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Utilization of Fractal Analysis to Assess
The Efficacy of Bisphosphonates in the Adjunctive Treatment of Chronic Periodontitis

by

Susan J. Hsieh

THESIS

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Utilization of Fractal Analysis to Assess the Efficacy of Bisphosphonates in the Adjunctive Treatment of Chronic Periodontitis
By Susan J. Hsieh

Abstract

The purpose of this study was to utilize fractal analysis, clinical assessments, and digital subtraction radiography to assess the efficacy of bisphosphonates (alendronate/risedronate) in the adjunctive treatment of chronic periodontitis. A randomized, double-blind, placebo-controlled clinical trial was performed in 73 subjects with moderate to severe chronic periodontitis to compare the treatment effects of conventional therapy plus bisphosphonate administration with that of conventional therapy plus placebo. Periodontal assessments of bleeding on probing (BOP), probing depth (PD), and clinical attachment level (CAL) were performed and vertical bitewing radiographs taken at baseline, 6-month, and 12-month timepoints. Fractal analysis was performed on the bitewing radiographs as a quantitative measure of trabecular bone patterning. Assessments of bone height and mass were performed at 12 months using digital subtraction radiography. BOP, PD and CAL all showed improvements over time in both groups, but a statistically significant greater improvement in CAL was found in the bisphosphonate group compared to the placebo. Bone mass and height did not show a statistically significant difference between the two groups. Change in fractal dimension also was not significantly different between the two groups, however, it was found to be significantly correlated to PD, CAL, and bone mass. Therefore, bisphosphonates administered in conjunction with conventional periodontal therapy did not appear to increase periodontal bone mass significantly more than conventional periodontal therapy alone, and this result was reflected in the fractal dimension.

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I. Introduction

Chronic periodontitis is an inflammatory disease of bacterial etiology that results in loss of alveolar bone and connective tissue attachment. Clinical signs include decreased alveolar bone height, increased tooth mobility, and eventual tooth loss.[1] Reports of prevalence rates in adults vary among epidemiological studies. According to the National Health and Nutrition Examination Survey I in the early 1970's, 20% of adults ages 18-80 had gingivitis and 34% had periodontitis (a total of 55% with disease). A National Institute of Dental and Craniofacial Research (NIDCR) study in 1985 cited an even higher prevalence: 44% of adults had gingivitis, and 14% had periodontitis (a total of 58% with disease). Risk factors for periodontitis that have previously been identified include: osteoporosis in women, cigarette smoking, host-response genetic factors, diseases such as diabetes and immunosuppressive disorders, and poor access to dental care.[1]

Pathogenesis of Periodontitis

Alveolar bone loss, as occurs in periodontitis, results from the alteration in the normal balance of bone formation and resorption. Regulatory mechanisms that couple bone turnover are disrupted in favor of bone resorption. Oral bacteria initiate the cascade of events that lead to alveolar bone loss by stimulating the increased production of cytokines by local immune cells, gingival tissues, and fibroblasts.[2] Cytokines (IL-1β, IL-6, TNF-α) have been identified at higher levels in sites with periodontitis than in healthy sites.[3] Cytokines upregulate the activity of osteoclasts, resulting in increased degradation of the extracellular matrix and periodontal bone. The specific mechanisms of how

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cytokines stimulate bone resorption are now just beginning to be understood. These involve highly regulated cell-cell interactions and extracellular matrix signaling molecules that lead to increasing osteoclast activity and increased bone loss. Given the etiology of periodontitis, treatment is typically directed at reducing the tooth-borne bacterial biofilms that cause the disease thereby resulting in decreased inflammation and preventing additional loss of alveolar bone.

Clinical Diagnosis of Periodontal Disease

Traditional diagnostic procedures are essentially comprised of clinical assessments of inflammation and damage to periodontal tissues. The strengths of the traditional diagnostic procedures are that they are easy to use and cost-effective. In addition, the findings are directly related to pathologic processes associated with periodontal infections, and the absence of clinical signs of disease is strongly related to the presence of a stable, healthy periodontium. However, clinical signs alone do not provide useful information regarding the severity, morbidity, or eventual outcome of periodontal infections, and cannot distinguish between nondestructive forms (gingivitis) and destructive forms (periodontitis). Additionally, physical assessments of damage to periodontal tissues, such as radiographic detection of bone loss, can only measure past episodes of destructive disease.[4]

Assessments of inflammation include observation of the amount of gingival redness, suppuration (exudate or pus), bleeding on probing (BOP), elevated gingival temperature, and examination of the components of gingival crevicular fluid. Assessment of damage to periodontal tissues includes probing depth (PD), clinical attachment level

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(CAL), and gingival recession. Probing depths measure the distance from the gingival margin to the base of the probeable crevice. Deep pockets are a major habitat for putative periodontal pathogens, and are sites potentially difficult for the patient to clean. The CAL is the distance from the cementoenamel junction (CEJ) to the base of the probeable crevice. This represents a clinical approximation of the amount of loss of connective tissue attachment from the root surface and is considered the "gold standard" of clinical outcome assessment [4].

Unfortunately, traditional clinical markers are not precise enough to detect small amounts of periodontal damage, so biochemical markers in the gingival crevicular fluid (GCF) have become a vital area of interest. Current research is focused on seeking markers which are present in periodontitis and/or gingivitis, and which can be related to the progression of periodontitis. Three general categories of markers in GCF are inflammatory mediators and products (e.g. PGE_2, cytokines, antibacterial antibodies, autoantibodies), host-derived enzymes (e.g. aspartate aminotransferase, alkaline phosphatase, elastase), and tissue-breakdown products (e.g. glycosaminoglycans, pyrridinoline cross-links, firbonectins) [4].

Radiographic Diagnosis of Periodontitis

Dental radiographs are the traditional means of assessing amount of bone, however this gives a limited two-dimensional view of an actual three-dimensional structure. Radiographs also inherently have a low level of sensitivity. Longitudinal radiographic experiments conducted on dry skulls found that the unaided eye is only able to detect radiographic changes when approximately 50% of the bone mineral has been

lost.[5] Thus, what is visible on the X-ray is actually an underestimation of the amount of bone lost.[4] Additionally, changes in x-ray angulations and exposure time can affect images.

Digital Subtraction Radiography

Digital subtraction radiography (DSR), takes serial digitized X-rays from different timepoints, superimposes them, and a composite image is generated.[6] Changes in the density and/or volume of bone can be detected as lighter areas (bone gain) or dark areas (bone loss). Previous studies have shown a good correlation between changes in alveolar bone determined by subtraction radiography and CAL changes in periodontal patients after therapy. Hausmann et al. (1992) detected differences in crestal bone height of 0.87 mm with good reliability.[7] Jeffcoat et al. (1992) showed a strong relationship between loss of clinical attachment and bone detected with digital subtraction radiography.[8]

Fractal Analysis

Recently, there has been much attention focused on the application of fractal techniques in the medical sciences. Theoretical biologists have found fractal organizations in many structures throughout the body. Not surprisingly, it has been found that trabecular patterns of alveolar bone have a fractal nature that can be defined by their fractal dimensions. Thus, fractal analysis may be a sensitive descriptor of bone structure and may provide a diagnostic tool to objectively characterize trabecular bone structure, and ultimately to discriminate between normal and diseased subjects.

Several studies have previously been undertaken to examine the fractal dimension in bone. Many showed results that underline the potential of fractal analysis as a diagnostic tool. Ruttimann et al. (1992) investigated the utility of fractal dimension in the characterization of structural changes in alveolar bone.[9] Ten dry mandibular bone segments were radiographed from controlled projection angles before and after acid-induced partial decalcification. Fractal dimension (computed by regression analysis of power spectra by Fourier transform) of selected regions of interest increased after acid-induced demineralization, irrespective of changes in radiographic projection angles. In the second portion of the study, *in vivo* fractal dimension was computed from randomly selected intraoral radiographs of six premenopausal and six postmenopausal women. A significantly higher fractal dimension was observed in the older postmenopausal group as compared to the younger group.

Along a similar vein, Southard et al. (1996) performed an *in vitro* study to examine radiographic fractal dimension changes in alveolar process bone during simulated osteoporosis.[10] Specimens of maxillary alveolar process bone were progressively decalcified, and radiographs of the specimens were digitized. The relationship between calcium loss and fractal dimension change was quantified. A strong correlation was found between generalized demineralization and decreasing fractal dimension. In every bone sample, fractal dimension changed significantly with angular change. The conclusion of the study was that fractal dimension does hold potential for detecting simulated osteoporosis in the maxilla under certain conditions, but the fractal analysis was found to be sensitive to small x-ray beam angular changes, contrary to the findings of Ruttiman et al. (1992)

In 1998, Shrout et al., using non-standardized clinical radiographs of mandibular alveolar bone, analyzed the fractal dimension in two groups of patients: one group had healthy gingiva and/or gingivitis and the other group had periodontitis[11]. They found that the fractal dimension in the two groups were significantly different. They concluded that fractal dimensions could be used to distinguish between gingivitis and periodontitis patient groups, and fractal dimensions could be calculated from non-standardized clinical radiographs.

A master's thesis by Khosrovi in 1995 examined the fractal dimension of bone in wrist radiographs of osteoporotic patients, and found that the fractal dimension was able to discriminate between healthy and osteoporotic patients [12]. The second portion of the study involved two separate groups of healthy and periodontally compromised subjects. First, it was determined that fractal dimension of trabecular bone structure showed a difference between the two groups. Second, fractal dimension of trabecular bone varied significantly between the anterior and posterior regions of the jaw. And finally, fractal dimension varied significantly between the maxilla and the mandible.

In a study performed by Majumdar et al. (1999), cubic specimens of human trabecular bone were obtained from the vertebrae and femur [13]. Three different fractal techniques were used to measure the fractal dimension. Statistically significant correlations were found between bone mineral density (as assessed by quanititative computed tomography), strength of the bone, and the fractal dimension, however these were direction and technique dependent. Using the power spectral method, the fractal dimension increased with bone mineral density when computed over a lower range of spatial frequencies. On the other hand, it decreased in the higher range of spatial

frequencies. For the surface area technique, the fractal dimension increased with increasing bone mineral density. Thus, fractal-based texture analysis of radiographs was found to be technique dependent, but the authors concluded that the fractal analysis might be useful in the quantification of bony trabecular structure.

Finally, a master's thesis by Lee (2001) described a longitudinal study on two groups of patients: periodontally healthy subjects and patients with untreated periodontitis.[14] Standardized vertical bitewing radiographs were obtained at baseline and after six months. Clinical changes were compared to the changes in fractal dimension. Digital subtraction radiography was used to characterize the boss loss (either positive change, negative change or no change). The baseline results revealed that fractal dimensions were significantly different between the healthy and diseased groups of patients. In the diseased group of patients, fractal dimension was significantly associated with radiographic bone loss as assessed by digital subtraction radiography.

In conclusion, several studies have previously demonstrated fractal analysis to be an indicator of bone changes, both *in vitro* and *in vivo*. Variations in results between studies may be due to differences in the areas of bone surveyed, method of image acquisition, spatial resolution of the image and specific fractal analysis technique chosen. However, further studies are necessary to define clearly the relationship between a given technique and its correlation with clinical assessments of periodontal disease.

Treatment of Periodontal Disease

Successful management of periodontal disease is focused on elimination of etiologic factors. Therefore, much emphasis has been placed on using methods that reduce and eliminate pathogenic bacteria that colonize the dentogingival interface.

Clinically, removal of bacteria and the concomitant reduction in inflammation are accomplished by mechanical treatment approaches, including scaling and root planing (SRP), oral hygiene measures, and surgical intervention.[1,15] Systemic and topical antimicrobial medications are also used with the intention of suppressing the bacteria that cause periodontitis.[16-18] In chronic periodontitis, a good clinical response usually occurs in response to SRP and maintenance care, exhibited by a reduction in gingival inflammation, reduction in PD, and modest gains in CAL. However, even when periodontitis is controlled in patients, little or no bone previously lost is regained through these conventional therapies. Therefore, newer approaches are being developed in hopes of stimulating regeneration of lost alveolar bone.

Bisphosphonates

A class of drugs known as bisphosphonates have shown potential in stimulating new bone formation in the periodontium, around teeth, and in surgically created osseous defects.[19] Bisphosphonates are structurally similar to pyrophosphate, a normal product of human metabolism present in serum and urine that has calcium-chelating properties.[20] Several mechanisms of action have been proposed, including bisphosphonate-mediated induction of osteoclastic apoptosis, [21] reduction of osteoclastic activity, [22] prevention of the development of osteoclasts from hematopoietic precursors,[23] and stimulation of production of an osteoclast inhibitory factor.[24] However, the main effect of the bisphosphonates is to reduce osteoclast activity and preventing formation of new resorption pits.

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Clinically, bisphosphonates have been shown to be effective in the prevention and treatment of osteoporosis. Etidronate and alendronate have been shown to increase bone mass and reduce fracture rates at the spine, hip, and other osseous sites in postmenopausal women.[25-27] Likewise, bisphosphonates may be potentially useful in the management of periodontal bone loss. This has been previously studied in animal models and more recently in clinical trials with encouraging findings. In a study in which experimental periodontal defects were created in monkeys, it was demonstrated that the bisphosphonate alendronate could retard bone loss around affected teeth compared to controls.[28] It was an interesting finding that although the bone loss was reduced with alendronate, the probing depths did not decrease. This finding suggests that although bone loss may be inhibited, the effects of bisphosphonate treatment may not be detected by clinical assessments of periodontal damage alone. Since this study utilized experimentally induced periodontitis in the monkey, the question remained whether the same effect could be found in naturally occurring periodontitis. This was examined in another study in the beagle dog, an animal that naturally develops periodontitis. The results of this study found similar results, although the bone loss inhibition was not as great in magnitude as the previously mentioned study. Again, there were no differences in signs of inflammation or pocketing, but there were still increases in bone mineral density.[29] These studies taken together are evidence that there could be a potentially important role for bisphosphonates in the management of periodontitis. Thus, a longitudinal prospective clinical study to determine if bisphosphonates can prevent or modestly increase alveolar bone mass in human periodontitis is warranted.

II. Hypothesis

First, a change in the fractal dimension of interproximal alveolar bone is associated with changes in clinical assesments of periodontal status (clinical attachment level, bleeding on probing, probing depth) and change in bone mass and bone height as assessed by digital subtraction radiography. Secondly, using change in fractal dimension as a diagnostic tool, bisphosphonates can be shown to be an effective adjunctive treatment in increasing alveolar bone mass in patients with moderate to severe periodontitis.

III. Specific Aims

1. To determine the correlation between clinical measurements of periodontal status (bleeding on probing, probing depth, clinical attachment level) and fractal dimension.

2. To determine the correlation between digital subtraction radiography and fractal dimension.

3. To use fractal analysis in conjunction with clinical periodontal assessments and digital subtraction radiography to understand the relative changes in bone and soft tissue in patients undergoing bisphosphonate therapy.

4. To determine if administration of bisphosphonates in conjunction with conventional periodontal treatment can significantly improve bone formation in individuals with moderate to severe chronic periodontitis compared with similar individuals who are treated with conventional periodontal therapy and a placebo.

IV.   Materials and Methods

A double-blind, randomized, placebo-controlled clinical trial was carried out to examine the effects of comparing the clinical outcomes of two groups of subjects: those taking bisphosphonate (alendronate/risedronate) and those taking a placebo.

Subject Recruitment

Subjects were recruited from patients being seen in the Periodontal Specialty Clinic of the University of California San Francisco Division of Periodontology. Patients with moderate and severe chronic periodontitis were included in the study under the following criteria:

- Moderate Periodontitis: Mean clinical attachment level (CAL) $\geq$ 1.4-2.4 mm or having $\geq$ 8 sites with CAL $\geq$ 3 mm distributed through at least 3 quadrants or at least 6 teeth (not counting straight buccal and lingual surfaces, and distal surfaces of the second molars).

- Severe Periodontitis: Mean CAL $\geq$ 2.5 mm with one or more sites in 3 out of 4 quadrants having CAL measurements of $\geq$ 5 mm.

In order to be included, participants must have agreed to have their periodontal disease treated by the attending periodontist, and return for maintenance visits every three months. In addition, participants must not have any physical conditions that would prevent them from receiving the proposed treatment regimens or from completing the study. The patient must be ambulatory and able to return to the site of the investigation at the specified times and study intervals. Finally, the patient must be willing to participate in the proposed study as indicated by signing an informed consent form as approved by

the UCSF Committee on Human Research. Out of the 203 patients who were screened, 130 did not meet the criteria and were excluded from the study. The remaining 73 subjects were randomized into the bisphosphonate and placebo groups.

Exclusion criteria:

1. Generalized disease of the bone other than that from chronic periodontitis or menopause in women including hyperparathyroidism, hypoparathyroidism, and Paget's disease of bone.
2. Diseases that may affect bone metabolism: alcoholism, inflammatory bowel disease, malabsorption, symptomatic peptic ulcer disease, hyperthyroidism, renal impairment (creatinine > 2.0 mg/dl) or hepatic impairment (SGOT levels greater than two times the upper limit of normal).
3. Chronic treatment with anabolic steroids, anticonvulsants, anticoagulants, pharmacological doses of vitamin A or D supplements within 1 year prior to the study.
4. History of drug abuse.
5. Previous use of bisphosphonates within one year prior to the study.
6. History of gastrointestinal intolerance to bisphosphonates.
7. Calcitonin treatment continuously or intermittently within 6 months prior to the start of the study or previous fluoride treatment for more than one month.
8. History of unstable cardiovascular disease or uncontrolled hypertension.
9. Senile dementia, paraplegia or quadriplegia.

Assignment of treatment groups and response to treatment

Study subjects were randomly assigned by two-to-one randomization to one of the following treatment groups:

(1) Bisphosphonate (10 mg/day of alendronate or 5 mg/day of risedronate), oral calcium (1000 mg of elemental calcium as calcium citrate), and 400 I.U. Vitamin

D3. The first half of the consecutively recruited subjects were administered alendronate, and the later half were given risendronate. Both are second generation aminobisphosphonates and have been shown to have equal efficacy.

(2) Placebo tablet, oral calcium (1000 mg of elemental calcium as calcium citrate) and 400 I.U. Vitamin D3 a day.

In the bisphosphonate group, the first half of consecutively treated patients were administered risedronate 5 mg/day and the second half of consecutively treated patients were administered alendronate 10 mg/day. Both are second generation aminobisphosphonates and have equal efficacy. Treatment with bisphosphonate or placebo continued for a 24-month period. Patients were recalled at 6, 12, and 24 months to obtain longitudinal clinical and radiographic measurements as follows:

Baseline evaluation:
1. Periodontal assessments (Plaque Index, BOP, PD, CAL)
2. Full-mouth series of radiographs with vertical bitewings under standardized conditions
3. Scaling and root planing (SRP)

Study medications were given at the baseline visit.

Recall visits at 3-month intervals for two years:
1. Periodontal assessments
2. Scaling and root planing

At 6-, 12-, and 24-month visits,
1. Vertical bitewings taken under standardized conditions

Physical Examination and Monitoring of Medical Status

At the baseline visit and specific recall visits, physical examination and blood testing was performed. Lab work was performed at baseline, 6-, 12-,18-, and 24-month follow-up visits and consisted of a complete blood count including platelet count, serum creatinine, serum aspartate aminotransferase, and fasting blood glucose in the two patients with diabetes mellitus, urine dipstick test using Multistix Reagent strips (Bayer Corp, IN) and pregnancy test for women of childbearing age. Urine samples were collected for deoxypyridinoline crosslinks (DpD/Cr). DpD is excreted unmetabolized in urine and is unaffected by diet, making it suitable for assessing bone resorption. DpD/Cr determination was performed at baseline, 6-, 12-, 18-, 24-month follow-up visits using the METRA Creatinine Assay Kit (Quidel Corp, San Diego, Ca).

For polymorphic IL-1 genotyping, 4 drops of blood were placed on a DNA-free amplicard. These were then coded and sent to the commercial lab of Interleukin Genetics, (Sheffield, England) for IL-1 genotype analyis. Gingival crevicular fluid was collected from periodontal pockets at 3 different sites using a 15x3 mm Periopaper Gingival Fluid Collection Strips (Pro Flow Inc, New York) at baseline, 12-month and 24-month follow-up visits. GCF volume was measured with a precalibrated Periotron 6000. The levels IL-1β, IL-6, and TNF-α in the GCF were measured with ELISA.

Assessment of Compliance

During each visit, i.e. at every 3-month recall, the following information was recorded in the case report forms: (1) intake of concomitant medications, dosage, start and end date of intake, and their indications for use, (2) adverse drug events (onset,

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symptoms, severity, action taken regarding the study drug [none, reduced, interrupted or discontinued], relation between study drug and adverse event, (3) number of study pills and Citracal+D caplets returned. At baseline, 12- and 24-month visits, a Calcium Intake Questionnaire was filled out and used to calculate the amount of calcium consumed by the patient per day. To measure urine bone markers, DPD/Cr, urine samples were collected.

Clinical Periodontal Examination

A full-mouth periodontal examination was conducted on six surfaces of each tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual) (See Appendix for clinical periodontal assessment form). Clinical measurements obtained included Plaque Index (PlI) [30], bleeding on probing (BOP) measured as 0 for no bleeding, and 1 for bleeding, probing depth (PD) measured in millimeters, and clinical attachment level (CAL) measured in millimeters. One calibrated examiner performed all examinations using a North Carolina periodontal probe (Hu-Friedy Mfg., Inc., Chicago). Intra-examiner variability was assessed by repeating full-mouth clinical measurements on a subset of patients. For all clinical assessments the examiner kappa statistic was ≥ 0.85.[31]

Periodontal Treatment

All enrolled patients received conventional nonsurgical periodontal therapy for chronic periodontitis. Therapy consisted of full-mouth scaling and root planing (SRP), plaque control instructions, and periodontal maintenance/recall visits at 3-month intervals. SRP was performed with both ultrasonic and hand instrumentation at the

discretion of the therapist. No time limit was placed on the SRP treatment; SRP was considered complete when the therapist considered the tooth surfaces to be clinically free of supragingival and subgingival calculus. At the maintenance/recall visits plaque control instructions were reinforced and all supragingival and subgingival surfaces were cleaned.

Fractal Analysis

A Fourier transform based fractal analysis technique was used to assess the texture of the trabecular bone. The vertical bitewings were scanned and digitized (8-bit gray scale, 1200 dpi) using the Epson Expression scanner (1600 model EU-35). The operator was blinded while selecting qualifying interproximal sites and performing the fractal analysis. In each patient, a minimum of one interproximal site and a maximum of eight interproximal sites were selected. A total of 176 interproximal sites were selected in all 73 patients, ranging from one to eight sites per patient depending on the inclusion and exclusion criteria as follows:

Inclusion criteria for sites chosen for fractal analysis:
- Interproximal sites of posterior teeth clearly visible on sequentially taken bitewing radiographs.
- Interproximal sites with two adjacent teeth present.

Exclusion criteria for sites chosen for fractal analysis:
- Root proximity of less than 2 mm measured at the crest of the alveolar bone.
- Inability to clearly define a region of interest.
- New interproximal restorations extending beyond the CEJ

- Unreadable films due to technical problems (e.g., blurred images due to patient movement).

The region of interest was defined as a circle with a radius limited by the cortical border between the roots of two teeth and the alveolar crest. The two-dimensional power spectrum of the region of interest was performed using the Fourier Transform method on the Sun Workstation developed by Research Systems, Inc. The two-dimensional power spectrum of each region of interest was quantified by averaging over all values at a given distance from the origin. Data were decomposed into a one-dimensional graph with the x-axis showing the radial frequency offset from the origin and the y-axis the average power at that spatial frequency. Fractal dimension is proportional to the slope of the linear portion of the logarithmic plot of the power spectrum versus spatial frequency.

The slopes of the frequency ranges of 0.5-0.99 and 1.0-1.49 were obtained and treated as separate fractal dimension values. In this paper, the fractal dimension measured in the frequency range of 0.5-0.99 will be referred to as the low-frequency fractal dimension (FDL), and the fractal dimension measured in the frequency range of 1-1.49 will be referred to as the high-frequency fractal dimension (FDH).

Calibration of fractal analysis technique was performed with another experienced examiner using ten sites in different patients taken from the baseline timepoint. To assess intra-examiner reliability, the same ten sites were measured again, one week later and the fractal dimension calculated. Inter-examiner and intra-examiner reliability was calculated using Lin's concordance coefficient and were found to be 0.729 and 0.822, respectively.

Digital Subtraction Radiography

One independent blinded examiner performed all digital subtraction measurements. The vertical bitewing radiographs taken at 0 months and 12 months were placed under a video camera and digitized with 512 x 480 pixels of spatial resolution and 8 bits (256 gray levels) of color resolution. Pairs of 0-month and 12-month timepoint radiographs were subtracted following correction for contrast and planar geometric discrepancies. The resultant subtraction image showed areas of bone loss (dark areas) and bone gain (light areas) against a neutral gray background and also contained a negative image of an aluminum reference wedge. The purpose of the wedge was to provide a reference for the calculation of amount of bone loss or gain.

The subtraction image was then isolated and changes in bone mass calculated using a morphologically aided technique that also removed background noise from the image. Statistical analysis of the subtraction image revealed the area of change (in mm^2) and the mean gray level. To convert the gray levels to lesion mass, the reference wedge was used. The thickness of the wedge that corresponded to the change in gray level observed in the lesion was determined. The mass of the lesion (relative) was calculated by multiplying area x thickness x aluminum density x aluminum-to-bone density conversion factor [32,33].

Statistical Analysis

The mean and standard deviation of clinical variables and fractal dimensions were calculated at each timepoint for the placebo and bisphosphonate groups. Longitudinal change in the clinical variables and fractal dimension was examined several ways. First,

mean change of clinical variables and fractal dimensions was calculated for three time periods: 0-6 months, 6-12 months, and 0-12 months and tested for significance using a Wilcoxon non-parametric test. Since this consisted of repeated measurements taken on the same data, a Bonferroni correction was used and p-value of less than 0.01 was treated as the standard of significance.

Next, the changes in clinical variables, fractal dimensions, and DSR data were compared between bisphosphonate and placebo groups using a mixed models analysis to correct for intra-patient and intra-tooth correlations. In this analysis, and in the subsequent analyses of correlation between fractal dimension and clinical variables, the sample size used reflected individual changes in the tooth surface as opposed to one interproximal site. That is, one interproximal site where a fractal dimension was obtained also corresponded to four tooth surfaces (the distobuccal and distolingual surfaces of the tooth located distal to the interproximal site, and the mesiobuccal and mesiolingual surfaces of the tooth located mesial to the interproximal site). Averaging these four surfaces and obtaining one clinical variable for that interproximal site would have washed out any tooth-specific or surface-specific effects. Thus, the fractal dimension (drawn from the region of interest at the interproximal bone of the alveolar crest) would be used in association with each one of the four tooth surfaces individually.

Next, the tooth surfaces that showed disease progression over time (greater than 2 mm change in PD or in CAL) were analyzed separately for mean change in fractal dimension. The differences between groups were analyzed using a Mann-Whitney test. Finally, to examine the relationship between change in (1) the clinical data and fractal

dimension, and (2) the DSR data and fractal dimension, a linear regression parameter

estimate was calculated between the variables.

V. Results

<u>Study Population</u>

A total of 203 patients were screened for the study and of these, 130 patients did not meet the screening criteria. This resulted in 73 patients who were enrolled in the study and randomized into one of two groups. Of the 73 patients that began the study, 16 patients dropped out between the baseline and 12-month visits, leaving 57 patients at the 12-month timepoint. At the conclusion of the study at 24 months, 47 patients remained. The reasons for withdrawal from the study are categorized and listed in Table 1. An adverse effect is defined as any event in which the patient reports discomfort, ranging everywhere from abdominal pain to tooth-related pain, and these may or may not be related to the study medication. The list of adverse events reported by each patient is shown in the Appendix.

Reason for Withdrawal from Study	# of patients
Voluntary withdrawal without an adverse event	12
Voluntary withdrawal with adverse event	1
Lost to follow-up	11
Protocol Violation	2
Total	26

Table 1: Number of patients and reasons for withdrawal from study

This paper serves to describe results up to and including the 12 month timepoint only. The complete results including the 24-month timepoint will be described in a later paper. Of the 73 patients originally enrolled in this study, 49 subjects had interproximal sites that met the inclusion criteria for fractal analysis and also had radiographs available for study at the time of the writing of this paper. Table 2 shows the demographics of the specific study population described in this paper (49 subjects), listed by age, gender,

smoking history and race. There was no significant difference in the distribution of smoking history between the two groups.

	Bisphosphonate Group (N=27)	Placebo Group (N=22)
Mean Age (years)	47	48
Standard Deviation (years)	12.55	8.59
Gender		
Female	15 (56%)	8 (36%)
Male	12 (44%)	14 (64%)
Total	27 (100%)	22 (100%)
Smoking History		
Never Smoked	7 (26%)	3 (14%)
Past Smoker	11 (41%)	12 (55%)
Current Smoker	9 (33%)	7 (32%)
Total	27 (100%)	22 (100%)
Race		
Asian	0 (0%)	1 (5%)
African-American	6 (22%)	6 (27%)
Caucasian	18 (67%)	13 (59%)
Hispanic	3 (11%)	2 (9%)
Total	27 (100%)	22 (100%)

Table 2: Demographics of the study population

However, when examining the sites selected for fractal analysis (a total of 176 sites), there was a significant difference in smoker status between the treatment and placebo groups (p=0.0005). Table 3 shows the distribution of smoker status amongst the 176 sites that were selected for fractal analysis. Table 4 shows the distribution of smoker status amongst the 183 sites that were selected for fractal analysis. There were a total of 106 sites that had fractal analysis and DSR readings performed on them.

Smoking History	Fractal Sites in Bisphosphonate Group		Fractal Sites in Placebo Group	
	N	Percent	N	Percent
Never Smoked	38	36%	7	10%
Past Smoker	36	34%	36	51%
Current Smoker	32	30%	27	39%
Total	106	100%	70	100%

Table 3: Distribution of smoking status amongst the sites selected for fractal analysis

Smoking History	DSR Sites in Bisphosphonate Group		DSR Sites in Placebo Group	
	N	Percent	N	Percent
Never Smoked	31	29%	8	11%
Past Smoker	47	44%	41	59%
Current Smoker	35	33%	21	30%
Total	113	100%	70	100%

Table 4: Distribution of smoking status amongst the sites selected for DSR

Clinical data and fractal dimension at baseline, 6-month, and 12-month time points

When examining the bisphosphonate and placebo groups at baseline, there were no differences found between the two groups in any of the clinical parameters or fractal dimension. Table 5 shows the mean clinical data and fractal dimensions in the placebo group compared to the bisphosphonate group at 0 months, 6 months, and 12 months. In this analysis, the mean values of the clinical variables were obtained by first taking the average of the four tooth surfaces adjacent to an interproximal site, then finding the mean of the interproximal sites collectively. Intra-subject, intra-tooth, or smoking effects were not taken into consideration in this analysis. At 12 months, the bisphosphonate group had a statistically significantly better CAL (2.24 ± 1.29 mm) compared to the placebo group (2.76 ± 1.40 mm) ($p=0.013$). There were no differences in mean PD, BOP, fractal dimension measured in the low frequency range (FDL) or fractal dimension measured in the high frequency range (FDH) found between the placebo and bisphosphonate groups at any of the timepoints.

Variable	Timepoint (months)	BISPHOSPHONATE					PLACEBO					p-value
		N	Mean	Std Dev	Min	Max	N	Mean	Std Dev	Min	Max	
BOP	0	106	0.53	0.36	0.00	1.00	70	0.47	0.41	0.00	1.00	N.S.
	6	106	0.29	0.32	0.00	1.00	70	0.26	0.31	0.00	1.00	N.S.
	12	106	0.25	0.30	0.00	1.00	70	0.31	0.34	0.00	1.00	N.S.
PD	0	106	4.20	1.48	2.00	9.00	70	4.13	1.22	2.25	8.00	N.S.
	6	106	3.46	1.13	1.50	6.75	70	3.61	1.14	2.00	7.50	N.S.
	12	106	3.40	0.97	2.00	6.50	70	3.68	1.25	1.75	7.75	N.S.
CAL	0	106	3.27	1.63	0.75	7.50	70	3.37	1.42	1.00	7.50	N.S.
	6	106	2.55	1.40	0.00	6.75	70	2.74	1.47	0.50	7.50	N.S.
	12	106	2.24	1.29	0.25	6.75	70	2.76	1.40	1.00	6.75	0.013
FDL	0	106	2.599	0.050	2.521	2.742	70	2.593	0.045	2.521	2.707	N.S.
	6	60	2.590	0.047	2.525	2.747	48	2.598	0.046	2.522	2.705	N.S.
	12	106	2.603	0.048	2.535	2.732	70	2.602	0.048	2.529	2.725	N.S.
FDH	0	106	2.806	0.041	2.728	2.940	70	2.817	0.040	2.749	2.908	N.S.
	6	60	2.813	0.041	2.732	2.893	48	2.824	0.044	2.743	2.950	N.S.
	12	106	2.809	0.037	2.714	2.900	70	2.820	0.050	2.737	2.968	N.S.

Table 5: Mean clinical data and fractal dimension at each timepoint, divided by groups

Longitudinal change in clinical data and fractal dimension

Table 6 shows the mean longitudinal change in clinical variables, fractal dimension, bone mass and bone height over the three different time periods: 0- to 6-month, 6- to 12-month, and 0- to 12-month intervals. BOP is represented as the percentage of sites that were positive for bleeding on probing. Change in PD, CAL, and bone height is measured in millimeters and bone mass measured in milligrams. Unlike the previous analysis in which the clinical variables of four tooth surfaces were averaged at each interproximal site, this analysis was based on the individual tooth surface and a larger sample size reflecting the increase in tooth surfaces was used. Graphical representation of the data in Table 6 is shown in more detail in the Appendix.

Variable	Time interval (months)	Bisphosphonate Group					Placebo Group				
		N	Mean Change	SD	SE	p-value	N	Mean Change	SD	SE	p-value
BOP[1]	0-6	556	-21.40%	53.30%	2.26%	<0.0001	336	-21.40%	48.40%	2.64%	<0.0001
	6-12	556	-4.70%	49.30%	2.09%	N.S.	336	5.90%	53.80%	2.94%	N.S.
	0-12	556	-26.10%	54.60%	2.32%	<0.0001	336	-15.50%	57.80%	3.15%	<0.0001
PD[2]	0-6	556	-0.65	1.18	0.050	<0.0001	336	-0.56	1.14	0.062	<0.0001
	6-12	556	-0.099	0.943	0.040	N.S.	336	0.054	0.96	0.052	N.S.
	0-12	556	-0.75	1.31	0.056	<0.0001	336	-0.506	1.38	0.075	<0.0001
CAL[3]	0-6	556	-0.69	1.25	0.053	<0.0001	336	-0.592	1.17	0.064	<0.0001
	6-12	556	-0.311	0.975	0.041	<0.0001	336	-0.006	0.92	0.050	N.S.
	0-12	556	-1.00	1.34	0.057	<0.0001	336	-0.599	1.19	0.065	0.0001
FDL[4]	0-6	240	-0.005	0.025	0.002	N.S.	192	0.0027	0.028	0.002	N.S.
	6-12	240	0.012	0.026	0.002	<0.0001	192	0.008	0.046	0.003	N.S.
	0-12	424	0.0038	0.028	0.001	0.0053	280	0.0094	0.043	0.003	0.0038
FDH	0-6	240	-0.0038	0.022	0.001	N.S.	192	0.0031	0.028	0.002	N.S.
	6-12	240	0.0054	0.029	0.002	N.S.	192	0.0017	0.025	0.002	N.S.
	0-12	424	0.0022	0.028	0.001	N.S.	280	0.003	0.031	0.002	N.S.
Bone height[5]	0-12	226	0.053	0.618	0.041	N.S.	140	0.056	0.36	0.030	N.S.
Bone mass	0-12	226	0.122	2.337	0.155	N.S.	138	0.233	1.39	0.118	N.S.

Table 6: Mean change in clinical variables, fractal dimension, and bone mass and bone height, by group

[1] For BOP, a negative sign indicates a decrease in the number of sites that exhibit bleeding on probing over time.
[2] For mean change in PD, a negative sign indicates that there was a reduction in probing depths over time.
[3] For mean change in CAL, a negative sign indicates that there was a gain in clinical attachment levels over time.
[4] For mean change in FDH and FDL, a negative sign indicates a decrease in the fractal dimension over time, whereas a positive number indicates an increase in fractal dimension over time.
[5] For bone mass and bone height, a positive number indicates an increase in bone mass and bone height over time.

Both groups showed significant reductions in BOP over the 0- to 6-month time interval and the 6- to 12-month time interval (p<0.01). However, in comparing the two groups and correcting for intra-subject and intra-tooth effects, there was no statistically significant difference between the bisphosphonate and placebo groups in mean change in BOP.

There were significant reductions in PD over the 0- to 6-month and the 6- to 12-month time intervals (p<0.0001). However, after correcting for intra-subject and intra-tooth correlations and adjusting for the plaque index, there were no differences found between the bisphosphonate and placebo groups in mean change in PD over any of the time intervals.

Both bisphosphonate and placebo groups showed significant changes in CAL over the 0- to 6-month and 0- to 12-month time intervals (p<0.0001). Significant differences between the bisphosphonate and placebo groups were noted in mean change in CAL over all time intervals (p=0.0161). After adjusting for the intra-subject and intra-tooth correlations as well as plaque index, the bisphosphonate group still showed a significantly greater clinical attachment gain than the placebo group over 0- to 12-months (p=0.0015). As mentioned previously, there was a significant difference in the distribution of smoking habit between the two treatment groups (p=0.0005). The placebo had significantly fewer subjects who had never smoked and more past smokers than the treatment group. When the model was further adjusted to remove the effects of the smoking category, and the treatment group still showed more gain in CAL in the 6-12 month interval (p=0.0019) and in the 0-12 month interval (p=0.0251).

There was a significant increase in FDL over the 0- to 12-month interval in both bisphosphonate and placebo groups (p<0.01). However, after adjusting for intra-subject and intra-tooth correlations, no difference in mean change in FDL was found between the bisphosphonate and placebo treatment groups. Both groups showed no significant change in FDH over any of the intervals. There was no difference in FDH found between the two treatment groups after considering the intra-subject and intra tooth correlation.

In both groups, there were no significant changes in bone mass or bone height over 12 months. When applying mixed models analysis to compare the bisphosphonate and placebo groups, there were also no differences found in change in bone mass or height as detected by DSR.

To further investigate the relationship between longitudinal change in soft tissue clinical parameters and bone change as measured by fractal dimension and DSR, the sites that exhibited greater than 2 mm improvement in PD or CAL over time were examined separately from those sites that remained unchanged (i.e., had less than 2 mm of improvement). Sites that worsened over time were defined as any site that had an increase in PD or additional loss of CAL greater than 2mm. Sites that improved were those that had a PD reduction or gain in CAL of greater than 2 mm. The results are shown in Table 7 through Table 9.

Change in fractal dimension	Time interval (months)	Reduction in PD during 12 months	N	Mean	Std Dev	Std Err	p-value
FDL	0-6	< 2mm	362	0.0018	0.0266	0.0014	
		≥ 2mm	70	0.0006	0.0277	0.0033	N.S.
	0-12	< 2mm	571	-0.006	0.0342	0.0014	
		≥ 2mm	133	-0.004	0.0385	0.0033	N.S.
FDH	0-6	< 2mm	362	0.0002	0.0252	0.0013	
		≥ 2mm	70	0.0034	0.0249	0.003	N.S.
	0-12	< 2mm	571	-0.003	0.0297	0.0012	
		≥ 2mm	133	-0.001	0.0272	0.0024	N.S.

Table 7: Comparison of mean change in fractal dimension between sites that showed reductions in PD over 12 months.

Change in fractal dimension	Gain in CAL during 6 months	N	Mean	Std Dev	Std Err	p-value
FDL (0-6 months)	< 2mm	350	0.0022	0.027	0.0014	
	≥ 2mm	82	-0.0009	0.0257	0.0028	N.S.
FDL (0-12 months)	< 2mm	565	-0.005	0.034	0.0014	
	≥ 2mm	139	-0.011	0.0386	0.0033	N.S.
FDH (0-6 months)	< 2mm	350	-0.0004	0.0251	0.0013	
	≥ 2mm	82	0.0058	0.0247	0.0027	0.043
FDH (0-12 months)	< 2mm	565	-0.004	0.0301	0.0013	
	≥ 2mm	139	0.0031	0.0248	0.0021	0.004

Table 8: Comparison of mean change in fractal dimension between sites that showed gains in CAL during the first 6 months versus sites that remained unchanged.

Change in fractal dimension	Gain in CAL during 12 months	N	Mean	Std Dev	Std Err	p-value
FDL (0-6 months)	< 2mm	329	0.0023	0.0268	0.0015	
	≥ 2mm	103	-0.0006	0.0268	0.0026	N.S.
FDL (0-12 months)	< 2mm	523	-0.005	0.0346	0.0015	
	≥ 2mm	181	-0.008	0.0363	0.0027	N.S.
FDH (0-6 months)	< 2mm	329	0.0001	0.0264	0.0015	
	≥ 2mm	103	0.0027	0.0205	0.002	N.S.
FDH (0-12 months)	< 2mm	523	-0.004	0.0308	0.0013	
	≥ 2mm	181	0.0006	0.0239	0.0018	N.S.

Table 9: Comparison of mean change in fractal dimension between sites that showed gains in CAL during the first 12 months versus sites that remained unchanged

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There was a significantly greater change in FDH over 6 months for sites which showed greater than 2 mm gain in CAL during the first 6 months (p = 0.043). A similar trend was also observed for change in FDH over 12 months (p=0.004). There were no significant differences found between sites in mean change in FDL. There were also no significant differences found between sites when categorized by amount of PD reduction.

Relationship between clinical data, DSR and fractal dimension:

A Pearson correlation coefficient was calculated to examine the relationship between the fractal data and the clinical variables and DSR over 12 months, shown in Table 10. There were significant correlations found between change in FDH and PD, CAL and bone mass (p<0.05). A linear regression was performed on these correlations that were significant and a parameter estimate was calculated (Table 11). A positive parameter estimate indicates a positive correlation, whereas a negative parameter estimate indicates a negative correlation.

| | Fractal Dimension in Low frequency range | | | Fractal dimension in High frequency range | | |
	N	Correlation Coefficient	p-value	N	Correlation Coefficient	p-value
BOP	704	-0.021	N.S.	704	0.021	N.S.
PD	704	-0.031	N.S.	704	0.080	0.0329
CAL	704	-0.003	N.S.	704	0.095	0.0114
Bone mass	210	0.023	N.S.	210	-0.191	0.0055
Bone height	210	0.023	N.S.	212	-0.114	N.S.

Table 10: Pearson Correlation Coefficients

Fractal dimension	Clinical variable/DSR	N	Parameter estimate	Standard error	p-value
FDH	PD	702	0.0018	0.00084	0.0329
FDH	CAL	702	0.00218	0.00086	0.0114
FDH	Bone mass	208	-12.95	4.6	0.0055

Table 11: Parameter estimates for correlation between fractal dimension measured in the high frequency range and PD, CAL, and bone mass.

The relationship between FDH and PD was positive, that is FDH increased, PD also increased. Likewise, between FDH and CAL, the correlation was positive as well. The Pearson correlation coefficient for FDH related to bone mass measured by DSR was statistically significant and indicated a negative correlation. That is as bone mass increased, FDH decreased. There was no significant correlation found between bone height as measured by DSR and FDH. Change in FDL over 12 months showed no significant correlation with any other variable. It is important to note that the correlations that were statistically significant were however not very large in magnitude.

VI.   Discussion

Clinical data and fractal dimension at each timepoint

In examining the clinical data and fractal dimension variables at baseline, there were no significant differences found between the bisphosphonate and placebo groups. When comparing the clinical data between groups at later timepoints, the mean CAL was found to be statistically significantly lower in the bisphosphonate group at 12 months as compared to the placebo group. This finding was consistent with later findings when examining overall change in CAL over time.

Longitudinal change in disease progression

When examining the change in clinical variables over 6 months and 12 months, there were reductions in BOP and PD in both groups. It is important to remember that in this study, both groups were receiving periodontal therapy, so some resolution of periodontal disease would be expected in both groups. The relevant question is whether the group with the adjunctive bisphosphonate treatment showed *greater* improvement than the placebo group. In this case, the reductions in bleeding on probing and probing depths were not significantly different between the placebo and bisphosphonate groups. However, there was a significantly greater gain in clinical attachment level in the bisphosphonate group as compared to the placebo group.

There have been several mechanisms proposed for bisphosphonate action, one of which is alteration of matrix metalloproteinase (MMP) production from human periodontal ligament cells. The inhibitory effect of bisphosphonates on the activity of

MMP-1 and MMP-3 have been shown in cultured periodontal cells [34]. If this is indeed one of the mechanisms of action of bisphosphonates, clinically, one would expect to see a inhibition of the inflammatory pathway with reduction in signs of inflammation, including a decrease in probing depths and levels of bleeding on probing, however this was not the case in our study. Furthermore, these findings are consistent with those of previous animal and human clinical studies [28,29].

Brunsvold et al. in 1992 administered alendronate biweekly in monkeys with ligature-induced periodontal disease and found that while bone loss was inhibited in the 0.05 mg/kg alendronate group, there was no significant difference in probing depths between the placebo and treated monkey [28]. Similarly, Reddy et al. in 1995 in a study using alendronate to treat periodontitis in beagle dogs found that plaque indices and probing depths showed no change between alendronate and placebo groups whereas there was a trend towards less attachment loss in the alendronate group [29]. Therefore, it does not appear from the standpoint of animal and clinical studies that the action of bisphosphonates involves inhibition of the inflammatory process which eventually leads to bone resorption.

Bone mass and bone height change as measured by DSR

Changes in the amount of alveolar bone can be detected in a number of ways. In this study, we have chosen to use digital subtraction radiography that is used to measure changes in bone level by superimposition of images from two timepoints. Bone height changes as measured by DSR provide some information about external alveolar crestal structure, but not the internal architecture. Measurement of bone mass by DSR is

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typically done by using an aluminum reference wedge incorporated in the X-ray film holder to provide a density reference. Thickness and area measurements of the subtraction image are used to calculate an index of bone mass change. Previous studies have shown that the error in repeatability of determination of areas was 4% and that calculated changes in bone mass correlated with actual changes in bone mass ($r^2 > 0.9$) [35].

When comparing change in bone height and bone mass over 12 months, both bisphosphonate and placebo groups showed increases, however the change between the two groups was not statistically significant. This finding was in contrast with previous studies which have all shown that bisphosphonates increase bone mass compared to a control group. In Brunsvold's study of ligature-induced periodontitis in monkeys, bone loss (bone density) was inhibited in the alendronate group as measured by a computer assisted densitometric system [28]. In Reddy's study in beagle dogs, bone mass and bone height was also measured by DSR and found to be greater at six months in the alendronate group [29]. And finally, Rocha et al. in 2001 performed a clinical double-blinded randomized control study in which diabetic periodontitis patients were given either alendronate or a placebo and followed for 6 months. They found that alendronate induced more improvement in alveolar bone crest height (determined by manual measurements of the change in bone height relative to the CEJ on radiographs) than the control group [36].

There may be several reasons for this contrast in results. In this study, DSR measurements were only performed utilizing the 0-month and 12-month radiographs. This is the first clinical study to examine change for a time period longer than six months. If there was a significant difference in bone mass or height which occurred anytime

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earlier than 12 months, this would not be detected in our findings, unless this difference persisted up to the 12-month timepoint. Secondly, it is important to keep in perspective the fact that the bisphosphonate was being administered in addition to ongoing conventional periodontal therapy (consisting of scaling and root planing every three months) which the placebo group was also receiving. In other studies, there was either no periodontal therapy being instituted in the control group [28,29] or the therapy was only being instituted at the baseline timepoint [36], but not continuously throughout the study. Thus, our findings do not preclude the possibility that the bisphosphonate therapy did in fact help increase bone mass, but perhaps did not increase bone mass to a great enough magnitude to be detected statistically when compared to a placebo group receiving conventional periodontal therapy every three months.

Of course, there are other possible confounding factors that may have played a role in the outcome of nonsignificance in the DSR data. In our analysis, we have already accounted for the intra-tooth and intra-subject correlations. Other confounding factors include localized effects such as a cracked tooth, poor restorations, or an abscess of periodontal or endodontic origin. When examining the number of patients and teeth that had abscesses, it was found that those numbers were not significantly different between the two groups. Furthermore, of the teeth that had abscesses, there were only three teeth with DSR readings. Of these, two were in the alendronate group, and one was in the placebo group. Of the two in the alendronate group, one had a decrease in bone height and bone mass and the other had an increase in bone height and mass. The tooth in the placebo group had no change in bone height or mass. Therefore, it appears that localized

effects, at least in terms of distribution of abscesses in the study population, does not appear to explain why the DSR result was not different between the two groups.

To be precise, three-dimensional internal changes in bone structure and quantity in the trabecular bone are more accurately assessed with imaging technologies such as quantitative computed tomography (QCT) and T2 MRI. These are standards by which fractal analysis could be compared to. However the large amount of radiation associated with QCT precludes this from being used in a longitudinal clinical trial, and T2 MRI is still a relatively new technology in developing stages for applications to imaging the alveolar bone.

Fractal dimension

FDH was directly correlated with probing depths and clinical attachment level. That is, as FDH decreased, probing depths decreased and clinical attachment level improved. Therefore, from a clinical standpoint, as FDH decreases, this is consistent with an improvement in periodontal health and decreased inflammation. Of the correlations that were statistically significant, the strongest correlation was found between FDH and bone mass as measured by DSR, whereas there was no correlation between fractal dimension and bone height. This is logical since fractal dimension measurements are an indicator of internal bone patterning and architecture and may be more directly related to bone mass, whereas bone height is more of an external bony measurement. The correlation between FDH and bone mass was found to be an inverse relationship, that is, as bone mass increases, FDH decreases. Again, this is consistent with the previous

statement that resolution of periodontal disease (in this case, increased bone mass) is consistent with a decreased FDH.

As mentioned previously, there have been a number of studies that have also come to the conclusion that fractal analysis could be used to distinguish between healthy and periodontally compromised patients [9-14]. Majumdar et al. performed an in vitro study on cubic specimens of human trabecular bone, and found that fractal dimension showed varying trends with bone mineral density changes, and that these trends also depended on the range of frequencies over which the fractal dimension was measured [13]. Using the Fast Fourier Transform power spectral method, the same method we have employed in this study, the fractal dimension decreased when computed over a higher range of spatial frequencies as BMD increased. Majumdar's findings of an inverse correlation between bone mineral density and fractal dimension computed in a higher frequency range are consistent with the findings in this study.

Although it is heartening to find some consistency between studies in the direction of correlation between bone mass and fractal dimension, it is important to remember that with all studies utilizing fractal dimension, there are always inherent technical variabilities that may occur. There are differences in image acquisition and scanning even before the actual fractal analysis is applied, not to mention differences in fractal analysis technique. Variations amongst studies utilizing a similar fractal analysis technique may even occur due to differences in spatial resolution of the images or selection of frequency ranges. And finally, differences in bone architecture may occur when specimens are taken from different anatomical locations. Because of these variations in technique, it is difficult to extrapolate findings of one fractal study to

another. In future research in this area, it will be important to clearly document step-wise the process by which the fractal dimension was obtained so that the study could be repeated identically by another independent examiner. In addition, the ultimate goal would be to determine a reliable, repeatable method for obtaining the fractal dimension. As well, in the Majumdar study, the amount of bone mineral density change was quantified by QCT, however in this study, quantification of bony change was performed by DSR.

Overall, there was no statistically significant change in fractal dimension (FDL or FDH) over time. Admittedly, there were relatively large standard deviations and fractal changes are measured in a very small scale, thus, any differences would be statistically very difficult to find. Again, if we assume that fractal dimension varies with change in bone mass, then the lack of difference in fractal dimension between groups is consistent with our original finding that the change in bone mass and height was not different between the bisphosphonate and placebo groups.

VII. Conclusion

From this prospective, randomized controlled clinical trial examining the use of bisphosphonates in the adjunctive treatment of chronic periodontitis, the following may be concluded:

1. Fractal dimension measured in the high frequency range was shown to correlate significantly with clinical attachment level, probing depth, and bone mass as measured by digital subtraction radiography. There was no difference in change in fractal dimension over time in the bisphosphonate group compared to the placebo group.

2. Bisphosphonates were shown to improve clinical attachment levels in a group of periodontally diseased subjects, as compared to a group of controls. However, it did not appear to significantly increase bone mass or height more than conventional periodontal therapy alone.

CLINICAL PERIODONTAL ASSESSMENT–FORM 1 (Maxilla)

CHR No.	Patient No.	Initials	Patient Chart #	Visit	Page
11-0815-166384-01	-		-	11	3

Was the exam performed? No ☐ Yes ☐   If "Yes", give the exam date: _ / _ / _ (Mm/dd/yyyy)   If "No", give reason:

#	site	2		3		4		5		6		7		8	
		DB	MB	DB	MB	DB	MB	DB	MB	DB	MB	DB	MB	DB	MB
		B	B	B	B	B	B	B	B	B	B	B	B	B	B
PlI																
BOP																
PD																
R																
CAL																
PlI																
BOP																
PD																
R																
CAL																
site		DL	ML	DL	ML	DL	ML	DL	ML	DL	ML	DL	ML	DL	ML
		L	L	L	L	L	L	L	L	L	L	L	L	L	L
#		2		3		4		5		6		7		8	

#	site	9		10		11		12		13		14		15	
		MB	DB	MB	DB	MB	DB	MB	DB	MB	DB	MB	DB	MB	DB
		B	B	B	B	B	B	B	B	B	B	B	B	B	B
PlI																
BOP																
PD																
R																
CAL																
PlI																
BOP																
PD																
R																
CAL																
site		ML	DL	ML	DL	ML	DL	ML	DL	ML	DL	ML	DL	ML	DL
		L	L	L	L	L	L	L	L	L	L	L	L	L	L
#		9		10		11		12		13		14		15	

Examiner's Signature _____   Date: _ / _ / _

Figure 1: Clinical periodontal assessment Form 1

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CHR No.	Patient No.	Patient Chart #	Initials	Visit	Page
16x15-16638-01	-	-		11	4

CLINICAL PERIODONTAL ASSESSMENT-FORM 2 (Mandible)

Was the exam performed? No ☐ Yes ☐ If "Yes", give the exam date: / / (MM/DD/YYYY) If "No", give reason:

#	site	18			19			20			21			22			23			24		
		DB	B	MB	DB	B	MB	DB	B	MB	DB	B	MB	DB	B	MB	DB	B	MB	DB	B	MB
PlI																						
BOP																						
PD																						
R																						
CAL																						
PlI																						
BOP																						
PD																						
R																						
CAL																						
site	DL	L	ML	DL	L	ML	DL	L	ML	DL	L	ML	DL	L	ML	DL	L	ML	DL	L	ML
#	18			19			20			21			22			23			24			

#	site	25			26			27			28			29			30			31		
		MB	B	DB	MB	B	DB	MB	B	DB	MB	B	DB	MB	B	DB	MB	B	DB	MB	B	DB
PlI																						
BOP																						
PD																						
R																						
CAL																						
PlI																						
BOP																						
PD																						
R																						
CAL																						
site	ML	L	DL	ML	L	DL	ML	L	DL	ML	L	DL	ML	L	DL	ML	L	DL	ML	L	DL
#	25			26			27			28			29			30			31			

Examiner's Signature   Date: / /
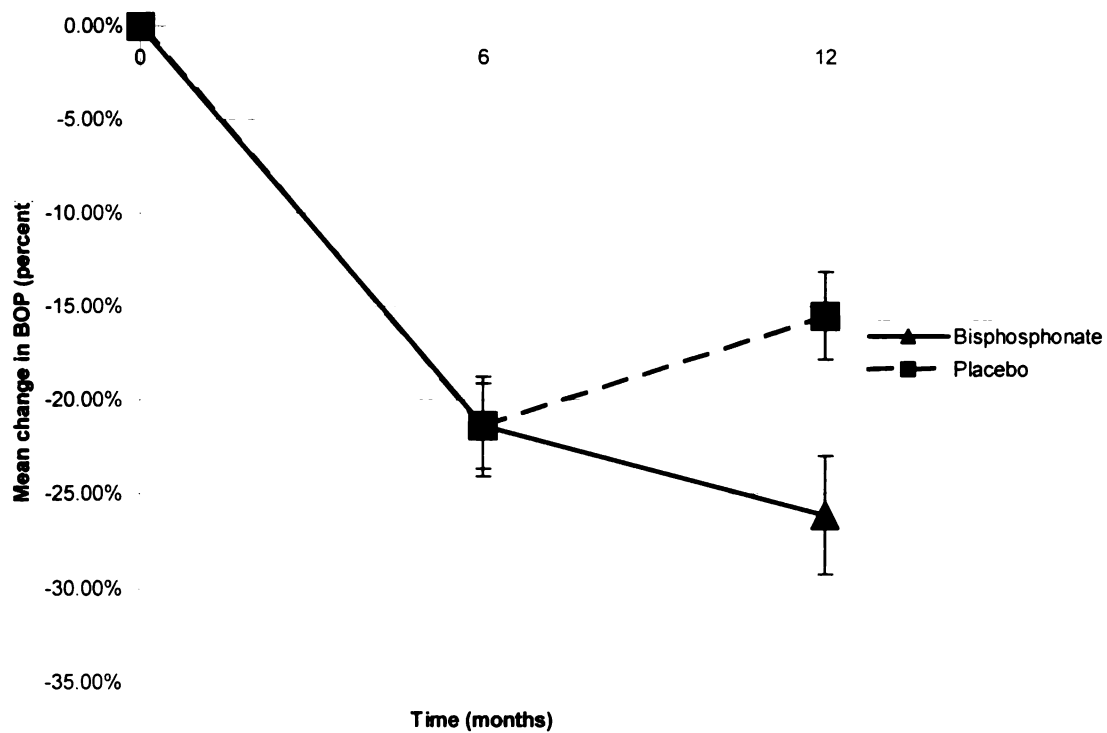
Figure 2: Clinical periodontal assessment Form 2

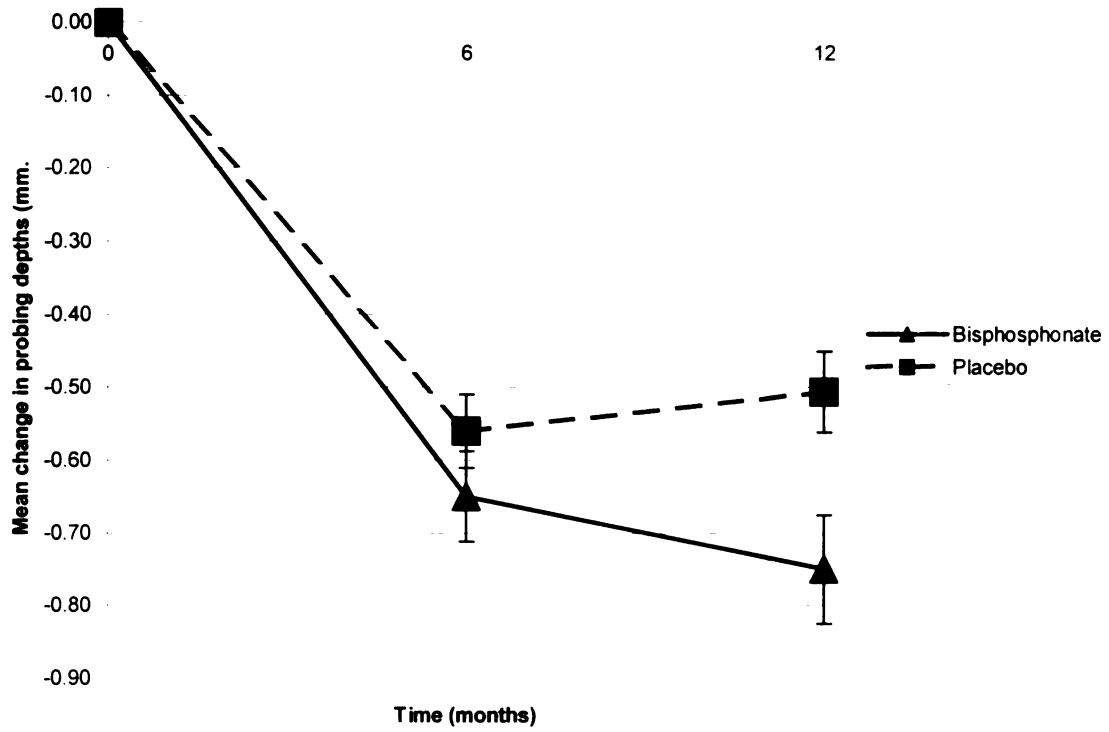Figure 3: Mean change in percentage of sites with BOP
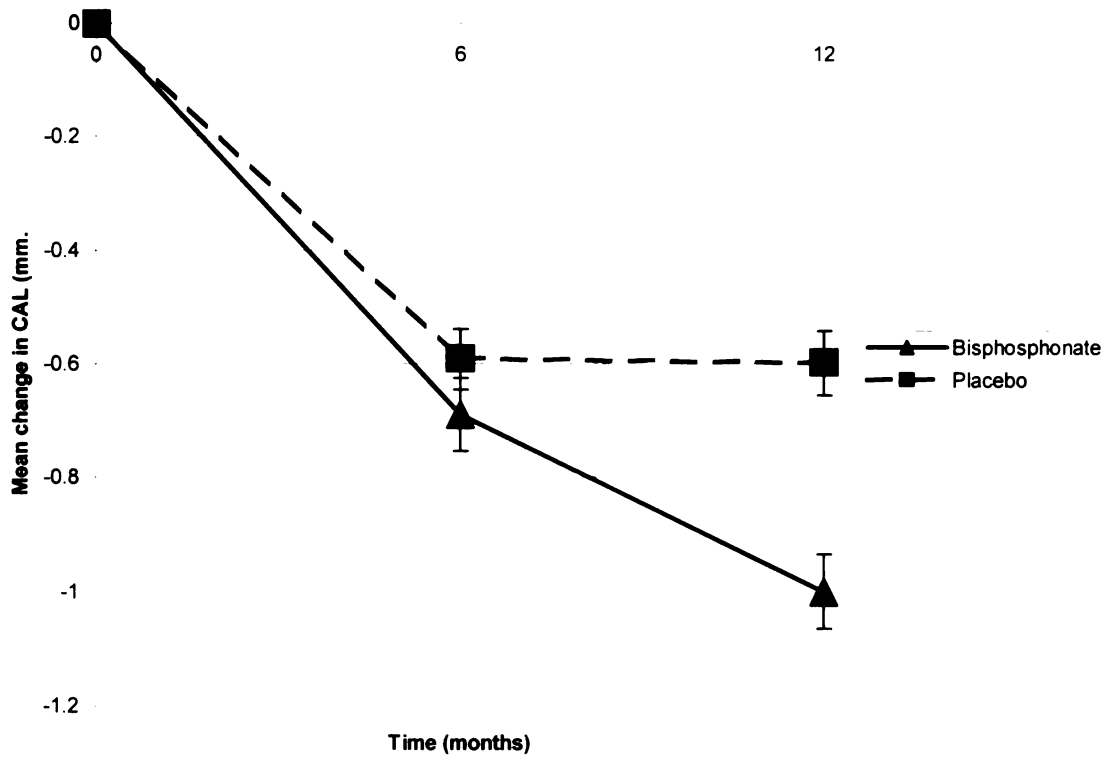
Figure 4: Mean change in probing depths

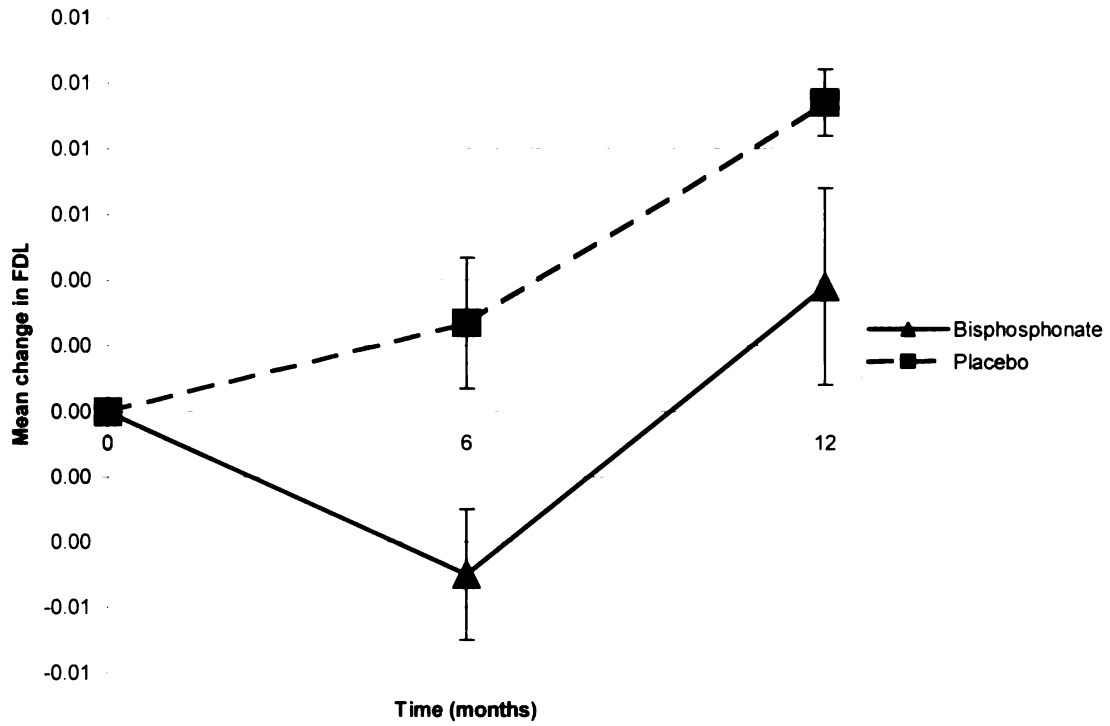Figure 5: Mean change in clinical attachment level

Figure 6: Mean change in fractal dimension measured in the low frequency range
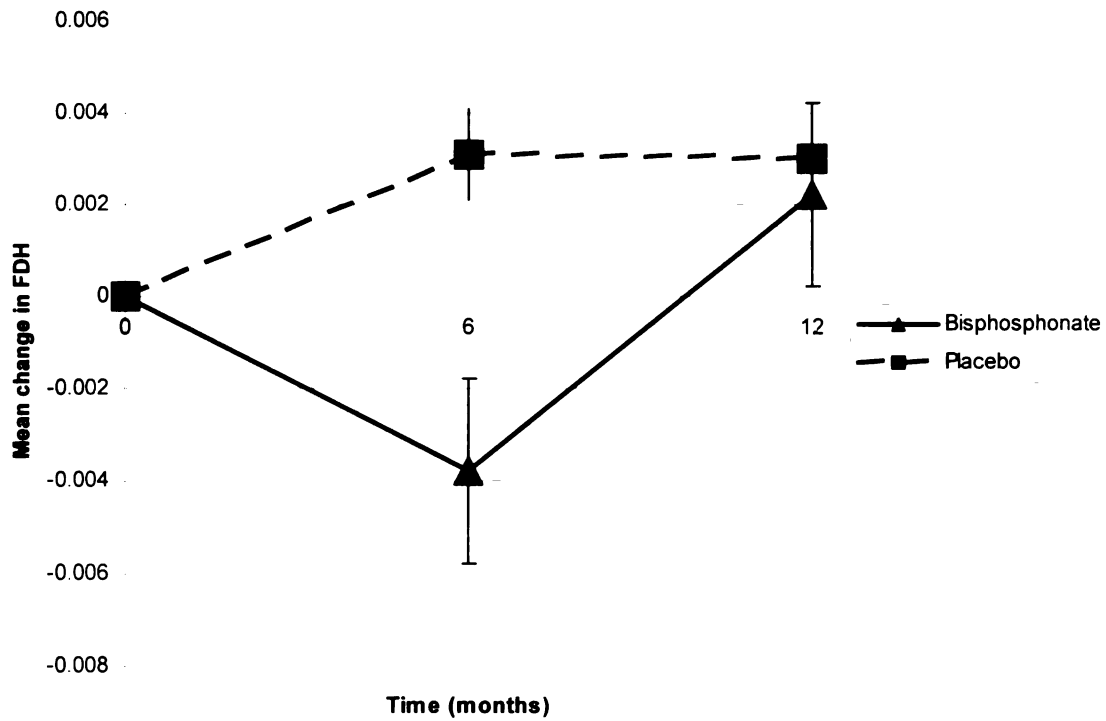
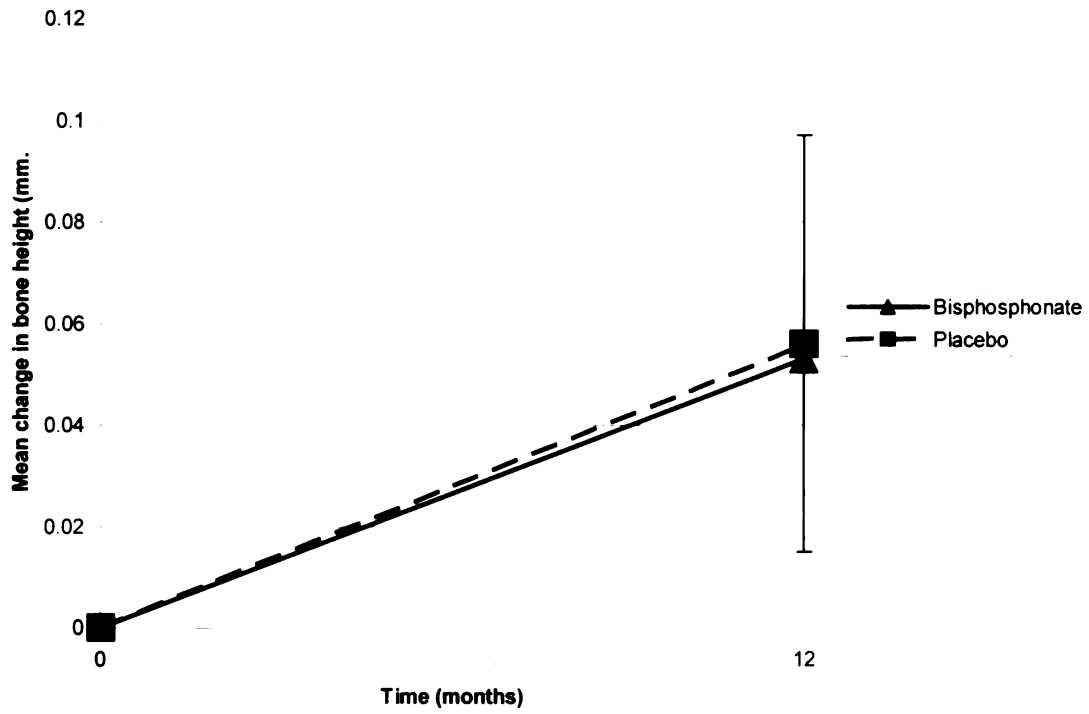Figure 7: Mean change in fractal dimension measured in the high frequency range

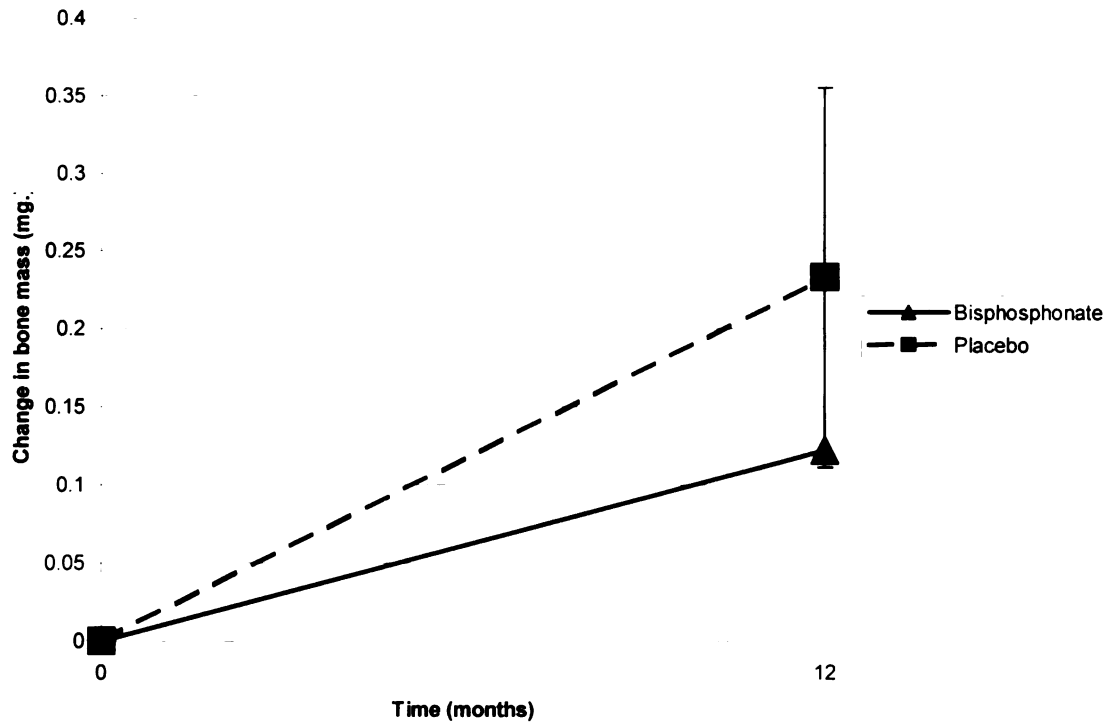Figure 8: Mean change in bone height

Figure 9: Mean change in bone mass

PT ID	PT INIT	Study Grp	ADVERSE EVENT	BODY SYSTEM	SEVERITY	RELATION
E-002	SJB	Bisphosphonate	chest pain	body as a whole	mild	probably not
E-002	SJB	Bisphosphonate	thyroid cyst	endocrine	mild	definitely not
E-002	SJB	Bisphosphonate	dermoid cyst	body as a whole	mild	definitely not
E-010	DMK	Bisphosphonate	URTI	respiratory	moderate	probably not
E-010	DMK	Bisphosphonate	nasal allergy	respiratory	mild	definitely not
E-012	RAG	Bisphosphonate	constipation	digestive	mild	probably not
E-013	J-W	Placebo	fatigue	body as a whole	mild	probably not
E-013	J-W	Placebo	tooth abscess	digestive	moderate	definitely not
E-013	J-W	Placebo	hyperlipidemia	body as a whole	moderate	definitely not
E-013	J-W	Placebo	EBV infection	body as a whole	mild	probably not
E-014	AJD	Bisphosphonate	fracture	musculoskeletal	severe	definitely not
E-017	JMG	Placebo	heartburn	digestive	mild	possibly
E-017	JMG	Placebo	strep throat infection	body as a whole	mild	probably not
E-019	HHH	Placebo	flare up of gout	musculoskeletal	moderate	probably not
E-019	HHH	Placebo	tooth abscess	digestive	mild	definitely not
E-020	T-R	Placebo	tooth abscess	digestive	severe	definitely not
E-020	T-R	Placebo	diarrhea	digestive	mild	possibly
E-021	IVF	Placebo	palpitations	cardiovascular	mild	definitely not
E-021	IVF	Placebo	hyperlipidemia	body as a whole	mild	definitely not
E-024	DDD	Bisphosphonate	sweet taste in mouth	special senses	mild	possibly
E-028	RDR	Bisphosphonate	calf & knee pain	musculoskeletal	mild	probably not
E-031	EAD	Placebo	parotid gland tumor	digestive	moderate	definitely not
E-033	HML	Bisphosphonate	toothache	digestive	mild	definitely not
E-034	CSW	Bisphosphonate	tooth abscess	digestive	moderate	definitely not
E-034	CSW	Bisphosphonate	low back pain	body as a whole	mild	probably not
E-036	P-J	Placebo	low back pain	body as a whole	mild	probably not
E-036	P-J	Placebo	near syncope	nervous	moderate	probably not
E-037	A-G	Bisphosphonate	epigastric discomfort	body as a whole	mild	possibly
E-038	JBF	Bisphosphonate	body rash	skin and appendages	mild	probably not
E-038	JBF	Bisphosphonate	blepharitis	special senses	mild	definitely not
E-042	ADS	Bisphosphonate	headache	nervous	mild	definitely not
E-042	ADS	Bisphosphonate	bilateral retroorbital pain	special senses	mild	definitely not
E-043	GHI	Placebo	abdominal pain	body as a whole	severe	possibly
E-043	GHI	Placebo	Pancreatitis	digestive	severe	probably not
E-043	GHI	Placebo	hip pain	musculoskeletal	mild	definitely not
E-044	ERC	Bisphosphonate	URTI	respiratory	mild	probably not
E-044	ERC	Bisphosphonate	Hypertension	cardiovascular	mild	definitely not
E-044	ERC	Bisphosphonate	tooth abscess	digestive	mild	definitely not
E-046	KMB	Placebo	stomach upset	digestive	mild	possibly
E-046	KMB	Placebo	tooth abscess	digestive	mild	definitely not
E-046	KMB	Placebo	diarrhea	digestive	mild	possibly
E-047	JAB	Bisphosphonate	fatigue	body as a whole	mild	probably not
E-048	E-M	Bisphosphonate	oral lesion	digestive	mild	definitely not
E-049	N-D	Placebo	heartburn	digestive	mil	possibly
E-049	N-D	Placebo	constipation	digestive	mild	probably not
E-049	N-D	Placebo	belching	digestive	mild	possibly

Table 12: List of Adverse Events (Part I).

PT ID	PT INIT	Study Grp	ADVERSE EVENT	BODY SYSTEM	SEVERITY	RELATION
E-051	R-F	Bisphosphonate	breast lump	breast	mild	definitely not
E-051	R-F	Bisphosphonate	osteopenia	endocrine	mild	definitely not
E-052	LMH	Bisphosphonate	heartburn	digestive	mild	possibly
E-053	TRZ	Bisphosphonate	eyelid pain	special senses	mild	definitely not
E-055	EBP	Bisphosphonate	Peyronie's disease	urology	mild	definitely not
E-057	CQS	Bisphosphonate	headache	nervous	moderate	probably not
E-057	CQS	Bisphosphonate	backache	musculoskeletal	mild	probably not
E-057	CQS	Bisphosphonate	exacerbation of hiatal hernia	digestive	severe	possibly
E-057	CQS	Bisphosphonate	shoulder pain	musculoskeletal	mild	probably not
E-058	MNZ	Bisphosphonate	constipation	digestive	mild	probably not
E-058	MNZ	Bisphosphonate	osteoporosis	endocrine	mild	definitely not
E-058	MNZ	Bisphosphonate	respiratory tract infection	respiratory	mild	definitely not
E-058	MNZ	Bisphosphonate	lower back/bottocks pain	musculoskeletal	mild	probably not
E-059	NGD	Bisphosphonate	nasal allergy	respiratory	moderate	definitely not
E-060	GMY	Placebo	loss of consciousness	nervous	moderate	probably not
E-061	WEP	Bisphosphonate	malar (facial) rash	skin and appendages	mild	probably not
E-061	WEP	Bisphosphonate	benign prostatic hypertrophy	urology	mild	definitely not
E-061	WEP	Bisphosphonate	tooth sensitivity	digestive	mild	definitely not
E-061	WEP	Bisphosphonate	jaw pain	musculoskeletal	mild	probably not
E-067	MBB	Bisphosphonate	tooth abscess	digestive	mild	definitely not
E-067	MBB	Bisphosphonate	tooth sensitivity	digestive	mild	definitely not
E-070	CLH	Bisphosphonate	tooth sensitivity	digestive	mild	definitely not
E-072	CFN	Bisphosphonate	respiratory tract infection	respiratory	moderate	definitely not
E-073	LAS	Bisphosphonate	heartburn	digestive	mild	possibly
E-073	LAS	Bisphosphonate	respiratory tract infection	respiratory	mild	definitely not

Table 13: List of Adverse Events (Part II).

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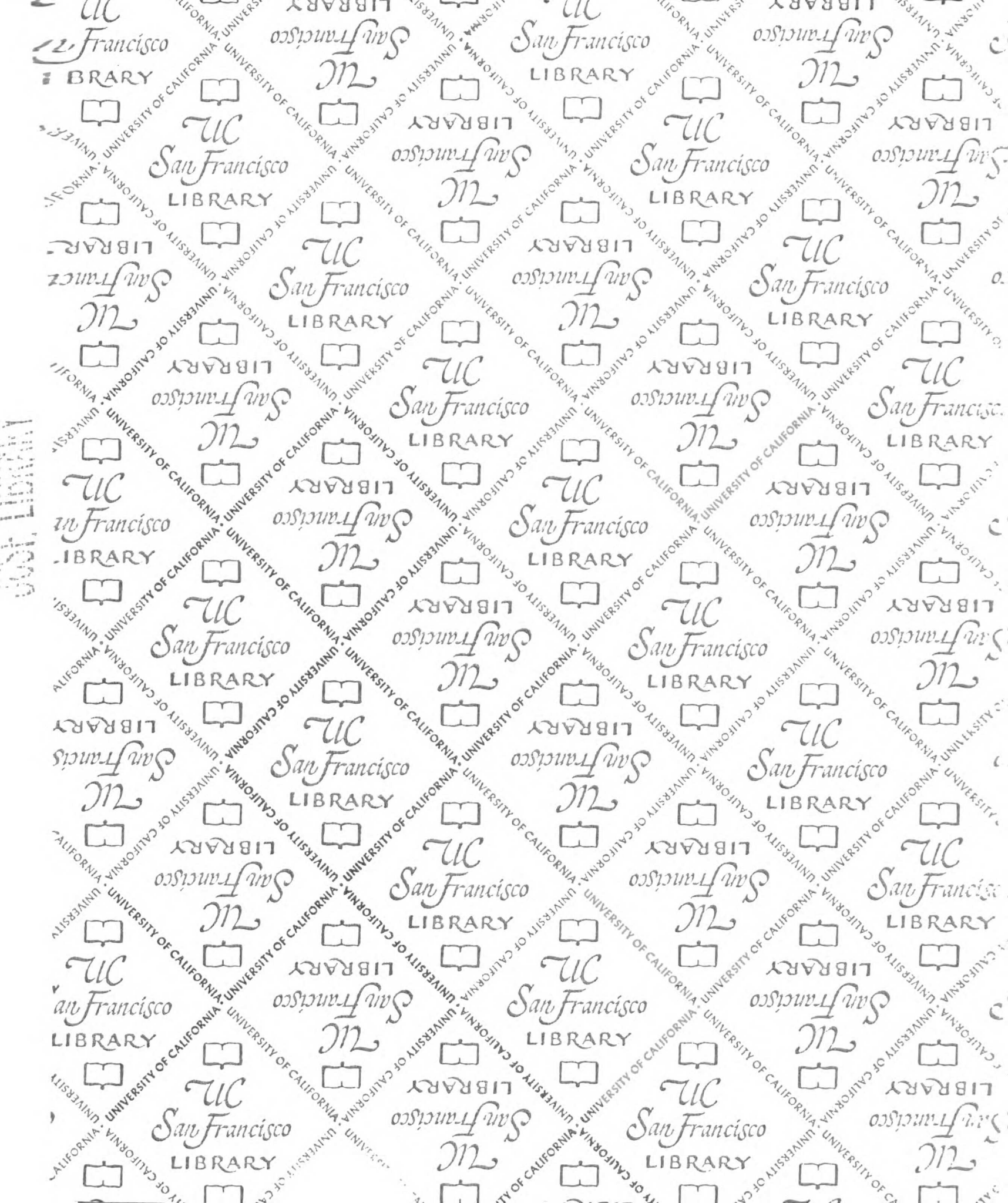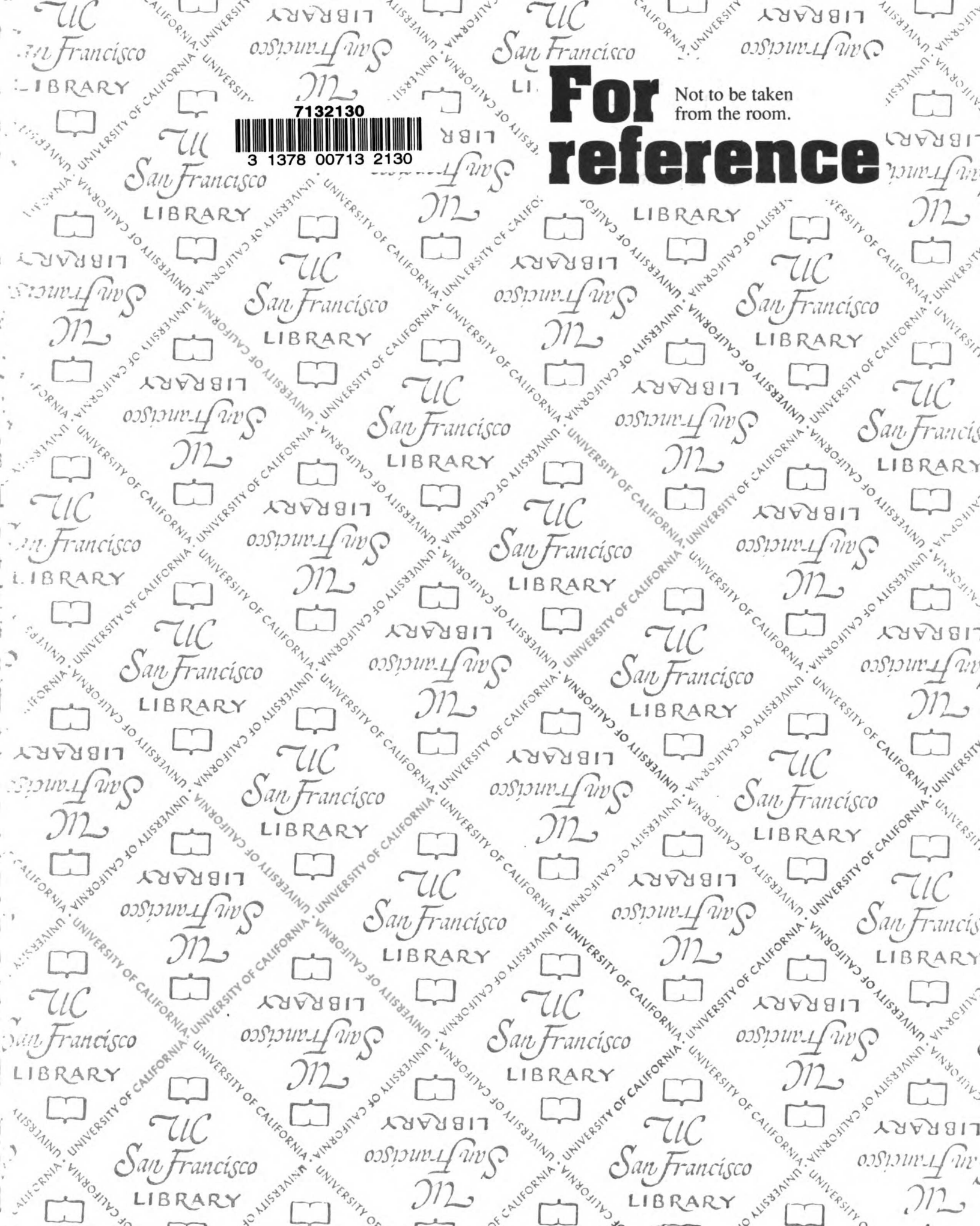
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