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Clinical Study Protocol

A randomized, parallel-group (autogenous *ex vivo* produced oral mucosa equivalent (EVPOME) vs. palatal oral mucosa (POM) safety and efficacy study in subjects requiring additional keratinized oral mucosa for dental rehabilitation with endosseous dental implants or around erupted teeth to restore periodontal health.

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SYNOPSIS

Study Title

A randomized, parallel-group (autogenous ex vivo produced oral mucosa equivalent (EVPOME) vs. palatal oral mucosa (POM)), safety and efficacy study in subjects requiring additional keratinized oral mucosa for dental rehabilitation or around erupted teeth to restore periodontal health.

Objectives

The study objective is to assess the safety and efficacy for use of human EVPOME for soft tissue intraoral grafting procedures compared to the "gold standard" palatal oral mucosa (POM) graft.

Design and Outcomes

This is a randomized, parallel-group phase II study to assess EVPOME vs. POM to determine differences in the primary efficacy measure of increased keratinized mucosa; secondary measures of graft contracture and Wound Healing Index; and ancillary outcome measures of tissue perfusion measured graft color and laser Doppler flowmetry, and postoperative pain.

Primary outcome measure: Clinical increase in zone (width) of keratinized mucosa (KM). For edentulous sites, KM width will be measured by determining the distance from the crest of the edentulous ridge to the mucogingival line to the nearest millimeter with a Castroviejo caliper. For patients with an existing implant, KM width will be measured from the implant mucosal margin to the mucogingival line. The amount of attached gingival tissue (keratinized) will be evaluated using a calibrated periodontal probe measured to nearest 0.5mm.

Secondary outcome measures: Graft contracture will be measured in mesio-distal and corono-apical directions. Post-surgical measurements of the graft site will be taken at visits 5, 6, 7, and 8, and will be compared to the original graft size to assess percentage of graft contracture. Wound Healing Index (WHI) will be recorded after surgery using the following criteria:

- Score 1= uneventful healing with no gingival edema, erythema, suppuration, patient discomfort, or flap dehiscence
- Score 2 = uneventful healing with slight gingival edema, erythema, patient discomfort, or flap dehiscence, but no suppuration
- Score 3 = poor wound healing with significant gingival edema, erythema, patient discomfort, flap dehiscence, or any suppuration

Ancillary outcome measures: Laser Doppler flowmetry (LDF) measurements will be used

to assess graft blood flow (tissue perfusion of the grafts).

Graft color is correlated to vascular perfusion and thus can give us an indication of graft vascularity. Finger pressure will be applied in the center of the graft for 15 seconds and/or until tissue blanching. The pressure will then be released and the time for the tissue to return to its previous color will be recorded. If the tissue returns to its previous color within 15 seconds, this will be recorded as positive graft vascularity. If not, then it will be recorded as negative graft vascularity.

Overall postoperative pain will be assessed via a visual analog scale (VAS).

Interventions and Duration

The outcomes data in patients treated with experimental EVPOME soft tissue intraoral grafting procedure will be compared to the outcomes in patients treated with POM, which is the standard of care procedure. Those randomized to the experimental group will undergo a small 6mm palatal punch biopsy. Keratinocytes extracted from the punch biopsy will be harvested and expanded *in vitro* then seeded onto the AlloPatch®. This process will take approximately 30 days depending on the period of time needed for cell amplification. EVPOME or POM will then be grafted onto the oral defect and secured with absorbable sutures. At Week 4, after grafting, a 2 mm punch biopsy will be taken to assess degree and maturity of keratinization and microvessel ingrowth.

Throughout the duration of the study, the subject will return for subsequent study visits to assess oral health and graft healing, contracture, and revascularization. The subjects will attend 7-8 scheduled study visits, one of which will be the grafting procedure, over a period of approximately 30 weeks. The post-operative follow-up period will include 5 study visits over approximately 24 weeks.

Sample Size and Population

Sixty (60) subjects, thirty (30) subjects per treatment group, will be randomized to receive either the experimental treatment, EVPOME (Group 1), or standard of care, the palatal oral mucosa (POM) graft (Group 2). The study population will include non-smoking adults (ages 18 and older) in need of additional keratinized oral mucosa.

Subjects will be recruited from UMHS through clinic referrals and the UMHS clinical research web portal for UMClinicalStudies as well as the University of Michigan (U-M) School of Dentistry, and their graduate periodontics clinic. Subjects may be also be recruited via Clinicaltrials.gov.

1. STUDY OBJECTIVES

1.1 Primary Objective

To assess the safety and efficacy for use of human EVPOME for soft tissue intraoral grafting procedures compared to the "gold standard" palatal oral mucosa (POM) graft.

Short-term impact:
Regeneration of oral mucosal tissue has not been previously addressed in craniomaxillofacial soft tissue injuries. At present, there are no successful means of repairing or regenerating soft tissue structures that contain mucosa, such as oral mucosa, or facial tissue units with a muco-cutaneous (M/C) junction, such as the lip. Damage to these tissues may occur in active military personnel during military operations. The lack of sufficient mucosal tissue is a reconstructive challenge that limits the ability of surgeons to reconstruct the oral cavity and other functional facial units that contain an M/C junction, such as the lips. This reconstructive challenge is frequently encountered by surgeons who treat traumatic avulsion injuries. Tissue engineering/regenerative medicine (TE/RM) offers a unique opportunity to address such reconstructive challenges using an innovative application of our proven technology and the surgical procedures proposed in this application.

The *in vitro* development of a tissue-engineered human oral mucosa is important because it can assist in intraoral reconstruction of the oral cavity and other mucosal structures such as the lips, eyelid, and anterior nares. For example, in the lip, the oral mucosa extends onto the vermillion border to unite with the skin of the face to form an M/C junction and is similar to the mucosa seen in the nasal cavity and inner aspect of the eyelid and the conjunctiva. Skin equivalents have been developed for treatment of burns and chronic wounds. The development of an *ex vivo* produced oral mucosal equivalent (EVPOME) has lagged behind that developed for skin.

An impediment to clinical use of TE/RM has been the lack of clinical validation trials and development activities. These are necessary to accelerate the transition of medical technologies, clinical practice guidelines, and standards of care into clinical capabilities. The clinical trials must be relevant to the definitive and rehabilitative care for patients. Of special concern are the injured war fighters who must be returned to readiness in terms of duty performance and quality of life. We will address the rehabilitative-reconstructive deficiency of contemporary options to regenerate mucosa and M/C junction. The proposed clinical trial is a compelling step to a solution.

Long-term impact and potential to change the standard of care:
The ultimate source of the cells to develop the soft tissue constructs will come from the patient, thus making the construct autochthonous (self to self). There are several advantages of using a TE/RM approach to reconstruct craniofacial soft tissue injuries:

1) Reduced donor site morbidity (we do not have to use extremities as a tissue source thus shortening the rehabilitation phase);
2) Improved restoration of function (rate and degree);
3) Reduced frequency of surgeries and operating room time;
4) Enhanced quality and shape of the regenerated soft tissue; and
5) Decreased hospital personnel and cost.

Potential to change the standard of care:
The results from this study will provide information to plan a Phase III clinical trial which will utilize larger wounds. A Phase III study will simulate the repair of major maxillofacial soft tissue intraoral defects that are in need of keratinized oral mucosa and will include medically compromised patients. The ability to assess keratinocyte metabolic activity and function prior to grafting is an essential component to assure a higher graft success rate. The potential phase III clinical trial could shift paradigms in surgical reconstruction of avulsion injuries to soft tissues containing oral mucosa and functional facial units with a mucocutaneous border (lips). The trial will also assist in the validation of a method to fabricate composite soft tissue grafts that will supplant facial transplants that require lifetime immunosuppression.

This Phase II clinical trial will be the first step in a paradigm shift on how soft tissue reconstruction of traumatic avulsion injuries of functional facial units will eventually be treated.

2. BACKGROUND

2.1 Rationale

The need for improved technology for craniofacial reconstruction is extremely relevant to military patients considering the high prevalence of combat-related craniomaxillofacial (CMF) injuries. A recent study analyzed the Joint Theatre Trauma Registry database for maxillofacial battle-injuries (BI) experienced by U.S. soldiers in the Iraq/Afghanistan conflicts to describe the type, distribution, and mechanism of injury. The study identified 7,770 BI of which 26% had maxillofacial involvement. The primary mechanism of injury was due to explosive devices (84%).

The study concluded that Maxillofacial BI account for a disproportionate number of injuries observed in Iraq and Afghanistan compared to previous American wars. Thus, the focus of this study on soft tissue reconstruction is especially pertinent given the significant number of soft tissue penetration injuries from explosive devices.

Craniofacial soft tissue defects that result from explosive munitions wounds to unprotected regions of the body, such as the face, present unique requirements among tissue engineering applications. Furthermore, the nature of explosive penetration wounds often creates extremely complex geometric and avulsion defects. The EVPOME technology will address these evocative challenges.

We can now fabricate human tissue-engineered oral mucosa (EVPOME). By conducting this planned Phase II clinical trial, we will improve and advance therapeutic technology for soft tissue craniofacial reconstruction which is profoundly relevant to military patients. The knowledge gained from this study will lead to a Phase III clinical trial where we will focus on larger maxillofacial intraoral defects that need oral mucosa.

This project will also be synergistic with another Department of Defense (DoD) project recently awarded, "Tissue Engineering Lips for Use in Repair of CMF Soft Tissue Injuries." One of the most difficult areas of the face to reconstruct after avulsion is the lips because they represent a composite tissue of mucosa, skin, and muscles. Significant loss of these structures is an obvious functional and esthetic concern because the neuromuscular control of normal lip structures is required for everyday activities of eating, drinking, talking, and social gestures. Avulsion of the lips is a survivable injury, but without functional lip reconstruction, life for these injured service members is burdened by drooling, food spillage while eating, unintelligible speech, and social rejection. Success of this study will enable movement of the "lip" project into the clinical arena sooner, as the methodology and protocols being used are quite similar. The success of the lip repair/reconstruction project is predicated by the ability to bring our tissue-engineered human oral mucosa into the clinical arena. Thus, this Phase II clinical trial will be a precursor for the successful implementation of a clinical trial for soft tissue reconstruction of the lips which incorporates the use of oral mucosa.

2.2 Supporting Data

A previous Phase I clinical trial demonstrated the safety and efficacy of EVPOME to increase keratinized gingival tissue without adverse events or local complications. This study achieved its main objectives and informed the design of the prospective clinical trial described here. For the phase I clinical trial, the researchers did not receive approval to have a control group (i.e, AlloDerm® without cultured primary oral keratinocytes), since the study was done to determine safety of the EVPOME and not efficacy. Thus, no post-operative biopsy specimens were obtained to assess vascularization of the tissue or persistence of the grafted cultured cells. Also note that while the previous study was performed using AlloDerm® as the scaffold upon which the EVPOME is constructed, this study will use AlloPatch® which is very similar to AlloDerm®. AlloPatch® must be used in this study because AlloDerm® is no longer available.

Previous studies showed that the grafted EVPOMEs had a deeper red hue than AlloDerm® grafts at post-operative day 6. This observation suggests that the grafted EVPOMEs revascularized faster than AlloDerm® grafts. In addition, there was histologic and immunohistochemical documentation of microvessel ingrowth into the underlying dermal component, AlloDerm®, of the EVPOME in a study of severe combined immunodeficient mice. It can therefore be reasonably stated that the grafted EVPOME had undergone revascularization by postoperative days 7 to 14.

The previously mentioned completed Phase I clinical trial was distinctive from other case studies using tissue-engineered products because it was conducted under our CBER/FDA-approved, investigator-initiated IND 10118. The IND required specific and rigorous cell testing and monitoring of the biologic product. Glucose consumption was selected as the functional test because it was considered a good indicator of the number of viable cells present on the EVPOME. Glucose consumption could detect cellular variations within EVPOME grafts, even in material from the same individual. As such, this functional test addresses the quality assurance/control and the release criterion mandated by CBER/FDA.

Overall, the cell culture protocols used in this clinical trial proved to be acceptable, since all EVPOMEs were successfully grafted into subjects with no signs of morbidity and/or contamination. The release criterion of glucose consumption correlated well with histologic examination of the EVPOME. Results showed a direct correlation between glucose consumption and the presence of a well-stratified oral mucosa epithelial layer. Therefore, the glucose consumption testing for this protocol will be similar to that of the earlier phase I study under IND 10118.

3. STUDY DESIGN

This safety and efficacy study will be a single site, randomized, parallel-group autogenous ex vivo produced oral mucosa equivalent (EVPOME) vs. the "gold standard" palatal oral mucosa (POM), in subjects requiring additional keratinized oral mucosa.

A total of sixty subjects will be randomized in a 1:1 ratio to autogeneous EVPOME vs. POM. If randomized to EVPOME, the subjects will be scheduled for a harvest biopsy (Visit 2 procedure). If randomized to the POM group, the subjects will be scheduled for the graft (Visit 3 procedure). See Table 1(Section 6.1) for the Schedule of Evaluations. Subjects will be non-smoking adults (18 years and older) needing increased keratinized oral mucosa.

4. SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.1.1 Adults ages 18 years and older

4.1.2 Deficient band (≤ 3 mm) of keratinized mucosa.

4.1.3 Surgery to increase width of keratinized mucosa is clinically indicated or requested by the patient to facilitate oral hygiene procedures or to improve esthetics.

4.1.4 Patients must be able to understand and provide informed consent for participation in the protocol.

4.1.5 Patients in need of a graft measuring up to approximately 15 x 10 x 20 mm in dimension.

4.1.6 Women who test negative on a urine pregnancy test.

4.2 Exclusion Criteria

4.1.1. Subjects with a known sensitivity to agents used in AlloPatch® production including: Gentamicin, Cefoxitin, Lincomycin, Polymyxin B, and Vancomycin

4.1.2. Subjects with potential medical complications such as evidence of clinically significant (as defined by investigators) renal, hepatic, cardiac, endocrine, hematologic, autoimmune, or any systemic disease which may complicate execution of the protocol and/or interpretation of results, i.e. interfere with wound healing or be a poor candidate for general anesthesia, uncontrolled diabetic, renal failure etc.

4.1.3. Documented history of syphilis, HIV, Hepatitis B or C virus

4.1.4. Pregnant women or women planning to become pregnant or unwilling to abstain or use double barrier contraceptives during the course of the study

4.1.5. Known or suspected allergy to bovine (cow) protein or iodine

4.1.6. Current radiation therapy or history of radiation therapy treatment to the intraoral donor biopsy site or recipient site for graft placement.

4.1.7. Smoking or use of tobacco products within 6 months prior to screening (subjects who quit smoking > 6 months prior to screening will be considered former smokers)

4.1.8. History of either alcohol or drug abuse in the past 5 years

4.1.9. Participation in another clinical trial within 30 days of screening

4.1.10. Subjects taking medications that can result in gingival enlargement/overgrowth (Cyclosporine, Dilantin, calcium channel blockers)

4.1.11. Current use of a medication used to treat a thyroid disease.

4.1.12. Current use of intravenous anti-resorptive therapies or a history of intravenous antiresorptive therapies during the past 5 years

4.1.13. Prior successful or attempted graft placement at the study defect site

4.1.14. Any physical or mental condition which in the opinion of the Investigator or Medical Monitor may interfere with the subject's ability to comply with the study procedures

4.3 Study Enrollment Procedures

The clinical treatment site will maintain a screening and enrollment log to track screen failures and enrollment (See Appendix A). A subject will not be screened until an Informed Consent Form has been signed. A subject's reason for ineligibility and reasons for nonparticipation or withdrawal from the study will be documented for inclusion in the Trial Master File (TMF).

Subjects who appear to meet the criteria for study participation will be informed of the study and, if interested, will review the study consent(s) with a qualified individual (approved by the PI to administer consent and listed on the Delegation of Authority Log).

During the informed consent process, study staff will detail the study procedures to ensure that the subject understands the study procedures and what is involved prior to signing the informed consent document. Subjects will be informed that they can withdraw from the study at any time and receive alternative care outside of the study. Subjects will be afforded as much time as needed to make an informed decision regarding willingness to participate in the study. Additionally, the study staff will provide contact information and will be available to discuss any questions or concerns potential subjects may have.

At the time of enrollment, immediately after written informed consent is obtained and before performing any study-related procedures, each subject will be assigned a unique sequential subject number for identification throughout the study by the study staff. This subject number will not be reused for any other participant in the study.

Once a subject has successfully met all inclusion and exclusion criteria, the subject will be randomized using the random number generator in a 1:1 ratio to EVPOME or POM via the web-based Treatment Assignment Tool U-M (TATUM) to obtain the subject's treatment assignment. The randomization schedule will begin with block sizes of 2, 4, or 6.

Upon randomization, an email will be sent to the Co-Investigator and/or any appropriate study staff. Randomization numbers will be assigned in the order in which subjects qualify for treatment, not in the order of study enrollment.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

5.1.1 Oral Examination

Study investigators will examine the subject as part of a comprehensive oral examination to evaluate the mouth, jaw, and teeth to assure that there is no cancer

recurrence and to verify the absence of other pathology that would mitigate subject study inclusion (i.e. dental infection).

5.1.2 Intraoral Photographs

Study staff, or medical residents aiding study staff will collect intraoral photographs for each subject randomized. Photographs will be taken of the graft site at a standard magnification (1:1 ratio) so that they can be compared from visit to visit to assess percent keratinization and contracture.

5.1.3 Laser Doppler Flowmetry (LDF)

LDF measurements will be taken at post-grafting for assessment of tissue perfusion.

Details of use and measurement of the LDF is described in Section 6.2.5.

5.1.4 Dental Impressions of Maxilla

All subjects will have an upper, maxillary, impression taken for a healing palatal stent that will be protective for the patient post-surgery at the graft donor site.

5.1.5 Harvest Biopsy

For subjects randomized to the EVPOME arm of the study, during visit 2, surgeon co-investigators will extract a single 6 mm circular punch biopsy for EVPOME manufacturing. Detailed handling of the EVPOME is described in Section 5.2.

5.1.6 Biopsy and Specimen Handling

All subjects will be prepped and draped in a sterile fashion prior to intraoral biopsies. Anesthesia will be obtained by local infiltrations and/or blocks, and tissue will be harvested using a 6 mm punch. If bleeding is not controlled under gauze tamponade, a hemostatic collagen sponge will be placed and a cyanoacrylic dressing will be applied topically over the wound. Subjects will be given routine post-operative instructions to include a prescription for analgesia. The 6 mm biopsy specimen will be placed in a specifically prepared transport medium containing 500.0 ml DPBS (Dulbecco's Phosphate Buffered Saline) w/o Ca^{2+} or Mg^{2+} and containing 0.54 g D-Glucose, 62.5 mg Gentamicin and 500.0 µg Fungizone. The biopsy will be immediately delivered to the Clinical Tissue Manufacturing Laboratory (CTML) for oral keratinocyte dispersion and culture. The specimen will be labeled with pre-printed labels from the CTML with protocol number and subject study number.

5.1.5 Grafting

After the subject is anesthetized, the surgical site will be prepared leaving the underlying periosteum intact. The EVPOME or POM grafts (harvested from the palate at this same visit) will be sutured and assessed to assure they are immobile. Patients will be given standard post-operative instructions, analgesics, and antibiotics per standard of care.

5.1.6 Assessment of Graft Contracture

At each follow-up visit, the percent (%) graft contracture will be determined by linear measurements taken from the periphery of the standardized size graft.

5.1.7 Study Laboratory Evaluations

Histology and immunohistochemistry will be performed on each 2 mm circular graft biopsy taken at Week 4 at the site after laser Doppler measurement has been taken. Histology will consist of standard hematoxylin and eosin stain, while immunohistochemistry will be used to assess microvessels within the dermal matrix.

5.1.8 Observation

At each follow-up visit, the percent (%) epithelial coverage will be determined by linear measurements taken from the periphery of the graft.

5.1.9 Postoperative pain

Pain following the grafting procedure will be evaluated using a visual analog scale (VAS) at the time of palatal biopsy for the EVPOME and at the time of harvesting and grafting of the POM grafts.

5.1.10 X-rays/radiographs

If a current panoramic radiograph or full mouth series of periapical radiographs are not available, new ones will be obtained. Close proximity of the mental nerve canal, periapical (endodontic) lesions or other pathologies to the anticipated surgical site may contradict surgery. Radiographs taken in the past two years are sufficient to determine if surgery is contradictory.

5.2 Handling of Study Interventions

All of the AlloPatch® used in this study will be procured and stored by UM Clinical Tissue Manufacturing Laboratory (CTML) for use on this study. When subjects are randomized to EVPOME arm, AlloPatch® will be available to the study team in the Clinical Tissue Manufacturing Laboratory where EVPOME manufacturing will take place.

For subjects randomized to the EVPOME arm of the study, during Visit 2, surgeon co-investigators will extract a single 6 mm circular palatal punch biopsy for EVPOME manufacturing. In the case in which the subject wears an upper prosthesis the harvest biopsy will be taken from the retromandibular trigone region in the mandible. A member of the EVPOME manufacturing team will provide pre-labeled transportation vessels, a primary vessel containing transportation media in which the biopsy will be placed, and a secondary containment vessel into which the primary transportation vessel will be placed.

The surgeon and the EVPOME manufacturing technician will document the chain of custody by completing the transportation of punch biopsy record. The manufacturing technician will transport the biopsy and record to the Clinical Tissue Manufacturing Laboratory and begin EVPOME manufacturing. EVPOME manufacturing procedures provide detailed instructions for the manufacture, control, and release of the EVPOME product.

On the day that the EVPOME engraftment is to occur, the Principal Investigator, Dr. Feinberg, or the Clinical Tissue Manufacturing Laboratory Manager will review the EVPOME manufacturing records and determine if the EVPOME meets required specifications (passing the sterility tests and satisfying the release criterion of glucose consumption over the last 24 hours) for engraftment. If the EVPOME is released by the Principal Investigator or the CTML Manager, the certificate of analysis is signed. The manufacturing staff will then transport the EVPOME to the surgeon in the surgical suite and attain site staff signature of receipt. If the EVPOME is determined to be unsuitable for engraftment, then there are two paths of accountability for the unused EVPOME. One of the following two paths will be used and documented on the EVPOME certificate of analysis:

1. The informed consent document allows research subjects to opt-in or opt-out of allowing excess tissues and EVPOME devices to be transferred to a University of Michigan tissue repository held by Dr. Feinberg (IRB approved HUM00035831). If a subject opts-in, then their unused cells and EVPOMEs will be de-identified, labeled, and transferred from the Clinical Tissue Manufacturing Laboratory to the Medical Science Research Building II where the PI, Dr. Stephen Feinberg, has a research laboratory and the oral mucosa bank is housed.

2. If a subject opts-out, then their excess cells and EVPOMEs will be destroyed as biohazard waste by processing in an autoclave.

5.3 Concomitant Interventions

5.3.1 Required Interventions

All standard surgical and post-operative standard medications and therapies will be performed.

5.3.2 Prohibited Interventions

- Consumption of alcohol or use of alcohol-containing products
- Smoking
- Other interventions which may be questionable for use should be discussed with the Principal Investigator and/or surgeon prior to use, if able.

5.4 Adherence Assessment

During each post-surgery visit, study subjects will be questioned on adherence to post-operative guidelines including dental hygiene, alcohol consumption, and smoking abstinence to assess correlation of these factors with graft contracture and revascularization.

6. CLINICAL AND LABORATORY EVALUATIONS

A defined table of study procedures by visit is listed in section 6.1. A narrative of these evaluations is described in section 6.2.

6.1 Schedule of Evaluations

Procedure	**Visit 1 Screening**	**Visit 2 Harvest Biopsy**	**Visit 3 Base-line Intra-oral Graft**	**Visit 4 Post-Op**	**Visit 5 Follow-up**	**Visit 6 Biopsy and Follow-up**	**Visit 7 Follow-up**	**Visit 8 End of Study**
Timeline (in reference to baseline)	**-1 to -90 days**	**-2 to -40 days**	**Day 0**	**Week 1 +/- 3 days**	**Week 2 +/- 3 days**	**Week 4 +/- 1 week**	**Week 8 +/- 1 week**	**Week 24 +/- 1 week**
Informed Consent and Inclusion/Exclusion	X							
Urine Pregnancy Test *females of child bearing potential only	X							
Randomization	X							
Medical/Dental History	X							
X-rays (if none have been taken in the past two years)	X							
Oral Examination	X			X	X	X	X	X
Pain Visual Analog Scale (VAS)	X	X	X	X	X	X	X	X
Clinic Assessments*	X				X	X	X	X
Intraoral Photographs	X	X~	X	X	X	X	X	X
Dental Impressions	X							
Tissue Harvest Biopsy~		X~						
Graft Placement			X∞					
Graft Biopsy						X		
Laser Doppler Flowmetry (LDF)					X	X	X	X
Laboratory Evaluations+						X		
Assess Adverse Events		X	X	X	X	X	X	X
Follow-up phone calls will occur at 9 and 12 months post-grafting.								

*Clinic assessments may include: PPD, CAL, BOP, Mobility, keratinization, graft contracture, graft color, wound healing index,
~Harvest biopsy will be performed only on subjects randomized to the experimental EVPOME arm of the study
+Laboratory evaluations include: histology, immunohistochemistry
∞ At this time the POM graft will be harvested from the donor palatal site and grafted to the recipient site

6.2 Evaluation Schedule

6.2.1 **Visit 1 Screening**

Subjects will be recruited through the Patient Admitting and Emergency Services (PAES) clinic in the U-M School of Dentistry in conjunction with MCOHR. All new patients are routed through PAES and assigned to specialty or undergraduate clinics. Most patients do not return to the PAES clinic, so we are not likely to have redundancy. PAES had 13,100 visits in 2003. It is estimated that 40% of the western population is missing 1 or more teeth (50% to be restored with implants for our purposes). Approximately 1/3 of them are in need of soft tissue oral mucosa grafts which is a very common procedure. This will give us a population from which to draw approximately 865 new patients per year. Of this group we need to recruit 60 subjects over two years from a potential pool of 1,730 patients. With an estimated enrollment of 1 out of 4 screened patients, we should have a pool of over 400 patients per year from which to select 30 subjects. Subjects will be recruited by full-time faculty members in the Department of Periodontics at the U-M School of Dentistry.

Informed consent will be obtained from each subject before any study-related procedures are performed. Screening and consenting procedures will be performed by the study staff on site at the clinic.

During the consenting process, the study staff will allow time for the subject to review the Informed Consent Form, ask questions, and make an informed decision (i.e. allow the subject to discuss with family etc.).

If the subject is willing and able to consent, the study staff will perform the study-specific procedures and evaluations to determine eligibility. Determination of eligibility will include review of subject-reported medical and dental history, an oral examination, and x-rays, if necessary. Subject weight will also be collected.

Medical and dental history will be questioned to determine any history of special conditions that may affect the subject during the study and its associated procedures. Potential study contraindications may include history of heart disease, relevant allergies, or the use of medications such as blood thinners. Concomitant medications will be updated throughout the trial. A visual analog scale of pain will be taken at this time for both group I and II,

Women of childbearing potential must agree to use a medically acceptable means of birth control and test negative on the Screening Visit urine pregnancy test. Intraoral photographs will also be taken.

Lastly, upper and lower jaw impressions will be taken for stent fabrication to protect the donor site and for use in LDF measurements

Subjects randomized to Group 1 (EVPOME), will be scheduled for Visit 2 to undergo a palatal punch biopsy/tissue harvesting which will be the source of autogenous cells used to make the cellular component of the EVPOME, within 14 days of determination. Subjects randomized to Group 2 (POM) will bypass Visit 2 and proceed to scheduling of harvesting of palatal donor tissue graft and intra-oral grafting at recipient site (Visit 3) upon completion of screening procedures.

6.2.2 Visit 2: Harvest Biopsy (EVPOME group only)

At this time, a 6 mm in diameter supra-periosteal circular biopsy will be harvested for fabrication of the EVPOME from Group 1 subjects. Co-Investigators will perform the biopsy procedure. This biopsy will be performed at the school of dentistry research clinic. Intraoral photographs will also be taken at this time. A stent will be placed at this time if needed.

Biopsy procedures: All subjects will be prepped and draped in a sterile fashion prior to intraoral biopsies. Anesthesia will be obtained by local infiltrations and/or blocks. Tissue will be harvested using a 6mm punch. If bleeding is not controlled under gauze tamponade, a hemostatic collagen sponge will be placed and a cyanoacrylic dressing will be topically applied over the wound. Subjects will be given routine post-operative instructions to include a prescription for analgesia.

Specimen handling: The 6 mm biopsy specimen will be placed in a specifically prepared transport medium, containing 500.0ml DPBS (Dulbecco's Phosphate Buffered Saline) w/o Ca^{2+} & Mg^{2+} containing 0.54 g D-Glucose, 62.5 mg Gentamicin and 500.0 µg Fungizone. The biopsy is immediately delivered to the CTML for oral keratinocyte dispersion and culture. The specimen will be labeled with pre-printed labels from the CTML with protocol number and subject study number.

A visual analog scale of pain will be taken at this time for group I and subjects will also be questioned about any adverse events.

6.2.3 Visit 3: Baseline: Intraoral Grafting of EVPOME or POM Grafts

Grafts will be applied as determined by the randomization schedule. Group 1 subjects will undergo intraoral grafting of EVPOME and Group 2 subjects will receive POM that will be harvested at this time from the donor site, palate. For Group 1 subjects, before release of the EVPOME for grafting from the CTML, glucose utilization will be measured and determined as percent of glucose utilized by the EVPOME during the final days of laboratory incubation. Gram stain analysis will be performed on culture media that the EVPOME was grown in during the final day of incubation. Preliminary results of in-process sampling to determine the sterility (aerobic, anaerobic, and fungal cultures) of the product will

be reviewed. Results of in-process mycoplasma testing will be reviewed. Analysis of in-process sampling for endotoxin will also be reviewed. If the device fabrication is unsuccessful, the subject will be given the opportunity to have a second palatal or retromandibular trigone (location is at the discretion of the principal investigator and the surgeon) biopsy taken for production of another EVPOME device.
Every effort will be made to maintain contact with the subject for safety data should the subject decline a second biopsy and withdraw from the study to pursue standard of care treatment. It is important to note that in our completed Phase I clinical trial we did not have to abort any procedure because of in-processing difficulties in not achieving our release criteria of passing the sterility tests and satisfying glucose consumption over the last 24 hours. Samples of spent media that the EVPOME was grown in may be stored and analyzed for proteins or other cell secretions in order to better understand graft success or failure.

EVPOME and POM grafts will be sutured and assessed to assure they are immobile. Subjects will be given standard post-operative instructions which may include analgesics and antibiotics per standard of care.

Intraoral photographs will also be taken at this time. A maxillary stent will be placed at this time for the POM subjects.

A visual analog scale of pain will be taken at this time for both group I and II and subjects will also be questioned about any adverse events.

6.2.4 **Visit 4: Post-operative (Post-grafting)**

Routine post-operative visits at Week 1 +/- 3 days post-grafting will include an oral examination. Intraoral photographs will also be taken at this time. A visual analog scale of pain will be taken, and subjects will also be questioned about any adverse events.

6.2.5 **Visit 5: Post-operative (Post-grafting)**

Routine post-operative visits at Week 2 +/- 3 days post-grafting will include an oral examination and clinical assessments, including degree of epithelialization and percent contracture.

Measurement of the maximum distance between the peripheral borders of the graft in both the x and y axis will be taken to assess percent of graft contracture. Laser Doppler flowmetry measurements will be taken with the pre-fabricated template at the approximate geometric center of the graft and on the contralateral side of the jaw as the graft placement. This will be done after stent removal.per this protocol:

LDF measurements will be with the subject resting in supine position, and at a

room temperature. A flexible probe (PR407, Perimed) will be used to obtain the measurements. The flowmeter will be calibrated before taking any measurements. Gingival blood-flow data will be reported in perfusion units (PU) and collected on the wideband setting. Voltage output values will be stored on an encrypted PC computer for storage and subsequent analysis.

Intraoral photographs will be taken. A visual analog scale of pain will be taken, and subjects will also be questioned about any adverse events.

6.2.6 Visit 6: Clinical Assessment and Biopsy of Grafts

Visit 6 will occur 4 weeks after the graft procedure +/- 3 days. Grafted sites of all subjects will be evaluated through clinical observation and photographs. Measurement of the maximum distance between peripheral margins of the grafts will be taken to assess degree of contracture as performed previously in Visit 5. Three variables of keratinization, contracture and micro-vascularization will be evaluated to assess outcomes

A 2 mm biopsy of the central portion of the surface of the graft will be obtained for evaluation, under local anesthesia, after the LDF measurements have been completed. Histology and immunohistochemistry evaluations will be performed on the biopsied tissue. Intraoral photographs will be taken. We expect that the EVPOME will have a higher number of microvessel ingrowth at the early stages after grafting compared to the POM. We also expect that epithelium will be present and will mature at an earlier stage after grafting compared to the POM.

Rationale for selection of 4 weeks for timing of biopsy: The first 2 weeks after grafting, a pressure dressing (surgical stent) is in place. At 3 weeks, the overlying mucosa is immature and friable. Based on previous experience, four weeks is the earliest time period we can detect a mature graft. If a graft fails, it will do so within the first 4 weeks. This supports the rationale for selection of this time period. Previous clinical studies took the biopsy at 6 months. We believe this time period is too far beyond the surgery date and would diminish the differences seen between the two types of grafts. The EVPOME graft is approximately 0.75-1.0 mm thick. It has been previously shown that it takes 10-11 weeks for a free mucosal graft of this thickness to reach maturity. Others showed that in 4 weeks, free grafts are keratinized.

Intraoral photographs will also be taken at this time. A visual analog scale of pain will be taken, and subjects will also be questioned about any adverse events.

6.2.7 Visit 7: Clinical Assessment

A clinical assessment and oral examination visit to measure subject comfort and

progress will be scheduled at Week 8 +/- 1 week. This visit will also include measurements for degree of graft contracture, as well as other periodontal measures for adjacent teeth and implant (if present). Intraoral photographs will be taken. A visual analog scale of pain will be taken, and subjects will also be questioned about any adverse events.

6.2.8 **Visit 8: Clinical Assessment and End of Study**

A clinical assessment and oral examination visit will be performed to measure subject progress at Week 24 +/- 1 week (6 months). This visit will also include measurements for degree of graft contracture, as well as periodontal measures for adjacent teeth and implants (if present). Intraoral photographs may be taken at the investigator's discretion. A visual analog scale of pain will be taken, and subjects will also be questioned about any adverse events. At this time point, the subject's participation in this study will be completed.

6.2.9 **Long-term Follow-up**

At 9 and 12 months, the subject will be phoned to discuss any Adverse Events or concerns with the graft site. At approximately 12 months, the subject will return for a standard of care visit and clinical examination with their primary oral surgeon/restorative dentist for further dental rehabilitation. Any issues with the study graft will be recorded in the patient dental records.

6.2.10 **Early Withdrawal/Study Discontinuation Evaluations**

Upon discontinuation, subjects will be evaluated following the Visit 8/ End of Study procedures listed in the Schedule of Evaluations (Table 1, Section 6.1).

6.2.11 **Off-Study Requirements**

Subjects who discontinue early from the study for any reason after Visit 3 will be asked to return to perform study procedures as described in Visit 8/End of Study All subjects who discontinue or complete the study will also be asked to report any Serious Adverse Events that occur within 30 days of completion/withdrawal from the study.

6.2.12 **Pregnancy**

Subjects who become pregnant will be followed until pregnancy completion and reported as per FDA reporting guidelines.

6.2.13 **Adverse Events**

Adverse Events will be assessed during all On-Study/On-Intervention Evaluation visits.

6.3 Special Instructions and Definitions of Evaluations

6.3.1 Informed Consent

The subject will be provided and allowed time to review the study informed consent form as outlined in the study Manual of Procedures and as described in section 6.2.1.

6.3.2 Documentation of Degree of Inefficient Zone of Keratinized Oral Mucosa

The study subject source documents will document the need for additional zone of keratinized oral mucosa for each subject. We will use a standard trapezoidal graft measuring up to approximately 15 x 10 x 20 mm for both arms of the study.

6.3.3 Medical History

The subject Medical and Dental history will be questioned and documented by the subject's recollection of history. Source documentation from hospital records may be collected and reviewed to determine subject eligibility or for inclusion/exclusion criteria.

6.3.4 Study Intervention Modifications

Any Study Intervention Modifications must be discussed with the Medical Monitor prior to implementation unless changes are required due to emergency needs of the subject. All modifications must be reported to the Medical Monitor within 24 hours of occurrence if not prior to the event. All study intervention modifications will also need to be reported to the IRB and FDA (if required per 21 CFR 312.30) prior to the change being made unless the change is intended to eliminate an apparent immediate hazard to the subjects.

6.3.5 Clinical Assessments

Clinical assessments include width of keratinized mucosa (KM) as determined by the distance between the crest of the edentulous ridge, the gingival margin of the tooth, or the implant mucosal margin and the mucogingival line to the nearest millimeter, graft contracture, graft color as determined by assessing tissue perfusion, postoperative pain, Wound Healing Index (WHI), color of graft tissue compared to surrounding

non-grafted tissue, and oral care follow-up. These assessments will be performed at post-operative visits 5, 6, 7 and 8. Periodontal measures include Periodontal Pocket Depth (PPD – measured from gingival margin to base of periodontal pocket to nearest millimeter), gingival recession measured from cemento-enamel junction on tooth to gingival margin, Clinical Attachment Level (CAL- determined by subtracting the gingival recession from PPD), and bleeding on probing (BOP) determined as present (1) or absent (0),

Visual analog scale for pain will be assessed for groups, I (at time of harvest of biopsy and EVPOME placement) and group II at time of harvesting of palatal donor graft and grafting at recipient site.

6.3.6 Laboratory Evaluations

Histology and immunohistochemistry evaluations will be performed on tissue collected via the Visit 6 biopsy. Histology will consist of standard hematoxylin and eosin stains to assess presence and maturity of the epithelial layer, while immunohistochemistry will assess the endothelial cells in microvessels within the dermal component.

6.3.7 Intraoral Photographs

Standardized intraoral photographs will be taken at each visit.

6.3.8 X-rays

If a current panoramic radiograph or full mouth series of periapical radiographs are not available, new ones should be obtained. Close proximity of the mental nerve canal, periapical (endodontic) lesions or other pathologies to the anticipated surgical site may contradict surgery. Radiographs taken in the past two years are sufficient to determine if surgery is contradictory.

7. MANAGEMENT OF ADVERSE EXPERIENCES

Expected, mild and routine adverse experiences including swelling, bruising, bleeding at the site, numbness at the site, lack of sensation, pain associated with the grafting site and lack of adherence of the graft after stent removal will not be considered adverse events, unless the magnitude or duration is greater than what is routinely expected. These events, however, will be documented in the medical record. If the magnitude or duration is greater than what is routinely expected, the experience will be considered an adverse event and will be recorded on the Case Report Form. Serious, expected and unexpected (serious or not serious) adverse events will be collected at every study visit and recorded on the Case Report Forms."

Quality Control procedures at the CTML will be in place to reduce the likelihood of EVPOME contamination. Comprehensive post-operative oral care instructions will be

distributed to study subjects. Adverse events will be reported as described in Section 10.4.

Possible risks to the subjects participating in this study include:

- Pain, swelling, numbness or discomfort from the biopsy of the roof of the mouth
- Contamination of the graft
- Graft failure
- Pain at the surgical site
- Possible unforeseeable harm to pregnant women, the embryo, or fetus
- Allergic reaction to the reagents used in the development of the EVPOME

8. CRITERIA FOR INTERVENTION DISCONTINUATION

Subjects who are diagnosed with HIV, cancer requiring radiation therapy, etc. will be discontinued from the study.

Those subjects who are discontinued will be asked to return for a final End of Study subject visit as described in Visit 8 study procedures.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This is a randomized, parallel-group (autogenous *ex vivo* produced oral mucosa equivalent (EVPOME) vs. palatal oral mucosa (POM)) safety and efficacy study in subjects requiring additional keratinized oral mucosa for dental rehabilitation. The primary hypothesis is that EVPOME is an equivalent intraoral grafting material when compared to POM in regards to keratinized tissue and graft contracture, but is superior to POM in regards to pain, color rendition, and microvessel infiltration.

The comparison will be done using primary efficacy measures of enhanced keratinization, and secondary measures of graft contracture and wound healing index, and ancillary measures of increased microvessel ingrowth. Clinical assessments will include postoperative pain. Intraoral photographs will be done at screening and at biopsy, 2, 4, 8, and 24 weeks after intra-oral grafting. Tissue biopsy will be conducted at week 4 and laser Doppler flowmetry at weeks 2, 4, 8, and 24. Primary assessment time will be at week 4, and final assessments for all subjects will be at week 24.

Subjects will be screened until we have the necessary 60 enrolled. It is likely there will be 4 screened subjects to 1 enrolled subject. All subjects will undergo informed consent prior to any screening procedures. It is expected that it will take 2.5 years to successfully enroll 60 patients. Subjects will be recruited through the Patient Admitting and Emergency Services (PAES) clinic in the U-M School of Dentistry in conjunction with MCOHR. All new patients are routed through PAES and assigned to specialty or

undergraduate clinics. Most patients do not return to the PAES clinic, so we are not likely to have redundancy. PAES had 13,100 visits in 2003. It is estimated that 40% of the western population is missing 1 or more teeth (50% to be restored with implants for our purposes). Approximately 1/3 of them are in need of soft tissue oral mucosa grafts which is a very common procedure. This will give us a population from which to draw approximately 865 new patients per year. Of this group we need to recruit 60 subjects over two years from a potential pool of 1,730 patients. With an estimated enrollment of 1 out of 4 screened patients, we should have a pool of over 400 patients per year from which to select 30 subjects. Subjects will be recruited by full-time faculty members in the Department of Periodontics at the U-M School of Dentistry.

9.2 Outcomes

9.2.1 Primary outcome

The primary outcome will be clinical increase in zone (width) of keratinized mucosa (KM). For edentulous sites, KM width will be measured by determining the distance from the crest of the edentulous ridge to the mucogingival line to the nearest millimeter with a Castroviejo caliper. For patients with an existing implant or tooth, KM width will be measured from the implant mucosal or tooth margin to the mucogingival line.

9.2.2 Secondary outcomes

The secondary outcome will include percent graft contracture and wound healing index. Graft contracture will be measured in mesio-distal and corono-apical directions. Post-surgical measurements of the graft site will be taken at visits 5, 6, 7, and 8 (Weeks 2, 4, 8, and 24), and will be compared to the original graft size to assess percentage of graft contracture. Photographs of the surgical site will be taken.

Wound Healing Index (WHI) will be recorded after surgery using the following criteria:

- Score 1= uneventful healing with no gingival edema, erythema, suppuration, patient discomfort, or flap dehiscence
- Score 2 = uneventful healing with slight gingival edema, erythema, patient discomfort, or flap dehiscence, but no suppuration
- Score 3 = poor wound healing with significant gingival edema, erythema, patient discomfort, flap dehiscence, or any suppuration

9.2.3 Ancillary outcomes

Ancillary outcome will include assessment of blood flow (tissue perfusion of the grafts) via laser Doppler flowmetry (LDF) evaluation, graft color, postoperative

pain, histology, and immunohistochemistry (IHC) to evaluate graft revascularization.

Graft color is correlated to vascular perfusion and thus can give us an indication of graft vascularity. Finger pressure will be applied in the center of the graft for 15 seconds and/or until tissue blanching. The pressure will them be released and the time for the tissue to return to its previous color will be timed. If the tissue returns to its previous color within 15 seconds, this will be recorded as positive graft vascularity. If not, then it will be recorded as negative graft vascularity.

Overall postoperative pain will be assessed via a visual analog scale (VAS).

9.3 Sample Size and Accrual

We propose to have 60 subjects to give clinical and histological endpoints of interest at the primary assessment time of 4 weeks post graft. We expect no more than 6 subjects to potentially drop out of the study before providing Week 4 data, and thus will enroll 66 patients in total. With an estimated enrollment of 1 out of 5 screened patients, we expect to have a pool of over 330 patients over two years from which to select 66 subjects (33 subjects per group over two years) that will be randomized equally to one of the two groups - EVPOME and POM grafts. The randomization list will be prepared electronically in advance by the biostatistician and transferred to the MICHR statistics group for incorporation into the TATUM system. Randomization will be stratified by smoking status.

The primary clinical endpoint is the degrees of keratinized tissue. Secondary outcomes are contracture and wound healing index. Ancillary endpoints include the presence or absence of a continuous epithelial layer, the degree of vascular ingrowth, the LDF measurement of gingival blood flow. The main aim is to show that EVPOME treatment give comparable clinical and histological results when compared with POM treatment. It is also expected that the EVPOME will cause less patient discomfort and pain, and have a better color rendition to the surrounding tissue than the POM. Hence, we expect EVPOME to show better outcomes for pain, color rendition, and microvessel infiltration than POM, while showing comparable outcomes on primary endpoints of keratinized tissue and graft contracture or histological endpoints compared with POM. Sample size is calculated to show near equivalence of the two groups for primary and secondary outcomes. Sample size calculation and power consideration are provided following the data analytic plan described for each outcome.

9.4 Data Monitoring

No interim data analysis is planned, but accrual rates and dropout rates will be monitored every 6 months. On-site data monitoring will occur at least annually.

9.5 Data Analyses

The main aim of the study is to determine by quantitative outcome measures that EVPOME will function as well as POM in terms of increased epithelialized tissue, decreased contracture, and increased microvessel infiltration. It is also expected that the EVPOME will have a closer adaptation in color rendition to the surrounding tissue. Our primary comparisons will be based on the observations at four weeks post-graft.

AIM 1 - Keratinized Tissue: Primary comparison will be based on week 4 assessment using an equivalence test of means. Background data on measurements of keratinized tissue for POM (standard of care) gave a mean measurement of 3.70 mm with an SD of 0.65 mm. The mean and SD were relatively constant over the follow up period from 3 to 12 months. If, in this study, both EVPOME and POM give comparable results, the distribution of the mean difference in keratinized tissue with 30 subjects in each arm will be approximately normal with a mean of 0 and standard error of 0.17, and a 95% confidence interval for the true mean difference between the two procedures will have a margin of error of about 0.33 mm. This confidence interval will exclude a value for the mean difference of 0.55mm or larger with probability at least 80%. Therefore sample size will be adequate to show near equivalence of the two treatments if the degree of keratinized tissue measurement of EVPOME is within ±0.55mm of POM. Statistical power will be even larger if non-inferiority of EVPOME to POM is desired, where true increase in keratinized zone in EVPOME group is no less than 0.55 mm compared with the increase of zone of keratinized tissue in POM.

AIM 2 - Shrinkage in Graft: The percent shrinkage (contracture) in graft will be expressed as 100*(1- (Area final/Area Day0)), and the comparison will be based on week 4 assessment using an equivalence test of means (with log transformation if the data are highly skewed upon visualization). Prior data for POM gave a mean shrinkage of 25% at 4 weeks post-op with a standard deviation (SD) of about 13%. If shrinkage is about the same for both EVPOME and POM, with a sample size of 30 subjects in each group, this translates into an estimate of the mean difference with standard error of about 3.4%. This gives a margin of error of 6.6% for a 95% confidence interval, and the 95% confidence interval excluding a value for the mean difference in shrinkage as large as 11% with probability of 80%.

Wound Healing Index (WHI): WHI will be analyzed via the following chart, and the index will be tabulated by treatment group at follow-up visits 5, 6, 7, and 8. Depending on the observed distribution of the index, the data will either be dichotomized or considered as an ordinal data.

Scores	Criteria
1	Uneventful healing with no gingival edema, erythema, suppuration, patient discomfort, or flap dehiscence
2	Uneventful healing with slight gingival edema, erythema, patient discomfort, or flap dehiscence but no suppuration

3	Poor wound healing with significant gingival edema, erythema, patient discomfort, flap dehiscence, or any suppuration

Ancillary Outcomes:
Histological Epithelial Coverage: The presence/absence of a continuous epithelialized tissue will be determined from the Week 4 biopsy using routine histology and staining. The percent of subjects with complete epithelialized layer between the two groups will be compared using non-equivalence of the two proportion test. If the EVPOME and POM groups give a common proportion of about 75% epithelial coverage, then the standard error of the estimate of the difference between the EVPOME and POM groups is about 11% based on 30 subjects per group. This gives a margin of error of about 22% for a 95% confidence interval. The primary test will be based on a two-proportion test where a lower bound for the power can be determined by the power associated with binomial comparisons with n = 30 in each group. If the true epithelial coverage of 75% is assumed at 4 weeks, and if we consider that a difference in epithelial coverage as large as 36.5% in favor of POM would still allow the EVPOME to be non-inferior, the proposed sample size of 30 per group would give 80% power to confirm non-inferiority and a one-sided confidence level of 97.5%.

Vascular Ingrowth: The degree of vascular in growth noted on the Week 4 biopsy specimen will be compared based on ordinal microvessel vascular in-growth from the IHC data. It is expected there will be utilization of the full range of the four point scale (labeled 1 through 4) and that a standard deviation of 1 unit might conservatively be expected. The margin of error for a 95% confidence interval will be 0.51 based on two sample sizes of 30 each. For the comparison between EVPOME and POM, the sample size will allow us to detect a difference as large as 0.72 units with 80% power using a 0.05 level test.

Laser Doppler Flow (LDF): LDF data will be compared based on gingival blood flow at Week 4 post-surgery at the graft site between the EVPOME and POM groups. One aspect of this study will be to examine and explore the usefulness of this measure. In particular, we will explore the use of control measures taken on normal tissue to help adjust for intra subject factors contributing to the variability. The primary investigation will compare gingival blood flow at the graft site at two weeks, four weeks, eight weeks, and 24 weeks post-surgery to the values in an analysis of covariance (ANCOVA) model. The corresponding differences at the control site will also be incorporated as a covariate to partially adjust for intra-individual variability. More detailed analyses will utilize ANCOVA methods for repeated measures. There is relatively little background data on the use of LDF to measure perfusion in the context being studied here though some preliminary data on measuring gingival blood flow can be found in Justus et al. where change in gingival blood flow from baseline to post surgery (over the period 7 to 24 .days) was found to have a mean of about -25 perfusion units (PU) with an SD of about 60 PU. The measurement is highly variable.

For repeatedly measured data such as pain and wound healing index, data will be

summarized by treatment groups at each follow-up time. In addition, linear mixed-effects models will be used to make the between group comparison while adjusting for potential within person correlation of the outcome variables. By using the modeling approach, it will be possible to incorporate all observations from both treatment groups and make between treatment group comparisons efficiently using a treatment dummy indicator. The model will allow us to not only compare the time-averaged means, but also allows us to explore the rate of decline between the EVPOME and POM groups such as the rate of decline in pain, using appropriate interactions terms. When appropriate, comparisons between treatments will be adjusted for surface area of the graft as well as measures of its geometry, and other variables including age, general health and alcohol use.

10. DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE REPORTING

10.1 Records to Be Kept/Case Report Forms

Data for this study will include subject source data for all clinical data assessments as described in the protocol and subject safety and efficacy data.

- Data will be stored in the study database and will be password protected. All research staff requiring access will be assigned a unique login name and password.
- Data for subjects, including documentation of informed consent, will be stored in a locked area accessible only by study research personnel.

The research team will develop paper Case Report Forms based on data collection requirements outlined in the protocol. These paper forms will serve as back-up to the electronic data entry screens that are created during development of the project database. The site coordinator will be responsible for completing paper forms and entering the results into the data management system. Alternatively, results may be entered directly into the project database via the data management system's web-based interface. The data collection instruments will undergo internal quality checks, and review and acceptance testing prior to their release for use. Paper Case Report Forms will be maintained at the study site and stored in compliance with Good Clinical Practice and relevant institutional policies.

Once completed, the study data will be archived in a secure location and documented by the Study Investigator. An Electronic Trial Master File (TMF) will be retained. All expected research data must be entered into the data management system and any data discrepancies resolved before the database is locked. Edit access to the study database will be restricted to read-only at this time. A read-only, time-stamped snapshot of the data will be created for analysis. Following data analysis, the database is frozen and access rights to the database are revoked. Only approved changes that significantly affect data

analysis are permitted once the database has been frozen. A snapshot of the frozen database is archived. Both the lock and freeze snapshots will be transmitted to the study team as password-protected files or via MiShare, a secure file transfer system supported by Medical Center Information Technology (MCIT).

10.2 Role of Data Management

The data management group will create a validated, 21CFR part 11 compliant database using the OpenClinica system. To ensure quality data, logic checks will be incorporated into the database. Discrepancy management will also be performed both manually and through the use of programmed validation checks within the system to ensure high quality data. Data extracts in the form of tables and reports will be exported from the database routinely to assess study progress, enrollment, safety, and efficacy. These reports are made available to the Investigator and the Statistician, as needed.

10.3 Quality Assurance

To ensure the highest level of data integrity and quality assurance, the study will be monitored by a qualified clinical trial monitor.

The purpose of monitoring is to ensure that the rights and safety of the participants are protected and the study is implemented in accordance with the protocol, Good Clinical Practice, applicable federal and local regulations, and the quality and integrity of the study data are maintained.

The clinical site monitoring plan will specify the frequency, procedures, levels of monitoring activities, and a monitoring communication plan. Monitoring activities will include the site initiation (pre-investigation) visit, interim site monitoring visits, and a close-out visit. The plan may also include for-cause visits. During the interim monitoring visits, the monitor will review participant medical and research records, consent documents, unanticipated problems, serious adverse events, site regulatory documents, etc. A summary of findings will be documented in a monitoring report and action items will be reviewed and followed until closed. Complete monitoring visit documentation will be provided to the study PI/Site PI and sponsor.

A site initiation visit will be scheduled prior to site activation and enrollment, and interim site visits will occur at least annually.

The sponsor or other regulatory officials may perform an audit or may accompany the clinical monitor on a monitoring visit.

The Medical Monitor, Dr. Christos Skouteris, will be available to review significant protocol deviations and AEs/SAEs on a scheduled and as needed basis.

10.4 Adverse Experience Reporting

Adverse Events (AEs) other than routine, mild and expected experiences, either observed by the Investigator or one of his/her medical collaborators, or reported by the subject, will be documented.. These AEs will be followed until the event is resolved, until the event is deemed chronic, or until 30 days after the subject’s participation in the study has ended.

Expected, routine, mild adverse experiences include: lack of sensation, numbness, swelling, bleeding, and pain associated with the grafting site and lack of adherence of the graft. These adverse experiences will be documented in the medical record.

Any Serious Adverse Event (SAE) that occurs must be reported to the University of Michigan IRB (IRBMED) according to the IRBMED reporting guidelines. Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form. All pertinent medical records and information collected during the treatment and follow-up of the subject should be maintained. The Investigator must assess the SAE relationship to investigational product. All SAEs will be reported to the FDA in compliance with the FDA reporting requirements as found under 21 CFR 312.32. This includes reporting to the FDA all unexpected, fatal or life-threatening adverse events assessed as related to the product by telephone or fax within seven days and the reporting in writing of unexpected serious adverse events assessed as related to the product within 15 days.

Expected Serious Adverse Events include allergic reaction, uncontrollable bleeding and/or pain or an infection requiring hospitalization.

Any death occurring through the end of the study (30 days following the intervention), regardless of the degree of relationship to study, must be reported as a Serious Adverse Event.

The investigator will submit an attribution for the relatedness of the adverse event to the test article or procedure. As far as possible, each AE should be evaluated to determine:

- the severity grade (mild, moderate, severe);
- its relationship to the study investigational product(s);
- its duration (start and end dates or, if ongoing, at final exam);
- action taken; and
- seriousness (yes/no)

The severity grade should be determined by the Investigator using the definitions below:

- Mild: Discomfort noticed but no disruption of normal daily activity;
- Moderate: Discomfort sufficient to reduce or affect normal daily activity; or
- Severe: Inability to work or perform daily activity.

The relationship of AEs to the investigational product should be determined by the Investigator using the definitions below:

- Definitely related: clearly associated with study drug/treatment;
- Probably related: likely associated with study drug/treatment;
- Possibly related: may be associated with study drug or other treatment;
- Unlikely to be related; or
- Definitely not related to the study drug/treatment.

For reporting purposes, an AE should be regarded as definitely or probably related to the regimen if the investigator believes that at least one of the following criteria are met:

a. There is a clinically plausible time sequence between onset of the AE and the administration of the study drug or treatment;

b. There is a biologically plausible mechanism for the study drug or treatment causing or contributing to the AE;

c. The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures; or

d. A potential alternative cause does not exist.

Expected adverse events are those adverse events that are listed in the study informed consent documents.

Unexpected adverse events are those that are not described in the study informed consent. This includes adverse events for which the specificity or severity is not consistent with the description in the informed consent.

Should an unanticipated problem occur during the investigation, the investigator will report them to IRBMED, the FDA, and DOD according to the reporting requirements of each entity.

An unanticipated problem is any incident, experience, or outcome that meets ALL three of the following conditions:

1. Is unexpected (in terms of nature, severity, or frequency) given the procedures described in the research protocol documents (e.g., the IRB-approved research protocol and informed consent document) and the characteristics of the human subject population being studied;
2. Is related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places human subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, even if no harm has actually occurred.

10.5 Data Safety Monitoring Board (DSMB)

A DSMB will be chartered to assure adequate protection of research subjects. The board will review Serious Adverse Events and other pertinent data according to the DSMB charter on a regular basis throughout the study. The DSMB will be comprised of at least three members with expertise in the dental/medical field and experience in the conduct of clinical trials or statistical knowledge. DSMB members will not have any conflicts of interest with the study.

11. HUMAN SUBJECTS

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol, the Informed Consent Form, and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for study oversight. A signed consent form will be obtained from the subject. For subjects who cannot consent for themselves, such as those below the legal age, a parent, legal guardian, or person with power of attorney must sign the consent form. Additionally, the subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, parent, or legal guardian, and this fact will be documented in the subject's record.

11.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the OHRP, the sponsor, or the sponsor's designee.

11.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the IRB, the sponsor, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

12. PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures developed by the Executive Committee. Any presentation, abstract, or manuscript will be made available for review by the sponsor prior to submission.

13. STUDY DISCONTINUATION CRITERIA

Stopping Rules for Safety reasons: The Data Safety Monitoring Board (DSMB) will review all Serious Adverse Events (SAEs) and make recommendations regarding the continuation or dis-

continuation of the study, as appropriate. SAEs that may necessitate possible discontinuation include:

- Infection of the graft site reported in 5 experimental subjects. Subjects will be treated with antibiotics and followed until resolution of the infection.
- Complete loss of the experimental graft occurring in 5 subjects
- Severe or excessive oral bleeding in 5 experimental arm subjects requiring transfusion
- Reported uncontrollable oral pain in 5 experimental arm subjects

All subjects will be followed until resolution of the adverse event and will also be contacted 9 and 12 months post-surgery to assess any possible long term safety issues.

Should the study be stopped because of safety concerns, the FDA and IRB will be promptly notified, and a comprehensive safety review will be initiated.

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Appendix A: SUBJECT SCREENING & ENROLLMENT LOG

Protocol Number:	**Investigator Name:**	**Study Coordinator Name:**

Screening Number	**Date Screened** (MM/DD/YY)	**Eligibility Criteria Met** (Yes/No)	**Subject Initials**	**Informed Consent Signed** (Date or N/A) Copy to subject	**Version of Consent**	**Surgeon**	**Reason for Exclusion** (Screen fail, refused to participate, etc.)	**Randomization Number Assigned**	**Treatment**