



Microbial Colonization of Spent Minocycline Strips - A Preliminary Report.

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ABSTRACT

This study investigated the colonization pattern of oral microbes on Minocycline strips used as an adjunct in non-surgical periodontal therapy. Minocycline (1.4 mg/strip) and control strips were applied into all residual pockets (PD \geq 5 mm, \geq 4 pockets/subject) of adult periodontitis patients one month after a course of non-surgical periodontal therapy. The clinical experiment was conducted in a double-blind randomised parallel fashion. Strips were inserted into the pockets for 3 days each time on 2 occasions. Chlorhexidine mouthrinses were used during the week of strip placement. Spent strips were randomly recovered from 10 of the 32 participants at Days 3 and 6, and were subjected to i) anaerobic culture using ETSA, ii) Coliforms culture using MacConkey agar, iii) yeast culture using Sabouraud's dextrose agar. The mean proportion of gram-positive species (% total cfu/strip) among all groups (test vs control; 3 vs 6 days) ranges from 65-89% with gram-positive cocci being the most predominant species isolated (52-74%). The corresponding values for gram-negative rods were 10-29%. Minimal amounts of gram-negative cocci were recovered and one yeast was isolated on only one occasion. No difference could be found regarding the prevalence and quantity of bacteria from all 4 groups as per anaerobic culture. However, significantly increased prevalence of coliform bacteria was found on day-3-spent-minocycline strips versus control (75% vs 0%, Fisher exact test, $p=0.03$). Such difference was not observable among day-6-spent strips (50% vs 80% $p=0.52$). The preliminary findings indicate that the micro-organisms colonizing the spent test Minocycline or control strips comprised flora compatible with periodontal health which probably reflect the ecology of the corresponding subgingival flora. The emergence of coliform bacteria warrants the removal of the minocycline strips on days 3 and 6 as practised.

INTRODUCTION

Local delivery of antimicrobial agents into periodontal pockets offers further possibilities in periodontal therapy. A considerable number of agents using different vehicles have been developed. Some clinical studies showed the agents tested to be as effective as conventional mechanical therapy, however in most studies the agents were adjunctive to mechanical therapy. A related project (Sun *et al.* 1999) was carried out to investigate if local delivery of minocycline strips could produce added clinical effects in residual periodontal pockets one month after a course of non-surgical periodontal therapy. The present investigation was carried out to study the short-term effects of the minocycline strip on subgingival microbiology.

MATERIALS & METHODS

Subjects:

- 10 adults patients randomly selected from a group of 32 periodontitis patients who participated in a double-blind randomized parallel clinical trial on a local delivery Minocycline Strip (Vehicle-polycaprolactone, Dong Kook Pharmaceutical Co., Seoul, Korea).
- Bleeding on probing (BOP) and probing depth (PD) of sample sites were measured using Florida Probe (Gainesville, FL).
- Strips (minocycline or control) were inserted into all residual periodontal pockets (PD \geq 5 mm) of the participants, 2 times for 3 days each, one month after a course of non-surgical therapy.
- Strips were retained by Coe-pack.
- Chlorhexidine mouth rinses twice daily were performed during the period of strip placement.

Laboratory investigations:

- Spent minocycline and control strips retrieved at days 3 and 6 were subjected to:
 - anaerobic culture using ETSA
 - culture for coliform bacteria using MacConkey agar
 - yeast culture using Sabouraud's dextrose agar

RESULTS



Figure 1. Minocycline strip used in the study. Left: new minocycline (test) strip; right: 3 day spent minocycline strip.

Table 1. Subjects and sites in the study

	Control	Test
Age (yr) (range)	47.2 \pm 7.6 (39-56)	49.1 \pm 11.1 (36-65)
n	6	4
Clinical parameters of sampled sites:		
- Plaque detectable	3/6	2/4
- BOP	2/6	1/4
- PD (mm) (range)	6.0 \pm 1.2 (4.6 - 7.8)	5.6 \pm 1.4 (4.6 - 8.0)

Table 2. Prevalence % of microbial morphotypes isolated from strips' culture

	Control		Test	
	day 3	day 6	day 3	day 6
Anaerobic culture*				
Gram-positive				
cocci	67	100	100	100
rods	33	60	0	50
Gram-negative				
cocci	17	0	0	50
rods	100	40	75	100
Lost	0	20	0	50
Coliforms	0	80	75 ^b	50
Yeasts	17	0	0	0

* No gram-negative curve rod or fusiforms isolated

^b Test day 3 significantly different from Control day 3 samples, Fisher Exact test, $p=0.03$.

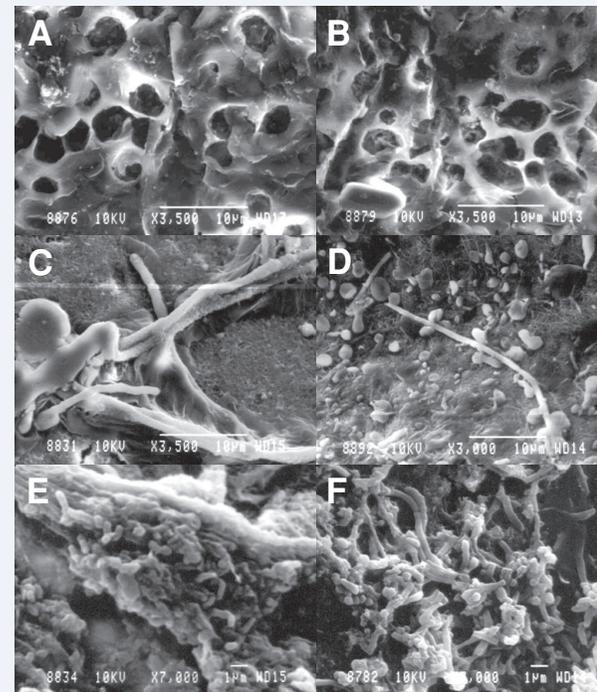


Figure 2. SEM pictures of Strips. Colonization by plaque bacteria such as cocci, rods and filaments on used control and test (minocycline) strips is readily observable. A: unused control strip, B: unused test (minocycline) strip, C: control strip retrieved on day 3, D: test (minocycline) strip retrieved on day 3, E: biofilm on control strip retrieved on day 6, F: biofilm on test (minocycline) strip retrieved on day 6.

Table 3. Morphotypes and counts of microbes isolated from strips' culture

	Control		Test	
	day 3	day 6	day 3	day 6
Anaerobic culture*				
Gram-positive				
cocci	1.3x10 ²	1.1x10 ²	8.5x10 ⁴	2.2x10 ⁴
rods	0 - 4.4x10 ²	1.7x10 ² - 1.7x10 ²	240 - 2.4x10 ⁴	2.5x10 ² - 9.1x10 ²
Gram-negative				
cocci	0	0	0	0
rods	0 - 377	0	0	0 - 3.6x10 ²
Lost				
cocci	0	0	0	0
rods	74 - 1.1x10 ³	0 - 5.8x10 ⁴	2.2x10 ²	1.5x10 ²
Total				
cocci	2.2x10 ²	0	1.3x10 ²	2.5x10 ²
rods	320 - 4.7x10 ²	5.6x10 ² - 2.0x10 ⁴	320 - 2.4x10 ⁴	1.7x10 ² - 1.5x10 ³
Coliforms				
cocci	0	0	4.3x10 ²	0
rods	0	0 - 1.1x10 ⁴	0 - 6.1x10 ⁴	0 - 2.3x10 ⁴
Yeasts				
cocci	0 - 720	0	0	0

* No gram-negative curve rods or fusiform isolated.

^a Shown are medians and range of cfu/strip.

CONCLUSION

- 1) Minocycline in polycaprolactone vehicle (1.4 mg/strip) suppressed but did not eliminate the subgingival survival or colonization of microbial plaque, species.
- 2) Predominant cultivable microbes from spent test and control strips were gram-positive cocci. Certain amount of gram-negative rods were also found colonizing spent strips.
- 3) *Enterobacteriaceae* colonization of spent test strip in the subgingival pocket environment was observed indicating that it might be in the best interest of the patients to remove the minocycline strips after a short period of time.
- 4) Minimal amount of yeast is recoverable from spent test and control strips within the study time period.
- 5) The microbiota colonizing the spent test and control strips from residual periodontal pockets of patients one month after non-surgical therapy is compatible with periodontal health.

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