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14. ABSTRACT

Microbial colonization is thought to play a critical role in the healing trajectory of wounds. The microbial load and community composition are both important variables that, historically, were extremely difficult to characterize. Under this award, we have developed a suite of cutting-edge molecular tools, databases, and analytical approaches to enable more accurate characterization of wound microbiota. In the past year, we developed and validated culture-independent molecular tools for quantifying and identifying wound fungi. We also initiated a prospective study to elucidate the impact of hyperbaric oxygen therapy (HBOT) on chronic wound microbiota, using our novel tools and approaches. We have also begun using whole genome sequence analysis to identify the genetic characteristics that enable *Staphylococcus aureus* to progress from simple skin and soft tissue infections to sepsis and endocarditis. We are confident that this work will lead to significant advancements in wound care and healing and human microbiome research in general.

15. SUBJECT TERMS

Burn wounds; Chronic wounds; Microbiota; Microbiome; Culture-independent microbial characterization; Quantitative polymerase chain reaction (qPCR); Fungi; Bacteria

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I. INTRODUCTION

Work has proceeded quite successfully in this grant year. A protocol to prospectively collect diabetic foot wound clinical samples has been approved through the respective IRBs of the clinical site, Banner Good Samaritan (BGSMC) Wound Care Center, and TGen. Recruitment for this study is ongoing at the Wound Care Center and three shipments of samples and clinical data have been sent to TGen. In addition, *Staphylococcus aureus* isolates have been obtained from a collaborator at Duke University and sequencing of the *S. aureus* genomes has commenced.

II. BODY

II A. Summary of Work Performed in Quarters 1 - 3.

Prospective clinical sample collection

IRB approval was received in the third quarter to initiate sample collection at the clinical site, the BGSMC Wound Care Center. Swab and curette samples were collected from subjects with diabetic foot wounds undergoing hyperbaric oxygen therapy (HBOT) as part of their standard of care treatment. In addition to these HBOT subjects, samples were also collected from control subjects, who were clinical candidates for HBOT, but who did not receive HBOT due to personal/time commitment or insurance reasons. Samples were collected during standard of care debridement procedures for both the HBOT and control subjects. In addition, a second set of samples were obtained from HBOT subjects after their HBOT session. All samples were immediately frozen on dry ice after collection and shipped to TGen on dry ice. Sample collection was launched on 5/28/12 and at the end of the third quarter, 1 HBOT subject and 2 control participants had been enrolled. A total of 52 samples were collected during this quarter: 26 swab samples and 26 curette samples.

S. aureus sample collection and analysis

A Material Transfer Agreement was completed with collaborators at Duke University and 200 *Staphylococcus aureus* isolates were shipped to TGen. In the third quarter, 27 of these *S. aureus* genomes were sequenced. Continued sequencing of isolates and analysis of all sequenced isolates is planned for the next grant year.

II B. Work Performed in Quarter 4.

Prospective clinical sample collection

Subject enrollment and sample collection continued at the BGSMC Wound Care Center. An additional HBOT subject and 3 additional control subjects were enrolled, for a total of 7 subjects enrolled. During the fourth quarter, 1 HBOT subject and 2 control subjects completed the study. Thirty one samples were collected in this quarter, for a total of 83 samples collected since the study began, of which 46 are curette samples and 39 swab samples. There have been three shipments of samples to TGen from the Wound Care Center and all samples are currently stored at -80C.

IV. REPORTABLE OUTCOMES

Article title: FungiQuant: A broad-coverage fungal quantitative real-time PCR assay
MS ID: 1794266190728898
Authors: Cindy M Liu, Sergey Kachur, Michael G Dwan, Alison G Abraham, Maliha Aziz, Po-Ren Hsu, Yu-Tsung Huang, Joseph Busch, Louis J Lamit, Catherine A Gehring, Paul Keim and Lance B Price
Journal: BMC Microbiology

V. CONCLUSION AND FUTURE DIRECTIONS

In the third year, we will continue to sequence *S. aureus* isolates obtained from collaborators at Duke University and to perform comparative genomic analyses on the samples. We will continue to prospectively collect samples from HBOT patients and controls with diabetic foot wounds. We will perform bacterial and fungal load analyses on all of the HBOT wound samples. Finally, we will complete the validation and integration of wound assays developed in the first year of this grant.

VI. APPENDICES

APPENDIX A. IRB-Approved Clinical Sample Collection Protocol

TRANSLATIONAL GENOMICS RESEARCH INSTITUTE

HUMAN SUBJECT PROTOCOL

1. General Protocol Information

Investigators:

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Glenn Silverstein, DPM
Banner Good Samaritan Medical Center (BGSMC)
Wound Care and Hyperbaric Medicine Center
Assurance #: FWA00002630
Assurance expiration date: 5/19/2013
IRB of record for subject recruitment and clinical procedures: BGSMC IRB
1012 East Willetta Street
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Clinical Research Coordinator:
Lora Nordstrom, PhD, BSN, CCRC (TGen)

Sponsor: Department of Defense (DOD) Telemedicine and Advanced Technology Research Center (TATRC)

Protocol Title: The Impact of Hyperbaric Oxygen Therapy on the Wound Microbiome, Wound Host-Microbe Interactions, and Patient Outcomes

Protocol Number: lprice11-023

2. Subject Issues.

(a) Number and brief description of subjects to be enrolled at TGen: No subjects will be enrolled at TGen. All subjects will be enrolled by clinical collaborators at Banner Good Samaritan Medical

Center (BGSMC).

(b) Number and brief description of subjects to be enrolled elsewhere: Patients with diabetic foot ulcers who are receiving care for their wounds as outpatients at the Wound Care Program at BGSMC will be screened, consented, and enrolled in this study. Up to 100 subjects will be enrolled in this study. It is expected that a certain percentage of participants will not complete the study due to missed study visits; thus, up to 100 participants will be enrolled in order to accrue 25 subjects actively receiving **both** standard-of-care wound care and adjunctive hyperbaric oxygen therapy and 25 diagnosis- and sex-matched control subjects, who are receiving standard-of-care wound care, but not adjunctive hyperbaric oxygen therapy.

The sample size is based on typical enrollment rates and the number of patients that would meet enrollment criteria within the Wound Care Program over the time frame of the study. Since this is the first study of its kind, a true statistical sample justification could not be determined.

☒ **Adults** ❑ **Children** ☒ **Males** ☒ **Females** **Age Range:** 18-88 years

❑ **Yes** ☒ **No** **Do TGen Personnel Interact with Living Individuals?**

☒ **Yes** ❑ **No** **Do Collaborating Investigators Interact with Living Individuals?**

☒ **Yes** ❑ **No** **Do Collaborating Investigators Provide Human Biospecimens?**

❑ **Yes** ☒ **No** **Do Collaborating Investigators Provide Information about Individuals?**

❑ **Yes** ☒ **No** **Is any genetic testing for clinical proposes involved?**

Please explain any "YES" responses. All wound biosamples and annotated clinical information will be collected by clinical collaborators at BGSMC. All biosamples and clinical data will be completely de-identified and assigned a study-specific code number. The key linking the study code to identifiable subject information will be kept by the clinical PI at BGSMC on a password-protected database. TGen investigators will be prohibited from accessing or attempting to gain access to the code.

3. Radiation or Bio-Safety Considerations

DESCRIBE ANY RADIATION USE OR BIO-SAFETY ISSUES SUCH AS USE OF INFECTIOUS AGENTS, REGULATED TOXINS, RECOMBINANT DNA, HUMAN GENE TRANSFER, ETC

No radiation will be used in this study. Standard Universal Bio-safety precautions will be taken when handling the human samples.

4. Regulatory Criteria for IRB Approval of Research.

FEDERAL REGULATIONS AT 21 CFR 56.111 AND 45 CFR 46.111 REQUIRE THE IRB TO DETERMINE THAT THE FOLLOWING CRITERIA ARE SATISFIED.

4.1 Risks are Minimized Through Sound Research Design

(a) Briefly state the hypothesis or the objectives of the proposed research.
(b) Briefly describe the background of the research, citing scientific literature as appropriate.
(c) Fully describe the research design and procedures (including statistical design).
(d) Briefly describe the research team's qualifications for performing the proposed research.

(a) Objectives.

Our overall objectives are to:
1) Elucidate the impact of hyperbaric therapy on the microbial ecology, host immunity, and host-microbe interactions in diabetic foot ulcers;
2) Determine the host immunological and microbial predictors of foot ulcer outcomes in diabetic patients.

Hypothesis: Hyperbaric oxygen therapy results in decreased absolute and relative abundance of anaerobic bacterial species in diabetic foot ulcers in comparison to standard of care diabetic foot ulcer management.

(b) Background.

Chronic wounds are wounds that require a prolonged time to heal, do not heal, or recur; and it is a major public health in the United States and globally. It has been estimated that 1% of the industrialized countries will experience a leg ulcer at some time. Broadly, chronic wounds encompass four major categories, which are diabetic foot ulcers, venous ulcers (i.e., associated with venous insufficiency), arterial ulcers (i.e., associated with limb ischemia), and pressure ulcers. In diabetes mellitus, the development of foot ulcers can usually be ascribed to peripheral neuropathy and/or peripheral vascular disease, which can be venous or arterial. The annual incidence of foot ulcers among people with diabetes have been estimated at 2.5-10.7%, with an annual incidence of amputation at 0.25-1.8%. It has also been estimated that 50% of diabetic foot ulcers become infected at some point, with 25% of the infected foot ulcers resulting in lower limb amputation, making wound infection the most important risk factor for amputation in

To address this challenging clinical group of disease, we need to better understand the pathophysiology underlying the chronicity and recurrence of chronic wounds. One of the major hypotheses of delayed wound healing in chronic wounds is hypoxia, which has led to the investigation of adjunctive hyperbaric oxygen therapy for chronic wound patients. Hyperbaric oxygen therapy is a non-invasive therapy that uses 100% oxygen under increased atmospheric pressure in a controlled body chamber. Another important knowledge gap is the role of the wound microbiome the resultant host-microbe interactions. While there have been several studies that have shown the potential benefit of adjunctive hyperbaric oxygen therapy in chronic wound patients, particularly in diabetic foot ulcers for reducing major limb amputations, there has been no study that investigates the impact of hyperbaric oxygen therapy on the wound microbiome and the wound host-microbe interactions in patients with diabetic foot ulcers. Therefore, in this collaborative study, we will perform a prospective, observational study using cutting-edge molecular genomic, transcriptomic, and proteomic techniques to study the molecular impact of hyperbaric oxygen therapy in patients with diabetic foot ulcers.

(c) Design and Procedures

Identification of Participants: Two groups participants will be identified:

- **Treatment Group:** Diabetic foot ulcer patients actively receiving both standard-of-care and adjunctive hyperbaric oxygen therapy for their wounds.

- **Control Group:** Diabetic foot ulcer patients receiving the standard-of-care wound treatment but who are **not** receiving adjunctive hyperbaric oxygen therapy.

Collection of Biospecimens: Biospecimens from wounds will be collected via swab and/or curette during routinely scheduled clinical visits to minimize any discomfort for the subjects. The most difficult-to-heal site will be selected and will be recorded and photographed at each sample collection. The site will be cleansed to remove surface dirt using a gauze pad saturated with sterile normal saline before sample collection. All swab and curette samples will be collected from the apposing leading edges of each wound (proximal and distal), as well as the center of each wound, if the wound is of sufficient size to obtain discrete samples from each area. With smaller wounds (those less than 2 cm in diameter) only a single curette and swab sample will be collected from the center of the wound. Swab samples will be collected using a swab pre-moistened in sterile saline. NOTE: swab samples should be taken from the wound base after curette debridement for the weekly debridement visits.

Curette samples will be collected from wounds until the treating physician makes a determination that collection of a curette sample would be detrimental to the wound healing process. Swab samples will be collected until the wound has healed completely.

Routine Sample Collection

- **Treatment Group**: Patients receiving adjunctive hyperbaric oxygen therapy have regularly scheduled clinic visits 5 days per week for approximately 6 weeks. As the standard of care for these wounds involves weekly debridement, samples will be collected at weekly debridement visits. After normal saline cleansing, both swab and curette samples will be collected during the debridement procedure, as well as after the adjunctive hyperbaric oxygen therapy. Thus, two sets of swab and curette samples will be collected during the debridement visits. The swab and curette samples will be collected at the same location on the wound (distal, proximal and center or center only, if the wound is small), with curette samples collected first, followed by the swab samples. All swab and curette samples will be immediately flash frozen on dry ice or in liquid nitrogen and stored at -80°C at BGSMC, until they can be shipped to TGen for analysis.

- **Control Group**: Patients who do not receive adjunctive hyperbaric oxygen therapy are scheduled for weekly clinic visits, at which time their wounds are debrided. Patients not receiving hyperbaric treatment usually have 7 – 8 weekly clinic visits. Matching swab and curette samples will be collected at the same locations (distal, proximal, center or center only, if the wound is small) during the weekly clinic visits from subjects in this group. The curette samples will be collected first, followed by the swab samples. All swab and curette samples will be immediately flash frozen on dry ice or liquid nitrogen and stored at -80°C at BGSMC, until they can be shipped to TGen for analysis.

  If a participant in the control group misses more than 2 regularly scheduled clinic appointments in a row, he or she will be withdrawn from the study, as his/her wound healing will likely be affected by the cessation of treatment.

Schedule of Sample Collection:

- **Treatment Group**

  Study visit 1:
  - Participant will be enrolled in the study after a thorough informed consent discussion
  - Initial clinical data will be collected
  - A photograph will be taken of the wound from which samples will be collected
  - The wound site will be cleansed with normal saline
  - Three curette samples will be collected during the debridement procedure, from the center, proximal and distal edges of the wound. **Note:** three samples will be collected for wounds greater than or equal to 2 cm in diameter. For wounds less than 2 cm in

diameter, single curette and swab samples will be collected.
    - Collection of curette samples will be followed by collection of three swab samples from the center, proximal and distal edges of the wound.
    - The participant will undergo standard of care hyperbaric therapy
    - Three curette samples will be collected after the hyperbaric treatment, from the center and proximal and distal edges of the wound. **Note:** three samples collected for wounds greater than or equal to 2 cm in diameter. For wound less than 2 cm in diameter, single curette and swab samples will be collected.
    - Collection of curette samples will be followed by collection of three swab samples from the center and proximal and distal edges of the wound.

  Subsequent weekly study visits:
    - Follow-up clinical data will be collected
    - A photograph will be taken of the wound from which samples will be collected
    - The wound site will be cleansed with normal saline
    - Three curette samples will be collected during the debridement procedure, from the center, proximal and distal edges of the wound. **Note:** three samples will be collected for wounds greater than or equal to 2 cm in diameter. For wounds less than 2 cm in diameter, single curette and swab samples will be collected.
    - Collection of curette samples will be followed by collection of three swab samples from the center and proximal and distal edges of the wound.
    - The participant will undergo standard of care hyperbaric therapy
    - The wound site will be cleansed with normal saline
    - Three curette samples will be collected after the hyperbaric treatment, from the center, proximal and distal edges of the wound. **Note:** three samples will be collected for wounds greater than or equal to 2 cm in diameter. For wounds less than 2 cm in diameter, single curette and swab samples will be collected.
    - Collection of curette samples will be followed by collection of three swab samples from the center, proximal and distal edges of the wound.

- **Control Group**
  Study visit 1:
    - Participant will be enrolled in the study
    - Initial clinical data will be collected
    - A photograph will be taken of the wound from which samples will be collected
    - The wound site will be cleansed with normal saline
    - Three curette samples will be collected during the debridement procedure, from the center, proximal and distal edges of the wound. **Note:** three samples will be collected for wounds greater than or equal to 2 cm in diameter. For wounds less than 2 cm in diameter, single curette and swab samples will be collected.
    - Collection of curette samples will be followed by collection of three swab samples from the center, proximal and distal edges of the wound (see Note above).

  Subsequent weekly study visits:
    - Follow-up clinical data will be collected
    - A photograph will be taken of the wound from which samples will be collected
    - The wound site will be cleansed with normal saline
    - Three curette samples will be collected during the debridement procedure, from the center, proximal and distal edges of the wound. **Note:** three samples will be collected for wounds greater than or equal to 2 cm in diameter. For wounds less than 2 cm in diameter, single curette and swab samples will be collected.
    - Collection of curette samples will be followed by collection of three swab samples from the center, proximal and distal edges of the wound (see Note above).

Collection of Clinical Data: In addition to biospecimens, annotated clinical information will be

obtained from each study participant (see attached data collection forms). Briefly, basic demographic data will be collected as well as medical history of the wound, including: history of wound care, medications and treatments received, and co-morbid conditions. In addition, data from transcutaneous oximetry measurements (TCOM), which will be provided as part of standard of care therapy for all study participants, will be collected.

All diabetic foot ulcer patients who receive adjunctive hyperbaric oxygen therapy are tested for blood glucose levels before and after treatment. This data will be collected for study purposes. Diabetic patients not receiving hyperbaric oxygen therapy do not routinely blood glucose testing performed at their clinic visits; thus, a research-specific blood glucose reading, collected via peripheral finger stick, will be performed at weekly sample collection visits, using a glucose monitor at the clinic (not the patient's personal glucose monitor). Hemoglobin A1C (HgbA1C) values will be collected from participants when performed as part of their standard of care treatment.

No PHI will be collected for this study. All clinical data will receive a bar-coded study number, which matches the collected biosamples from each subject. The key linking the study code to identifiable subject information will be kept by the clinical PI or delegee on a password-protected database. No TGen employees may access or attempt to gain access to this key.

Laboratory Methods:

Once samples are transferred to the Pathogen Genomics Division at TGen, they will be processed and analyzed as follows:

1) Isolate and purify total DNA, RNA, and Protein from each sample
2) Analyze the microbial community composition by sequencing analysis
    a. 16S targeted approach for community-level bacterial characterization
        i. Amplify the 16S rRNA gene or cDNA generated from16S rRNA using fusion PCR primers.
        ii. Pool the barcoded amplicon for sequencing
        iii. Sequence the amplified 16S rRNA gene region.
        iv. Process the resultant sequences using an in-house data processing pipeline to assay each sequence to its sample source.
        v. Assign taxonomic classification to each processed sequence.
        vi. Generate community matrix data for comparative ecological analyses including: rarefaction, species accumulation curve, diversity calculations, and other ecological community visualization and analyses.
        vii. Sequences that could not be classified taxonomically will be analyzed phylogenetically and full-length 16S rRNA gene sequence analysis may also be performed.
    b. Non-targeted metagenomic approach
        i. Shear total DNA or RNA content
        ii. Separate human DNA or eukaryotic RNA from the microbial contingent by subtraction technique
        iii. Ligate barcoded sequencing adapter to sheared DNA or RNA
        iv. Sequence the resultant DNA or RNA shotgun library using next-generation sequencing technology.
        v. The resultant sequences will be analyzed using a combination of BLAST, MEGAN, and other computational tools to elucidate the DNA and RNA metagenome.
    c. Non-targeted RNA-based transcriptome approach
        i. Separate human RNA from bacterial RNA
        ii. Isolate RNA from each component
        iii. Create human and microbial cDNA mRNA libraries
        iv. Sequence transcriptome on next-generation sequencing platforms.

d. Non-targeted Proteomics approach
    i. Perform lysis and extraction for proteins from each sample.
    ii. Perform additional enzymatic digestions to isolate desired protein contingents
    iii. Proteome sequencing and analysis

3) Quantify specific target organisms using species/strain specific quantitative PCR assays.
    a. Measure associations between quantities of specific pathogens and wound healing.

4) Measure diabetes-related inflammatory and wound healing proteins and gene expression among the subjects
    a. Measure associations among proteins, gene expression, wound microflora and wound healing.
    b. Expression will be assessed by microarray analysis, mass spectrometer, sequencing, qPCR and/or ELISA.
    c. Host genotyping

4.2 Risks Are Reasonable in Relation to Anticipated Benefits

(a) Fully describe the reasonably foreseeable (physical, psychological, social) risks, side effects, and discomforts to the subject of the proposed research.
(b) Describe steps taken to minimize these risks (e.g., instituting specific protections, choosing specific techniques, or relying on procedures already being performed for other purposes).
(c) Describe the reasonably anticipated benefits of the research to subjects and the importance of the knowledge that may be reasonably expected to result.
(d) Demonstrate that the risks are reasonable in relation to these benefits and/or resultant knowledge.
(e) Justify the use of a placebo control group if one is included in the proposed research.

(a) Risks. There is a slight risk of pain, infection and/or bleeding involved in taking samples from open wounds. Aseptic technique will be used at all times during the collection process and only study staff who are trained in collection of samples from wounds will be involved in collection procedures. All sample collections from wounds will be done at the same time as normal clinical procedures/assessments. Analgesis will be administered as needed during sample collection. The Wound Care Clinic utilizes the following options for analgesia:

- Topical 4% Lidocaine solution
- Topical 5% Lidocaine gel/ointment
- Injected 1% and 2% Lidocaine plain
- Injected 0.5% Marcaine plain

Possible side effects of these medicines include: skin irritation, fatigue, weakness, dizziness, blurred vision, numbness, and or tingling.

The decision to use hyperbaric therapy, in addition to standard of care treatment, for diabetic foot ulcers is entirely dependent on the study participants' decisions of whether or not to have hyperbaric treatment. This decision is made in consultation with the participant's physician. There are some known risks involved in this treatment. The most common side effect is barotrauma to the ears and sinuses due to the change in pressure. To minimize this effect, the hyperbaric technicians will work with the patients to show them techniques for ear-clearing. A rare side effect is oxygen toxicity. All patients will be continuously monitored during the procedure by wound care staff and are given "air breaks" at scheduled times during the procedure to prevent oxygen toxicity.

In addition to the physical risks outlined above, there may be some emotional anxiety for subjects, regarding the fact that subjects will not directly benefit from this study, nor will they receive any

results from the analysis of there samples.

(b) Risks Minimized. As described above, every effort will be made to make certain that sample collection uses aseptic technique and occurs at the same time as clinical procedures, to decrease any discomfort associated with the procedure. Hemostasis will be ensured after each sample collection. No additional samples will be collected from subjects exhibiting a coagulopathy. If any subject requires immediate medical care related to a study-related procedure, emergency medical care will be provided at the hospital. No subjects will receive compensation for a research-related injury.

To minimize any emotional discomfort, the voluntary nature of the study will be stressed at all times and measures to keep study data confidential will be communicated to study subjects.

(c) Benefits. There will be no direct benefit to subjects participating in this study. The subjects may, however: feel good about participating in research that may help wound patients in the future.

(d) Risks Reasonable. The physical risks outlined above may be lessened to a great degree with meticulous sample collection techniques. Given these facts, the risks are minimal compared with the potential to benefit subjects in the future with wounds in terms of improved prognostics and treatments.

(e) Placebo. There will be no placebo group for this study.

4.3 Selection of Subjects is Equitable

(a) Describe the procedures for recruitment of subjects, including any advertising for subjects.
(b) Describe the inclusion and exclusion criteria for each subject population. Provide justification for excluding any subject populations or groups from the research.
(c) Describe how subjects will be assigned to experimental and control groups.
(d) Describe any compensation or other inducements that will be offered to subjects for participating in the research, and the schedule/procedure for conveying such inducements.
(e) List any costs to the subject associated with participation in the research.
(f) Describe any compensation or other inducements that will be offered to investigators for conducting the research, and the schedule/procedure for conveying such inducements

(a) Recruitment. Potential subjects will be recruited for this study when they present for care at the BGSMC Wound Care Clinic. Subjects will be approached by the clinical PI or sub-investigator and a detailed informed consent discussion will take place using the informed consent form as a guide for the discussion. No advertising will be used to recruit subjects for this study.

(b) Inclusion/Exclusion Criteria.

Treatment Group

Inclusion Criteria:

- Eighteen years of age or older
- Has been diagnosed with diabetic foot ulcer
- Has diabetes mellitus
- Patient is a candidate for adjunctive hyperbaric oxygen therapy and will receive adjunctive hyperbaric oxygen therapy
- Patient has received TCOM as part of standard of care treatment.

Exclusion Criteria:

- Prisoners
- Pregnant women

Control Group

Inclusion Criteria:

- Eighteen years of age or older
- Has been diagnosed with diabetic foot ulcer
- Has diabetes mellitus
- Patient is a candidate for adjunctive hyperbaric oxygen therapy but will NOT receive adjunctive hyperbaric oxygen therapy
- Patient has received TCOM as part of standard of care treatment.

Exclusion Criteria:

- Prisoners
- Pregnant women

(c) Assignment to Groups. Subjects will be assigned to groups with different collection procedures based on their personal decisions for their clinical treatment course (i.e., the addition of hyperbaric oxygen therapy to their normal diabetic foot ulcer care). As this study uses convenience sampling, we will have knowledge of whether each potential subject meets the inclusion criteria for either the treatment or the control group. Therefore, during consent and enrollment, all potential study subjects will be assigned and enrolled into the appropriate study group, as they will have already consulted with their physician regarding treatment options.

(d) Compensation. Subjects will not be compensated in any way for participation in this study.

(e) Costs. Subjects will not incur any additional costs for participation in this study.

(f) Investigator Inducements. Study investigators will not receive any inducements for participation in this study.

4.4 **Informed Consent Process and Documentation**

(a) Describe the informed consent process. If the consent process will occur in a context that might involve lowered comprehension (e.g., emergency room setting, crisis state, under sedation), explain how comprehension will be assured. If children are involved in the research, describe the process for obtaining the permission of parents and the assent of the child-subjects.

(b) If Informed Consent will not be obtained, or if an informed consent document will not be used, explain why. Show how obtaining consent would not pose greater than minimal risks to subjects, would not adversely affect their rights and welfare, and would not be practicable.

(a) Consent. A complete informed consent discussion will take place for all subjects, lead by the clinical P, a sub-investigator or clinical research coordinator. The discussion will include the risks and benefits of study participation, as well as the voluntary nature of the study. Subjects will be given ample time to ask questions and to determine whether they would like to participate in the study.

(b) Waiver of Consent. All subjects will be required to provide written, informed consent in order to participate in this study. A waiver of consent will not be sought.

4.5 Monitoring of Data Adequate to Ensure Safety of Subjects

(a) Describe any local or study-wide data monitoring procedures.

(b) Explain how the IRB will be kept informed of the results of such monitoring.

(a) Monitoring.

MEDICAL MONITOR STATEMENT

The medical monitor may be assigned to assess one or more of the following phases of research project: volunteer recruitment, volunteer enrollment, data collection, or data storage and analysis. The medical monitor will provide an independent evaluation of serious adverse events and unanticipated problems involving risk to subjects or others to the IRB and the HRPO. The medical monitor may be assigned to discuss research progress with the principal investigator, interview volunteers, consult on individual cases, or evaluate adverse event reports. Medical monitor, Dr. Michael Berman, DO will promptly report discrepancies or problems to the IRB and the HRPO. He shall have the authority to stop a research study in progress, remove individual volunteers from a study, and take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor's report.

Although this study is slightly higher than minimal risk, the sample collection procedures are similar to standard of care procedures performed daily at the Banner Wound Care and Hyperbaric Medicine Center. The research personnel obtaining the samples have extension experience in collecting samples from diabetic foot wounds. At the start of each sample collection procedure and throughout the procedure, the hemostasis and pain control of the subject will be assessed. Any Serious Adverse Events (SAEs) will be reported promptly to the medical monitor, the BGSMC IRB and the HRPO. The medical monitor for the study will be Dr. Michael Berman, DO, who is a physician in the clinic. Although he is not involved in this research study, Dr. Berman has extensive experience in treating wounds.

All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

(b) Communication with IRB. A copy of the approved continuing review report and local IRB approval notification will be submitted to HRPO as soon as these documents become available. A cop of the approved final study report and local IRB approval notification will be submitted to the HRPO as soon as these documents become available. If any issues arise with study subjects or with biospecimen or data collection, the IRB will be promptly notified.

(c) Protocol Modifications. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report (if applicable) to the ORP HRPO for acceptance.

(d) Review of Research Records. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command. These representatives are authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

(e) ORP HRPO Final Approval. The protocol will be conducted in accordance with the protocol submitted to and approved by the ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the ORP HRPO.

(f) Compliance: The knowledge of any pending compliance inspection/visit by the FDA, DHHS-OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to ORP HRPO.

(g) Protocol Deviations. Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the ORP HRPO as soon as the deviation is identified.

Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command. These representatives are authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

4.6 Privacy of Subjects and Confidentiality of Data are Adequately Protected

(a) Describe procedures for ensuring the privacy of subjects and the confidentiality of data, including procedures for protecting electronically stored data.

(b) If identifiable private information will be obtained from anyone other than the target subject (eg, subjects' family members, classmates, friends), please explain and justify.

(a) Protections. All biospecimens and clinical data collected for this study will be completed de-identified. All data will be identified by a study-specific code. The key, linking identifiable subject information to the code, will be kept by the clinical PI or delegee on a password-protected database. Only de-identified data, containing the study code, will be sent to TGen for analysis. The TGen PI and any study staff at TGen will be prohibited from accessing or attempting to access the key to the code. Clinical data will be entered into a password-protected electronic database at TGen. As described earlier, no identifiers will be present in the database. Clinical data and biosamples will be stored at TGen indefinitely.

(b) Non-Target Subjects. No non-target subjects will be approached for information about the subject.

4.7 Safeguards for Vulnerable Subjects.

Describe safeguards to protect the rights and welfare of any subjects who may be vulnerable to coercion or undue influence (e.g., children; pregnant women; persons with cognitive, mental, economical, educational, or social disadvantages).

No vulnerable subjects will be approached for study participation. All subjects must be able to provide consent to participation in this study.

6. Principal Investigator's Statement of Commitment

The proposed investigation involves human subjects. I certify that I am knowledgeable about and

will follow applicable federal regulations, TGen requirements, and IRB determination s for the conduct of human subject research. I agree to:

- ***a. Obtain the voluntary informed consent of subjects (or of subjects' legally authorized representatives) to the extent required by federal regulations and by the determinations of the IRB.***
- ***b. Report to the IRB any serious or unexpected on-site or off-site adverse events or unanticipated problems involving risks to subjects or other within the appropriate reporting period.***
- ***c. Cooperate fully with the IRB in the timely continuing review of this project.***
- ***d. Obtain prior approval from the IRB before amending or altering this research project or implementing changes in the approved informed consent document.***
- ***e. Maintain informed consent documents and progress reports as required by institutional policies, IRB requirements, and federal regulations).***
- ***f. Accept responsibility for the conduct and supervision of this research and the protection of human subjects as required by state and federal law and regulation, and as documented in the TGen Federalwide Assurance, guidelines of the TGen-designated IRB(s), and TGen policies and procedures.***

Signature of Principal Investigator **Date**