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The influence of periodontopathogenic microorganisms and locally-delivered antimicrobials on the efficacy of guided tissue regeneration surgery

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The influence of periodontopathogenic microorganisms and locally-delivered
antimicrobials on the efficacy of guided tissue regeneration surgery

by

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THESIS

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Introduction

Bacterial plaque is the primary cause of periodontitis. Studies have shown that a susceptible host is a risk factor to periodontal destruction; however, periodontopathogens are the triggers that initiate the inflammatory response and subsequent destruction of the periodontium.

Prior to popularization of guided tissue regeneration (GTR), the treatment protocol for a diseased periodontium was to reduce the accumulated plaque, and establish a periodontal environment that is readily available for oral hygiene access by means of pocket reduction and/or ostectomy. Although the protocol slows or stops the disease progression, patients often have to live with reduced periodontal support surrounding dentition and an inevitable esthetic compromise.

Periodontal regeneration is defined as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum. (Garrett, 1996) The basis for guided tissue regeneration as developed by Björn (1961) is to exclude the epithelium from taking part in the healing process. Ellegaard et al. (1974) used free gingival grafts over intrabony defects in an attempt to retard the apical migration of the epithelium. Prichard (1983) used a denudation technique as epithelial exclusion to treat vertical bony defects. Melcher (1976) stated, "From the clinical standpoint, the design of surgical procedures that will allow colonization of wounds coronal to the alveolar crest by cells derived from periodontal ligament and bone rather than by cells derived from

the lamina propria of gingiva or bone alone could provide a fruitful field for investigation."

GTR has been investigated using many different techniques and materials. Various GTR techniques – involving different types of membranes for epithelium exclusion as well as combining solid material (e.g., demineralized freeze-dried bone allograft material and tricalcium phosphate) to assist space maintenance – have been investigated in the hope of complete periodontal defect regeneration. Molecular enhancements with growth factors such as fibronectin, were also studied and resulted in mixed conclusions. (Caffesse et al. 1988; Alger et al. 1990)

Complete regeneration of periodontal defects is rarely obtained. Becker et al. (1986) reported that upon surgical re-entry at 9 to 16 months using Prichard's technique, the mean percentage fill was 47.5%, with more than a 50% defect volume fill in 50% of the defects. Gottlow et al. (1986) - using ePTFE membranes as a barrier for epithelium exclusion - reported 2.8 to 4.5 mm of new attachment versus no new attachment in control sites in a series of case reports. Becker et al. (1988) reported a 4.5mm gain in three-wall intrabony defects treated with ePTFE. Blumenthal and Steinberg (1990) found 93% of defects – at one-year re-entry – had 50% or more of fill using a combination of demineralized bone-collagen gel with collagen membrane barriers. Schallhorn and McClain (1988) used citric acid root conditioning in addition to the ePTFE membrane and combined these with a mix of autogenous bone and either tricalcium phosphate or

demineralized freeze-dried bone allograft to treat furcation defects. The complete fill of defects was observed in 72% of the sites.

Obstacles that prevent complete regeneration have been investigated. Stahl et al. (1990) studied histologic results of guided tissue regeneration in intrabony lesions treated with ePTFE membranes and proposed that topography of the bony lesion may be the key controlling factor determining regeneration. Cortellini et al. (1993) also reported less fill in the 1-wall treated defects versus the 2- and 3-wall defect lesions. Klein et al. (2001) stated that narrow and deep infrabony defects respond radiographically and - to some extent - clinically more favorably to GTR therapy than wide and shallow defects.

To overcome this problem, the uses of reinforced membranes as well as a filling material have been suggested. Paolantonio (2002) suggested using a filling material in combination with barrier membranes for intrabony defects characterized by an unfavorable architecture. Choice of filling material can include autogenous grafts, allogenic bone grafts, mineralized freeze-dried bone allografts (FDBA), decalcified freeze-dried bone allografts (DFDBA), and synthetic materials. Synthetic materials – regardless of absorbable or non-absorbable characteristics – demonstrate that the grafts tend to be encapsulated by connective tissue with little bone formation histologically. (Stahl and Froum 1986, 1991; Carranza et al. 1987; Baldock, 1985)

Autogenous grafts can be extraoral or intraoral. Extraoral autogenous grafts are not used for periodontal procedures due to the second surgical site and observation of

root resorption. (Hiatt et al. 1978; Schallhorn, 1972) Intraoral autogenous grafts are generally obtained from the maxillary tuberosity, healing extraction sites, or the coagulum from bone recontouring around the surgical site. The histologic results show new tissue attachment with connective tissue above notches in the calculus. (Froum et al. 1983) The limitation of intraoral autogenous graft is that the donor source is not always available.

Many studies showed greater bone fill in sites treated with DFDBA than non-grafted sites. Mellonig (1984) showed a 65% defect fill with DFDBA compared to a 30% defect fill with controls. Borghetti et al. (1993) reported a 60% defect fill compared to a 29% fill for non-grafted sites. The use of DFDBA was preferred over FDBA. (Urist, 1965; Urist et al. 1968) The demineralization of cortical bone allograft enhances its osteogenic potential by exposing bone morphogenetic proteins (BMPs), proven to be osteoinductive, while FDBA proved to be osteoconductive for new bone formation.

Another possible reason for the incomplete regeneration maybe the continuing bacterial contamination of the surgical site and the ability of periodontopathogenic bacteria to inhibit bone formation. Simion et al. (1995) showed that membranes used in guided tissue regeneration were completely penetrated by bacteria approximately four weeks after placement. Nowzari and Slots (1994) were able to associate microflora recovered from an expanded polytetrafluoroethylene membrane to different outcome levels. Clinically, Machtei et al. (1994) showed less favorable

regeneration results associated with poor oral hygiene, gingivitis, *Actinobacillus actinomycetemcomitans*, and the marked absence of connective tissue cells on retrieved membranes. Rudiger et al. (2003) showed colonization of periodontal pathogens at the site treated by guided tissue regeneration correlates with the same pathogen before surgery.

The association of periodontal disease with specific bacterial species was first discussed in the early 1960's. Since then, numerous researchers have investigated the specific pathogens that contribute to periodontal disease. The presence of certain specific periodontal pathogens is a significant predictor in these multivariate models. Slots (1977) stated that Gram-negative anaerobic rods are the predominant organisms inhabiting deep periodontal pockets. Armitage et al. (1982) found a significant increase in the relative percentage of spirochetes noted when the probing depth and attachment loss are greater than 3 mm. Moore et al. (1983) found differences in the relative proportions of some of the periodontal pathogenic species relative to moderate chronic periodontitis and healthy subjects. Based on the presence of *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Treponema denticola*, Socransky et al. (1998) categorized these oral microorganisms as "the red complex" - the microbes most strongly associated with periodontal disease.

More recently, Dewhirst et al. (2000) - using the 16Sr RNA PCR technique - found a greater diversity of *Treponema* species in the subgingival environment than

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previously believed. Timmerman et al. (2001) found the composition of subgingival microbiota to be the most important parameter related to disease progression.

Using periodontal pathogens as parameters to predict the magnitude of periodontal destruction has been disappointing. It has been suggested that only about 9 to 16% of the variability in disease expression can be explained by the levels of specific microbes. (Offenbacher, 1996) Low rates of association are due to several factors: smoking, emotional stress, and genetics make significant contributions to the expression of periodontal disease.

Another explanation for the low magnitude of association of the microbial flora with disease expression is that not all strains of pathogenic organisms possess the same virulence traits. For example, Zambon et al. (1996) found that the presence of a highly leukotoxic genotype of *Actinobacillus actinomycetemcomitans* is associated with localized aggressive periodontitis. Furthermore, certain virulent strains of *Porphyromonas gingivalis* can cleave the Fc regions off the bacterial-bound IgG, preventing phagocytosis and killing of the neutrophil. It was identified that these strains of *Porphyromonas gingivalis* have a prtH gene, which is responsible for this enzymatic capacity (Schenkein 1988, Schenkein et al.1995). Additionally, other components of the pathogen can function as virulence factors toward the enzymatic capacity of virulent strains of periodontal pathogens. Fimbrial adhesions, lipopolysaccharides (LPS), and the capsule of *Porphyromonas gingivalis* are able to cause inflammation without the live pathogen.

Demonstrably, *Porphyromonas gingivalis* extracts, including proteinaceous as well as non-proteinaceous products (e.g., LPS or endotoxin), caused inhibition of osteogenesis in a 17-day old chick periosteum model system. There was decreased alkaline phosphatase activity when the osteoblasts were treated with extracts of *Porphyromonas gingivalis*. When osteoblasts were exposed to the bacterial extracts from day 0 to 2, alkaline phosphatase activity, calcium levels, and collagen amounts were less than those of the controls. This study therefore suggests that once inhibition of osteogenic activity occurred, the formation of bony tissue was less than with osteoblasts that were not exposed to bacterial extracts during this period. (Loomer et al. 1994, 1995, 1998)

Putative pathogens have also been shown to cause bone resorption in vitro and in animal models. Lino and Hopps (1984) showed that lipopolysaccharides (LPS) from *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Capnocytophaga ochracea* induced bone resorption; this study further strengthens the conclusions from the Scheinkein 1988, Schenkein et al.1995 work. Additionally, and applicable to bacterial virulence factors, secondary mediators of inflammation and connective tissue destruction such as histamine, serotonin, kinins, tissue activating factors, matrix metalloporteinases (MMPs), nitric oxide, platelet activating factor, leukotrienes, and prostanoids make the periodontal destruction process complicated. Birkedal-Hansen (1993) proposed a pathway model demonstrating that MMPs mediate bone resorption; this indicates that the regulation component of these mediators leads to tissue destruction in periodontal diseases.

Many studies have found an increased prevalence of *Bacteriodes forsythus* in diseased sites versus healthy sites. Christersson et al. (1992) found that if pocket depths were more than 5 mm, *Bacteroides forsythus* was 14 and *Porphyromonas gingivalis* 6.3 times greater than those of the control group. Kamma et al. (1994) found that sites with a greater loss of attachment possess a higher prevalence of *Bacteroides forsythus* and *Porphyromonas gingivalis* in young adults with aggressive periodontitis. The implications of these findings suggest that *Bacteroides forsythus* could be more virulent than *Porphyromonas gingivalis* and other microbes; or the combination of *Porphyromonas gingivalis* and *Bacteroides forsythus* might be more destructive than either found separately.

The control of bacterial plaque, therefore, is essential for maintenance of periodontal health; the interruption of their pathogenic effects is crucial to improvement of the periodontal condition.

Since bacterial effects on the periodontium is the primary cause of periodontal disease, the use of antibiotics to control the disease has been investigated, however, with contradictive conclusions. Nowzari et al. (1995) found Augmentin 500mg tid for 8 days starting immediately prior to the membrane placement improved probing attachment gain in guided tissue regeneration procedures. Vest et al. (1999) showed administration of post-surgical antibiotics (e.g., metronidazole 250 mg tid and ciprofloxacin 250mg bid for 1 week, followed by doxycline 50mg qd for 7 weeks) did not produce superior

osseous healing in guided tissue regeneration. Loos et al. (2002) did not detect a significant difference between systemic amoxicillin and a metronidazole regiment compared to no antibiotic for the guided tissue regeneration procedure using polylactic acid membranes.

The use of antibiotics systemically yields contradictive conclusions and may be due to several factors, including the antimicrobial spectrum and the pharmacokinetic characteristics of the drug in the local environment. The drug-binding property, the total bacterial load relative to the maximum achievable antibiotic concentration, effectiveness of the host defenses, and influence of biofilm phenomena, have all have been known to impose on the efficacy of the antibiotic. The most unpredictable factor would be the biofilm in each individual local environment. The concentration of the antimicrobial agent in the crevicular fluid under the dynamic of the healing process following surgical treatment is currently a field lacking research data. This unknown effect of the antimicrobial agent translates into the various antibiotic regimens used in the treatment of periodontitis.

Addressing this concern, Newman (1993) in his review paper suggested the following:

1. Intraoral infection control including the elimination of active periodontitis;

2. Thorough intraoperative root debridement;

3. Establishment of intraoral infection control including the elimination of active periodontitis;

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4. Establishment of good oral hygiene stressing the importance of compliance;

5. Precise surgical techniques including a thorough understanding of the benefits and limitations of specific techniques (products) and materials;

6. Consideration of presurgical systemic antibiotics when antibiotic use is anticipated and based on the clinician's assessment of the patient's dental and systemic factors;

7. Use of preoperative "degerming" with chlorhexidine rinses with follow-up rinsing continuing at least 1 month following surgery;

8. Close post-surgery monitoring and evaluation;

9. Removal of exposed regenerative materials associated with implants;

10. Removal of all obviously infected regenerative material; and

11. Comprehensive and frequent Supportive Periodontal Treatment with the introduction of adjunctive oral hygiene instructions as needed.

The additional use of a local antimicrobial agent in the surgical area may improve the overall clinical response to GTR therapy. Frandsen et al. (1994) found decreased numbers of bacteria and lower median proportions of black-pigmented anaerobic rods in the surgical site following the local administration of metronidazole gel. The result – with the additional topical metronidozole applied at the time of the guided tissue surgery – has demonstrated a 92% defect resolution compared to a 50% resolution for the control group at 6-month follow-up. (Sander et al. 1994) When Kurtis et al. (2002) loaded metronidazole on polylactide/glycolide membranes to treat osseous defect in dogs, the result showed marked improvement in the overall clinical response to

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GTR therapy. However, the effect of locally delivered metronidazole was not statistically significant.

Ciprofloxacin, a broad-spectrum bactericidal agent that inhibits DNA gyrase needed for the replication of bacterial DNA, may be better used in the treatment of periodontal disease. Incorporating ciprofloxacin in the local environment might reduce the bacterial contamination that inhibits bone formation, therefore achieving a higher regeneration of periodontal tissue.

The barrier membranes that could be used to promote selective cell population of the root surface and facilitate tissue regeneration are numerous. The original e-PTFE material had large nodes of PTFE spaced 100 to 300 μm apart by thin fibrils. (Scantlebury, 1993) Biomaterial porosity can be varied to either encourage or discourage tissue growth, having been produced in a variety of porosities for various medical applications.

In 1985, W. L. Gore & Associates, Inc. began working on a two-part material specifically for periodontal use: 1) an open microstructure collar that could be implanted subgingivally, ingrown with connective tissue, and which would limit epithelium migration; and 2) an occlusive portion that would still attach to stabilize the wound area, separate cell types for GTR, provide a strong structure to retain sutures around the tooth, be easy to cut and shape with no sharp edges to perforate tissue, and in the event of complication, allow the membrane to be easily removed. In 1988, the material was

redesigned with a space-making property that stiffened the center portion of the membrane to support it and resist collapse. (Scantlebury, 1993)

Polylactide/acetyltributyl citrate (Guidor®) is made of amorphous polylactic acid and is softened with a citric acid ester to accomplish malleability while facilitating clinical handling. The resorption process is programmed to ensure barrier function for a minimum of 6 weeks, after which it slowly resorbs without interfering with the regenerative healing process. Bioresorption of the material is normally completed within 12 months. The matrix barrier is designed to prevent or minimize epithelial downgrowth adjacent to the barrier by integration of the device with the gingival connective tissue. This is accomplished through a double-layer design in which the large perforations of the outer layer allow rapid ingrowth of gingival tissue into the interspace between the two layers. Epithelial downgrowth along the barrier is thereby prevented or minimized. The inner layer perforations are adapted to retard the penetration of connective tissue and to achieve proper barrier function.

Collagen was tested as another possible barrier material based on the following facts: it is physiologically metabolized; it is chemotactic for fibroblasts which may enhance cells migration; it is reported that collagen prevents migration of gingival cells in vitro; it is hemostatic; and it is a weak immunogen. (Pitaru, 1988)

Resolut™ (polyglycolic and polylactic acid) membranes are made from a synthetic copolymer of glycolide and lactide. The absorption of these membranes is

accomplished by hydrolysis through the Krebs cycle as carbon dioxide and water. The absorption is minimal for approximately 6 weeks, and is essentially complete by 8 months. (Brady et al. 1973) Polylactic acid membranes (Vicryl Mesh®) are made of a woven mesh of polyglactin 910, a co-polymer of polyglycolic acid and polylactic acid. It has a resorption time of 30 to 90 days. (Fleisher et al. 1988; Quiñones et al. 1990) According to the manufacturer's manual, the woven mesh is preferred over the knitted version for periodontal application due to the tightness of the weave. The pore size allows for the passage of critical fluids while providing a barrier to larger invasive tissue cells.

Studies using Atrisorb® (Polson et al.1993, 1995; Rosen et al. 1998; Rosen and Reynolds, 1999) have demonstrated some favorable clinical responses. Atrisorb® is composed of a polymer of lactic acid; the polymer is dissolved in a biocompatible carrier N-methyl-2-pyrrolidone. The polymer exists as a fluid and transforms to a solid upon contact with water or other aqueous solutions. The solidified membrane is then absorbed via hydrolysis. Facility of use is a clear advantage of Atrisorb® over other membranes. The fluid quality of the material also makes it an ideal choice of membrane to incorporate the testing of antibiotics into the immediate local environment where bacterial influence is of concern. A recent study even suggests that the N-methyl-2-pyrolidine component of Atrisorb® may be a source of its antibacterial activity against *Prevotella nigrescens* and *Enterococcus faecalis*. (Chogle and Mickel, 2003)

Hypothesis:

1. The composition and quality of the local microflora influences the outcome of GTR therapy; and

2. The addition of locally delivered ciprofloxacin improves the GTR therapeutic outcome.

The purpose of this study is:

1. To evaluate whether the level and quality of microflora have any effect to the success of GTR therapy outcomes; and

2. To investigate if the addition of ciprofloxacin incorporated with Atrisorb® in the GTR procedure improves clinical outcomes.

Materials and Methods

A. Clinical Protocol

This pilot study was conducted on 12 subjects recruited from those patients with chronic periodontitis undergoing active periodontal treatment at the University of California at San Francisco School of Dentistry dental clinic. This group consisted of 7 females and 5 males; average age being 47 years old. All subjects had radiographic evidence of at least one narrow interproximal periodontal defect, and had completed initial periodontal therapy consisting of scaling and root planing, oral hygiene instruction, and re-evaluation. The interproximal intrabony defects were defined by radiographic appearance as a narrow triangular-shaped bone defect having vertical bone loss of 3-6 mm on one proximal side of the tooth with minimal bone loss on the other side (less than 1 mm) and a maximum width of the crest defect of 4 mm.

As each subject enrolled in the study, they received a periodontal examination on the sites to be treated as well as reviewing and signing the informed consent form approved by the UCSF Committee on Human Research. This exam included measures of probing depths, Gingival Index, (Löe and Silness, 1963), Plaque Index (Silness and Löe, 1964), and clinical attachment loss using preselected reference points such as the cementoenamel junction or crown margin with a North Carolina Probe. Vertical bitewing radiographs for each potential site were taken. Guided tissue regeneration surgical treatment was then scheduled within 2 weeks of the baseline exam. The inclusion and exclusion criteria for patient selection are listed in the appendix.

On the day of the surgical procedure, a bacterial sample was obtained prior the surgery. After the periodontal defects were surgically exposed, the area was scaled and planed. The Atrisorb®[1] material and 250mg of ciprofloxacin, or Atrisorb alone, decided by coin toss, were placed at the time of defect site treatment. The ciprofloxacin was used in its pure form, no placebo treatment used with the control group. The defects were then filled with human freeze-dried and demineralized ground cortical bone[2], the membrane was applied to the surgical site, and sutured. Postoperative instructions were given to rinse with a 0.12% chlorhexidine mouthrinse twice a day for 2 weeks.

Following the surgical procedure at the 1- and 2-week, and 1-, 2-, 3-, 4-, 6-, and 12-month appointments, each subject received a prophylaxis to remove newly formed plaque and calculus accumulations found at the surgical site. Sutures were removed at two weeks. Probing depths and clinical attachment levels were measured at the 2-, 3-, 4-, 6-, and 12-month visits. Follow-up radiographs of the surgical site were taken at the 6- and 12-month visits for subtraction radiographic analysis. The schedule for measurement parameters is presented in Table 1.

B. Bacterial Plaque Sample Collection

Prior to surgery, subgingival bacterial plaque samples were collected from the site of interest following a thorough supragingival plaque debridement. Plaque samples for the University of South California Oral Microbiology Testing Laboratory (OMTL) were

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[1] CollaGenex Pharmaceuticals, Inc. 41 University Drive, Newtown, PA 18940
[2] Lifenet, 5800 Ward Cr., Virginia Beach, VA 23455

collected with paper points inserted into the periodontal pocket of the study site for 5 seconds. The paper point samples were preserved in reduced transfer fluid for anaerobic bacteria provided by the OMTL for bacterial culture testing, while paper point samples for DNA analysis were stored in sterile Eppendorf tube. Both samples were then shipped to the laboratory via same-day delivery.

N-benzoly-DL-arginine-2-napthylamide (BANA) is a substrate that is degradated by trypsin-like enzymes produced by *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Treponema denticola*. BANA testing was performed at chairside with PerioScan[3] for the presence these bacteria using bacterial plaque samples collected with a 4R/4L curette. The sample was then transferred onto a BANA testing strip for the BANA test. The BANA test incubator was set at 35^0 Celsius for 10 minutes, the results were recorded in either negative or positive depends on the presence of blue-black color indicator on the testing strips.

Subgingival bacterial plaque samples were also studied under a darkfield microscope. From the bacterial plaque sample collected with a 4R/4L curette, the sample was then transferred into an Eppndorf tube filled with sterile saline and subsequently disrupted by vortexing ten times. The diluted plaque sample was then studied under darkfield microscopy. At the 400X power, five random fields were selected for bacterial counts categorized into 1) mobile rods, 2) cocci, 3) spirochetes, and 4) any unusual findings. The bacterial counts from all fields were then averaged according to the above categories.

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[3] PerioScan (BANA Test) Oral B Laboratories, Edwood City, CA.

C. Data analysis

Clinical attachment level, probing depth, plaque, and gingival indices of each individual – at baseline, 3-, 6-, and 12-month intervals – were analyzed for significance with Student's t-test by comparing within the test and control groups as well as between the groups at each allocated time point.

The bacterial sample results were categorized as low (less than 1%), medium (1-5%), and high (greater than 5%) according to bacterial counts and DNA analysis reports provided by the University of Southern California Oral Microbiology Testing Laboratory. The bacterial sample results were mapped with their clinical results in regard to the use of ciprofloxacin in the GTR procedure.

Note: Only *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Bacteroides forsythus* were independently analyzed in this study.

The vertical bitewing radiographs of the surgical sites were taken with size 2, E-speed Kodak[4] intraoral two-film packets at baseline and the 6- and 12-month post-operative junctures; the same x-ray machine was used and set at 15 mA. After importing the radiographic image by scanning the original radiograph using a UMAX Astra 2200 scanner with transparency cover UTC-2100 model for 2200 SU use at 600 bpi resolution, the radiographs were analyzed using a digital subtraction program in Emago-Advanced diagnostic radiography software[5].

The subtraction analysis would ultimately provide information on the changes in vertical bone height identified on the radiographs.

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[4] Eastman Kodak Company
[5] Emago®/Advanced v 3.2

Discussion:

I. Summary of results

A. Presurgical levels of *Porphyromonas gingivalis* and *Prevotella intermedia* appear to inversely influence the outcomes of guided tissue regeneration. The presurgical levels of *Bacteroides forsythus* seem to positively influence the outcome of guided tissue regeneration.

B. The Plaque Index was not statistically significantly different throughout the study; meaning that oral hygiene did not influence the differences between the test and control groups. The Gingival Index was statistically significant between both test and control groups at the 3- and 12-month intervals with significance approached at 6-months.

C. Clinical attachment level in the test group significantly and statistically improved at the 6- and 12-month junctures compared to the control group. Probing depth improved significantly in both the test and control groups at the 3-, 6-, and 12-month intervals. However, the differences between the test and control groups regarding probing depth were uncertain.

II. Study Population

Due to the small sample size, the significance of the outcome may be magnified. The subject variations were also of concern in this study. Since host factors are of major importance in the pathogenesis of periodontal disease, the ethnic background of the subjects in this study may impose too much variation and make the significance of the results questionable. For the same reasons, the wide range of age differences between subjects was also another shortcoming of this study.

The inclusion/exclusion criteria of the study were followed for patient selection, however, as is common to all clinical studies, compliance toward follow-up appointments was a problem.

Loss of data points due to the lack of adherence with the recall schedule could also affect the results of this study. One drawback to the inclusion/exclusion criteria is that it failed to consider the smoking status of test subjects. As the subjects were recruited, all subjects were confirmed non-smokers. For example, one subject – at the 6-month interval – failed the smoking cessation effort and failed to return for follow up until the 12-month follow-up. This patient was in the test group and received ciprofloxacin for the guided tissue regeneration procedure. There was a 1.5mm average attachment gain; one might be able to expect more gain if the smoking cessation had been successful. Based on numerous studies, (Ehmke et al. 2003; Klein et al 2001; Eickholz and Hausmann, 1998; Trombelli et al. 1997; Tonetti et al. 1995) smoking was the

strongest predictor negatively affecting alveolar bone gain following GTR. In a recent publication, Machtei et al. (2003) stated that with local anti-infective therapy, class II furcations treated with GTR in smokers resulted in a more favorable outcome. This single incident is not enough evidence for confirmation of local anti-infective therapy for smokers, but it is another interest of further study.

III. Microbiological Data

The chairside BANA test is sensitive to the presence of *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Treponema denticola*. In this collective analysis of plaque samples, the results revealed that, in both groups, the addition of ciprofloxacin improved the attachment results in all time points except in the BANA-positive group at 6 months. Interestingly, the BANA-positive group had better gain in the control group.

This could be translated as the influence of bacterial plaque encouraging the attachment gain. The same result was observed in the test group. There might be a substrate of *Bacteroides forsythus* that encourages attachment gain independent of the influence of ciprofloxacin.

To independently analyze the result of each individual bacterium from the chairside BANA test, the attachment changes were compared with the bacterial culture reports. The outcome suggested that the presurgical level of *Porphyromonas gingivalis*, *Prevotella intermedia*, inversely influences a guided tissue regeneration outcome, while

the presurgical level of *Bacteroides forsythus* positively influences a guided tissue regeneration outcome. After understanding the bacterial influence on the healing tissue, the adverse effects of *Porphyromonas gingivalis* and *Prevotella intermedia* on clinical outcomes were expected. The influence of *Bacteroides forsythus* on the guided tissue regeneration outcome, however, was contrary to study expectations. The addition of locally delivered ciprofloxacin overcame the difference in outcome of guided tissue regeneration relative to *Porphyromonas gingivalis* and *Prevotella intermedia*. The average attachment gain is achieved at the 6-month interval, as opposed to 12-months in the control group. In regard to *Bacteroides forsythus*, the addition of ciprofloxacin brought the outcome of guided tissue regeneration closer, but not as close as in *Porphyromonas gingivalis* and *Prevotella intermedia*.

The collective bacterial analysis with the BANA test and specific single bacteria analysis were reported in this study. Previous studies support *Porphyromonas gingivalis* and *Prevotella intermedia* as detrimental to periodontium, but *Bacteroides forsythus* might benefit by stimulating regeneration. In one published reference, Smith (2001) showed the substrate of *Bacteroides forsythus* stimulates bone formation *in vitro*, and the results supports this pilot study observation. An ongoing study (Loomer, 2003) found that substrate of *Bacteroides forsythus* increases gene expression of BMP and alkaline phosphatase activity of osteoblast cells (MG63 cell-line). These findings were contradictory to the conclusion of previous studies (Kamma et al. 1994; Haffajee et al. 1991; Listgarten et al. 1993; Grossi et al. 1995) on the destructive influence of *Bacteroides forsythus*. These evidences suggested the mixed understanding of influence

of *Bacteroides forsythus* on periodontium. Further *in vitro* investigation as well as clinical studies on change of *Bacteroides forsythus* levels in relation to alveolar responses to periodontal therapy will prove more information to clarify the role of *Bacteroides forsythus*, and possibly isolate the substrate that responsible of the up-regulation and regeneration observed in the more recent studies.

Two test subjects tested positive for spiroches (one *Treponema denticola* with DNA probing, one with darkfield microscopy) and experienced no attachment gain at all three measured time points. While the test sample is too small to draw the conclusion that *Treponema denticola* inhibits regeneration, both control and test groups proved that surgery had proven to improve the clinical attachment level and it is statistically significant when compared to the baseline. The significance of *Treponema denticola*, therefore, cannot be overlooked.

With more reports of additional unseen species in the subgingival plaque (Paster et al. 2001), the biofilm phenomenon gets increasingly complex. Additional questions need to be explored are the role of virus and fungi, as well as any other possible microorganisms in the subgingival microflora that could affect study outcomes.

IV. Clinical Data

The differences in the Plaque Index were not statistically significant throughout the study, meaning that the oral hygiene of subjects did not change, either within the

group or between the groups. The Plaque Index between control and test groups at baseline showed that the subjects were evenly distributed.

Changes in the Gingival Index were not significant in the control group, but were statistically significantly improved in the test group after guided tissue regeneration. Comparing the test and control group, the Gingival Index was statistically significantly better in the test group both at the 3- and 12-month intervals, while approaching significance at 6-months. This improvement of gingival inflammation seems to be contributed to the addition of local antibiotics at the guided tissue regeneration site.

The outcome of GTR on clinical attachment is statistically and significantly improved at 6- and 12-months for both the control and test groups when one compares the baselines. Between the control and the test groups, there were no significant differences at the baseline; but differences were significantly higher for the test group at the 6- and 12-month revisits.

This is an interesting observation, as all subjects in the test group experienced membrane exposure at postoperative appointments, a complication associated with a less favorable outcome. According to Machtei (2001), exposure of membrane in GTR procedures yields statistically significant differences in regenerative responses. As all subjects in the test group experienced membrane exposure, it appears that the antibiotic effect of ciprofloxacin may also exert a negative effect on healing of the gingival flap. However, the results suggested the in spite of membrane exposure, the addition of locally

applied antibiotic seems to reverse the negative effect of membrane exposure and achieve more attachment gain then the control group.

Probing depth improved significantly when compared to the baseline in both the test and control groups at the 3-, 6-, and 12-month intervals. The differences between the test and control groups regarding probing depth were uncertain however, due to the uneven distribution of subjects under this clinical mearsurement at the baseline. The test group started with significantly less probing depth than the control group. With the small sample size of this study and the grouping of subjects by chance of coin-toss, it is beneficial that the other clinical parameters were evenly distributed between the control and test groups. In order to avoid this problem, a larger sample size is required.

Considering the outcome of this pilot study, it is suggested that the presurgical bacterial level and composition influence the guided tissue regeneration outcome. The high levels of *Porphyromonas gingivalis* and *Prevotella intermedia* appear to result in lower clinical attachment gain, while the *Bacteroides forsythus* level appears to positively correlate with clinical attachment gain. The addition of locally delivered ciprofloxacin improves clinical parameters after a guided tissue regeneration procedure, possibly by reducing microbial contamination.

Toward the observation that membrane exposure occurred with all subjects in the test group, one possible explanation is that the ciprofloxacin may discourage the adhesion of the gingival flap. Yet, based on the improved clinical attachment gain in the test group

despite the membrane exposure, one has to ask how important the closure of gingival tissue is to the for GTR outcome. Wikesjö et al. (2003) used created periodontal defects on the mandibular premolar of beagle dogs to compare macroporous or occlusive ePTFE membranes on GTR results. The animals experienced membrane exposures were excluded from the data analysis, but substantial periodontal regeneration was observed. Ling et al. (2003) reported 26.3% of the membrane exposure sites had zero attachment gain, other exposed membrane sites had smaller clinical attachment gain and significantly greater marginal tissue recession. Hung et al. (2002) reported that the bacterially contaminated GTR membranes would affect PDL cells attachment, which may subsequently alter healing following membrane exposure.

In this pilot study, none of the subjects had *Actinobacillus actinomycetemcomitans* in the bacterial culture report. The observed clinical attachment gain may contribute to the effect of ciprofloxacin as well as to the lack of *Actinobacillus actinomycetemcomitans* in the subjects' subgingival flora.

V. Radiographic Data

The digital subtraction results with the Emago-Advanced diagnostic radiography are from the radiographs that were available for analysis. Statistical analysis was not performed due to the small sample size. Results of the radiographic data do not reflect attachment gain from the result of the clinical data, since attachment gain can also originate from soft tissue attachment gain. The percentage of subjects who experienced

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gain vs. no gain reflected that more subjects experienced radiographic gain in the control group. It is possible that most of attachment gain in the clinical data from the test group is in the form of long junctional epithelium attachment.

DSR software program has an advantage over Emago-Advanced diagnostic radiography software in that it can measure mass changes and can be applied for statistical comparison. Suggestion for further study would be using the DSR software program in conjunction with bite registration during radiographic exposure for more accurate images.

VI. Overview

In general, the result of this pilot study suggested that the level and quality of microflora might have an influence on GTR outcomes, and a greater improvement in guided tissue regeneration outcome might be expected with the use of a locally delivered ciprofloxacin as opposed to not using antibiotic therapy at all. The results of this pilot study suggest further investigation for the role of *Bacteroides forsythus* in relationship to periodontal tissue regeneration might be an area of interest and should be conducted with larger sample sizes as well as more uniformly controlled in subject variations. The combined effect of different bacteria should be analyzed for further study as well as a single specific bacterium.

Conclusion

In conclusion, the results of this study suggest that both presurgical bacterial levels and composition influence the outcomes of guided tissue regeneration. High levels of *Porphyromonas gingivalis* and *Prevotella intermedia* appear to result in lower clinical attachment gain, while high *Bacteroides forsythus* levels appear to be positively associated with clinical attachment gain. The addition of locally delivered ciprofloxacin improves clinical parameters after the guided tissue regeneration procedure, possibly by reducing microbial contamination. This conclusion is drawn based on a single bacterial comparison to clinical attachment. More study and analysis are needed on groups of bacteria in different combinations to compare with the clinical attachment outcomes.

Future studies

Continue study with larger sample size.

Analysis on broader spectrum of bacteria.

Digital subtraction analysis with DSR program.

Appendix A: Inclusion/ Exclusion Criteria for Patient Selection

Inclusion criteria:

1. Be 18 years of age or older;
2. Be in general good health, with no contraindications to periodontal surgical therapy;
3. Based on the investigator's observations and opinion, patient should qualify as a subject who can be expected to comply with the entire protocol;
4. Present with one or more narrow interproximal infrabony defects in one or more teeth. For this study, a narrow interproximal defect would be defined - by its radiographic appearance - as a narrow triangular shaped bone defect incorporating vertical bone loss of 3-6 mm on one proximal side of the tooth, with minimal bone loss on the other side (less than 1 mm loss), and a maximum defect width of 4 mm at the crest;
5. Have good oral hygiene -OH Index (O'Leary et al. 1972) should measure less than 25%;
6. Have no allergy to ciprofloxacin; and
7. Be willing to attend the clinic for several appointments.

Exclusion criteria:

1. Require antibiotic premedication;
2. A history of allergy, sensitivity, or any other form of adverse reaction to local anesthetics of the amide type, e.g., epinephrine, ciprofloxacin, or chlorhexidine;
3. A history of specific systemic illness (e.g., liver, renal, cardiovascular, blood dyscrasias, and so on) that would preclude administration of a local anesthetic or vasoconstrictor such as epinephrine;
4. A history of systemic illness that would interfere with normal healing responses (e.g. liver disease, blood dyscrasias, uncontrolled diabetes, and so on);
5. Current systemic medication which interferes with healing responses or microbial colonization;
6. Current systemic medication which contraindicates the use of local anesthetic or epinephrine;
7. Pregnant or lactating females; processes which contraindicate the use of local anesthetics;
8. Received an antimicrobial agent within 60 days prior to screening or therapy appointments; and
9. Acute infections or conditions in the oral cavity requiring immediate or emergent treatment.

Appendix B: Table and Graph

Procedure	Baseline Exam	Surgical Appointment	Week 1	Week 2	Month 1	Month 2	Month 3	Month 4	Month 6	Month 12
Radiograph	X								X	X
Probing	X						X	X	X	X
Plaque Removal			X	X	X	X	X	X	X	X
Plaque Index				X	X	X	X	X	X	X
Gingival Index				X	X	X	X	X	X	X

Table 1. Periodontal measurement parameters

**Schedule for measurement of clinical parameters

	Control	Test	Total
Sex: Female	4	3	7
Male	2	3	5
Age: mean	43.5 ± 13.22	49.8 ± 3.82	46.7 ± 9.85
range	21-59	47-56	21-59
Race: Caucasian	2	4	6
African American	2	0	2
Asian American	2	2	4
Defect: 1-2 walls	2	3	5
2-3 walls	3	1	4
3-walls	1	2	3
Clinical attachment loss	6.64 ± 1.08	6.0 ± 1.71	6.32 ± 1.44
Probing depth	6.43 ± 1.34	5.14 ± 1.29	5.79 ± 1.45
Plaque index	1.07 ± 0.73	0.86 ± 0.53	0.96 ± 0.64
Gingival index	1.36 ± 0.63	1.29 ± 0.47	1.32 ± 0.55

Table 2: Subject distribution
Note: Where applicable, each value represents a mean of the group ± standard deviation

Category	Control:	Test:
Rods:	2.06/cm ± 1.79	4.63/cm ± 3.79
Cocci:	5.01/cm ± 3.52	3.59/cm ± 2.64
Spirochetes:	0	1 patient
Amoebae:	1 patient	0
Fungi:	0	1 patient

Table 3: Darkfield microscopy
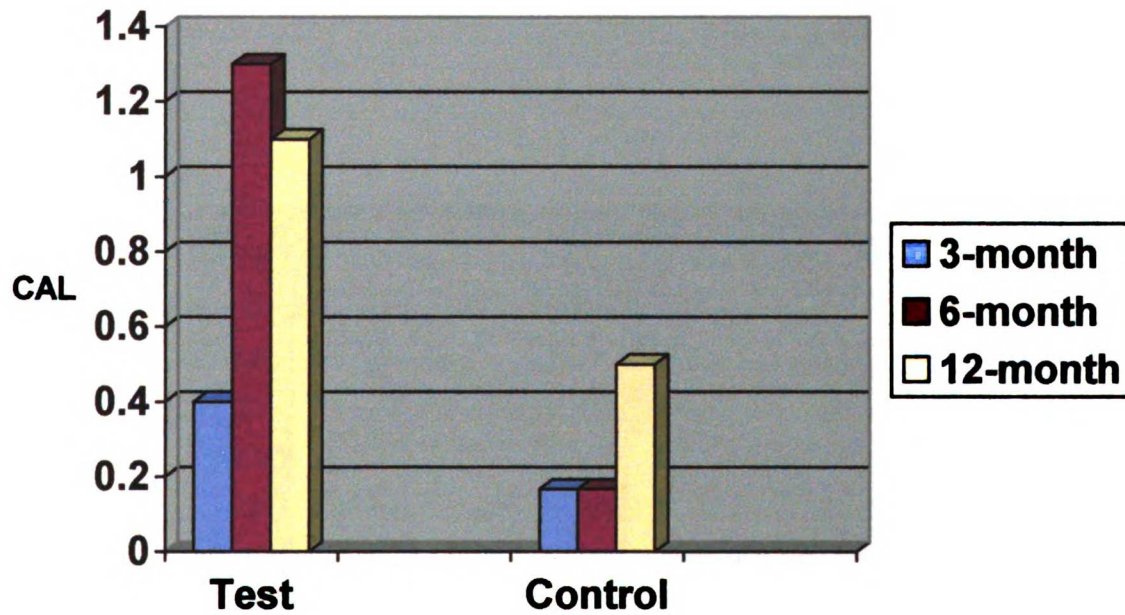Note: Where applicable, each value represents a mean of the group ± standard deviation

BANA	Test	Control
Positive	1	4
Negative	5	2

Table 4: Summary of BANA test results

CAL	Test	Control
3-Month	0.4 ± 0.65	0.167 ± 1.04
6-Month	1.3 ± 0.42	0.167 ± 0.58
12-Month	1.1 ± 0.58	0.5 ± 0

Table 5: Attachment changes in BANA-negative group
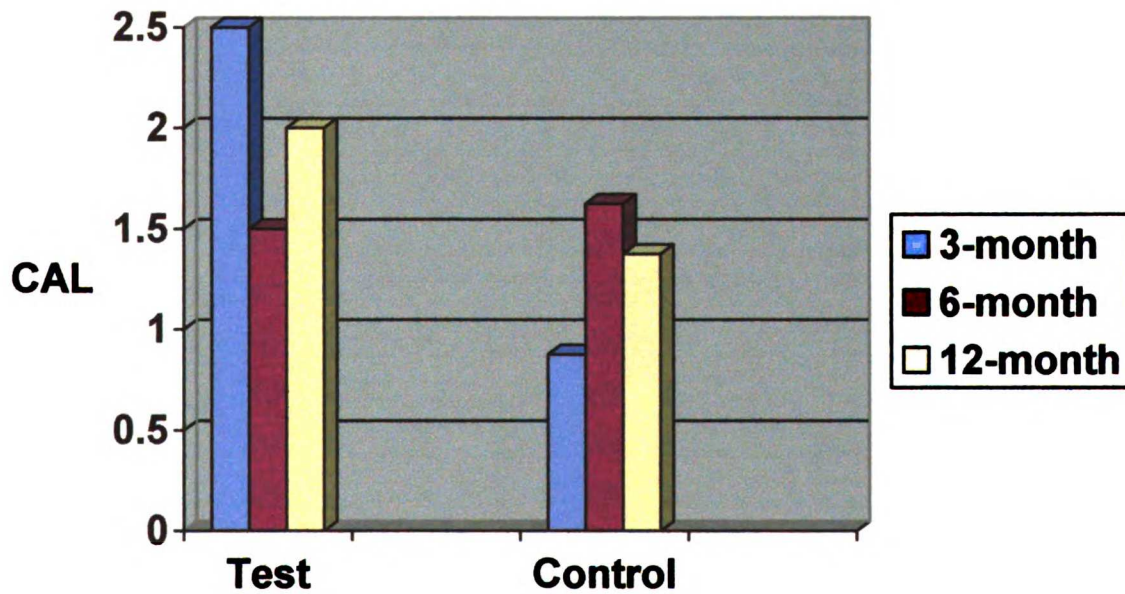Note: each value represents a mean of the group ± standard deviation

Graph 1: Attachment changes in BANA-negative group

CAL	Test	Control
3-Month	2.5	0.875 ± 1.65
6-Month	1.5	1.625 ± 1.38
12-Month	2	1.375 ± 0.95

Table 6: Attachment changes in BANA-positive group
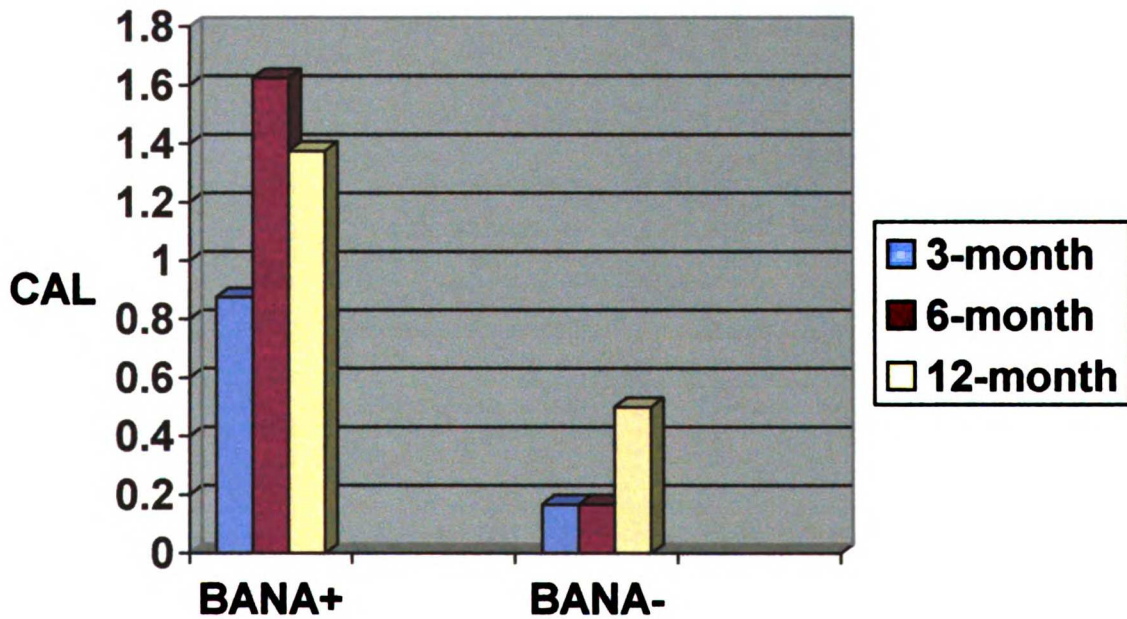Note: Where applicable, each value represents a mean of the group ± standard deviation

Graph 2: Attachment changes in BANA-positive group

CAL	BANA +	BANA -
3-Month	0.875 ± 1.65	0.167 ± 1.04
6-Month	1.625 ± 1.38	0.167 ± 0.58
12-Month	1.375 ± 0.95	0.5 ± 0

Table 7: Attachment changes in control group.
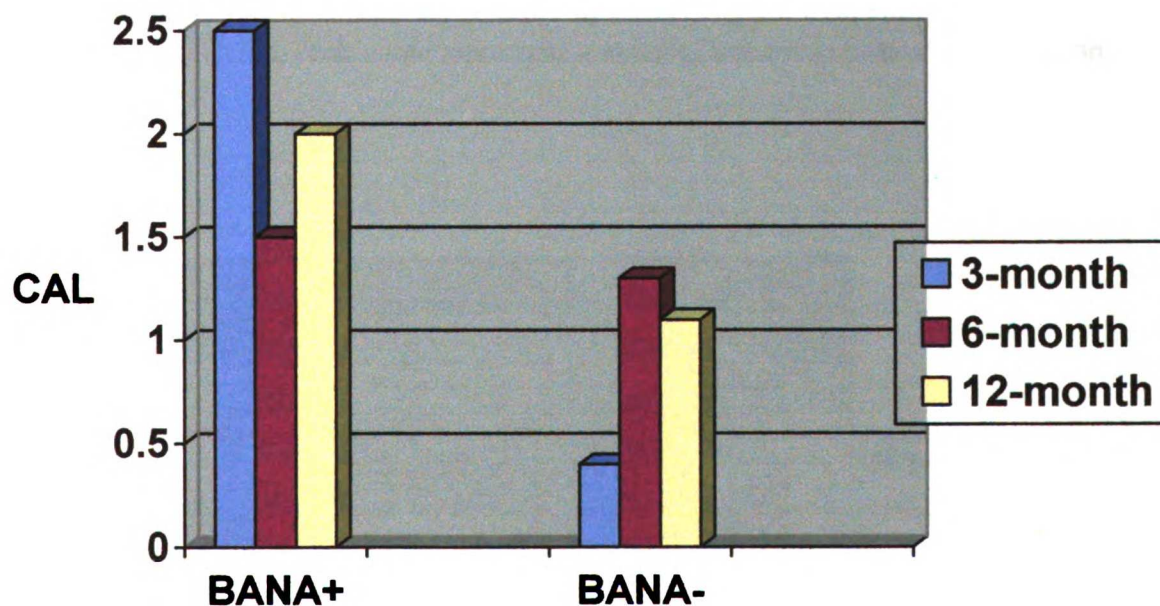Note: each value represents a mean of the group ± standard deviation

Graph 3: Attachment changes in control group

CAL	BANA +	BANA -
3-Month	2.5	0.4 ± 0.65
6-Month	1.5	1.3 ± 0.42
12-Month	2.0	1.1 ± 0.58

Table 8: Attachment changes in Test group
Note: Where applicable, each value represents a mean of the group ± standard deviation

Graph 4: Attachment changes in Test group

| Culture	%	Culture	%
A.a.	0	*P. micros*	4.4 ± 2.99
P.gingivalis	1.7 ± 3.7	Enteric G(-) rods	1.5 ± 4.15
P.intermedia	2.7 ± 3.66	*β-hemolytic Streptococci*	2.2 ± 5.65
B.forsythus	5.1 ± 3.88	Yeast	0
Campylobacter	9.7 ± 7.44	*E. corrodens*	0
Eubacterium	3.5 ± 3.87	*Staphylococcus*	0
Fusobacterium	9.7 ± 3.67	*D. pneumosintes*	0.6 ± 2.08

Table 9: Bacterial Culture Report
Note: Where applicable, each value represents a mean of the group ± standard deviation

Control	Low*	Medium**	High***
3-Month	0.5 ± 1.48	0	1.0 ± 0
6-Month	1.25 ± 1.21	0	-0.5 ± 0
12-Month	1.2 ± 0.91	0	0.5 ± 0

Table 10: CAL vs. Level of *Porphyromonas gingivalis* –Control group
*low: less than 1%; ** medium: between 1-5%; ***high: more than 5%
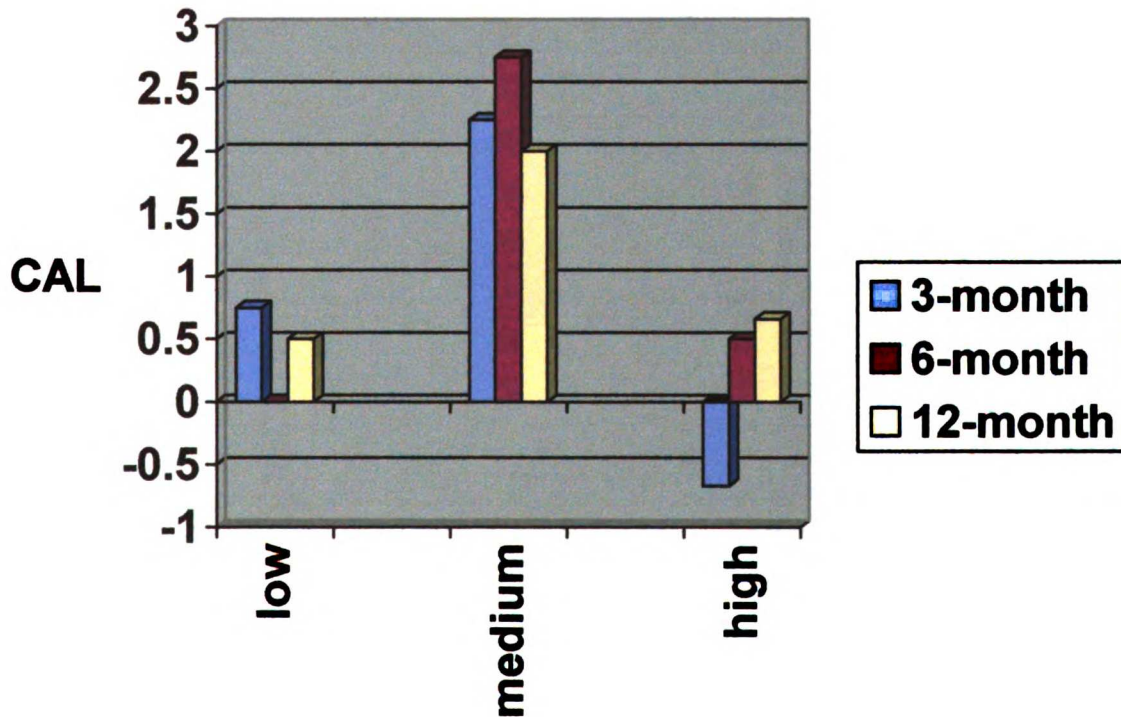Note: Where applicable, each value represents a mean of the group ± standard deviation
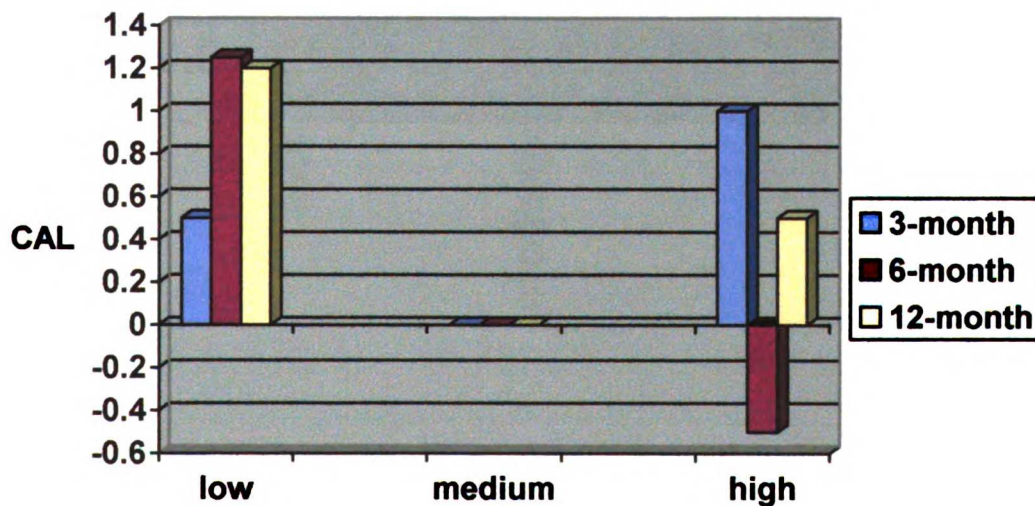
Graph 5: CAL vs. Level of *Porphyromonas gingivalis* –Control group

Control	Low*	Medium**	High***
3-Month	0.75 ± 0.35	2.25 ± 0.35	-0.67 ± 0.58
6-Month	0.0 ± 0.71	2.75 ± 0.35	0.5 ± 0.5
12-Month	0.5	2.0 ± 0	0.66 ± 0.76

Table 11: CAL vs. Level of *Prevotella intermedia* -Control group
*low: less than 1%; ** medium: between 1-5%; ***high: more than 5%
Note: Where applicable, each value represents a mean of the group ± standard deviation

Graph 6: CAL vs. Level of *Prevotella intermedia* -Control group

Control	Low*	Medium**	High***
3-Month	1	-0.25 ± 1.06	0.875 ± 1.65
6-Month	-0.5	0.5 ± 0	1.626 ± 1.38
12-Month	0.5	0.5	1.375 ± 0.95

Table12: CAL vs. Level of *Bacteroides forsythus* –Control group
*low: less than 1%; ** medium: between 1-5%; ***high: more than 5%
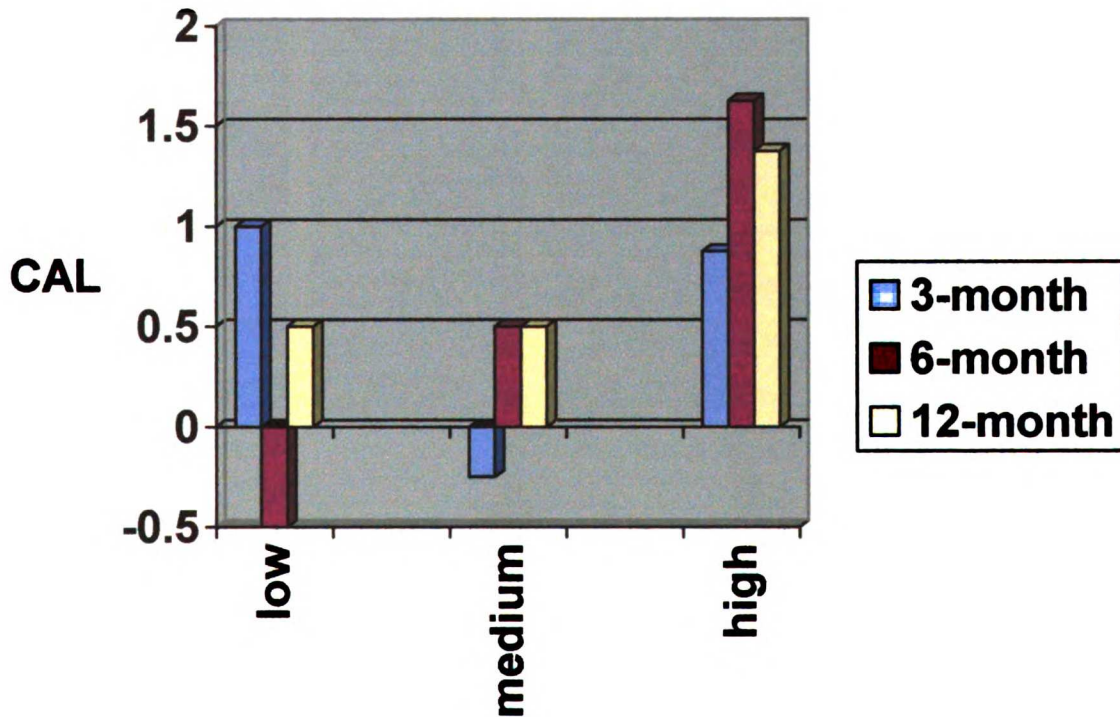Note: Where applicable, each value represents a mean of the group ± standard deviation

Graph 7: CAL vs. Level of *Bacteroides frosythus* –Control group

Control	Low*	Medium**	High***
3-Month	1.0 ± 1.32	1.5	x
6-Month	1.375 ± 0.25	x	x
12-Month	1.5 ± 0.41	1.5	x

Table 13: CAL vs. Level of *Porphyromonas gingivalis* –Test group
*low: less than 1%; ** medium: between 1-5%; ***high: more than 5%
Note: Where applicable, each value represents a mean of the group ± standard deviation
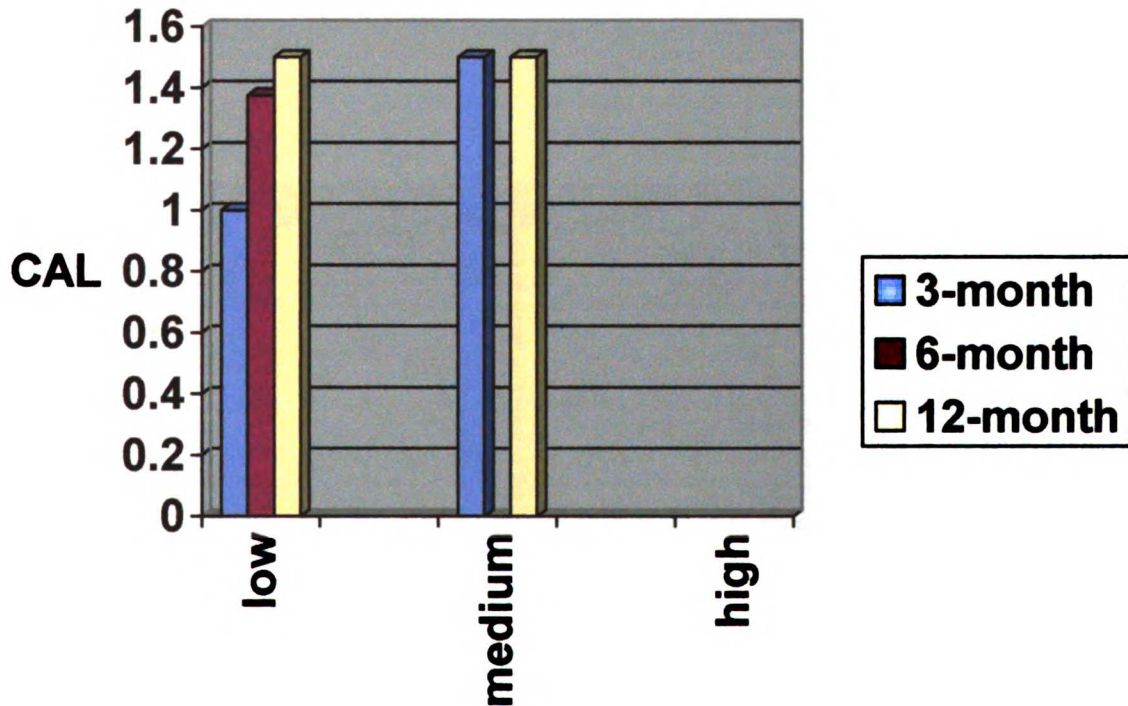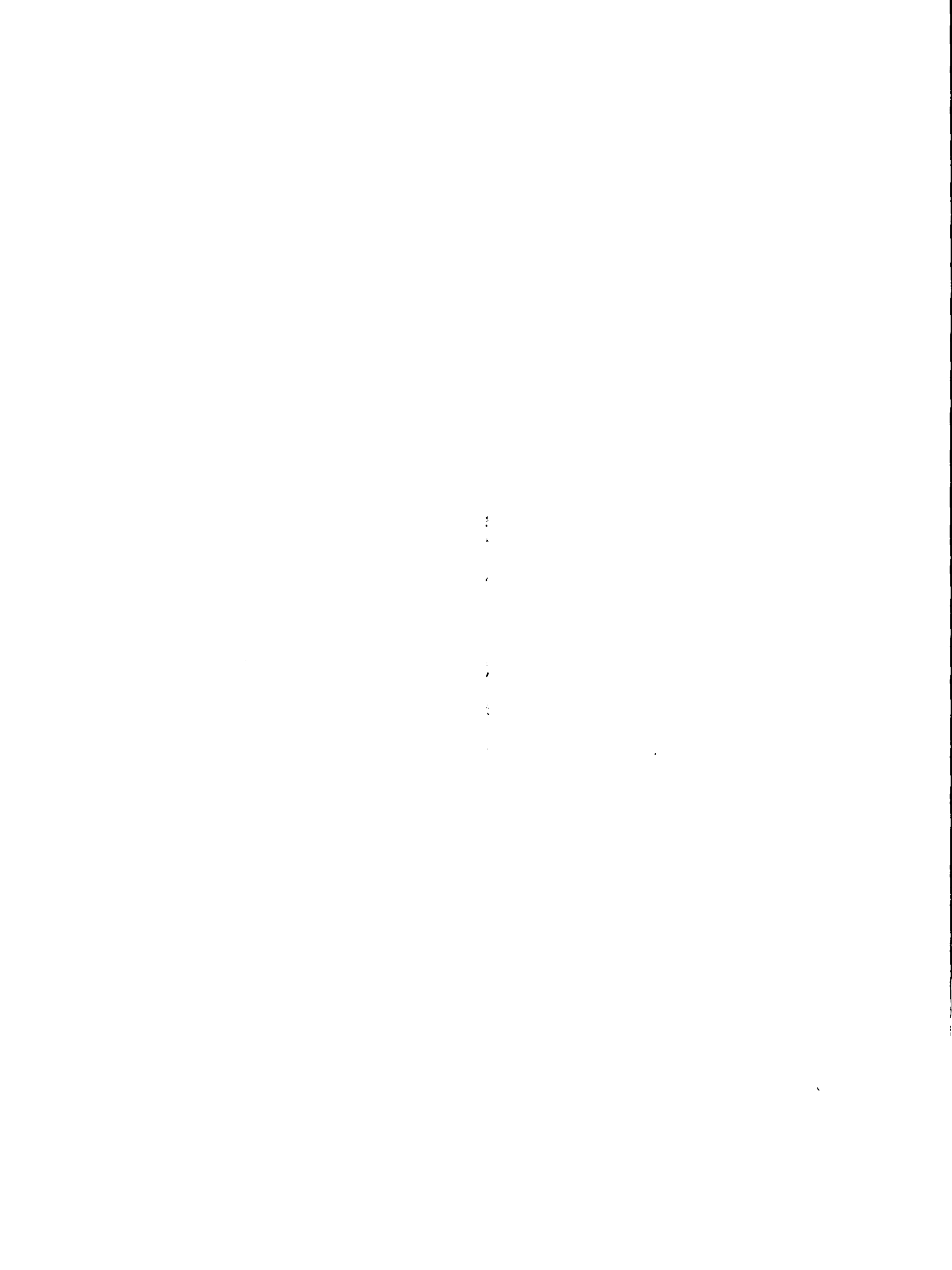

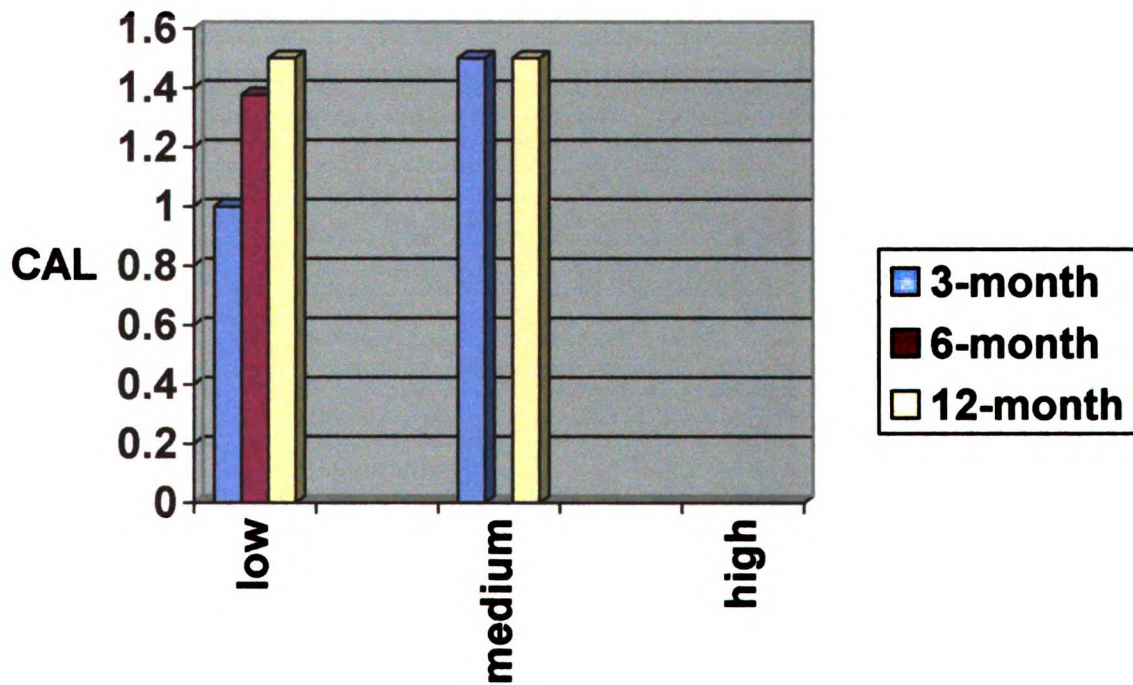Graph 8: CAL vs. Level of *Porphyromonas gingivalis* –Test group

Control	Low*	Medium**	High***
3-Month	1.0 ± 1.32	1.5	x
6-Month	1.375 ± 0.25	x	x
12-Month	1.5 ± 0.25	1.5	x

Table 14: CAL vs. Level of *Prevotella intermedia* –Test group
*low: less than 1%; ** medium: between 1-5%; ***high: more than 5%
Note: Where applicable, each value represents a mean of the group ± standard deviation

Graph 9: CAL vs. Level of *Prevotella intermedia* –Test group

Control	Low*	Medium**	High***
3-Month	0.5	x	1.33 ± 1.25
6-Month	1.25 ± 0.35	x	1.5 ± 0
12-Month	1.25 ± 0.35	x	1.67 ± 0.29

Table 15: CAL vs. Level of *Bacteroides forsythus* –Test group
*low: less than 1%; ** medium: between 1-5%; ***high: more than 5%
Note: Where applicable, each value represents a mean of the group ± standard deviation

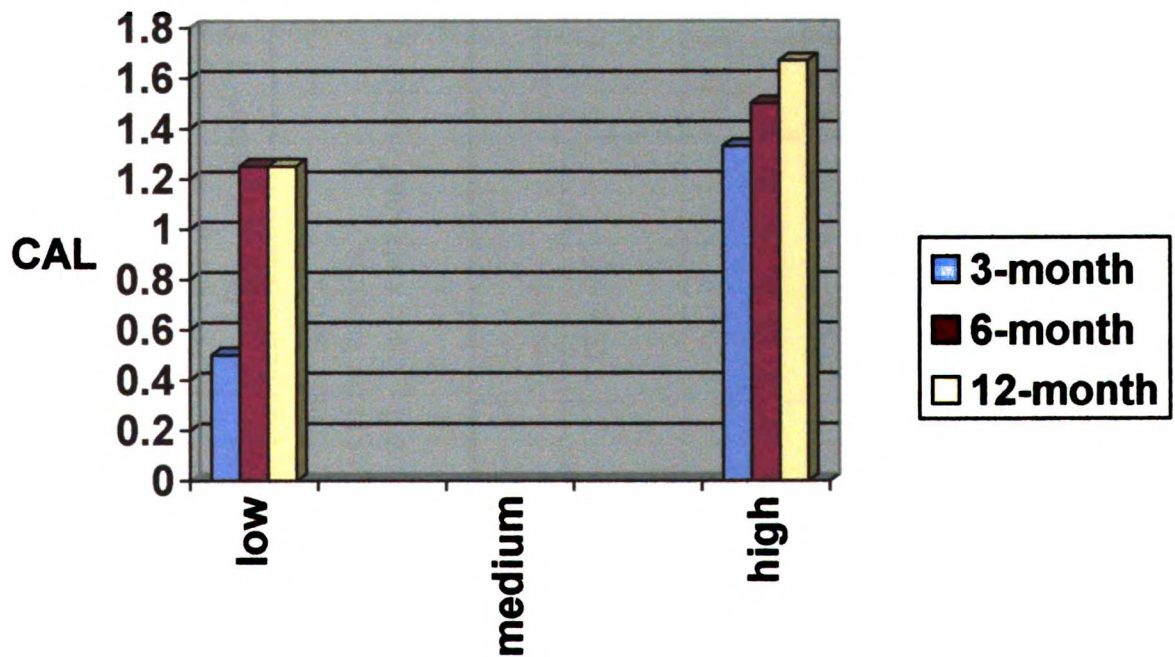Graph 10: CAL vs. Level of *Bacteroides frosythus* –Test group

Table 19a (Control and Test):

Control	Baseline	3-month	6-month	12-month	Test Baseline	3-month	6-month	12-month
	6.43 ± 1.34	5.14 ± 0.95	5.00 ± 1.30	5.00 ± 1.54	5.14 ± 1.29	3.58 ± 1.38	3.25 ± 0.87	3.36 ± 1.22
P value*		P= 0.003	P= 0.004	P= 0.009	P value*	P= 0.003	P= 0.0001	P= 0.0004

Table 19a: Probing depth, compared to baseline

* P value at each time point compares to the baseline

Note: Each value represents a mean of the group ± standard deviation

PD	Baseline	3-month	6-month	12- month
Control	6.43 ± 1.34	5.14 ± 0.95	5.00 ± 1.30	5.00 ± 1.54
Test	5.14 ± 1.29	3.58 ± 1.38	3.25 ± 0.87	3.36 ± 1.22
P value*	P= 0.008	P= 0.001	P= 0.0003	P= 0.003

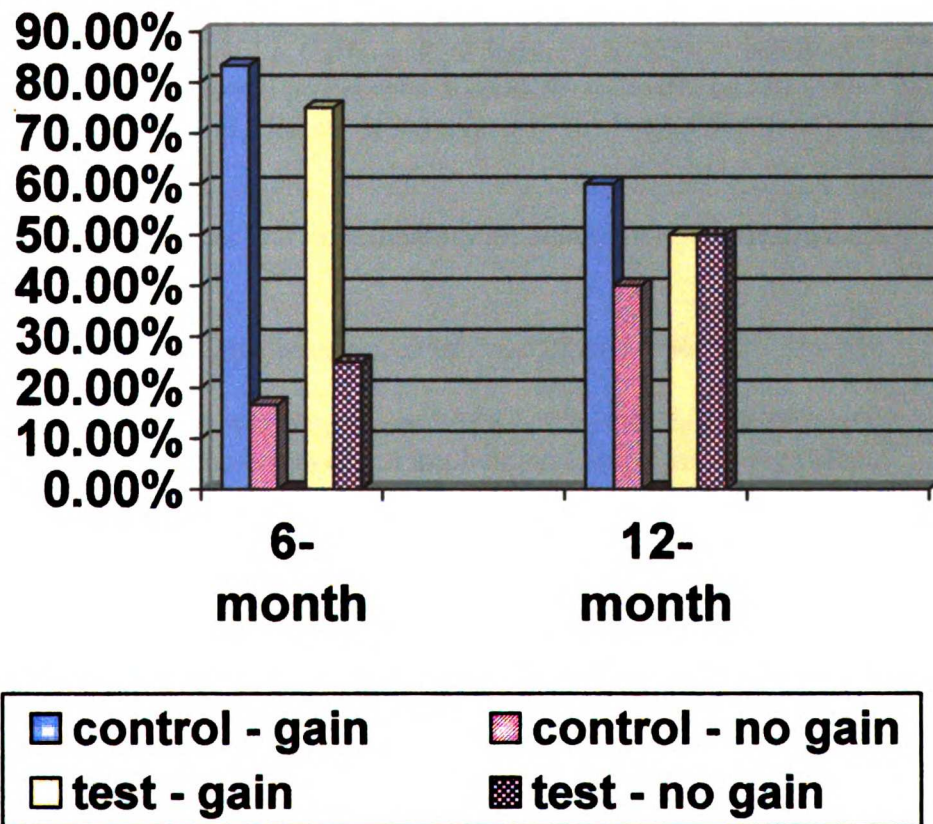Table 19b: Probing depth, between control and test

* P value is the significance between the control and test

Note: Each value represents a mean of the group ± standard deviation

	Control -gain	No gain	Test -gain	No gain	Total
6-Month	5	1	3	1	10
12-Month	3	2	3	3	11

Table 20: Radiographic Results

Graph 11. Radiographic results in percentage of subjects

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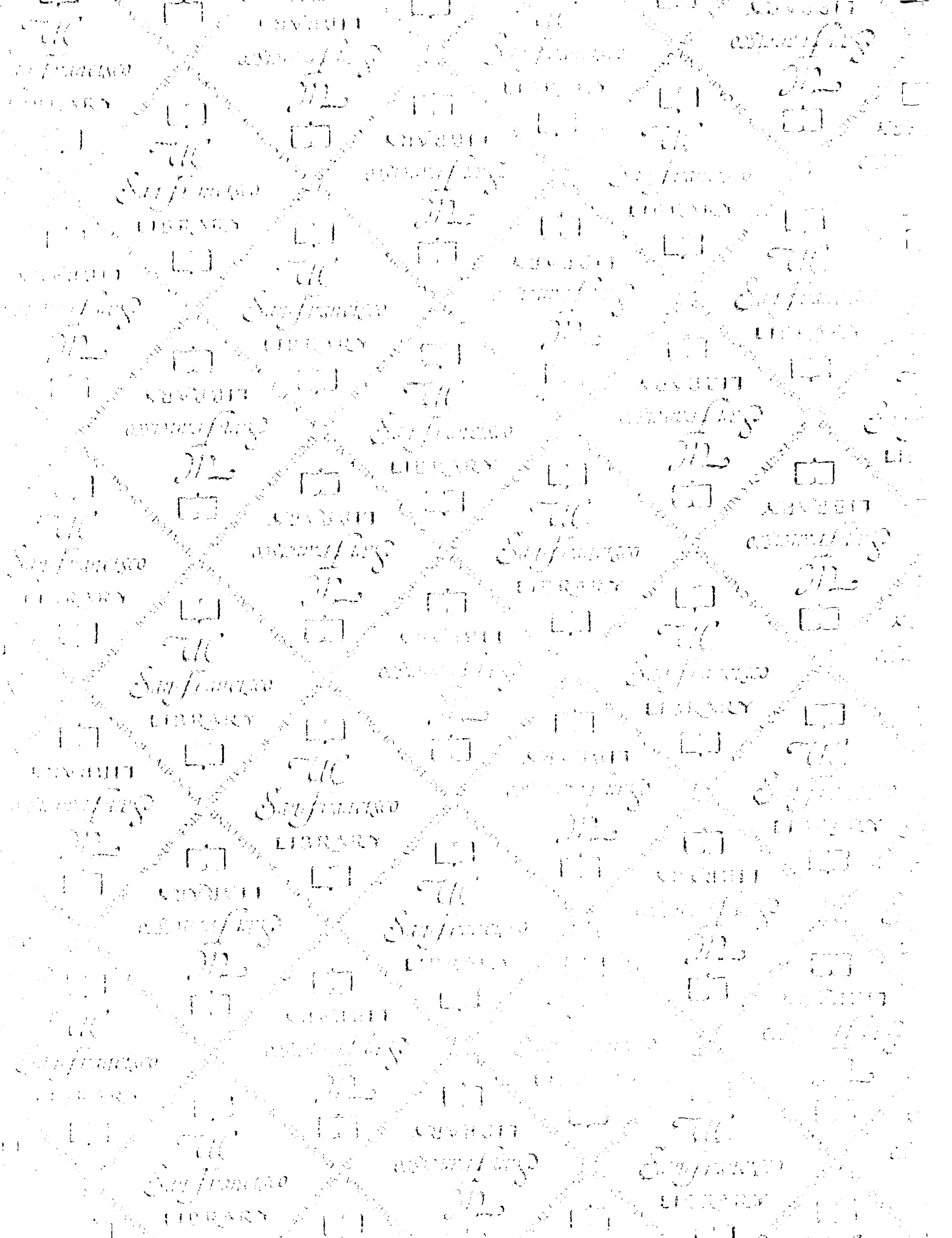Garrett S. Periodontal regeneration around natural teeth. *Ann Periodontol* 1996; 1: 621-666.

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