PHOTOBIOMODULATION FOR THE MANAGEMENT OF TEMPOROMANDIBULAR DISORDER PAIN

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Protocol

1. Project Title:

PHOTOBIOMODULATION FOR THE MANAGEMENT OF TEMPOROMANDIBULAR DISORDER PAIN

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3. Abstract:

Given the paucity of effective treatments for TMD and the overuse of pain medication, well-designed studies are needed to evaluate pharmacological alternative to treat this chronic pain condition. Our goal in this study is to conduct a double blinded, sham controlled, randomized clinical trial of multimodal Photobiomodulation (PBM) for TMD pain. Also, we propose to determine if PBM-induced changes in inflammation and pain sensitivity contribute to PBM's analgesic effects. A total of 120 TMD participants will be recruited through community-based advertisements. Participants will complete a computer-assisted telephone screening (CATI). Eligible participants will be age 18 and older with pain intensity of ≥30 on a visual analog scale (0-100). Participants will be excluded if: a) starting a new daily prescription medication for the management of pain within 30 days before TS; b) use of injection therapy (e.g., tender or trigger point injections, steroid injections) for the management of pain within 2 weeks before the CATI; c) starting occlusal appliance therapy within 30 days before CATI; d) history of facial trauma or orofacial surgery within 6 weeks before CATI; e) active orthodontic treatment; f), psychiatric hospitalization within one year before the screening. Participants eligible after CATI will be scheduled for a pre-randomization visit (V0), six treatment visits (V1 to V6). V0 will include informed consent, completion of a detailed medical history and the Pain, Enjoyment, General Activity (PEG) Scale, followed by a clinical exam to confirm TMD status according to the Diagnostic Criteria for TMD (DC/TMD), Pressure Pain Sensitivity (PPT) and blood draw. V1 will be the randomization visit to either receive PBM or Placebo, during which the first treatment will be delivered. There will be 5 subsequent treatment visits. At the final visit, a blood draw, a standard movement task to evaluate the range of motion of the mouth and PPT will be performed. PBM/Placebo treatment: We will use three types of active/Placebo probes; A) Single Laser 810 NM 200 mw; B) Laser Cluster of 810 NM equivalent to 1 WATT and; C) LED Cluster, 34 X 660nm at 10 mw and 35 850nm, 30mw 1390mw total applied to multiple craniofacial sites. Analyses will determine treatment effects on the primary outcome (pain intensity) and multiple secondary outcomes, and will examine whether changes in inflammation and pain sensitivity mediate treatment response. Findings from this rigorously designed trial will provide the most definitive evidence to date regarding the effectiveness and mechanisms of PBM for treating TMD pain.

4. Background

According to the National Institute of Dental and Craniofacial Research (NIDCR), in the United States, up to thirty-nine million Americans suffer from Temporomandibular joint and muscle disorder (TMD). Among them, more than ten million Americans suffer from chronic TMD pain [(1)] . In 2001, TMD resulted in 17.8 million lost working days yearly for every 100 million working adults in the United States and that financial costs are in the billions of dollars [(2)]. TMD is, therefore, the 4th most common pain condition in the US population; it is a complex musculoskeletal disorder characterized by muscle and joint pain and limited jaw function. TMD treatment is of limited efficacy. Evidence-based treatments for TMD are lacking, and the most common treatments for TMD, intraoral appliances and pain medication, including opioids, provide suboptimal pain control and can produce treatment-limiting side effects[(3-5)] . Therefore, as recently highlighted in a consensus report from the National Academies of Sciences Engineering

and Medicine, [6] the development of new safe and effective therapy is crucial to improve the quality of life of patients suffering from this painful condition.

Recent years have witnessed a burgeoning interest in the therapeutic potential of Low-Level Laser Therapy (LLLT) and Light-emitting Diodes (LED), recently named Photobiomodulation (PBM), in pain and inflammation. LLLT and LED therapies are both designed to deliver energy to target tissues to impact biological processes through photobiomodulation. Briefly laser and LED light stimulates photoreceptors in the target tissue(s), activating secondary mediators and thereby influencing multiple biological processes, including gene expression, cell signaling, cellular metabolism and cytokine release[7] . Therapeutic LLLT and LEDs are often manufactured to emit similar wavelengths in the red or near-infrared spectrum; however, these two modalities differ in important ways. First, laser light is both columnated and coherent (i.e. organized), which allows lasers to deliver light to a narrowly focused area of tissue and with deeper tissue penetration. Also, laser light is delivered at a single wavelength, allowing it to target biological processes that respond only to specific wavelengths (e.g. mitochondria) [8-10] . In contrast, LEDs emit light beams that are neither coherent nor columnated and are of lower power than lasers, and these properties limit LED penetration to more superficial tissues. Notably, multiple LEDs can be arranged into arrays, which increases the area of tissue that can be stimulated. [7] Thus, while these two forms of PBM share similarities, there are important differences that render them potentially highly complementary in the treatment of musculoskeletal pain.

PBM therapy has been demonstrated to have several benefits to muscles. It can prevent muscle damage after exercise, including delayed onset muscle soreness; and increase capacity of muscle workload, improving fatigue resistance, functional and activity and fastening muscle recovery. These benefits to muscle function could potentially improve TMD pain, which commonly originates in muscle. Another mechanism implicated in TMD pain is inflammation. Indeed, increased circulating levels of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, tumor necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) has been observed in patients with TMD for both myalgia and arthralgia. This systemic inflammation can influence the response properties of nociceptive afferents, resulting in peripheral sensitization [11] which can increase the intensity of TMD pain. [12] Over time, this increased nociceptive activity can produce changes in the response properties of spinal and trigeminal brainstem neurons, leading to central sensitization that can produce more widespread hyperalgesia and allodynia. Interestingly, PBM therapy has been shown to decrease the release of several cytokines (e.g.; IL6, TNF-α, MCP-1, etc.)[13-17], which could, in turn, reduce TMD pain.

Finally, TMD has been associated with peripheral and central sensitization, partially mediated by glutamate, N-methyl-D-aspartate (NMDA)[18, 19] as well as with increased oxidative stress[20, 21] .Interestingly, following injuries to the nervous system (both peripheral and central), PBM has been shown to promote axonal growth and nerve regeneration, as well as a change in the redox state of the cell, reducing oxidative stress and consequently reducing excitotoxicity. Because excitotoxicity involves the abundant release of the excitatory neurotransmitter glutamate, this could continue the cycle of glutamatergically-mediated central sensitization. Therefore, PBM can reduce TMD pain by reducing oxidative stress and subsequent excitotoxicity.

Moreover, existing evidence suggests that PBM therapy may reduce pain associated with multiple chronic pain conditions that share some common features to TMD pain, such as whiplash injury[22], tendinitis [23], osteoarthritis[24, 25], rheumatoid arthritis, neck, and back pain[26], epicondylitis [27], fibromyalgia [28], post-herpetic neuralgia [29], and trigeminal neuralgia [30, 31]. While these studies support the potential benefits of PBM in treating chronic pain, these studies have not combined PBM modalities, nor have they implemented the credible placebo condition proposed in this trial.

Given the multiple contributing mechanisms (e.g., muscle dysfunction, peripheral & systemic inflammation, peripheral and central sensitization), we hypothesize that treatments targeting multiple

pathophysiological pathways may be more effective in treating TMD. Therefore, we propose to rigorously test a promising non-pharmacologic approach to treat chronic TMD pain via PBM with both LLLT and LED, targeting several potential pathophysiological pathways for TMD pain. We hypothesize that the proposed protocol has the potential to improve symptoms and reduce reliance on pain medication in the future.

5. Hypotheses and Specific Aims:

SA1: To investigate the **analgesic efficacy of multi-wavelength PBM protocol [810nm 200mw; 810nm 1W aggregate and 660nm (10mwx34) aggregate 850nm (30mw x 35) LED] versus placebo PBM in patients with chronic TMD.** Therefore, we will test the following hypothesis: **H1a**: compared to a credible placebo condition, the proposed PBM protocol will significantly reduce TMD pain.

SA2: ***To investigate whether multimodal PBM changes inflammatory responses and mechanical pain sensitivity in patients with TMD and to determine the association of these changes with the analgesic response***. The majority of studies investigating the biological effects of PBM therapy have been conducted in preclinical models using a single wavelength in an injured site, and these findings suggest that PBM can reduce the inflammatory response. Our goal is to evaluate, in humans, the effect of the multi-wavelength PBM protocol on the inflammatory response and on mechanical pain sensitivity among individuals with TMD. Therefore, we will test the following hypothesis: **H2a**: the PBM protocol used will reduce the amount of circulating pro-inflammatory cytokines and will increase mechanical pain thresholds. **H2b** PBM-induced changes in inflammation and pain sensitivity will be associated with the magnitude of reductions in clinical pain following PBM.

6. Research Plan:

This study proposes to validate the use of an existing device that does not have FDA approval for use in treating chronic pain related to TMD. However, this device is already FDA approved for relaxation of muscles and relief from muscle spasms, temporary relief of minor muscle and joint aches, pain and stiffness, temporary relief of minor pain and stiffness associated with arthritis, temporarily increase blood circulation. Because we are studying individuals with muscle and joint pain due to TMD, the condition we are studying falls within the scope of conditions for which the device is FDA approved. (R#3006747388,https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfRL/rl.cfm?start_search=&OwnerOperatorNumber=9054807).

6.1 Overall Study Design: This study will be a double-blind, sham-controlled, randomized trial testing the efficacy of photobiomodulation (PBM) for pain related to TMD. Figure 1 depicts the study flow chart and each activity for each visit. After initial eligibility screening, potential participants will complete a clinical visit (V0) to confirm eligibility and verify their TMD status via clinical examination using the Diagnostic Criteria for Temporomandibular Disorder (RDC/TMD). Enrolled patients will complete an electronic daily symptom diary (DSD) for one week prior to the randomization visit (V1). V1 will include assessment of pain during movement and Pressure Pain Threshold, and a blood draw for measurement of inflammatory markers. Participants will then be randomized to either active or sham PBM, with the first treatment applied at V1. This will be followed by five more clinical visits, such that all participants will complete two to three treatment visits per week for two to four weeks. PPT, jaw range of motion and a blood draw will be repeated at the final visit. Likewise, Daily Symptom Diaries will be completed for one week midway through treatment and leading up to the final visit.

6.2 Participants: A total of 120 participants with chronic TMD, age 18 years and older will be enrolled at the University of Florida, College of Dentistry. As in our prior studies, recruitment will be community-based, including advertising across multiple prints and electronic media (e.g., posted flyers, radio, newspaper, social media, etc.). These methods have been highly effective in our prior studies. All participants that contact us will complete the pre-screening computer assisted telephone interview

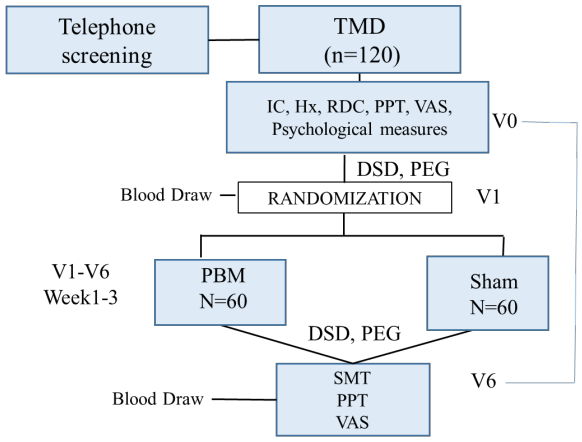

Figure 1: Study Flow Chart. IC: Informed Consent; Hx: Medical history; RDC: Research Diagnostic Criteria; PPT: Pressure Pain threshold; VAS: Visual Analog Scale; SMT: Standard Movement Task; DSD: Daily Symptom Diary; PBM: Photobiomodulation; V1- V4: Visit 1 to Visit 4; V5-V8: Visit 4 to Visit 8; PEG: Pain, Enjoyment, General Activity Scale

(CATI). Participants eligible to attend an enrollment visit will be those with facial pain for at least 3 months and an average pain intensity rating for the week preceding CATI, of ≥30 on a visual analog scale VAS (0-100).

6.3 Exclusion Criteria: Participants will be excluded if: a) starting a new daily prescription medication for the management of pain within 30 days prior to treatment session; b) use of any injection therapy (e.g., tender or trigger point injections, steroid injections) for the management of pain within 2 weeks prior to the CATI; c) starting occlusal appliance therapy within 30 days prior to CATI; d) history of facial trauma or orofacial surgery within 6 weeks prior to CATI; e) active orthodontic treatment; f), psychiatric hospitalization within one year prior to screening; g) hypersensitivity to PBM following patch test.

Prescreening may occur by telephone or at a clinic visit, and it may be combined with the Screening and Baseline Visit (Visit 0). After obtaining verbal consent, a brief prescreening interview script will be administered and basic eligibility information, specific points of medical history, and contact information will be recorded. If a participant expresses interest in the study and if the participant is eligible, the participant will be scheduled for Visit 0.

6.4 Clinical visits – Those participants eligible after CATI will be scheduled for a pre-randomization visit (V0), six treatment visits (V1 to V6) as described in Figure1 and Table 1. For female participants of child bearing age, before the PBM therapy is delivered (V1 to V6), a pregnancy test will be performed. If the pregnancy test shows a positive result, the participant will be withdrawn from the study. The costs of these tests will be covered by the study sponsor. The procedures to be completed in each visit are depicted in Table 1

V0 – Pre-Randomization visit will include informed consent and completion of a detailed medical history to confirm, followed by a clinical exam to confirm TMD case status according to the Diagnostic Criteria for TMD (DC/TMD). The patient will continue in the study if they meet the criteria for myalgia, with or without arthralgia. As part of the DC/TMD, all participants will perform a Standard Movement task (SMT) to determine their range of motion and pain upon movement. These tasks are standardized components of the DC/TMD and include measures of pain-free mouth opening, maximum unassisted mouth opening, and maximum assisted mouth opening. Pain is assessed during the last two maneuvers using a visual analog scale (VAS 0-100).

Following the clinical exam, an experienced trained lab technician will perform a well-validated psychophysical measure of pressure pain sensitivity. *Pressure Pain Threshold (PPT)* will be assessed with a handheld algometer bilaterally at temporalis, masseter, TMJ, trapezius, and ulna as in our prior studies. We will also conduct a patch test of the PBM treatment to ensure that the participant shows no hypersensitivity to PBM. This will involve brief application of the PBM treatment at a few different skin sites, and participants will be asked to report any adverse effects at 15 minutes and 48 hours after the test. Individuals who experience moderate to severe skin sensitivity, nausea, fatigue or dizziness after the patch test would be excluded from the study. All participants will be instructed to fill out a week-long Daily Symptom Diary over the phone or on the computer. They will rate their average pain intensity and their worst pain intensity each day on a numeric rating scale (0 = no pain, 100 = worst pain imaginable), as well as their pain interference. Also, they will be instructed to log any rescue medication used to control pain related to TMD.

V1 – Randomization Visit. The following criteria will be required for trial eligibility after the week prior to randomization: (1) a minimum of 4 of 7 diary entries were completed, and (2) the mean worst pain intensity score for the week was ≥30 of 100.

Participants who meet the criteria will then be block randomized to Active versus Sham PBM. The intervention will then be delivered by a trained operator that will be blind to the TMD diagnosis and the type of treatment to be delivered.

V2 - V5 – Treatment Visits. The patient will receive one minutes of the assigned treatment in each of the 42 points/areas (see *Photobiomodulation therapy protocol section for more information)*. Whenever possible, V2 will be scheduled within 5 days from V1 and V3 within 5 days from V2.

Table 1. Table of Events

Procedure	CATI	V0	V1	V2	V3	V4	V5	V6
Inclusion/exclusion criteria	X	X						
Informed consent		X						
Medical history		X						
RDC		X						X
SMT		X						X
PPT		X						X
Pregnancy test (women only)			X	X	X	X	X	X
DSD between visits		X						X
PEG Scale		X	X	X	X	X	X	X
PBM/Placebo			X	X	X	X	X	X
Blood draw			X					X

V6 – Final Treatment and Post-Treatment Assessment. The final treatment will be administered, followed by completion of the Pain, Enjoyment, General Activity (PEG) Scale and the same movement and PPT measures as in V0. Also, the post-treatment blood draw will be collected at this session, and the participant will be reminded about the follow-up assessments.

6.5 Outcome Measures (see Table 2)

The primary outcome measure will mean one-week pain intensity from the Daily Symptom Diary, comparing the one-week average prior to randomization to the one-week average for the week preceding V6.

Secondary outcome measures will include:

a) Pain during jaw function, which will be evaluated by comparing the pain Intensity score from the Standard Movement Task (SMT) from V1 with the pain intensity score from the SMT at V6.

b) To assess clinical pain and interference, participants will complete the Pain, Enjoyment, General Activity (PEG) Scale, a three-item instrument assessing average pain intensity and interference of pain with general activity and enjoyment of life over the past week.

Table 2 – Outcome Table

	Control Variables
Primary outcome:	
Pain Level Change with PBM treatment Average daily pain from Daily Pain & Symptom Dairy over one week prior to V1 will be compared to the average daily pain one week prior to V6	• Demographics (age, race, sex) • Concomitant medication
Secondary outcome	
TMD Function Pain Intensity during SMT will be compared between V1 and V6	• Demographics • Concomitant medication
Pain sensitivity PPT during V1 will be compared to PPT during V6	• Demographics • Concomitant medication
Effect of PBM on circulating cytokines Levels of circulating cytokines will be measured using Milliplex assay with blood collected at V1 (Baseline) and V6 (Post PBM treatment)	• Cortisol • Time of visit • Concomitant medication
Mediator Variables	
Inflammation	Change in cytokine pre-post treatment
Pain Sensitivity	Change in PPTs pre-post treatment

PBM: Photobiomodulation; SMT: Standard Movement task; PPT: Pressure Pain Threshold.

c) The effect of PBM on circulating cytokines measured using Milliplex assay with blood collected at V0 (Baseline pre-randomization) and V6 (Post PBM treatment). The control variables for each outcome and Mediator Variables are depicted in Table 2 (also, see the Data Analysis section).

6.6 Photobiomodulation Therapy Protocol

PBM has been used clinically in the treatment of musculoskeletal and other pain conditions for over 30 years. Despite the low quality of the existing evidence, PBM has been increasingly used in other countries for the treatment of TMD. However, in the US PBM is not widely used for the treatment of TMD pain. Due to the multifactorial nature of chronic TMD pain, we propose that a multimodal PBM protocol targeting multiple pathophysiological

mechanisms will be the optimal approach for PBM implementation in patients with TMD. Therefore, we are proposing to use a 30-45 minute protocol developed by Dincher, ME and Carrol, James (Thor CEO and Consultant to this project) which recommends the following sequence (Table 3): we will first apply PBM (LLLT and LED) to neuronal targets to suppress nociceptive peripheral afferent input to the spinal cord and trigeminal nucleus by treating the dorsal root ganglia associated with the dermatome(s) where the pain is experienced, including ganglia both rostral and caudal to the affected dermatome. Also, we will target autonomic ganglia in the cervical chain, blood, and lymphatic tissue areas. Treatment of the sympathetic ganglia is intended to modulate the sympathetically-mediated enhanced pain. Also, treatment of these ganglia has been shown to reduce inflammation. Finally, PBM/LLLT will target the painful musculoskeletal tissue associated with palpable tender points. Tender points will be located by digital palpation of the muscle that is the primary source of pain for each individual, and a tender point will be identified as a taut band that produces familiar pain upon palpation. We hypothesize that this multi-wavelength PBM protocol that targets different pathophysiological mechanisms will produce significant and long-lasting analgesic effects.

PBM parameters*:* We chose the THOR® laser system given because their active treatment arm uses both coherent laser and monochromatic LED light. Therefore, we will use three types of active probes in this investigation including, A) Single Laser 810 NM 200 mw; B) Laser Cluster of 810 NM equivalent to 1 WATT and; C) LED Cluster, 34 X 660nm at 10 mw and 35 850nm, 30mw 1390mw total. As detailed in Table 3. We propose to use these three PBM probes in concert for the treatment of TMD pain. *Laser A (Single Diode Laser)* is designed for isolated trigger points and superficial muscles. *Laser B (Cluster Laser)* is designed for a more diffuse treatment area, targeting analgesia, anti-inflammatory, and deep tissue repair. *Laser C (LED Cluster)* is purportedly designed for the presence of diffuse inflammation. Another reason that we propose the use of THOR®, it is because of their newly manufactured Clinical Trial Device, which includes a credible sham condition, which effectively maintains the blinding of both the patient and the interventionist.

Sham PBM*:* When applying PBM therapy, there are some heating elements in the treatment device, and most of the sham treatment devices available do not offer this feature, which increases the likelihood of unblinding both the patient and the interventionist. The THOR® LX2.3 PBM machine includes this new feature, such that the sham condition mimics the heating activity of the active treatment. Treatment Codes: Another feature of the THOR® LX2.3 is that the machine comes with a Sham Switch Box that allows the assignment of numeric codes for sham and active treatment. The interventionist simply enters the code but is unaware of whether it is assigned to active or sham treatment, as the statistician maintains the code list. Sham Concealment Goggles: Proper protective eyewear that absorbs the damaging radiation will be worn by staff as well as by participants during both active and placebo therapy. The patient goggles also emit LED light inside (behind the lenses), which prevents them from discerning sham versus active treatment. There is a lead which plugs into a socket on the Placebo Switch Box to power the LED when the treatment device turns on. Therefore, only the THOR goggles will be worn, no substitutes. They meet the necessary laser safety requirements and they prevent potential “unblinding” of treatment assignment.

Table 3; Laser Devices and Parameters

Type of probe	Dose and Dose Rate	Treatment Intend
Single laser –Laser A: 810nm 200mw	6-12 Joules (J) continuous, firm contact	Tender points
Laser Cluster –Laser B: 810nm 1W	6-12 J continuous, firm contact	Broad muscle, neural blockage if ganglions, 3-5 cm depth
LED Cluster – LED: 660nm (10mw x 34) aggregate 850nm (30 mw x 35)	6-20 J, 20Hz firm contact	Blood, lymphatic areas and superficial neural targets

Areas of treatment: Target areas for the treatment will include trigger points on temporalis, masseter, sternocleidomastoid, occipital and trapezius (Laser A); Neuronal blockage of trigeminal area, spinous process of C2-C6, broad muscle trapezius and occipital (Laser B); and finally blood and lymphatic areas of preauricular, occipital, and superficial and deep cervical (LED). These treatment targets are

based on the most common locations of pain reported by patients with TMD and the putative underlying neuronal and inflammatory mechanisms, and they were recommended in the recently developed multi-wavelength protocol[32] .

6.7 Treatment Assignment Procedures

Randomization Procedures
Prior to randomization, the inclusion and exclusion criteria will be reviewed, and only participants who meet eligibility criteria will continue in the trial. Participants will be randomized in a 1:1 ratio of active Photobiomodulation to Sham-PBM within prespecified blocks. Randomization will be accomplished by a web-based randomization system developed and maintained by the study statistician. At the time of randomization, study staff, who will remain blinded to treatment assignment, will access the system, enter the participant's study identification number, and verify that the participant is eligible for randomization. The system will assign a randomization code that will be entered in the randomization box in the PBM machine. The code will determine whether the participant receives sham or active PBM treatment according to the participant's treatment assignment.

Blinding Procedures
All study staff, including the study clinicians and investigators, will be blinded to the participants' treatment assignments throughout the data collection period. The THOR® LX2.3 PBM machine includes this new feature, such that the sham condition mimics the heating activity of the active treatment. Treatment Codes: Another feature of the THOR® LX2.3 is that the machine comes with a Sham Switch Box that allows the assignment of numeric codes for sham and active treatment. The interventionist simply enters the code but is unaware of whether it is assigned to active or sham treatment, as the statistician maintains the code list.
Sham Concealment Goggles: Proper protective eyewear that absorbs the damaging radiation will be worn by staff as well as by participants during both active and placebo therapy. The patient goggles also emit LED light inside (behind the lenses), which prevents them from discerning sham versus active treatment. There is a lead which plugs into a socket on the Placebo Switch Box to power the LED when the treatment device turns on. Therefore, only the THOR goggles will be worn, no substitutes. They meet the necessary laser safety requirements and they prevent potential "unblinding" of treatment assignment. The project staff will remain blinded through the analysis for the primary objective. If oversight boards, such as the IRBs or Data and Safety Monitoring Board (DSMB), request an unblinded data report during the data collection period, the unblinded biostatistician will generate the report.

Unblinding Procedures
Unblinding before the study is completed will occur only if a participant's well-being is threatened and is necessary to protect the participant. Study participants will be provided with instructions and contact information for emergency situations.

6.8 Inflammatory markers: We propose to assay a panel of circulating cytokines pre vs. post treatment (V1, V6). Based on work performed by Slade et al, we plan to examine multiple inflammatory mediators, such as MCP-1, IL-1ra, and IL-8, IL-6, IL-1β, IL-10 and TNF-α, as these inflammatory mediators are considered to play a crucial role in TMD pathophysiology and pain sensitivity. Also, we also plan to measure cortisol level as evidence suggests that cortisol can influence the immunological response. Therefore, we plan to include cortisol levels as a control variable when analyzing the cytokine data. Given that our biomarker assays will be completed at the end of the project, the final panel of biomarkers to measure will be determined at that time based on the best available evidence.

6.9 Strategies for Recruitment and Retention

We propose to enroll 120 individuals 18 years and older who meet DC/TMD criteria for myalgia, with or without arthralgia. We will recruit using community-based recruitment as well as clinic-based recruitment. Our recruitment strategy will be developed and implemented with support from the Recruitment Center of our Clinical and Translational Science Institute (CTSI). The CTSI Recruitment

Center assists with drafting recruitment plans, developing recruitment materials (e.g. flyers, online ads), using social media for recruitment and linking with other local and national recruitment resources. We have worked with the CTSI Recruitment Center on several previous projects, including our SOPPRANO clinical trial Study.

Community-Based Recruitment: Alachua County, Florida has a population of 269,956 people, 72% of which are 18 years and older. Racial/ethnic diversity is substantial, with 20.6% of residents being African American, 10.3 Hispanic, and 60.8% non-Hispanic white. The University of Florida has a large footprint in the community, which facilitates recruitment into research protocols. Our community-based recruitment methods may include any of the following strategies. We will advertise around our local institutions as well as throughout the local communities, including advertisements in local retail establishments, at bus stops and on buses, and in local print media. Also, we will participate in community health fairs and education programs sponsored by entities within our University. We have participated in many such events in the past, which have been highly successful. Fourth, we will leverage existing CTSI recruitment resources, including HealthStreet and UFHealth Study Listings. HealthStreet is a community portal of entry for linking and navigating underrepresented populations to opportunities to collaborate with the research community through town halls, focus groups, individual interviews, library use, individual health assessment, and navigation to appropriate research. HealthStreet's Community Health Workers engage with residents to assess health needs and enroll interested individuals in a research registry.

Clinic-Based Recruitment: Patients may also be recruited from the UF College of Dentistry Faculty Practice and Student Clinics, where patients with TMD are often evaluated and treated. In addition, we will identify potential participants through the CTSI's Integrated Data Repository, which contains de-identified data from the electronic health record. This allows for cohort identification, and UFHealth has implemented consent-to-share, such that a large proportion of patients seen in our clinics have provided consent to be contacted about research. If a sufficient number of individuals are identified in the IDR, we may request a list of all individuals with a TMD-related diagnosis who have provided consent to share, whom we can then contact directly to determine their eligibility and interest for participation in the study.

Retention: We are overenrolling by 20% to account for attrition. Retention in our previous SOPPRANO Study was 87%, even though this was a more intensive and lengthy protocol. That is, SOPPRANO included 6 study visits over a 12-week period, with daily medication intake and completion of daily diaries throughout the protocol. Thus, given the significantly shorter duration of the current protocol, and 80% retention rate is quite conservative.

6.10. Data Analysis

Sample Size Justification. For the primary endpoint VAS, we assume the standard deviation (SD) is 1.96 cm and effect size is 2.5 cm. We plan to recruit 120 subjects (60/group). Assume 15% attrition rate, we will have 102 subjects (51/group) for the analysis at the end of the study. The statistical power with two-sided type I error rate of 0.05 is 100% based on a two-sample T test. For the secondary endpoint IL6, we also have 100% statistical power to detect the effect size of 2.2 (on the natural-log scale) estimated from pilot data.

Overall Data Analysis: Descriptive statistics, specifically measures of central tendency (mean, median) and dispersion (variance, interquartile range), will be calculated for all continuous measures. Descriptive statistics will be calculated by group and histograms will be used to compare the distributions. Intent-to-treat analysis will be used to test the treatment efficacy. Missing data (if any) will be handled with multiple imputation and sensitivity analysis. The data analysis for each aim is described below:

***SA1: To investigate the* analgesic efficacy of multi-wavelength PBM protocol [810nm 200mw; 810nm 1W aggregate and 660nm (10mwx34) aggregate 850nm (30mw x 35) LED] versus placebo**

in patients with chronic TMD. H1a: compared to a credible placebo condition, the proposed PBM protocol will significantly reduce TMD pain.

For **H1a**: To evaluate the change in TMD related pain after PBM/Placebo treatment, the variable will be assessed using a 0 to 10 numerical rating scale (NRS, 0 = no pain, 10 = worst pain imaginable) for the average pain. Average daily pain from Daily Symptom Dairy averaged over one week prior to randomization will be treated as the baseline variable. The average daily pain one-week prior to Visit 6 will be treated as the primary endpoint. Linear regression model will be employed to test the association between VAS and treatment group (PBM vs Placebo). This approach will allow us to control for potential confounders (e.g. psychological factors). We will also try linear mixed effect model (LMM) to handle repeated measurements. An advantage of LMM is that it can provide valid estimates if there are missing data that are missing at random. An alternative to LMM is GEE to analyze the repeated measurements in relation to the treatment groups. Interactions between group and baseline inflammatory response will also be explored.

SA2: To investigate whether PBM changes inflammatory responses and mechanical pain sensitivity in patients with TMD and to determine the association of these changes with the analgesic response. **H2a**: the PBM protocol used will reduce the amount of circulating pro-inflammatory cytokines and will increase mechanical pain thresholds. **H2b** PBM-induced changes in inflammation and pain sensitivity will be associated with the magnitude of reductions in clinical pain following PBM.

For **H2a**: These secondary endpoints including cytokine and pain sensitivity will be analyzed in the same way as the primary endpoint in H1a where baseline values and potential confounders such as psychological factors and cortisol level will be adjusted in the model. For **H2b**, we will use potential-outcomes (PO, i.e., counterfactual-outcomes) mediation analysis approach to estimate and test the mediation effect of the inflammation and pain sensitivity. PO mediation analysis is more flexible than traditional mediation analysis approach in terms of allowing interaction between independent variable and mediators and non-linear indirect effects. In the mediation model, treatment is the independent variable, inflammation and pain sensitivity will be the mediators and the outcome variable is VAS. Baseline values and potential confounders will be adjusted in the mediation model.

7. Possible Discomforts and Risks:

RDC Exam. Assessments of muscle and TMJ sensitivity to digital palpation during the TMD examination, are designed to evoke brief pain or discomfort during application of the stimulus; however, the results are not long lasting or damaging to the affected tissues. This increase of pain normally is short lived and controlled by over the counter pain medication.

Pressure testing. Pressure is delivered by a hand-held algometer (spring-controlled device delivering calibrated pressure via a flat 10mm diameter rubber tip). Pressure is delivered at an approximate rate of 30 kPa/sec. Participants will be instructed to signal by pressing a button when the pressure sensation first becomes painful at which time the researcher removes the algometer. There is little opportunity for bruising or other transient trauma from this procedure. All tests will end upon completion of the modality or upon the participant's request, whichever comes first.

Questionnaires. A participant may experience discomfort associated with being asked personal questions about her or his health history, symptoms, or emotional feelings. Participants will be told that they may choose not to answer any questions that cause discomfort

PBM therapy. The therapy should produce minimum discomfort other than a light warmth or heat on the area where the light is being applied. If the patient feels uncomfortable, he/she can stop at any time. All interventionists and patients will be provided with goggles to protect their eyes from the light. Treatment on the head and neck with high irradiance laser may cause pain as the melanin in the fine superficial hair follicle absorbs a lot of the laser energy. If treatment becomes painful, we will remove the treatment probe from contact and treat ~15mm from surface of skin.

Blood Collection. There is a possibility of mild pain and bruising associated with a blood draw. Trained personnel will perform the blood collection using standard procedures.

Adequacy of Protection against Risks.

Our inclusion and exclusion criteria are designed to minimize risks to participants.

A total of 120 adults with TMD, (Masticatory Muscle Disorders, 1A: Myalgia) age 18 and older will be enrolled. Similar to our previous work, the inclusion criteria for participants are:

- Provides a signed and dated informed consent form
- Is at least 18 years of age (male or female and any race or ethnicity)
- Meets diagnostic criteria for TMD, (Masticatory Muscle Disorders, 1A: Myalgia)
- Has experienced facial pain for at least 3 months
- At Screening and Baseline Visit (Visit 0), reports an average pain intensity rating over the past week of ≥ 30 on a numerical rating scale (0-100)

Participants will be excluded if they have any concurrent medical conditions that could confound interpretation of outcome measures, pose a safety risk for any of the assessment or intervention procedures, or preclude successful completion of the protocol. Specific exclusion criteria are:

- Starting a new daily prescription medication for the management of pain within 30 days prior to treatment session;
- Use of any injection therapy (e.g., tender or trigger point injections, steroid injections) for the management of pain within 2 weeks prior to the CATI;
- Starting occlusal appliance therapy within 30 days prior to CATI; d) history of facial trauma or orofacial surgery within 6 weeks prior to CATI;
- Active orthodontic treatment;
- Psychiatric hospitalization within one year prior to screening.
- Has known hypersensitivity to laser therapy.
- Currently being treated with chemotherapy or radiation therapy
- Has been treated with another investigational drug or treatment within 30 days prior to the Screening and Baseline Visit
- Is pregnant or nursing
- Anything that, in the opinion of the investigator, would place the participant at increased risk or preclude the participant's full compliance with or completion of the study

Protection against Risk.

*Protection against risk to confidentiality***.** Information collected as part of this research protocol will be maintained in locked filing cabinets and password protected databases accessible only to study personnel. All study staff will be trained in handling human subject information to maintain privacy and confidentiality. Procedures for allowing access to investigators to use this information for research will be under the authority of the PI and will follow HIPAA compliant guidelines for the release of PHI.

No results will ever be reported in a personally identifiable manner. All research data will be entered directly into a web-based survey that is maintained by the University of Florida CTSI (REDCap). The data will be stored on secure servers at the University of Florida and will be accessible only to trained study personnel.

Protections of risks related to study questionnaires. To minimize any risks related to emotional responses to questionnaires, persons will be informed about the types of questions included in the surveys, which are similar to the types of questions persons might be asked by their doctor in a clinical setting. They will be informed that they can refuse to answer any questions if they so choose.

Protection of risks related to PBM Laser. PBM therapy and the Thor system has shown minimal side effects. THOR lasers have divergent beams but are potentially harmful if viewed directly from a distance of less than 1.1 meters. Proper protective eyewear that absorbs the damaging radiation will be worn by staff as well as by participants during both active and placebo therapy. Participants will be instructed not to take the goggles off until the treatment is completely off. In addition, the laser will be tested in a small area before proceeding to insure the participant's skin is not sensitive to the light. To minimize risk associated, participants will be monitored throughout treatment sessions and asked to report any discomfort. If they experience any uncomfortable sensations the treatment will be stopped. All PBM sessions will be administered and continually supervised by a trained experimenter. The above symptoms have only been reported when participants are in the active treatment group.

Data and Safety Monitoring Plan

The Study team will meet at least quarterly to review adverse events and enrollment progress.
The Study Team will closely monitor all adverse events (AEs) on a continuous basis and we will report any AEs to the IRB per current policies and guidelines.

8. Possible Benefits:

There may or may not be direct benefit to subjects for participation in the study. Some patients could benefit from reduction of pain during the trial and this pain may return after the trial is over. In an attempt to reduce pain related to TMD as well as decrease the overuse of pain medication, this search for an efficacious, safe, and affordable treatment may benefit many TMD patients in the future.

Importance of the Knowledge to Be Gained

The information obtained will provide novel and important information regarding the benefits non-pharmacological treatments for TMD

9. Conflict of Interest

The PIs of this research, Dr. Margarete Ribeiro-Dasilva and Dr. Roger Fillingim do not have any conflict of interest.

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