

The Effect of Chlorhexidine on Early Healing Stage of Guided Tissue Regeneration

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I. Introduction

The clinical application of the barrier membranes to facilitate new connective tissue attachment following regenerative surgery has been well accepted.

Animal experiments and human clinical studies have demonstrated that it is possible to favor regeneration of new connective tissue attachment on denuded root surfaces by placing an ePTFE membrane underneath the repositioned mucogingival flaps¹⁻³). The remarkable healing results seen in studies in dogs and monkeys using ePTFE and other membrane materials may be partly explained by the experimental procedure which has involved coronal repositioning of the flaps or complete submersion of the teeth. In clinical use, however, the membranes may be easily exposed during early healing state, become contaminated by oral microorganisms, and form a pathway for infection which may jeopardize connective tissue regeneration. Thus, it appears that the overall healing result under clinical circumstances may be less favorable

than indicated by studies in animal experiments⁴).

The membranes are left in situ for 4 to 6 weeks, and is then removed by a second surgical procedure. At this time, the surgical site may often show some exposure of the membrane with bacterial plaque and a visible inflammatory reaction in the adjacent gingiva⁵). Physical properties of ePTFE ; i. e., porous structure and surface texture and protein binding capacity may inhibit epithelial proliferation along the substrata⁶). The placement of the ePTFE provides a space between the root surface and the barrier. Another space is created between the barrier and the overlying mucogingival flap. During the healing stage, these two spaces may become contaminated by oral pathogens.

Today there is general agreement that the most significant etiologic factors for periodontal disease are microorganisms. Therefore, it would appear that the arrest of periodontal disease and the repair and regeneration of periodontal defects following treatment would necessitate the elimination or at least a significant

reduction of those organisms implicated in the development of the pathological condition. Recent studies have shown that putative periodontal pathogens can be found in numerous oral sites.

Although it has not been possible to identify the exact relationship between bacteria and periodontitis, some microorganisms such as *Bacteroides forsythus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Eikenella corrodens*, and *Fusobacterium nucleatum* are commonly found in association with periodontitis. The presence of these pathogens could be detrimental or minimize the outcome of the regenerative procedure. In guided tissue regeneration, infection is a major complicating factor and the information on site-specific bacterial colonization associated with the guided tissue regenerative procedure is limited⁷⁾. High proportions of *P. gingivalis* in operating sites turned net loss of clinical attachment after membrane removal^{8, 9)}. The placement of a membrane for guided tissue regeneration may create a subgingival environment conducive for anaerobic microbial growth¹⁰⁾.

So, anti-infective therapy may be one method that may favor early healing of periodontal tissues following guided tissue regeneration procedures¹¹⁾.

Chlorhexidine (CHX) is a strong cationic base of the bisbiguanides group. It possesses antibacterial characteristics and has, since its development over 40 years ago, had several applications in the medical and dental professions.

CHX has been used primarily to control plaque in situations when achieving ordinary

plaque control by mechanical measures is not feasible. CHX has been used short-term after surgical procedures. In addition, CHX has been employed for long-term use to promote periodontal health in orthodontic patients, in overdenture wearing patients, in patients receiving chemotherapy such as immunosuppressive agents.

As subgingival irrigant, CHX has been studied extensively and widely accepted as the agent of choice. Newman and co-workers have used CHX in their simplified oral hygiene regime since the early 1980's.^{12, 13)} These studies showed clinical improvement, especially with concentrations at or above 0.1%.

Beside rinsing time and frequency,^{14, 15)} the dose of CHX appears of considerable relevance to the efficacy of mouthrinse formulations¹⁶⁾. CHX mouthrinse products in Europe have been largely recommended 0.2% solutions in 10-ml volumes (20-mg dose), and in USA, 0.12% solutions in 15-ml volumes (18-mg dose) are recommended. Jenkins concluded that a distinct dose-response pattern was seen for CHX rinses on plaque accumulation. Maximum plaque inhibition was achieved with the 0.2% CHX solution supporting the recommendation of 2 daily rinsing with doses of this antiseptic at or around 20mg. However, the considerable plaque inhibition at much lower doses indicates that adjunctive benefits to oral hygiene and gingival health are possible with lower concentration¹⁷⁾.

The purpose of this study is to compare the antimicrobial effects of the two different oral rinsing CHX concentration during the early healing stages of guided tissue regeneration

by scanning electron microscope and anaerobic bacterial culture.

II. Materials and methods

1. Study population and sites

Thirty volunteer patients (17 males and 13 females; ages 35 to 57 years, mean age 42.6) requiring therapy for moderate adult periodontitis participated in this exploratory clinical trial. All were systemically healthy and none had received a dental prophylaxis or scaling and root planing during the past years. And none had taken antibiotics within the last 6 months nor used antimicrobial oral rinses. Experimental teeth were screened for the following inclusion criteria: Class II or Class III furcation involvement of the mandibular 1st or 2nd molars with no clinically or radiographically detectable interproximal intrabony defects and they are all clinically sound without any restoration and periapical lesions. All patients received an initial therapy comprising oral hygiene instructions and full mouth scaling and root planing. Following the hygienic phase, the patients were received guided tissue regenerative surgery. At the time of the surgery, a full-thickness mucoperiosteal flap was reflected, extending from the mesial aspect of the adjacent mesial tooth to the distal aspect of the neighboring distal tooth. The exposed root surfaces were thoroughly planed using hand instruments. Following the open-flap debridement, ePTFE membranes were fitted over the defects extending 3mm apically over the alveolar crest and approximately 1mm coronally onto tooth

structure, and were secured in this position with sling sutures. The flaps were repositioned to cover the membranes with interrupted sutures. Antibiotics (amoxicillin/clavulanate potassium 375mg t.i.d) were prescribed for 5 days and two different concentration of chlorhexidine gargling solution was also randomly prescribed for 20 test group patients (ten were given 0.1% chlorhexidine solution and another ten were given 0.2%) and 10 patient was not given any gargling solution. The two test groups were instructed to use twice a day for 30 seconds during the whole experimental period.

Sutures were removed 2 weeks after surgery and the barrier membranes were retrieved after 4 to 6 weeks at which time gross plaque removal was performed coronal to the newly formed tissue.

The retrieved membranes were sectioned vertically in half for microscopic study and microbiological cultivation and the halves placed immediately into fixative or reduced transport medium respectively.

2. Scanning Electron Microscope preparation

Upon removal, membranes were briefly rinsed in saline to remove adherent blood and then immediately fixed in 2.5% glutaraldehyde, 0.1M cacodylate buffer (pH 7.4). In preparation for SEM, the specimens were postfixed in 2% osmium tetroxide in 0.2M cacodylate buffer, dehydrated with graded ethanol, critical point dried with CO₂, sputter coated with 20nm gold-palladium and mounted on specimen stubs to allow SEM analysis. Specimens were examined in a JSM-840A (Japanese Scanning Microscope)

operated at 20KV. Every specimen was oriented to allow examination of the inner surface of the membrane. The tooth facing surface of each membrane was examined at 20 magnification. 9 randomly selected microscopic fields at 500 magnification were analyzed in each membrane:3 in the cervical collar area, 3 in the middle portion, and 3 in the apical portion. In each microscopic field, the proportion of membrane surface covered by organic deposit was evaluated. When majority of the examined field was covered by deposit, the magnification was increased up to 5000 in order to determine the nature of the deposit, i.e., bacteria or host cells. Only when bacteria accounted for the majority of the deposit, the microscopic field was considered positive for bacterial colonization. Conversely, the microscopic field was considered negative when other structures (host cells or unidentified material) were predominant.

3. Microscopic procedures

The other half of the retrieved membrane were dropped into vials containing VMGA-III. The vials were transferred into an anaerobic chamber(Coy Laboratory Products Inc., MI, USA). The oxygen-free atmosphere in the chamber consisted of 80% N₂, 10% H₂, 10% CO₂. Each sample was dispersed by vortexing for 30s. The resulting suspensions were 10-fold serially diluted in VMGA-I anaerobic solution. Using a sterile bent glass rod, 0.1 ml aliquots from appropriate dilutions were plated onto non-selective 4.3% brucella agar(BBL Microbiology System, Cockeysville, MD) supplemented with 0.3% Bacto-agar,

5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin and 0.00005% menadione for total viable counts and proportion of specific species, onto TSBV(Tryptic Soy Serum Bacitracin Vancomycin) medium for *Actinobacillus actinomycetemcomitans*. The non-selective blood agar was incubated at 37°C in Coy anaerobic chamber for 7days. After 7days' incubation, brown or black colonies were randomly selected and incubated for another 7days. Organisms were identified using API kit(bioMerieux: rapid ID 32A: ID of anaerobe). TSBV medium was incubated in 10% CO₂ in air at 37°C for 4days. Organisms identified were *Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*.

4. Indirect Immunofluorescence

Samples were dispersed by vortexing at maximal setting for 30 seconds. Heat-fixed, bacterial smears were prepared from these suspensions on the slide glass and stored at -70 °C until tested by indirect immunofluorescence method.

For immunofluorescence tests, the bacterial smears were reacted with 20 l of diluted rabbit antibacterial serum(*P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*) for 30 minutes at 37°C. After washing with PBS(Phosphate Buffered Saline) for 15 minutes, the slides were reacted with 20 l of fluorescein-labeled, goat anti-rabbit IgG (Cappel Lab., Cochranville, PA, USA) for 30 minutes at 37°C and then washed again in PBS for 1 hour and examined with Olympus BH-2 fluorescence microscope(Olympus Inc.

Co., Osaka, Japan) equipped with incident light. The fluorescence intensity of the bacteria were graded from 0 to 4+, with 4+ indicating the greatest fluorescence intensity. Bacterial cells exhibiting 3 to 4+ fluorescence with well-defined outlines and dark or lightly fluorescing centers that were morphologically characteristic for the target species positive. The specific fluorescent bacteria obtained by immunofluorescence and total cell counts obtained by dark-field microscopy were counted in four fields. The percent of each specific microorganisms in the total cell count was then determined. Slides were read by one individual who was unaware of the clinical findings, 0:no fluorescence

1+:bare fluorescence with single cells not distinguishable

2+:faint fluorescence with single cells visible, no definition of cell shape

3+:moderate fluorescence with good cell envelope definition and a dark cell center

4+:brilliant fluorescence with good cell envelope definition and a dark cell center

5. Data analysis

The total viable counts on anaerobic blood agar plate were transformed into colony forming units per ml(CFU/ml) using predetermined conversion factors to account for dilution and the size of the evaluated surface on the plate. The data was analyzed by t-test.

III. Result

1. Scanning Electron Microscope

The microscopic analysis of the inner surface of the retrieved membranes revealed that all fields examined in the coronal portion were positive for bacterial colonization (Table 1, 2, 3). In the mid portion of the membranes, 33% fields were positive for bacterial colonization in 0.1% chlorhexidine, 23% in 0.2% chlorhexidine, 63% in control.

Table 1 Positive(+) & Negative (-) microscopic areas with bacterial colonization when using no gargling agent

membrane	collar	middle	apical
1	+++	++-	---
2	+++	+++	++-
3	+++	+++	++-
4	+++	++-	++-
5	+++	++-	---
6	+++	++-	---
7	+++	++-	++-
8	+++	++-	++-
9	+++	++-	---
10	+++	++-	++-
% positive fields	100%	63%	27%

Table 2 Positive(+) & Negative(-) microscopic areas with bacterial colonization when using 0.1% chlorhexidine

membrane	collar	middle	apical
1	+++	++-	++-
2	+++	---	---
3	+++	++-	---
4	+++	++-	---
5	+++	---	---
6	+++	---	---
7	+++	++-	---
8	+++	++-	++-
9	+++	++-	++-
10	+++	++-	---
% positive fields	100%	33%	10%

Table 3 Positive(+) & Negative(-) microscopic areas with bacterial colonization when using 0.2% chlorhexidine

membrane	collar	middle	apical
1	+++	++-	---
2	+++	+-	---
3	+++	---	---
4	+++	+-	---
5	+++	---	---
6	+++	+-	---
7	+++	+-	---
8	+++	---	---
9	+++	---	---
10	+++	---	---
% positive fields	100%	23%	7%

In the most apical portion of the membrane, 10% in 0.1% chlorhexidine, 7% in 0.2% chlorhexidine, 27% in control.

Dense aggregates of bacterial deposits were present near the cervical portion of the membranes(Fig. 1, 4, 7). These aggregates consisted mainly of cocci, filaments, and short curved rods. More apically, on the unexposed portion of the membranes, filaments and long curved rods dominated. Bacterial colonies in some instances extended into the mid-third of the membrane(Fig. 2, 5, 8). In most cases bacterial colonization was limited to the cervical, open pore-structured portion of the membrane but some specimens showed a scatter of bacterial aggregates and single microorganisms deep to the contiguous bacterial plaque. Below the open pore-structured cervical portion, the mid-portion and deep portion of the membrane surface exhibited bands of fine, highly-oriented fibrils.

Fibrous structures and structures which were interpreted as blood vessels were seen mid-and deep-portion of the membrane(Fig.

8, 9). Structures which were interpreted as blood vessels were also seen in the coronal portion of the membrane(Fig. 7). Red blood cells and white blood cells were seen(Fig. 5). A limited amount of connective tissue cells was present in the deep 1/3 of all membranes. Varying amounts of connective tissue cells adhered to the mid-portion of the membrane surface as well. The predominant cell type had a fibroblast-like appearance. Flattened cells, presumably fibroblasts, were attached to the ePTFE membrane. Horizontally arranged, parallel fibers represent



Figure 1 A scanning electron microscope of coronal portion in control ×5000

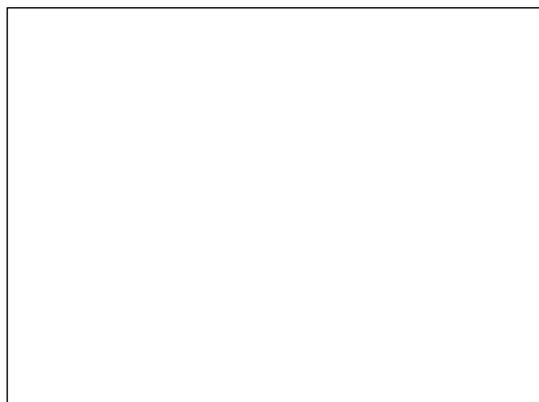


Figure 2 A scanning electron microscope of middle portion in control ×5000



Figure 3 A scanning electron microscope of apical portion in control $\times 5000$



Figure 4 A scanning electron microscope of coronal portion when gargling with 0.1% chlorhexidine $\times 5000$

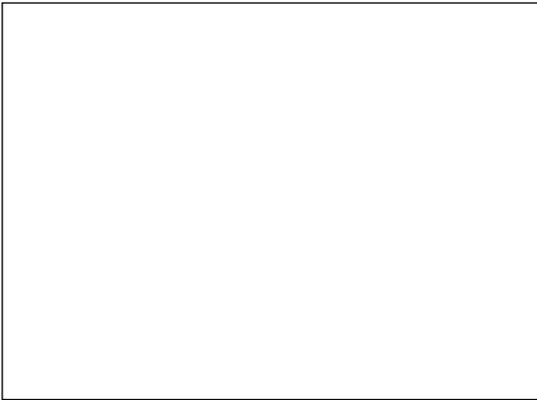


Figure 5 A scanning electron microscope of middle portion when gargling with 0.1% chlorhexidine $\times 5000$

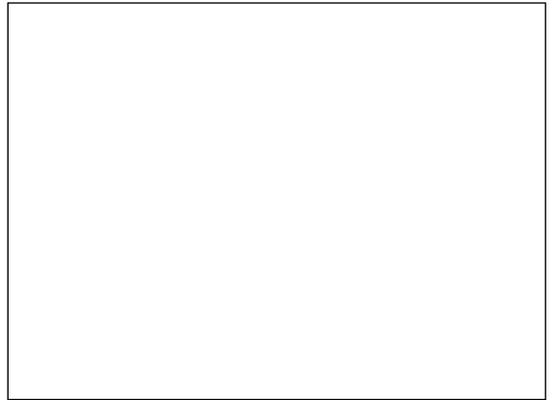


Figure 6 A scanning electron microscope of apical portion when gargling with 0.1% chlorhexidine $\times 2000$

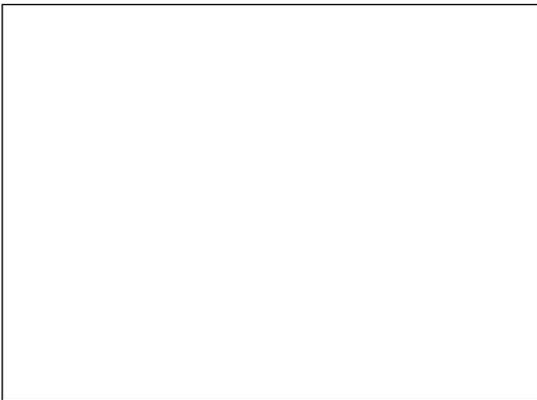


Figure 7 A scanning electron microscope of coronal portion when gargling with 0.2% chlorhexidine $\times 5000$

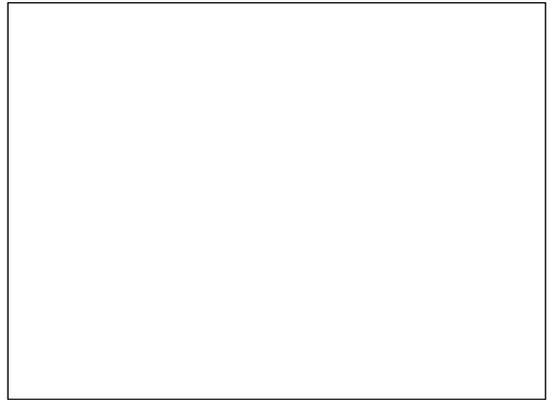


Figure 8 A scanning electron microscope of coronal portion when gargling with 0.2% chlorhexidine $\times 5000$

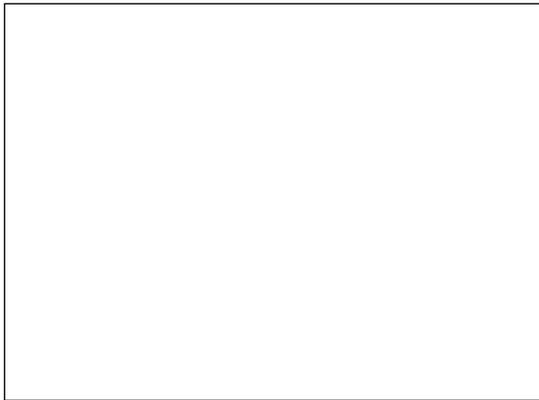


Figure 9 A scanning electron microscope of apical portion when gargling with 0.2% chlorhexidine × 500

membrane material. Fibrous structures suggestive of collagen fibers were also present.

Generally, however, the occlusive portion of the membrane was characterized by a sparseness of adherent tissue elements. No

distinguishable difference in bacterial colonization was seen among 0.1% chlorhexidine, 0.2% chlorhexidine rinse, and control in SEM.

2. Cultural Data

Table 4 presents that total viable counts of group A (no gargling), group B (0.1% chlorhexidine), and group C (0.2% chlorhexidine). Mean of group A is 170.1×10^3 , group B is 56.9×10^3 and group C is 68.2×10^3 .

There is no statistical significant difference between group B and group C ($P < 0.05$). 0.1% chlorhexidine and 0.2% chlorhexidine gargling showed similar results and found no differences. But when not used gargling, bacterial contamination was greater than the

Table 4 Microbiota of Barrier Membrane: Total viable counts ($\times 10^3$)

no gargling(A)	0.1% chlorhexidine(B)	0.2% chlorhexidine(C)	
1	96.8	52.2	1.65
2	230	2.00	54.0
3	87.0	34.0	260
4	210	220	0.350
5	135	1.76	73.2
6	93.2	65.0	230
7	156	72.2	5.30
8	217	80.0	21.6
9	175	10.4	20.5
10	301	31.1	15.3
mean S.D.	170.1 70.0	56.9 64.0*	68.2 96.3*

* $P < 0.05$

Table 5 Frequency of *P.gingivalis*, *P.intermedia*, *A.actinomycetemcomitans*(%)

	no gargling(A)	0.1% chlorhexidine(B)	0.2% chlorhexidine(C)
<i>P.gingivalis</i>	50	20	30
<i>P.intermedia</i>	50	30	30
<i>A.actinomycetemcomitans</i>	30	20	10

other two garglings. Statistically significant differences were found between group A and group B ($P < 0.05$) and between group A and group C ($P < 0.05$).

P.gingivalis was found 50% in group A and 20% in group B and 30% in group C, *P.intermedia* was found 50% in group A and 30% in group B and 30% in group C, and *A.actinomycetemcomitans* was found in 30% in group A and 20% in group B and 10% in group C (Table 5).

IV. Discussion

The purpose of this study was to compare the antimicrobial effect of 0.1% chlorhexidine and 0.2% chlorhexidine as a oral gargling agent in guided tissue regeneration by scanning electron microscope and anaerobic cultivation. The results of this study demonstrated that these two agents showed similar effects in scanning electron microscope and had no statistical difference in cultural data.

The SEM study confirmed that the ePTFE membrane material had been integrated in the healing connective tissue. Although the material consists of alternating bands of compact and finely porous structure, it had only been permeated by cells and fibrous connective tissue structures to a limited extent. The retrieved membranes impressed more by the sparseness than by the abundance of adherent tissue elements.

Most of the cervical portion of the membrane was heavily colonized by bacterial plaque at the time of removal. Cocci, rods, filaments, spirochetes were seen, and all cases the cervical portion was positive for

microorganisms. It was obvious that not only the function of the collar as a preventer of epithelial downgrowth through the ingrowth of connective tissue was lost, but also that the collar became a retention site for plaque bacteria. This result may raise questions as to the beneficial effect of the collar structure on the outcome of regenerative periodontal treatment. The presence of bacterial plaque in the apical portion of the membrane showed that the chronic inflammatory condition was spread over along the membrane. This was because of true microbial colonization during the healing period. Also, cervical plaque had been displaced during the retrieval process or contamination by saliva may have occurred. However, the interpretation of SEM images was a subjective evaluation of the true nature of the observed deposits. Exposure of the occlusive portion of the membrane allowed colonization and down-growth of bacteria. Bacteria colonized the most coronal portion of the membrane first, then grew downward along the membrane surface and through the pores of the ePTFE to areas of the membrane that remained covered by tissue. It has been suggested that the barrier material itself may form an environment for bacteria due to its structure and texture characteristics. SEM analysis left the impression that bacterial plaque, when present, adhered strongly to the membrane material. In addition to bacterial cells which had invaded the membrane in situ, it may be argued that marginal accumulations of plaque may have been smeared over the membrane during removal.⁵⁾ Selvig et al.⁴⁾ in a scanning electron microscopic study of ePTFE membranes following retrieval found only small

numbers of adherent cells located predominantly on the most apical one-third of the membrane.

In 1994, Nowzari and Slots⁸⁾ investigated the importance of microbial colonization of the barrier membrane for the regeneration of a new periodontal attachment. Some organisms seemed to be particularly inhibitory to guided tissue regeneration. *Porphyromonas gingivalis* was detected in sites that had experienced a net loss of probing attachment after membrane removal. *A. actinomycetemcomitans*¹⁸⁾, *P. gingivalis*¹⁸⁾, *P. intermedia*¹⁸⁾ and *P. micros*¹⁹⁾ constitute important putative pathogens in human periodontitis. These studies suggested that suspected periodontal pathogens play an important role in failed GTR.

The detrimental role of these bacteria in GTR may be the result of specific virulence factors that are capable of inhibiting coronal migration of periodontal fibroblasts on the tooth surface. *P. gingivalis* elaborates collagenase and other proteolytic enzymes that have the potential to degrade key periodontal tissue constituents²⁰⁾ and to kill human gingival fibroblasts²¹⁾. *A. actinomycetemcomitans* possesses a fibroblast inhibitor and other toxins that have tissue damaging potential.^{22, 23)} *P. micros*, *Fusobacterium* species, and *B. forsythus* generate hydrogen sulfide, and *P. gingivalis* and *P. intermedia* produce methyl mercaptan and hydrogen sulfide in serum.²⁴⁾ These toxic, volatile, sulfur compounds can inhibit protein formation and periodontal healing. Several other bacterial-derived enzymes and toxins can impair GTR as well²⁰⁾. The inflammatory reactions induced by periodontal pathogens can also inhibit periodontal tissue regeneration. Microorganisms

in tooth-associated membranes probably originate from residual microbial foci in the treated periodontal lesions and other sources. Microorganisms in deep furcation areas are difficult to eradicate by mechanical or chemotherapeutic means and can constitute a reservoir for pocket recolonization and membrane infection²⁵⁾. Dentinal tubules or cracks in the tooth root can serve as a reservoir for potential pathogens, and the eradication of microorganisms in these areas can be difficult²⁶⁾. Organisms might also seed to the barrier membranes from other periodontally involved teeth in the dentition²¹⁾. Salivary contamination during membrane placement can also be a source of microbial colonization. Nowzari⁹⁾ showed that barrier membrane can become contaminated with *P. gingivalis*, *P. intermedia*, *B. forsythus*, or other major suspected periodontal pathogens within 3 minutes of intraoral membrane manipulation. Also, Nowzari⁹⁾ determined the distribution of periodontal pathogens on the apical parts of barrier membrane surfaces facing the gingiva and the tooth. The organisms studied included the putative pathogens *A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *B. forsythus*, *C. rectus*, *Fusobacterium* species, *P. micros*, *Capnocytophaga* species, β -hemolytic *Streptococcus* species, *Staphylococcus* species, *Enterobacteriaceae* species, *Pseudomonadaceae* species, and *Candida* species. Microbes on the tooth-facing surface of the membrane played a particularly important role in failing GTR. The particular importance of pathogens on the tooth-facing membrane surface is probably a result of their close proximity to the periodontal ligament cells and cells

originating from the endosteum.

In 1994, Nowzari et al⁸) found that inverse relationship between microbial counts and gain of probing attachment. 80% of teeth with membranes with less than 108 total viable counts gained 3mm or more in probing attachment, whereas teeth with membranes harboring more than 108 total viable counts either lost attachment(50%) or showed small attachment increases from 1 or 2 mm(50%). Also they suggested that subgingival microorganisms interfere with optimal healing with guided tissue regeneration.

Black-pigmented anaerobic rods, *A. actinomycetemcomitans* and *P. micros* seemed to be particularly detrimental to healing.

In this study, 103 levels of microorganisms were found. This level was lower than other studies. There may have been some errors in the anaerobic incubation procedures.

Adjunctive antimicrobial therapy with guided tissue regeneration should aim at eradicating periodontal pathogens prior to membrane insertion and maintaining a pathogen-free environment during the healing period. But systemic augmentin therapy and chlorhexidine rinse did not prevent microbial contamination of barrier membranes²⁷).

Augmentin was prescribed because of the common use of beta-lactam drugs in oral surgery. However, Augmentin used alone may not arrest all cases of disease-active adult periodontitis. Since metronidazole/Augmentin combination drug regimens²⁸), doxycycline/augmentin serial drug regimens²⁹), or other systemic or topical antibiotic therapies³⁰) may be more proficient in suppressing periodontal pathogens, they may be more effective infection with barrier membranes around

implants.

The antimicrobial activity of chlorhexidine in the mouth appears due to adsorption of the antiseptic to the oral surfaces from which a prolonged bacteriostatic action is produced³¹). Such activity on the tooth surface alone also may explain the plaque inhibitory effect of chlorhexidine³²). Substantivity of chlorhexidine can be demonstrated, by recording effects of single rinses on salivary bacterial members which may reduced by greater than 75% for in excess of 12hours³³).

0.12% chlorhexidine oral rinse during the entire healing period of membrane retention may be useful in controlling bacterial colonization of ePTFE.³⁴ Local administration of chlorhexidine was considered necessary, but was limited to the first post-operative week when patients would most likely have difficulties with their oral hygiene. The administration of chlorhexidine could have affected post-operative bacterial colonization.

This administration would have minimal effects on the microflora present within the preserved furcation space due to the limited subgingival penetration of mouthrinses. The systemic administration of an antibiotic may not significantly affect bacterial colonization inside the membrane. Although the use of systemic antibiotic demonstrated efficacy, an antibiotic incorporated into the ePTFE providing a site-specific, slow release of the drug would probably enhance the ability to prevent microbial colonization of the ePTFE. The potential for periodontal regeneration could be affected if putative periodontal pathogens associated with active periodontal disease are present on the ePTFE membranes⁶).

In a comparative experimental gingivitis investigation the 0.1% product had greatly reduced activity by comparison with 0.2% product³²). In 1996, Asari et al³⁴) there was no clinical or statistical difference between 0.1% chlorhexidine(Eludril, Pierre Fabre, France) and 0.2% chlorhexidine(Corsodyl, then ICI, U.K.) used as subgingival irrigants in a simplified oral regimen in the treatment of chronic adult periodontitis. A clear dose-response pattern was seen for chlorhexidine with mean plaque scores decreasing with increasing dose. Even at 0.01%, chlorhexidine showed considerable and significant plaque inhibition compared to control, so low concentration chlorhexidine rinses as adjuncts to oral hygiene¹⁷).

Khoo and Newman¹³) noted reductions in motile organisms and spirochetes following daily irrigation with 0.2% chlorhexidine as compared with single session of scaling, root planing and oral hygiene instruction. Chlorhexidine achieves plaque inhibition as a result of an immediate bactericidal action during the time of application and a prolonged bacteriostatic action as a result of adsorption to the pellicle coated enamel surface³²).

The results of this study suggested that 0.1% chlorhexidine and 0.2% chlorhexidine showed similar antimicrobial activities by scanning electron microscope and anaerobic cultivation.

V. Conclusion

The purpose of this study was to compare the antibacterial effect of 0.1% chlorhexidine and 0.2% chlorhexidine in guided tissue

regeneration by scanning electron microscope and anaerobic incubation.

1. 0.1% chlorhexidine, 0.2% chlorhexidine, and no rinses exhibited similar bacterial contamination in scanning electron microscope.
2. 0.2% chlorhexidine generally exhibited lower anaerobic bacterial counts than 0.1% chlorhexidine, but that difference was not statistically significant. No rinses showed higher bacterial counts than the other two rinses.(P<0,05)
3. 0.1% chlorhexidine, 0.2% chlorhexidine, and no rinses showed similar percentages of P.ginigvalis, P.intermedia, A.actinomyetemcomitans.

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조직유도재생술의 초기치유에 있어서 클로르헥시딘의 영향

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본 연구의 목적은 조직유도재생술의 초기치유시에 구강양치액으로 사용되어지는 0.1% 클로르헥시딘과 0.2% 클로르헥시딘을 사용했을 경우, 양치액을 사용하지 않았을 경우의 세균감염 정도를 비교하는 것이다.

30명의 성인형 치주염에 이환되어진 사람을 대상으로 하였다.

초기치료(Scaling/Root planing/Oral hygiene instruction)를 시행한 후에 한 사람에 한 군데씩 선정하여 2급이나 3급의 치근이개부를 가지고 임상적으로 혹은 방사선학적으로 치간골내낭을 보이지 않는 치아에 통법에 따라 Gore-Tex™를 위치시켰다.

술후 5일간 항생제(Unasyn™ 375mg tablet p.o.tid)를 투여하고 차폐막을 제거할 때까지(4주 혹은 6주) 10명의 환자에게는 0.1% 클로르헥시딘을, 다른 10명의 환자에게는 0.2% 클로르헥시딘으로 구강양치를 하게 하고, 또 다른 10명의 환자에게는 구강양치액을 사용하지 않도록 하였다. 또 1주일에 한번씩 전문가구강위생술식을 실시하였다. 4주나 6주 후에 차폐막을 제거하고 주사전자현미경, 혐기성 세균배양을 이용하여 세균감염정도를 비교하였다.

1. 주사전자현미경으로 관찰시에 0.1% 클로르헥시딘을 사용했을 경우와 0.2% 클로르헥시딘을 사용했을 경우, 클로르헥시딘을 사용하지 않은 경우에 별 차이를 발견할 수 없었다.
2. 혐기성 세균배양시에 0.2% 클로르헥시딘을 사용했을 경우, 0.1% 클로르헥시딘을 사용했을 경우보다 적은 수의 세균 수를 보였으나 통계적으로 유의할 만한 차이는 보이지 않았다. 클로르헥시딘을 사용하지 않은 경우에는 다른 두 경우에 비해 통계적으로 유의할 만한 차이를 보였다.($P < 0.05$)
3. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*를 인지한 경우에는 세 경우 모두 비슷한 비율로 발견되었다.

주요어: 0.1% 클로르헥시딘, 0.2% 클로르헥시딘, 조직유도재생술, 세균감염