

# The Effect of Minocycline-loaded Polycaprolactone Film to the Subgingival Microflora of Adult Periodontitis

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= 국문초록 =

## 성인형 치주염에서 국소약물 송달제제의 치은연하 세균에 대한 항균 효과에 관한 연구

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본 연구의 목적은 고분자 소재인 polycaprolactone film에 항생물질인 minocycline을 함유시킨 국소약물 송달제제가 성인형 치주염 환자에서 치은연하세균에 대한 효과를 평가함에 있다. 15명의 성인형 치주염 환자를 대상으로 치주낭 깊이가 5mm 이상인 60개의 치주낭을 선택하여 그중 30개는 실험군으로, 나머지 30개는 대조군으로 사용하였다. 0, 7일째 각 환자에게 구강위생 교육과 치은연상 치석제거술을 시행한 후, 실험군에는 minocycline이 함유된 polycaprolactone film을, 대조군에는 항생물질이 들어있지 않은 polycaprolactone film을 각각 치주낭에 넣어 1주일 후에 제거하였다. 미생물학적 검사로는 위상차 현미경 검사와 세균배양 검사를 각각 0, 7, 14, 28, 56일째 시행하여 다음과 같은 결과를 얻었다.

1. Minocycline film 투여후 나선형의 세균과 운동성 막대형 세균의 상대적 분포가 4주동안 유의하게 감소하였으며, 구균형 세균의 상대적 분포는 4주 동안 유의하게 증가하였다.
2. Minocycline film 투여후 총 혐기성 세균과 총 호기성 세균 및 *E. corrodens*의 세균 집락수는 2주동안 감소하였으며, *W. recta*는 투여후 4주동안, black-pigmented *Bacteroides*와 *F. nucleatum*의 세균집락수는 투여후 8주동안 감소하였다.

**Key Words**: 국소약물 송달제제, Minocycline, Polycaprolactone film, 성인형 치주염, 치은연하 치태세균.

## INTRODUCTION

It has been shown that the bacterial flora of the gingival crevice is important in the etiology of periodontal diseases<sup>27,42,45</sup>. Thus, periodontal therapy is directed at controlling the microflora. For this purpose, conventional therapy has relied almost exclusively upon the mechanical debridement of the tooth

surface. Although this method has proven to be fairly successful, there is still high recurrence rate. There is also problem of motivating people to maintain good oral hygiene following therapy and throughout their lives.

Recent studies indicate that various forms of periodontal diseases are associated with relatively specific microorganisms<sup>24,39,44</sup>. Under the hypothesis that these diseases may be caused by different specific microorganisms, interest has grown regarding the use of anti-

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biotics to aid in the selective elimination or suppression of the presumed periodontopathic microorganisms. Systemically administered antibiotics appear to lead improvements on both clinical and microbiological levels<sup>10,24,44</sup>). However, after the discontinuation of therapy, periodontal pathogens could return to the pockets and the disease might recur. Long-term antibiotic therapy has been tried, but the potential dangers associated with this form of therapy, including the development of resistant strains, superinfection and side-effects, don't warrant its serious consideration. To solve these problems, local application of antibacterial agents in the periodontal pockets has been studied and some interesting therapeutic effects were reported. Local delivery systems, such as mouth rinses, have been found to control the supragingival plaque and gingivitis<sup>8</sup>). However, such rinses were not effective against periodontal diseases involving pocket formation, presumably due to insufficient drug penetration<sup>4,37</sup>). Even direct irrigation with a syringe and a blunt needle at the interdental gingival margin failed to achieve penetration of the drug into the pocket deeper than 3mm<sup>16,37</sup>). Thus, it is not possible to control the subgingival microflora in deep pockets by the usual local drug delivery methods, such as mouth rinsing or irrigation into the pocket.

An important site for antibacterial drug delivery would seem to be from within the periodontal pocket, where local concentrations can be attained and maintained at a desired level for the duration required. From this point of view, a controlled release suppository form of drug placed within the pocket could be a highly effective method of administering antibacterial agents. Recently, many kinds of controlled-release intrapocket delivery systems have been developed and their effects have been reported<sup>1,9,12~15,18,22,32,36,47,49</sup>) since Goodson et al. developed cellulose acetate hollow fibers filled with tetracycline hydro-

chloride, which have a dramatic effect on the periodontal microflora and clinical signs of gingival inflammation<sup>12,22</sup>). Furthermore, Goodson et al. found that a therapeutic effect was obtained by the local administration of less than 1/1,000 of the amount of tetracycline that would have been used for systemic therapy. Addy et al.<sup>1</sup>) proposed acrylic strips containing the antibacterial agents such as chlorhexidine, tetracycline or metronidazole for local drug delivery into periodontal pockets. Goodson et al. adapted the hollow-fiber technique, using monolithic fibers of various polymers<sup>13,14</sup>). Friedman and Golomb developed an ethylcellulose strip as a nondegradable drug delivery device<sup>9,47</sup>). Collagen film<sup>32</sup>), resorbable polymer<sup>15</sup>), cross-linked protein matrix<sup>21</sup>) and hydroxypropyl cellulose<sup>36</sup>) have been developed as degradable intrapocket drug delivery devices.

In previous study<sup>18</sup>), 30% minocycline-loaded polycaprolactone film was developed as a controlled-release intrapocket delivery system and the release kinetics and antimicrobial activity *in vitro* was tested. Tests indicated that it is feasible to obtain sustained release of the drug for seven days and should be useful tool for elimination of pathogenic microflora from the periodontal pocket.

The purpose of the present study is to evaluate the microbiological effects of 30% minocycline-loaded polycaprolactone film(MC film), in patients with adult periodontitis.

## MATERIALS AND METHODS

### Subjects & Experimental Design

Fifteen patients with adult periodontitis, 6 males and 9 females, participated in the study. Their mean age was 39.5 years old (range 26-60 years). Diagnosis was done by macroscopic and radiographic examinations and probing pocket depth of all teeth. There were neither systemic complicating factors nor history of taking antibiotics for the pre-

vious six months. None of the patients had had any periodontal therapy for the previous six months, or a history of an allergic reaction to tetracycline. None of the women were pregnant.

As test teeth, a total of 60 teeth which showed radiographic evidence of bone resorption and which had periodontal pockets deeper than 5mm, were selected using a split-mouth design. Of the four periodontal pockets selected from each patient, two randomly selected pockets were treated by the insertion of 30% minocycline-loaded polycaprolactone film as the experimental group and the remaining two with a minocycline-free polycaprolactone film as the control group.

Following the initial examination, all patients were instructed in proper oral hygiene techniques and received supragingival scaling on days 0 and 7 (Fig. 1). For each pocket in both groups, two MC films or two placebo films were inserted on day 0 and removed one week later. On day 7, the same procedure was performed. Clinical and microbiological examinations were performed on days 0, 7, 14, 28 and 56. Oral hygiene instruction and supragingival scaling were done after the clinical examination and microbiological sampling. The films were inserted in the desired sites and covered with periodontal dressing.

### Microscopic study

Phase-contrast microscopic examination of subgingival plaque samples was performed by direct enumeration of morphologically distinct forms. Samples taken by a sterile No. 1/2 Gracey curette from the pocket were transferred to 100 $\mu$ l of sterile saline and dispersed by vortex mixing for 10 seconds. One drop of the homogenized bacterial suspension was placed on a microscopic slide, covered with a slip and examined by a phase-contrast microscope\* at a magnification of 400 $\times$ . Four bac-

terial groups, spirochetes, motile rods, non-motile rods and coccoid cells, were differentiated in randomly selected microscopic fields and the percentage of each morphologic type was determined.

### Bacterial-culture study

Subgingival plaque samples were collected from the two experimental sites and the two control sites for each patient by the following procedure: supragingival plaque was carefully removed from the isolated teeth and three fine sterile paper points (#35) were inserted into the periodontal pocket until a resistance was met. They were kept in place for 30 seconds, then transferred into a tube containing 2ml of sterile Ringer solution. The bacteria were dispersed by vortex mixing for 60 seconds and serially diluted in 10-fold steps in a 37 $^{\circ}$ C anaerobic chamber containing 85% N<sub>2</sub>, 10% CO<sub>2</sub> and 5% H<sub>2</sub>.

Samples of 100 $\mu$ l of suitable dilution were placed on selective media for *Actinobacillus actinomycetemcomitans*<sup>43)</sup>, *Actinomyces* species<sup>56)</sup>, *Eikenella corrodens*<sup>53)</sup>, *Fusobacterium nucleatum*<sup>52)</sup>, *Streptococcus* species, *Wolinella recta* and non-selective media for black-pigmented *Bacteroides* species, *Capnocytophaga* species<sup>30)</sup> and total anaerobic and aerobic bacteria. *A. actinomycetemcomitans* was identified on the basis of colony morphology, catalase activity and Gram staining characteristics after 2 to 3 days of incubation in a 10% CO<sub>2</sub> incubator. The total number of aerobic bacteria, *Capnocytophaga* species and *Actinomyces* species were enumerated in the same way. They were identified on the basis of Gram staining characteristics, colony morphology and bacterial motility with a phase-contrast microscope. *W. recta*, *E. corrodens* and *F. nucleatum* were identified on the basis of colony morphology, Gram staining characteristics and biochemical tests after 5 to 7 days incubation in an anaerobic chamber. *Streptococcus* species were identified on the basis of colony mor-

\* Olympus BH-2, Dental Scientific System, Inc., Virginia, U.S.A.

Fig. 1. Periodontal treatments consisted of oral hygiene instruction and supragingival scaling at Time 0 and 1. MC film was applied weekly at Time 0 and 1 and clinical and bacterial examination was done at Time 0, 1, 2, 4 and 8.

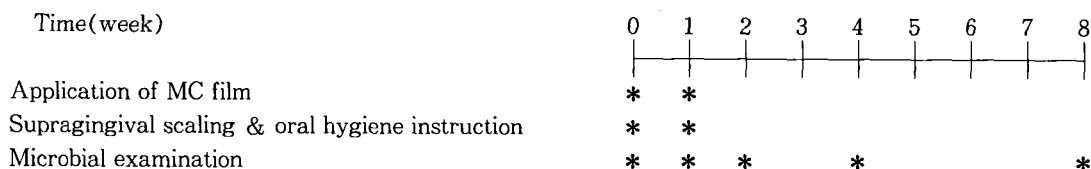


Table 1. Bacterial morphotypes(Mean percent ± S.E.)

Treatment		0	1	2	4	8(week)
Minocycline	Spiro.	15.3±11.8	9.5± 7.8*	3.7± 1.0***++	9.1±15.3*	12.8± 5.4
	Mot. rod	11.4± 7.3	4.5± 6.0***++	6.9± 2.7**	4.9± 7.4**	8.1±11.1
	Non-mot.	15.6± 7.9	18.3±14.3	18.3±11.5	18.6±11.3	20.3±15.8*
	Cocci	57.7±18.6	67.8±20.3**	71.0±19.0**	67.3±22.0*	58.7±17.4
Placebo	Spiro.	14.8±10.0	12.2± 8.3	11.8± 5.3	12.6± 9.5	15.1±12.7
	Mot. rod	10.8± 7.7	13.1±11.4	14.1±11.1	6.3± 6.0	8.3±11.9
	Non. mot.	16.1± 7.7	17.6±12.8	13.7±11.4	21.3±18.5	19.1±12.6
	Cocci	58.2±20.3	57.1±19.7	60.4±22.4	59.7±20.1	57.5±16.8

Note: \*,\*\* Significantly different from Time 0(p<0.05, p<0.01)

+,++ Significantly different from relevant placebo group(p<0.05, p<0.01)

phology, catalase activity and Gram staining characteristics after 2 to 3 days incubation in an anaerobic chamber.

The total number of anaerobic and black-pigmented *Bacteroides* colonies on the blood agar plates were enumerated after 10 days of incubation in an anaerobic chamber. Black-pigmented *Bacteroides* colonies were identified by colony morphology, Gram staining characteristics, anaerobiosis and biochemical tests.

### Statistical Analysis

Intergroup comparison and intragroup changes(Time 0 vs. 1, time 0 vs. 2, time 0 vs. 4, time 0 vs. 8) were analyzed by a paired t-test.

## RESULTS

### Microbiological Parameters

In the minocycline-treated group, there was a marked decrease in the proportion of spirochetes and motile rods and a corresponding increase in cocci one week after the insertion of MC film. This change in the na-

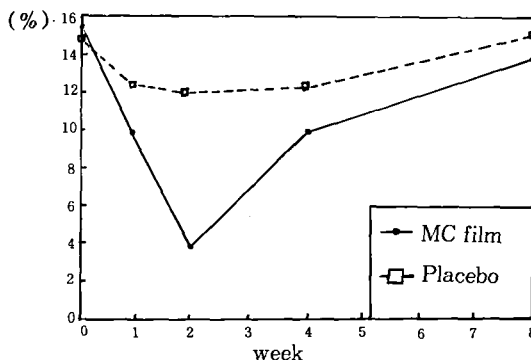


Fig 2. The changes with time in the mean % of spirochetes in the subgingival plaque.

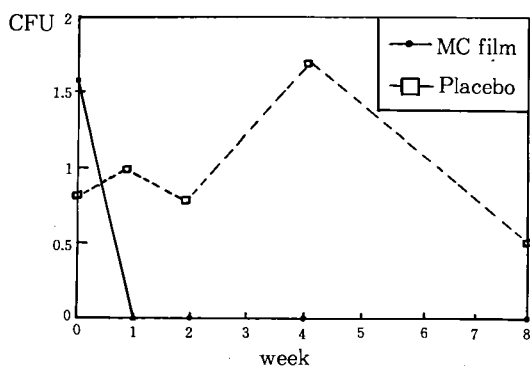
ture of the microflora was maintained up to the fourth week. At the eighth week, the flora tended to return to the levels seen at the start of the experiment. In the placebo sites, however, there was no significant change in the proportion of cocci, non-motile rods, motile rods or spirochetes during the experimental period. When the minocycline-treated group was compared to the placebo group at both the first and second weeks, a significantly greater percentage of cocci was found along with a significantly lower percentage of motile rods

**Table 2.** Total anaerobic & aerobic bacterial counts(CFU±S.E.)

Time(wk)	Minocycline		Placebo	
	Anaerobic	Aerobic	Anaerobic	Aerobic
0	95.4±14.9	54.4± 7.4	89.6±24.4	73.9±23.1
1	35.8± 5.1****	20.5± 5.2**	115.3±33.4	56.6±16.2
2	31.7± 4.7****	28.2± 7.3**	93.6±21.1	51.4±19.1
4	51.6± 7.2	49.2± 8.1	111.8±45.5	52.0±23.9
8	92.3±19.3	64.4±17.3	81.9±17.7	70.3±13.8

Note : \*,\*\* Significantly different from Time 0( $p<0.05$ ,  $p<0.01$ )

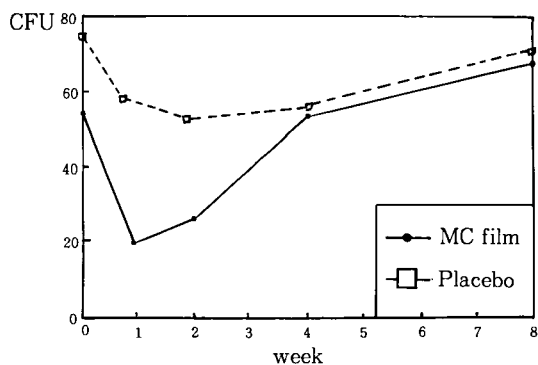
+,++ Significantly different from relevant placebo group( $p<0.05$ ,  $p<0.01$ ).



**Fig. 3.** The changes with time in the mean CFU of total anaerobic bacteria in the subgingival plaque.

and spirochetes in the MC film-treated group. After four weeks the differences in the microflora between both groups was not statistically significant (Table 1, Fig. 2).

At baseline, no statistically significant differences were found between the two groups in the mean viable counts (CFU/sample) of the bacteria cultured. There were significant reductions in the total anaerobic bacterial counts during the first two weeks in the minocycline-treated group, but these tended to return to the base line value at the eighth week, whereas no statistically significant reductions were found in the placebo group. Similar results were found for the aerobic bacteria during the observation period in the minocycline-treated group. In the placebo group, total aerobic bacteria tended to decrease at the one-, two- and four-week intervals, although no statistically significant



**Fig. 4.** The changes with time in the mean CFU of total aerobic bacteria in the subgingival plaque.

changes were observed (Table 2, Fig. 3, 4).

MC film induced a significant decrease in the mean viable counts of the black-pigmented *Bacteroides* species (Fig. 5). In the minocycline-treated group, they were reduced below detectable levels at the one-, two-, four- and eight-week intervals. Similar results were found for *Wolinella recta* except at the eighth-week examination where there was a trend toward the pretreatment value. In the placebo group, no statistically significant changes were found in both bacterial groups, although *W. recta* tended to decrease after periodontal therapy. *Capnocytophaga* species and *A. actinomycetemcomitans* were not detected in the samples with the exception of *Capnocytophaga* species in the placebo group at the first week examination. *E. corrodens* was reduced below detectable levels at the first two weeks after MC film insertion. It reappeared at the fourth

week examination but its bacterial counts were lower than the baseline value. *F. nucleatum* was significantly reduced during the eight-week period after MC film insertion. But there were no significant changes of both bacterial counts in the placebo group. In the bacterial counts of *Actinomyces* species and *Streptococcus* species, there were no statistically significant changes during the observation period in both groups (Table 3).

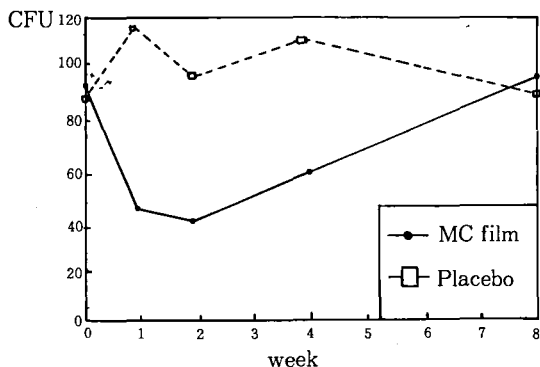


Fig. 5. The changes with time in the mean CFU of black-pigmented *Bacteroides* in the subgingival plaque.

## DISCUSSION

Minocycline is a semi-synthetic tetracycline with greater lipid solubility and longer serum half life. Lower urinary excretion than the parent compound was found by Ciancio et al.<sup>7)</sup>. Minocycline was found to be concentrated in the gingival fluid five times higher than in the serum. Minocycline showed not only marked antibacterial activity against periodontopathic bacteria including *Bacteroides* species, *F. nucleatum*, *A. actinomycet emcomitans* and *Capnocytophaga* species<sup>6,5)</sup> but also higher substantivity than tetracycline<sup>5)</sup>. The substantivity is considered an important criterion in the selection of an agent for topical usage in the oral cavity. Furthermore, minocycline has been shown to inhibit gingival collagenase activity<sup>11,46)</sup>. Thus minocycline may be one of the more suitable antimicrobial agents for the management of periodontal disease.

Table 3. Cultivable microflora (CFU ± S.E.)

Treatment		0	1	2	4	8 (week)
Minocycline	<i>B.P.B.</i>	1.6 ± 0.6	N.D.*	N.D.*	N.D.*	N.D.*
	<i>Capno.</i>	N.D.	N.D.+	N.D.	N.D.	N.D.
	<i>A.a.</i>	N.D.	N.D.	N.D.	N.D.	N.D.
	<i>W. recta</i>	0.3 ± 0.0	N.D.**	N.D.**	N.D.**	0.2 ± 0.0
	<i>E. corro.</i>	0.8 ± 0.1	N.D.*	N.D.*	0.2 ± 0.0	0.3 ± 0.0 <sup>+</sup>
	<i>F. nuc.</i>	0.7 ± 0.1	0.1 ± 0.0*	N.D.*	0.2 ± 0.0*	0.1 ± 0.0*
	<i>Act ino.</i>	0.1 ± 0.1	N.D.	N.D.	0.1 ± 0.0 <sup>++</sup>	0.1 ± 0.0
	<i>Strep.</i>	0.1 ± 0.0	N.D.	N.D.	N.D.	N.D.
Placebo	<i>B.P.B.</i>	0.8 ± 0.7	1.0 ± 0.5	0.8 ± 0.7	1.7 ± 1.6	0.4 ± 0.2
	<i>Capno.</i>	N.D.	0.4 ± 0.3	N.D.	N.D.	N.D.
	<i>A.a.</i>	N.D.	N.D.	N.D.	N.D.	N.D.
	<i>W. recta</i>	1.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
	<i>E. corro.</i>	0.9 ± 0.3	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.8 ± 0.2
	<i>F. unc.</i>	0.9 ± 0.5	0.2 ± 0.0	0.1 ± 0.0	0.6 ± 0.2	0.3 ± 0.0
	<i>Act ino.</i>	0.1 ± 0.0	0.1 ± 0.0	N.D.	0.9 ± 0.8	0.4 ± 0.2
	<i>Strep.</i>	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0

Note : \*,\*\* Significantly different from Time 0 (p < 0.05, p < 0.01)

+ , ++ Significantly different from relevant placebo group (p < 0.05, p < 0.01)

*B.P.B.* : Black-pigmented *Bacteroides* species

*Capno.* : *Capnocytophaga* species

*A.a.* : *Actinobacillus actinomycetemcomitans*

*W. recta* : *Wolinella recta*

N.D. : Not detected

*E. corro.* : *Eikenella corrodens*

*F. nuc.* : *Fusobacterium nucleatum*

*Actino.* : *Actinomyces* species

*Strep.* : *Streptococcus* species

Minocycline-loaded polycaprolactone film has been recently developed as a local drug delivery device, containing 30% minocycline as an antimicrobial agent. A previous study has demonstrated that the average minocycline concentration measured at the end of a seven-day period was 4–8  $\mu\text{g}/\text{ml}$ , which is higher than the minimal inhibitory concentration for most anaerobic bacteria. It also had antimicrobial activity against periodontopathic bacteria up to the seventh day *in vitro*<sup>18)</sup>. In this study, therefore, MC film was applied weekly in the periodontal pockets. Polycaprolactone film itself was not found to have an effect on the growth of fibroblasts in the cytotoxicity test. The procedure of placement of MC film was not painful to the patient.

The present study was designed to evaluate the microbiological effects of weekly application of MC film without subgingival mechanical debridement. The microscopic observations have revealed that the application of MC film in the periodontal pockets could induce significant qualitative changes in the subgingival microflora, i.e., reductions in the mean percentages of spirochetes and motile rods and an increase in the mean percentage of coccoid cells. There was individual variation in the composition of the pocket flora examined after treatment with MC film, but in many of the pockets, bacteria could not be identified. These findings are in agreement with the result of Lindhe *et al.*<sup>22)</sup> who demonstrated a marked reduction in spirochetes and other motile organisms from the subgingival plaque flora after the local administration via a hollow fiber device filled with tetracycline hydrochloride. Goodson *et al.*<sup>32)</sup> found a reduction of spirochetes and motile rods over a one-month period using a monolithic fiber delivery system. Similar observations were reported by Kimura *et al.*<sup>19)</sup> and Noguchi *et al.*<sup>36)</sup> who used PT-01 containing ofloxacin and hydroxypropyl cellulose filled with tetra-

cycline, respectively.

It has been shown in the previous studies that the proportions of motile rods and spirochetes were significantly higher at the disease sites when compared to healthy sites and that they decreased after periodontal treatment.<sup>23,25,35,41,51)</sup> Thus, spirochetes and motile rod in the subgingival microflora are frequently used as indicators for monitoring the efficacy of a periodontal therapy or for estimating disease activity.<sup>23,25,41,51)</sup> Therefore, it seems reasonable to use them as indicators of the effect of a topical antibiotic therapy, as was done in the present study.

In most of previous studies on the effect of subgingival antimicrobial treatment, only dark-field or phase-contrast microscopy has been used for the evaluation of the microbiological effects.<sup>17,20,22,32,38,47,54)</sup> The present study used both phase-contrast microscopic assessments and cultures of the subgingival microflora. The present findings in the culture study revealed that periodontal pathogenic bacteria were significantly reduced by the insertion of MC films in the periodontal pockets. The total anaerobic and aerobic bacteria, black-pigmented *Bacteroides* species, *W. recta*, *E. corrodens*, *F. nucleatum* were significantly decreased after the insertion of MC films in periodontal pockets. These findings are in agreement with the result of Kimura *et al.*<sup>19)</sup> who found a marked reduction in total bacteria, aerobic bacteria, black-pigmented *Bacteroides* and *Fusobacterium* species by the application of PT-01 in periodontal pockets. Addy *et al.*<sup>1)</sup> also reported that considerable number of *Fusiform* species, *Bacteroides* species and *Streptococcus* species were reduced by using polyethylmethacrylic strip containing 40% chlorhexidine. Thus it was suggested that local drug delivery therapy using MC film in combination with supragingival scaling and oral hygiene instruction could induce quantitative changes in the subgingival microflora.

However, a slight reduction of the total aerobic bacteria, *W. recta*, *E. corrodens* and *F. nucleatum* was also observed in the placebo group, although it was not statistically significant. A likely explanation may be that the supragingival plaque control could induce some quantitative changes in the subgingival microflora. This observation is inconsistent with findings by Listgarten et al.<sup>24)</sup> and Lindhe et al.<sup>22)</sup> who did not find an important change of the composition in the subgingival plaque flora, although the patients exercised meticulous supragingival plaque control.

In the present study, *A. actinomycetemcomitans* was identified in no subgingival plaque samples. *Capnocytophaga* species were not identified in any of the subgingival plaque samples except for the placebo group's at the first week. Similar findings were found in the study of Kimura et al.<sup>19)</sup> who couldn't identify the colonies of *A. actinomycetemcomitans*. It has been suggested that *A. actinomycetemcomitans* and *Capnocytophaga* species are present less frequently in the lesions of adult periodontitis than in that of juvenile periodontitis<sup>34, 35)</sup>. Moore et al.<sup>33)</sup>, in a cross-sectional study, found the mean subgingival concentration of *A. actinomycetemcomitans* to be only 0.1% of the flora in the affected sites of 26 patients with adult periodontitis. In cross-sectional studies<sup>28, 29, 39)</sup>, *Capnocytophaga* species has been proven to have no correlation with periodontal diseases. Therefore it can be speculated that the present results regarding *A. actinomycetemcomitans*, *Capnocytophaga* species substantiate the previously reported observations. However, more information on the efficacy of the different sampling methods of the subgingival plaque is needed.

It is possible, however, that the effect of MC film may have been masked by the improved oral hygiene in all patients. This hypothesis is supported by the microbiological results in this study. These results showed that there was a definite change in the sub-

gingival flora in the minocycline-treated group, while there was no significant change in the placebo group. However MC film had a transient effect on attachment gain, most marked at the fourth- and eighth-week examinations. Therefore the findings of the present study revealed that the insertion of MC film had only a minor effect on the clinical parameters examined. Consequently, the present experiment demonstrated that the use of minocycline-loaded polycaprolactone film (MC film) had the following results: (1) to markedly change the composition of the subgingival flora at the sites with advanced periodontal disease and, (2) to reduce gingival inflammation transiently.

It was not determined whether local administration of antimicrobial agent in the periodontal pocket is effective when combined with subgingival mechanical debridement. Therefore, it will be necessary to study the long-term effects of MC film when used in combination with conventional periodontal therapy. In addition, the optimum frequency and duration of the use of MC film needs to be clarified.

## CONCLUSIONS

This study was performed to determine whether 30% minocycline-loaded polycaprolactone film (MC film) has an effect on adult periodontitis in microbiological aspects.

The conclusion are as follows :

1. The relative proportions of spirochetes and motile rods were significantly reduced and the proportion of cocci was correspondingly increased for the first four weeks following the MC film therapy.

2. Total anaerobic and aerobic bacterial counts and the *E. corrodens* count were decreased up to the second week, the *W. recta* count up to the fourth week and the black-pigmented *Bacteroides* and *F. nucleatum* counts up to the eighth week following the



MC film therapy.

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