
day 6	11.3 ± 13.3	2.0 ± 3.3
Anaerobic count ($\times 10^4$)		
day 0	5.3 ± 8.6	13.1 ± 14.2
day 3	117.2 ± 286.5	1.4 ± 2.9
day 6	9.9 ± 16.8	4.3 ± 8.1
Selective culture for aerobic and facultative anaerobic gram-negative rods and yeasts		
day 0	0.0 ± 0.0	23.0 ± 64.0
day 3	5826 ± 9098	1030 ± 2555
day 6	295 ± 481	104 ± 293

[a]day 0: strip inserted, left for 30s removed; day 3: strips inserted and retained from day 1-3; day 6: strips inserted and retained from day 4-6.

Table 3 Prevalence of microbes isolated and the corresponding mean percentage isolation from control and used test minocycline strips[a].

	Control			Test		
	day 0	day 3	day 6	day 0	day 3	day 6
Gram-positive						
Facultative anaerobic cocci						
Gemella haemolysans	33.3	33.3	16.7	37.5	0	37.5
Gemella morbillorium	50	33.3	50	62.5	62.5	37.5
Granulicatella adiacens	0	0	0	0	0	25
Micrococcus spp.	16.7	0	16.7	25	0	0
Staphylococcus auricularis	0	0	33.3	12.5	12.5	0
Staphylococcus lentus	0	0	16.7	0	25	0
Streptococcus constellatus	0	16.7	33.3	0	0	0
Streptococcus equinus	0	16.7	0	25	0	12.5
Streptococcus intermedius	16.7	0	33.3	25	12.5	25
Streptococcus mitis biovar 1[b]	16.7	0	0	50	37.5	37.5
Streptococcus oralis	33.3	66.7	16.7	50	62.5	37.5
Anaerobic cocci						
Anaerococcus prevotii[c]	0	0	16.7	62.5	25	12.5
Peptostreptococcus micros	0	0	0	37.5	12.5	12.5
Facultative anaerobic rods *Actinomyces naeslundii*	50	0	33.3	12.5	0	0
Gram-negative						
Anaerobic cocci						
Veillonella spp.	0	0	0	0	25	25
Facultative anaerobic rods						
Enterobacter cloacae[b,d,e]	0	50	50	0	12.5	12.5
Kingella kingae	0	0	0	25	0	0
Anaerobic rods						
Campylobacter gracillis	33.3	16.7	0	25	12.5	0
Prevotella melaninogenica	0	0	0	25	12.5	0

Facultative anaerobic fusiforms							
Capnocytophaga gingivalis	66.7	33.3	16.7		25	0	12.5
Anaerobic fusiforms							
Fusobacterium necrogenes	16.7	0	0		25	0	12.5
Non-oral[e]	33.3	66.7	83.3		50	50	50
Lost/unidentified spp.[b]	66.7	83.3	50		50	12.5	37.5

[a] Only species with frequency of isolation > 20% in any sample are included.
[b] Significantly different prevalence of isolation from test than control strips after use, Fisher exact test, $P < 0.05$. Prevalence data of days 3 and 6 pooled together.
[c] Significantly greater prevalence of isolation from test than control strips at baseline (day 0), Fisher exact test, $P = 0.03$.
[d] non-oral species
[e] isolation by MacConkey agar and/or anaerobic culture

Legend

Figure 1. Relative mean proportion of predominant cultivable bacterial types from used test minocycline and control strips. Significantly lower proportions of total gram-negative species or gram-negative rods and fusiforms were observed on day 6 test minocycline strips than on corresponding controls (P = 0.026, Bonferroni multiple comparison).

Figure(s)

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