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"Hyperbaric Oxygen Therapy as Adjunctive Therapy to Scaling and Root-Planing in the Management of Periodontitis in Poorly Controlled Diabetics Type 2"

LLU Dental School Seed Grant Application
The Department of Pre-doctoral Periodontics
Loma Linda University School of Dentistry
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INVESTIGATORS

Principal Investigator: Dr. Zahra Mohammadzadeh

Co-Investigators and Research Mentors:
Dr. David Bland (Medical School)
Dr. Ahmed Khocht (Dental School)
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Dental Student Investigators:
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ROLES OF INVESTIGATORS

Dr. Mohammadzadeh as PI will be involved in periodontal clinical evaluations, microbial sample collection, non-surgical periodontal therapy (Scaling and Root Planing), obtaining subject consent, ensuring the well-being of subjects, management of subject recruitment and mentoring and guiding the student investigators with the various aspects of the project.

Dr. Bland and his designees will be involved in medical evaluations, subject recruitment and hyperbaric oxygen therapy.

Dr. Khocht will provide guidance in the overall management of the project including periodontal clinical evaluations, bacterial sample collection, processing/storage of collected samples, monitoring the consent process and managing the safekeeping of consent documents, as well as facilitate the conduction of the project.

Role of student investigators:
Student investigators will be involved with all aspects of the project including periodontal clinical evaluations, microbial sample collection, non-surgical periodontal therapy, and microbial sample processing.

The proposed research brings together a multidisciplinary team of experts in dentistry, medicine and microbiology to investigate the adjunctive effect of hyperbaric oxygen therapy to scaling/ and root planing in the management of periodontitis in not well controlled type 2 diabetics. Drs. Mohammadzadeh and Bland (along with his designees) at their respective Loma Linda University institutions have access to patient populations that would ensure the successful recruitment of subjects for the study.

PLAN TO OBTAIN EXTERNAL FUNDING TO CONTINUE PROJECT

Diabetes and Periodontitis are both prevalent diseases affecting millions of Americans. Periodontitis is prevalent among Diabetics. Furthermore, Periodontitis and associated inflammation can increase insulin resistance in Diabetics and worsens the condition. Managing Periodontitis in not well controlled type 2 diabetics is difficult. The National Institute of Health (NIH) as well as other Health Foundations would be interested in innovative therapies to manage and control Periodontitis in Diabetics. This proposed project is critical to collect preliminary data to support a proposal to seek external funding. We need to show that our hypothesis has merit for significant additional funding; we also need to demonstrate that we can collaborate and work together, we need to demonstrate that our laboratory assessments are appropriate and sensitive for the type of samples we will be collecting and we need to show that we have access to subject

populations that would sustain the project. Without this seed funding we would not be able to obtain this essential information and we would not be able to seek external funding with a realistic chance to succeed.

Abstract

Introduction: Diabetes and Periodontitis are both prevalent diseases affecting millions of Americans. Periodontitis is prevalent among Diabetics. Furthermore, Periodontitis and associated inflammation can increase insulin resistance in Diabetics and worsens the condition. Managing Periodontitis in not well controlled diabetics is difficult. There is need for innovative therapies to improve periodontal treatment outcome in poorly controlled diabetics. Hyperbaric Oxygen Therapy (HBOT) has the potential to improve periodontal treatment outcome in poorly controlled diabetics. The proposed pilot study is a longitudinal observational study to compare periodontal treatment (SRP) outcome between 2 main diabetic type 2 patient groups receiving medical care treatment: either Conventional Wound Therapies (CWC) with or without adjunctive Hyperbaric Oxygen Therapy at LLU Health. This project aims to investigate the effect of hyperbaric oxygen as adjunctive therapy to non-surgical periodontal therapy in the management of Periodontitis in not well controlled type-2 diabetics with chronic wounds.

Method and Materials: A total of 24 not well controlled diabetic mellitus (DM) type 2 patients (HbA1c =>7%) with Periodontitis will be recruited. All subjects must have generalized moderate to severe chronic periodontitis. All subjects will be recruited from LLU Health. Subjects will be divided into two group based on their medical needs: HBO therapy and Non HBO therapy.
For all subjects demographic data (age, gender, ethnicity, smoking history, alcohol use history, BMI, current medication list) and oral health habits will be obtained. Blood samples for HbA1c determinations, clinical periodontal measurements (plaque index, probing measurements including pocket depth, attachment levels, gingival index and bleeding-on-probing) and subgingival microbial samples will be obtained at baseline and end of the study. Subgingival microbial samples will be collected from three randomly selected sites with probing depth =>5mm and pooled together in a single tube. Both groups will receive full-mouth SRP. The HBOT group will also receive oxygen therapy protocol.

Microbiological Analysis: Collected microbial samples will be processed for DNA extraction and stored at -80°, then sent to the Forsyth Institute (Cambridge, MA) for HOMINGS analysis (The Human Oral Microbe Identification Microarray (HOMIM) technology for detection of about 300 of the most prevalent oral bacterial species, including many that cannot be grown in the laboratory using 16S rRNA gene sequencing).

Statistical analysis: Differences in periodontal clinical outcomes and bacterial profiles from baseline to 12 weeks between non-hyperbaric therapy group and hyperbaric therapy group will be analyzed using the repeated measures ANCOVA (Analysis of Covariance) procedure for paired observations. Periodontal clinical measures at baseline and later time-points will comprise the dependent variables. The ANCOVA procedure will help to account for patient differences in demographics and other clinical factors at baseline while also adjusting for effects of confounding factors.

Hypothesis: "The combination therapy of HBOT and SRP in patients with not well controlled DM type2 and periodontal disease will improve the periodontal clinical parameters and reduce subgingival microbial load."

Time line and Milestones:

We anticipate a period of one year to complete the proposed research.
Protocol Submission and Approval: September 2015
Preparations, team training and calibration: 10/1/15-10/30/15
Patient recruitment, examination visits, medical evaluation and commencement, lab tests, data entry, treatment, follow-up: 11/01/2015-05/30/2016
Data analysis, manuscript writing, and final report preparation: 06/01/2015-08/30/2016

REASERCH PROPOSAL

PROJECT TITLE:

"Hyperbaric Oxygen as Adjunctive Therapy to Scaling and Root-Planing in the Management of Periodontitis in Poorly Controlled Diabetics Type 2."

Specific Aims (Statement of Purpose):

Our central hypothesis is based on the fact that hyperbaric oxygen (HBO) therapy expose patients to 100% oxygen at an accompanied high pressure (at 2-3 atmospheres absolute, ATA) to induce a state of hyperoxygenation which has an immediate effect (occurring while the patient is within the HBO chamber) and many other delayed effects (which occurs many days or weeks after the initiation of the course of therapy). Immediate effect involves increased oxidative killing by neutrophils and direct killing of anaerobic organisms and organisms that use oxygen as a substrate, due to high-pressure oxygen saturation in their cytoplasm. The delayed effect (which manifests between 8-10 treatments) involves: the release of growth factors such as PDGF and VEGF, and promoting neovascularization along with hypergranulation that would enhance wound healing. We hypothesize that these effects would improve the gingival tissue healing response following non-surgical periodontal therapy and would also decrease the subgingival microbial load.

We plan to test our central hypothesis and thereby accomplish the objective of this application by pursuing the following specific aims:

1) **Evaluate the adjunctive effect of HBO therapy on the clinical outcomes of non-surgical periodontal therapy (scaling/root planing, SRP).**

   Our working hypothesis, based on previous evidence: SRP as the current standard of care is not fully successful in restoring periodontal tissue health in not well controlled diabetics. HBO therapy as an adjunctive treatment with SRP would bring significant enhancement on tissue healing and improve clinical periodontal outcomes.

2) **Examine the adjunctive effect of the HBO therapy on subgingival microbial profile.**

Our working hypothesis, based on previous evidence: HBO therapy as an adjunctive treatment with SRP would cause a significant reduction in levels of anaerobic bacteria, furthermore the effect would persist for an extended period (in 3 months).

Background (Review of Literature):

Periodontitis is a chronic inflammatory disease that affects periodontal tissues and finally causes tooth loss (Casanova et al., 2014; Preshaw et al., 2012; Xiong et al., 2013).The prevalence of clinically defined periodontal disease is very high. It affects about 47-58% of adults in the United States (about 21.8% have mild form and 12.6% have moderate or severe form of the disease). Clinically defined periodontal disease has considerable impacts on personal and community health level and the treatment is costly: the dental care cost is the fourth highest cost of all diseases that uses between 5-10% of all healthcare resources (Garcia et al., 2015). Periodontitis has a complex nature and analytical epidemiological studies have identified multiple risk factors for the disease onset and progression. Diabetes is one of the strongly addressed risk factors of periodontal disease (Casanova et al., 2014; Christgau et al., 1998; Garcia et al., 2015; Michalowicz et al., 2014; Navarro-Sanchez et al., 2007; Xiong et al., 2013)

Diabetes mellitus (DM) refers to a group of metabolic diseases in which the level of blood sugar (glucose) is high due to defects in insulin secretion, insulin action or both. Diabetes affects 29.1 million people or 9.3% of the population in the United States; 21.0 million of them are diagnosed and 8.1 million are still undiagnosed 27.8% (CDC, 2014
; Garcia et al., 2015). Three well-known symptoms of high blood sugar are: polyuria, polydipsia, and polyphagia. Diabetics can have a range of complications (acute or chronic) such as: diabetic ketoacidosis, cardiovascular disease, chronic non-healing wounds, foot ulcers and periodontal disease (Casanova et al., 2014; Kidambi and Patel, 2008). Several studies documented a two-way biological relationship between diabetes and periodontal disease (Bascones-Martinez et al., 2014; Cirano et al., 2012; Garcia et al., 2015; Kumar et al., 2014; Santos et al., 2009; Tan et al., 2006; Xiong et al., 2013; Zhou et al., 2015) and periodontitis was designated as the sixth complication of diabetes (Bascones-Martinez et al., 2014; Casanova et al., 2014; Navarro-Sanchez et al., 2007). Longstanding DM and poor metabolic control can affect micro vascular blood circulation, PMN functions (bacterial killing, chemotaxis, phagocytosis), and collagens synthesis. Thickening of vascular basement membranes due to long-lasting hyperglycemia causes reduction in tissue nourishment and leukocytes migration (Christgau et al., 1998). Several studies reported that the mechanisms involved between DM and periodontitis are related to an altered hyper-inflammatory response (large secretion of inflammatory mediators, mainly pro-inflammatory cytokines) (Christgau et al., 1998; Cirano et al., 2012; Kumar et al., 2014; Navarro-Sanchez et al., 2007).The combination of changes in blood supply, local immune response, hyper-inflammation, and tissue structure alterations increase the risk of periodontal disease and tissue wound healing in diabetic patients (Navarro-Sanchez et al., 2007). The healing outcome of non-surgical periodontal therapy in non-controlled or poorly controlled diabetic patients is compromised (Casanova et al., 2014; Christgau et al., 1998; Navarro-Sanchez et al., 2007).

Hyperbaric Oxygen therapy (HBOT) is a mechanism that exposes patients to high pressure (2-3ATM equivalent to 33 to 66 feet under sea water (fsw)) to induce a state of hyper oxygenation, and has two states of effects: initial and delayed (Devaraj and Srisakthi, 2014). Immediate hyper oxygenation

effect (a direct effect) is against anaerobic organisms that cannot survive in an oxygen rich environment. Another initial effect is Enhancement of oxidative burst killing of the neutrophils in organisms that use oxygen as a substrate; also reduced adhesion molecules (Beta 2 integrin) of the neutrophils to destroy tight junctions between cells, and hence, reduce cellular and tissue destruction. "Direct killing of anaerobes" due to high-pressure oxygen saturation in their cytoplasm is another initial effect. The delayed stage (which manifests between 8-10 treatments) is: at first, promoting in growth factors such as PDGF and VEGF; furthermore, neovascularization along with hyper granulation occurs which enhance wound healing (Kindwall and Whelan, 1999) (Shinde et al., 2014),HBOT is a valuable adjunctive therapy for diabetic foot ulceration healing due to the above mentioned effects as well as increasing the tissue oxygen concentration (10 folds increase in general) and propagation and permeation of oxygen molecules to ischemic tissues up to 3 fold to maintain cellular level activities for surviving of those tissues. There is evidence to show that HBOT not only enhances healing of diabetic foot ulcers but also improves limb salvage (Goldman, 2009; Lipsky and Berendt, 2010). In addition, there is some evidence to suggest that HBOT can also improve the outcome of non- surgical periodontal therapy (SRP) and decrease the subgingival microbial profile (Chen et al., 2002; Chen et al., 2003; Chen et al., 2012; Devaraj and Srisakthi, 2014; Nogueira-Filho et al., 2010; Shinde et al., 2014; Signoretto et al., 2007)

In summary: DM is a growing chronic health problem in the United States and the world. It is a well-established risk factor for periodontal disease. Periodontitis in turn can increase insulin resistance and potentially aggravates diabetes. Managing periodontitis in diabetics is important. However, poorly controlled diabetics do not respond favorably to periodontal therapy. HBOT as an adjunctive therapy, along with SRP, has promise to improve the outcomes of non-surgical periodontal therapy and reduce subgingival microbial load in poorly controlled diabetics. It may also have greater impact on the subgingival microbial profile than SRP alone. The main objectives of the research are investigating the combined effect of both therapies vs. the effect of SRP alone on the periodontal clinical outcome as well as the subgingival microbial profile in poorly controlled diabetics.

Hypothesis: "The combination therapy of HBOT and SRP in patients with not well controlled DM and periodontal disease will improve the periodontal clinical parameters and subgingival microbial profiles due to HBOT immediate and delayed effects on neovascularization, growth factors, osteogenesis, PMN's killing function, and oxygen saturation in anaerobic bacterial cytoplasm."
Alternative hypothesis: Combination therapy of HBOT and SRP in patients with poorly controlled DM and periodontal disease will not have a significant improvement on the periodontal clinical parameters and subgingival microbial profiles.

RESEARCH DESIGN AND METHODS

The Approach:

Study Design: The proposed pilot study is a longitudinal observational study to compare periodontal treatment (SRP) outcome between 2 diabetic patient groups receiving medical care at LLUMC either with or without adjunctive Hyperbaric Oxygen Therapy. The results will provide essential preliminary data to plan for an R21 NIH study that will be powered to comprehensively assess treatment outcomes and better understand the mechanisms involved.

Subject Characteristics:
All subjects will be recruited from the diabetic patient pool at LLU Health. Drs. Bland and Mohammadzadeh at their respective institutions will work together to identify and refer prospective subjects to the study. All subjects must have not well controlled DM type 2 (as defined below). In addition, all subjects must have generalized moderate to severe chronic periodontitis (as defined below).
A total of 24 not well controlled type 2 diabetic subjects with Periodontitis presenting for medical care at LLU Health will be recruited for the study (see power analysis under statistical section). An additional 6 subjects will also be recruited to compensate for subject attrition. Subjects will be divided into two group based on their medical needs: HBO therapy and Non HBO therapy. For all subjects demographic data (age, gender, ethnicity, smoking history, alcohol use history, BMI, current medication list) and oral health habits will be obtained. HBOT subjects will be recruited first and control subjects (non HBO group) will be recruited after to match the HBO subjects on all parameters including HbA1c level to minimize bias.

Inclusion Criteria:

Diabetic patients must meet the following inclusion criteria:

1. An established diagnosis of DM type 2 (HbA1c =>7%).
2. Subjects must have a minimum of 10 permanent teeth.
3. Age range 35 year or older.
4. All subjects must be registered patients at LLU Health.

Exclusion Criteria:

Individuals with the following conditions may NOT participate in the study:

1. Well-controlled type 2 diabetics (HbA1c <7%) and type 1 diabetic patients.
2. Other systemic conditions with known association with periodontitis, e.g. AIDS, Down's syndrome, current smoker, head and neck radiation, Xerostomia, currently under chemotherapy.
3. Intra-oral conditions present which might interfere with accuracy of periodontal probing, e.g. gingival overgrowth, orthodontic wires, excessive calculus.
4. Not a registered patient at LLU Health.
5. Overweight/obese individuals (BMI greater than 45 %)
6. Pregnancy or lactation

QUESTIONNAIRE:

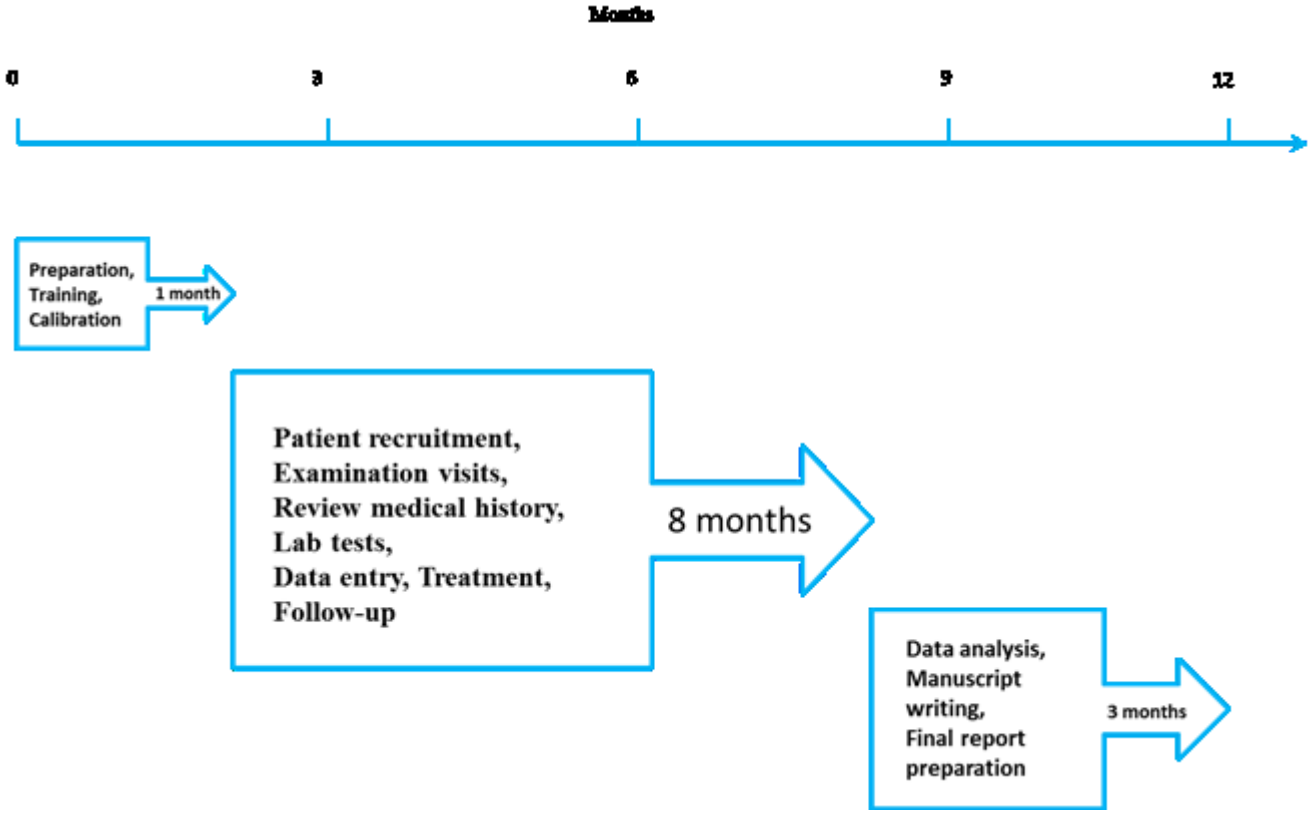
Oral Health Interview: subjects will be interviewed regarding personal and professional dental care habits and cigarette smoking habits.

Study Plan:

Group	**Not eligible for HBOT N=15**	**Eligible for HBOT N=15**
Medical History Review Medical Assessments	**Yes**	**Yes**
Baseline HbA1c	**Yes**	**Yes**
Baseline Periodontal Evaluations	**Yes**	**Yes**
SRP	**Yes**	**Yes**
HBO Therapy	**No**	**Yes** (20-40 daily treatments for 8 weeks)
Final Periodontal Evaluation (12 weeks after SRP)	**Yes**	**Yes**
Final HbA1c (12 weeks after SRP)	**Yes**	**Yes**

Note: subjects in both groups will be receiving any needed medical care

Study **Time** **Frame**



CLINICAL EVALUATIONS

Medical Assessments:
Determination of diabetes status: review of medical history and Hemoglogin A1C testing will be done under the direction of Dr. Bland or qualified residents. All subjects entered in the study will go through a comprehensive medical evaluations and blood testing to determine the diabetic status.

Dental/Periodontal Examination:
Dr. Mohammadzadeh will direct and supervise all dental/periodontal examinations. The dental examiners will be blinded to HBOT therapy status. At initial examination and 4 weeks after HBOT, all subjects will undergo comprehensive oral evaluations followed by recoding of the Loe 1967 gingival index (GI), periodontal probing measurements (PPD), bleeding on probing (BOP), clinical attachment loss (CAL), and Quigley & Hein plaque index (PI). Average scores will be computed for each measure per subject.

Periodontal Probing Measurements:
All fully erupted teeth present, excluding third molars, will be measured. A pressure controlled periodontal probe (Williams Probe, with 1mm marking, Hu-Friedy) will be used to determine probing depth at six sites per tooth: mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual. The position of the gingival margin in relation to the cementoenamel junction will also be recorded at the same 6 sites per tooth to the nearest mm. A negative sign will be given

when the gingival margin is coronal to the cementoenamel junction and a positive sign will be given when it is apical. Clinical attachment level (CAL) will be calculated by adding probing depth to position of gingival margin. Percentage of teeth with at least one site having loss of attachment (CAL) =>5 mm will be calculated for each subject. Also, the presence or absence of gingival redness, suppuration, bleeding on probing (BOP), and supragingival plaque accumulation will be recorded. Clinical measurements will be taken at baseline and at 3 months post-therapy.

Periodontitis diagnosis:
Periodontal diagnosis will be based on the 1999 AAP Classification of Periodontal Diseases. Diabetic individuals with Chronic Periodontitis will be recruited for the study. Selected subjects must have 5% or higher of scored teeth with attachment loss =>5 mm and probing depth =>5 mm.

Treatment Protocol:
Subjects in both treatment groups will receive full-mouth scaling and root planing (SRP) under local anesthetic, and instruction in proper homecare procedures at Prince Hall School of Dentistry at LLU. Five dental student investigators will provide full-mouth SRP. Wound care management will be provided to all subjects at LLUHealth. The hyperbaric treatment group will also receive their oxygen therapy protocol as planned in the medical center. Each session of HBOT would be given in LLU Health Hyperbaric Treatment Center per order of hyperbaric clinician.

Calibrations:
Dr. Mohammadzadeh (as mentioned in page2) is sole examiner and assessor of all steps, and would be blinded regarding the status of the patients. Dr. Khocht is an experienced examiner who has been repeatedly calibrated in performing the measures described above. Before data collection begins and periodically throughout the study period, Dr. Khocht calibrates the dental examiners to insure reliability of clinical measurements, and prevent bias introduced by "examiner drift".

Sample Collection

Blood Sampling:
Blood will be collected by a single venipuncture. The initial blood sample will be collected at the baseline appointment and the final blood sample will be collected at the last appointment (3 months after completing SRP).

Microbiological Sampling:
Microbial samples will be collected by same examiner at baseline and 3 months after SRP. Samples will be taken from the same sites at all visits. Subgingival samples will be collected from 3 randomly selected sites with probing depth => 5mm. The sample sites will be isolated with cotton rolls and air-dried. Supragingival plaque will be removed with sterile cotton pellets and scalers. Subgingival plaque samples will be taken separately from each site using sterile Gracey curettes. All three samples will be pooled in 1.5-mL tube containing 50 mL TE (50 mM Tris-HCl and 1 mM EDTA [pH7.6]) (Khocht et al., 2012).

Microbiological Analysis:

Collected microbial samples will be processed for DNA extraction and stored at Loma Linda University in the research laboratory of Dr. Li, then sent to Alexis Kokaras (HOMINGS) at the Forsyth Institute (245 First Street, Cambridge, MA). A total of 300 of the most prevalent oral bacterial species, including many that cannot be grown in the laboratory will be assessed by Dr. Bruce Pastor (Forsyth Institute, Boston, MA).

The following technique will be used:
For bacterial DNA extraction, 44 mL of each sample will be taken, and 0.5% polysorbate 20 and 1 mL of protease K (10 mg/mL) will be added. The samples will be heated at 55_C for 2 hours and at 95_C for 5 minutes for inactivation of protease K. All samples will be stored in a freezer at -80_C before polymerase chain reaction (PCR) amplification. The HOMIM methodology will be used here was described in detail in a previous study. Briefly, a total of 400 16S rRNA-based, reverse-capture oligonucleotide probesii targeting >300 bacterial taxa. 16S rRNA genes are PCR amplified from DNA extracts using 16S rRNA universal forward and reverse primers and labeled via incorporation of cyanine 3–deoxycytidine triphosphate in a second nested PCR. The labeled 16S amplicons will be hybridized overnight to probes on the slides. After washing, the microarray slides will be scanned and crude data will be extracted using software for microarray image analysis. Data will be normalized by comparing individual signal Data will be normalized by comparing individual signal intensities to the average of signals from universal probes(Ahn et al., 2011; Colombo et al., 2012; Lourenco et al., 2014)

Statistical Analysis:
Udochukwu Oyoyo, MPH, Assistant Professor, Loma Linda University School of Dentistry, Department of Dental Research, will perform statistical analysis.

Power Analysis:
To detect a difference of 2 mm in average pocket depth between the Non HBO+SRP and HBO+SRP treatment groups and assuming a standard deviation of 1mm, the study would need to have 12 subjects in each group to have an 80% chance of detecting the stated effect, at an alpha level of 0.05.

Hypothesis Testing:
We plan to test our central hypothesis and thereby accomplish the objective of this objective of this application by pursuing the following specific aims:

1) Evaluate the effect of HBO therapy on clinical outcomes of SRP.
Our working hypothesis, based on previous studies on SRP as a current standard of care in the non-surgical periodontal therapy, is that SRP alone is not fully successful in restoring tissue health and clinical outcomes such as GI, PPD, CAL, and BOP. The HBO therapy with SRP may have a synergic effect on enhancing blood supply, osteogenesis, and promoting growth factor production and can cause significant enhancement on tissue healing and clinical periodontal outcomes.

Statistical analysis:

Differences in pocket depth from baseline to 12 weeks between the Conventional Wound Healing group and Hyperbaric Therapy group will be analyzed using the repeated measures ANCOVA (Analysis of Covariance) procedure for paired observations. Periodontal clinical measures at baseline and later time-points will comprise the dependent variables. The ANCOVA procedure will help to account for patient differences in demographics and other clinical factors at baseline while also adjusting for effects of confounding factors.

2) Examine the effect of the HBO therapy on subgingival microbial profile.
Our working hypothesis, based on initial effect stage of HBO therapy, is significant reduction and low levels of anaerobes bacteria persist after 3 months of combination therapy compare with only current standard periodontal treatment.

Statistical analysis:
Differences in bacterial counts from baseline to 12 weeks between the Conventional Wound Healing group and Hyperbaric Therapy group will be analyzed using the repeated measures ANCOVA (Analysis of Covariance) procedure for paired observations. Bacterial counts as measured at baseline and later time-points will comprise the dependent variables. The ANCOVA procedure will help to account patient differences in demographics and other clinical factors at baseline while also adjusting for effects of confounding factors.

Potential difficulties and limitations:

Antibiotic medications:
Antibiotic medications used in conventional wound care may influence the clinical and microbial factors we are measuring. To control for their potential influence, we will keep a log of all medications and doses. Subjects enrolled in the proposed study will not be asked to refrain from their antibiotics and other medications as part of their wound healing treatment prior to examination and sampling. We will collect our baseline data after the subjects start their medication regimen. Thus, the subgingival bacterial samples and clinical parameters recorded at baseline will account for antibiotic use prior to periodontal intervention. Furthermore, in our statistical analysis we will adjust for the number and class of medications.

Study Design: We elected to go for a longitudinal observational design because of budget limitation. Our objective is to collect pilot data to enable us to seek external founding for a fully powered “Randomized Clinical Trial”.

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