POCKET-X GEL CLINICAL STUDY

TRIAL PROTOCOL

Protocol Number: PX-01

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Sponsor: Tree of Life Pharma Ltd.

Version Number: 06

Protocol approved by:

Name and Position	Date	Signature
Danny Rosenbaum, Head of the Board Tree of Life Pharma Ltd.	27.6.2019	

Summary of Changes from Previous Versions:

Version No.	Summary of Revisions Made
01	N/A
02	Added a clearer group allocation, added names of study sites.
03	Added more detailed information regarding the study and the studied devices, changed group allocation method, added additional optional efficacy measurements, added additional optional treatment sessions, added more participants in each group, added additional inclusion and exclusion criteria, added sample size rationale, changed statistical plan.
04	Removed blood tests from inclusion criteria.
05	Clarification of ethics committee approval procedure.
06	Removed Pocket-X Chip group from the protocol, added more background information, added more information regarding study rationale, removed PGE2 and MMP8 measurements, added more information regarding the studied device, added more information on the bacterial testing, added an abbreviations section.

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1. ETHICS COMMITTEE APPROVAL

The study will be conducted in accordance with this protocol, following its re- approval by the manager of Mayanei Hayeshua Medical Center. The study will be conducted in accordance with Procedure No. 14 ("Clinical Trials Procedure") of the Department of Pharmaceutics, Ministry of Health, Israel. Approval of both the protocol and the consent form will be obtained before any participant is enrolled.

2. ABBREVIATIONS

SRP – Scaling and root planing

RSD – Root surface debridement

PW – Peptone water

3. INTRODUCTION

3.1. BACKGROUND

Periodontal disease, affecting large proportion of adult population[1,2], both females and males, can lead to formation of spaces between the gingiva (gums) and teeth, called periodontal pockets, which occur due to loss of attachment between the gingiva and the teeth, mainly due to the inflammatory reaction to bacterial colonization on the teeth and gingiva[3,4,5].

Bacteria, which penetrate the pockets once they are formed, cause further inflammation and may deepen the pockets by creating further damage to the tissues and bones around the teeth[3,5]. Probing the depth of the periodontal pocket is a good indicator for diagnosing the extent of periodontal disease. In a healthy periodontium, there is no loss of epithelial attachment or pocket formation, and the space between the gingiva and the teeth is less than 2 mm deep[6].

Currently available conventional treatment for periodontal pockets includes root surface debridement (RSD) or scaling and root planing (SRP), which remove subgingival plaque and tartar, thus removing the causative factor for periodontitis while allowing the natural healing of the gums and sealing of periodontal pockets. However, new bacteria can infiltrate the pockets after RSD or SRP and inhibit the healing process.

In order to improve the healing of the gingiva and prevent relapse of inflammation within the periodontal pockets, adjunctive treatments have been developed, intended for application following RSD or SRP. These methods include antimicrobial laser therapy, host-modulating agents that inhibit host collagen-degrading enzymes which cause tissue loss due to inflammatory response, or use of antibiotic or antibacterial agents that assist in the inhibition of bacterial re-colonization inside the periodontal pockets. The latter are available either as rinses, toothpastes or sustained-release products, which are inserted into the periodontal pockets and remain inside for days or weeks. Additionally, systemic antibiotics may be administered with or without topical treatment. Other adjunctive treatment option

is use of barrier agents that fill the periodontal pockets and prevent infiltration of bacteria from the oral cavity into the pockets.
While the efficacy of SRP has been proven to be statistically significant in numerous clinical studies[7,8], various adjunctive treatments have been shown to provide greater improvement in clinical parameters such as probing pocket depth and clinical attachment level[9,10,11,12,13].

3.2. STUDIED DEVICE

The studied device, Pocket-X Gel, manufactured by Israeli-based Tree of Life Pharma Ltd., is an *in-situ* gelling device, used as physical barrier against bacterial re-colonization of the periodontal pockets following root surface debridement or scaling and root planing. Pocket-X Gel is provided in a pre-filled syringe along with 2 tips. It is applied to the periodontal pocket by a dentist using the provided syringe bearing one of the tips. It is applied in the form of a liquid, which transforms into a gel once placed in the periodontal pocket, due to contact with body temperature. The gel fills the periodontal pocket and prevents bacterial infiltration into the pre-cleaned pocket. One syringe can be used for multiple periodontal pockets present in the mouth of the same patient. The syringe or tip cannot be used on a different patient.

Pocket-X Gel contains the following components: Water, Poloxamer 407, Hyaluronic Acid, Phenoxyethanol, Octenidine Dihydrochloride.

The device is biodegradable. It remains within the periodontal pocket for a period of 1-3 weeks, afterwards it is either degraded or ejected from the pocket following natural sealing of the pocket as part of the gums healing process.

Pocket-X Gel only contains materials that are commonly used in oral products and are considered safe for oral use based on available literature. Additionally, *in-vivo* animal experiments conducted with the product at a GLP-certified laboratory indicated no irritation or sensitization. it is therefore expected that treatment with Pocket-X Gel will present a high safety profile during the study.

Furthermore, the product's application in the form of a liquid and its transformation into a gel only after its application allows to the product to perfectly fit the pocket's dimensions and shape. In addition, the product is applied through a thin and flexible plastic tip that does not traumatize the surrounding tissues. It is therefore expected that the product and its administration process will cause no discomfort to the patient.

3.3. STUDY DESCRIPTION AND RATIONALE

This study is a prospective open-label study in which Pocket-X Gel will be applied to periodontal pockets in one/two mouth segments of participants following scaling and root planing on the entire mouth, while the contralateral segment(s) of the mouth will serve as control.

The purpose of this study is to examine the safety and efficacy of Pocket-X Gel in the treatment of periodontal pockets, following scaling and root planing procedure.

The study's hypothesis is that Pocket-X Gel, when inserted into periodontal pockets following scaling and root planing procedure, would lead to greater improvement in periodontal clinical measurements such as pocket depth, bleeding upon probing, clinical attachment level, etc., in comparison to periodontal pockets which have undergone scaling and root planing only.

Pocket-X Gel is inserted into the periodontal pockets as a liquid. Following application, the product quickly transforms into a gel, due to contact with body temperature, and adheres to the inner lining of the periodontal pockets. This *in-situ* transformation from liquid form to gel allows Pocket-X Gel to fit into periodontal pockets of all sizes while completely filling the pockets. It is hypothesized that Pocket-X Gel, once inside the periodontal pocket, would act as a physical barrier against infiltration of bacteria originating from the oral cavity into the pocket, thus contributing to the greater improvement in the clinical state of the periodontal pocket.

The preceding procedure, scaling and root planing, is a conservative treatment which is considered the gold standard treatment of periodontitis in the majority of cases. As part of this procedure, the patient undergoes mechanical debridement which removes subgingival plaque and tartar, resulting in significant reduction of the bacterial load in the periodontal pockets. Subsequently, the inflammation in the area subsides, which allows the gingiva to initiate its natural healing process and seal the pockets.

However, if the periodontal pockets remain exposed following completion of scaling and root planing, bacteria in the oral cavity may return to the pockets and create new plaque, which may inhibit the beneficial effect of the procedure and prevent utilization of its full beneficial potential. This is especially crucial in the first few days following scaling and root planing, during which the patient may find it difficult to maintain proper oral hygiene due to gingival pain, bleeding and swelling resulting from SRP.

Pocket-X Gel is intended to prevent this situation and physically shield the periodontal pockets from external bacteria, which would allow the gingiva to promote its healing process and start sealing the pockets.

The study's hypothesis is supported by an *in-vitro* study, which was conducted in order to examine Pocket-X Gel's ability to physically block infiltration of oral pathogens. The oral pathogen chosen for this study was Porphyromonas gingivalis, which is considered one the main pathogens involved in periodontitis. In order to determine whether the bacterial-barrier activity of Pocket-X Gel is derived merely from its physical barrier properties, Pocket-X Gel used for the purpose of this study had both of its preservative agents, Octenidine Dihydrochloride and Phenoxyethanol, removed from its formula. The only components included in the studied formula were Pocket-X Gel's physical-acting components, which are responsible for creation of the viscous gel at 37° C: Poloxamer 407 and Hyalouronic Acid, as well as water as a solvent.

As part of this study, four sterile tubes were filled with sterile Peptone Water (PW), under aseptic conditions. Cell strainers were placed upon all four tubes. Two tubes were assigned to the test group and two tubes were assigned to the positive control group. One full

syringe of Pocket-X Gel was then applied upon each cell strainer of the test group tubes. The control group was left untreated. Next, 0.1 ml of Porphyromonas gingivalis at a concentration of 1.0 x 10^6 CFU/mL was applied to each of the cell strainer upon all four tubes. All tubes were then incubated at 37° C for 6 hours under anaerobic conditions. After 6 hours, all cell strainers and tube contents were tested by membrane filtration method. The membrane filters were placed onto TSA Petri plates, which were incubated at 30 - 35° C for 5 days. Results indicated that PW in test group tubes contained no more than 10 CFU per 40 ml, as opposed to CFU values of 4.7 x 10^3 and 5.3 x 10^3 per 40 ml of PW in the control group tubes. Cell strainers CFU value was high in both the test and control groups, with values ranging from 1.4 x 10^3 to 2.2 x 10^3. This study indicates that Pocket-X Gel was able to block the infiltration of P. gingivalis through the cell strainers and prevent the contamination of the PW in the test group tubes.

4. OBJECTIVES

The overall objective of this study is to assess the safety and efficacy of Pocket-X Gel in the improvement of gingival healing and natural sealing of periodontal pockets in patients with periodontal disease, following scaling and root planing. Safety will be evaluated in accordance with the adverse events reported by participants and oral pathologies recorded by the investigators, compared between segments treated with the studied device and segments untreated with the studied device (in case of localized adverse events) and to adverse events attributed to scaling and root planing based on available literature. Efficacy will be determined in respect to the differences between the measurements taken from segments treated with the studied device and segments that were not treated with the studied device, also taking into account efficacy measurements from similar studies and studies on scaling and root planing, based on available literature.

5. STUDY DESIGN

5.1. OVERALL DESIGN

This study is a single arm, open-label, split-mouth study.

All enrolled participants will initially undergo root planing and scaling on the entire mouth. Following the completion of scaling and root planing, each participant will have one/two of his/her mouth segments (quadrants) treated with Pocket-X Gel. The contralateral segment(s) of the Pocket-X Gel-treated segment(s) will serve as control.

The study hypothesis is that patients treated with Pocket-X Gel following scaling and root planing will exhibit better periodontal pocket healing on their treated segment(s) than their untreated segment(s) as well as compared to data available in the literature concerning the efficacy of scaling and root planing.

The total duration of the study for each participant will be 6-7 months. The study will include a total of 5-7 visits, depending on the number of scaling and root planing sessions required,

based on the severity of each participant's periodontal disease. Follow-up visits will occur 4, 12 and 24 weeks after completion of scaling and root planing and first application of the studied device. Detailed description of each visit is provided under section 5.5 – Visits and Follow-Ups.

5.2. STUDY ARMS AND INTERVENTION

The study will include a single arm.. Participants will undergo the following intervention and procedures:

- Examination of the gingiva and periodontal pockets and study measurements recording – the following measurements will be taken: pocket probing depth, bleeding index, recession level, clinical attachment level, mobility grade and furcation. Additionally, sub-gingival biofilm samples may be taken from some participants periodontal pockets for the purpose of bacterial testing to quantify three main periodontal pathogens: Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola. Samples will be tested at the periodontal clinic by the investigator or his/her assistants, using a BANA-Zyme test kit (OraTec Corp., USA).

- 1-4 sessions of scaling and root planing (SRP) on the entire mouth.

- Dividing the mouth into segments (split-mouth) – one/two segments (quadrants) that will be treated with Pocket-X Gel following SRP and two/three segments (quadrants) that will not undergo any further treatment beyond SRP. The number of treated segments and untreated segments will be decided by the investigators and is dependent on the clinical symmetry between the contralateral segments of each participant.

- 1-2 prophylaxis and oral hygiene instruction sessions.

- 1-3 sessions of treatment with Pocket-X Gel, which will be applied to periodontal pockets present in one/two of the pre-determined mouth segment(s) – administration will be performed by applying the product into the periodontal pockets, through a thin plastic tip attached to a syringe containing Pocket-X Gel. More information regarding Pocket-X Gel administration can be found under section No. 5.3 of this protocol.

Section 5.5 of this protocol provides information regarding the interventions and procedures that will be performed at each study visit.

5.3. METHOD OF ADMINISTRATION

Pocket-X Gel will be applied to the periodontal pockets in the pre-determined mouth segments of study participants, by means of a pre-filled syringe bearing a thin plastic tip, provided by Pocket-X Gel's manufacturer. The syringe tip will be inserted into each periodontal pocket and Pocket-X Gel will be applied until the pocket is completely filled by

the product. Each syringe will contain 1 ml of the product. A maximum of 1 syringe will be used on 1 patient per session. Each syringe is intended for use on a single patient but may be used on multiple periodontal pockets of the same patient at each treatment session. In total, a maximum of 3 syringes will be used on each patient throughout the study. The product's syringe and tips will be discarded after use.

5.4. NUMBER OF PARTICIPANTS

A total of approximately 34 participants will be included in the study, taking into account 20% dropout/lost to follow-up, thus allowing complete data collection from a sufficient number of participants to make a reliable assessment.

5.5. VISITS AND FOLLOW-UPS

Participants will enroll into the study after undergoing screening and signing an informed consent form. The study will include 4-7 visits, depending on the amount of sessions required for scaling and root planing. A list of the interventions and procedures that participants will undergo during each visit as well as visit schedule is presented in table 1 below. Further details regarding study interventions and procedures can be found under section 5.2 of this protocol.

Table 1 – Study visits plan

Visit No.	Procedure/Intervention	Schedule
Visits 1-4	• Physical examination, obtaining samples from periodontal pockets for bacterial testing [optional] and recording of study measures. • Scaling and root planing on the entire mouth. • Dividing the mouth into segments, with one/two segments designated to undergo treatment with Pocket-X Gel throughout the study, and two/three that will not undergo any treatment beyond SRP. • Single administration of Pocket-X Gel to each periodontal pocket in the pre- determined segment(s), after completion of scaling and root planing. • Instructions on maintaining proper oral hygiene.	Week 0 – Week 4

Follow-up 1	• Physical examination, obtaining samples from periodontal pockets for bacterial testing [optional] and recording of study measures. • Single administration of Pocket-X Gel to each periodontal pocket in the pre- determined segment(s) [optional]. • Instructions on maintaining proper oral hygiene (if further instructions are needed).	4 weeks following completion of scaling and root planing and first product application (± 14 days)
Follow-up 2	• Physical examination, obtaining samples from periodontal pockets for bacterial testing [optional] and recording of study measures. • Single administration of Pocket-X Gel to each periodontal pocket in the pre- determined segment(s) • [optional].	12 Week following completion of scaling and root planing and first product application (± 14 days)
Follow-up 3	• Physical examination, obtaining samples from periodontal pockets for bacterial testing [optional] and recording of study measures.	24 Week following completion of scaling and root planing and first product application (± 14 days)

5.6. RESTRICTIONS

In order to obtain reliable safety and efficacy results from the study, participants will be requested to refrain from undergoing any additional unnecessary periodontal treatments during the course of the study, aside from those provided as part of the study.

In cases that such additional treatment is deemed necessary, the participants will be requested to report the treatment details to the study investigators, who will record them.

Participants will also be requested to follow the instructions on maintaining good oral hygiene they receive from the investigators at the beginning of the study, and refrain from changing this routine. An exception will be made during the 2 days following treatment with Pocket-X Gel, during which participants treated with the product will be requested to brush their teeth very gently and not use dental accessories such as dental floss, in accordance with the manufacturer's instructions.

6. STUDY POPULATION

6.1. INCLUSION CRITERIA

In order to be found eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form
2. Male or female, aged 18 and above
3. Provision of up-to-date full mouth periapical x-rays
4. Participants suffering from active moderate to severe chronic periodontitis with at least 3 sites of periodontal pockets of 5 mm or greater depth in each quadrant.
5. Participants with a minimum of 6 teeth with periodontal pocket depth of more than 5 mm.
6. Participants with a minimum of 20 teeth

6.2. EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Known hypersensitivity to any of the device's components as listed on the user leaflet
2. Pregnancy or lactation
3. Smoking of more than 9 cigarettes per day
4. A concurrent dental disease, except for periodontitis, or planned treatment that may interfere with the study or study intervention, such as dental surgery, teeth implantation, etc.
5. Chronic disease such as diabetes mellitus or rheumatoid arthritis
6. Aggressive periodontitis
7. History of radiotherapy or chemotherapy

8. Mucosal immune disease
9. Mental disorders
10. Parafunctional habits such as bruxism
11. Participant has taken antibiotics during the previous 6 months
12. Participant has undergone periodontal treatment during the previous 12 months

6.3. SCREEN FAILURE

Screen failures are defined as participants who consent to participate in the clinical trial but do not meet one or more criteria required for participation during the screening procedures. These participants will not be included in the study.

6.4. INFORMED CONSENT

Participants will be requested to sign an informed consent form, which will be pre-approved by the study's ethics committee, prior to enrollment into the study. Only participants who signed the informed consent form may be enrolled.

7. PARTICIPANT DISCONTINUATION/WITHDRAWAL

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reason:

- Participant meets one or more of the exclusion criteria
- Participant has an interproximal Plaque Index of more than 30% after up to two initial sessions of prophylaxis and instruction, indicating that the participant does not follow investigator's dental hygiene instructions
- Serious adverse event
- Medical condition that prohibits the patient from continued participation in the study

8. LOST TO FOLLOW-UP

A participant will be considered withdrawn due to lost to follow-up if he or she fails to show up for one of the visits and is unable to be contacted by the study site staff/investigators.

Before a participant is deemed lost to follow-up, the site staff/investigators will make every effort to regain contact with the participant and reschedule the missed visit.

9. STUDY ASSESSMENTS

9.1. EFFICACY ASSESSMENT

The following measurements will be recorded during the study, and will be used to assess the efficacy of the intervention:

- Plaque index
- Pocket probing depth
- Bleeding index
- Recession level
- Clinical attachment level
- Mobility grade
- Furcation
- Specific periodontal bacteria quantification [optional]

The above measurements will be recorded at baseline and at follow-up visits for each participant. The values will be recorded separately for each periodontal pocket. .

Efficacy data from follow-up visits will be compared to the baseline values in order to obtain the efficacy results. The efficacy results will be compared between treated periodontal pockets and control periodontal pockets and to data obtained from available literature.

9.2. SAFETY ASSESSMENT

Safety assessment will be conducted based on adverse events reported by the study participants as well as any new pathologies observed by the investigators during visits that did not exist at baseline or have worsened since baseline. Such observations will also be regarded as adverse events.

Safety assessment will be conducted by analyzing the adverse events, their quantity, severity, duration and their connection to the studied device. In case that the adverse event is localized, these parameters will be compared between the segment(s) treated with Pocket-X Gel and the untreated segments of the same patient. Adverse events will also be compared to adverse events reported during scaling and root planing studies, based on available literature.

10. CALIBRATION OF EXAMINERS

The study's investigators – two experienced periodontists, will be calibrated for scaling and root planning procedures and recordings of clinical data to ensure that all clinical procedures will be standardized according to the study protocol and to minimize both intra and inter-examiner variability across the time points. The two examiners will be calibrated initially for PPD and CAL according to the method of Jeffcoat et al. (1). Briefly, as part of the calibration exercise, each of the 2 examiners will measure PPD and CAL 2 time at 10 sites (PPD>5 mm) in each of 5 patients. For both examiners and all 25 sites, the mean absolute will be <0.5 mm for both parameters.

11. STUDY SITES

The study will take place at Dr. Ariel Hirsch's, Dr. Roni Kolerman's and Dr. Alon Sabon's (study investigators) dental clinics in Tel Aviv, Israel.

12. ADVERSE EVENTS

During each visit, participants will be asked whether they experienced or experience any adverse events. In case that an adverse event has occurred, investigators will record all details of the event and will assess whether the event may be related to the studied device.

Any new pathologies observed by the investigators that did not exist at baseline or have worsened since baseline, will be considered adverse events and recorded accordingly.

Participants will be requested to report any serious adverse events immediately to the investigators, without waiting for the next visit.

12.1. DEFINITION OF ADVERSE EVENTS

Adverse event means any unfavorable or unintended medical occurrence associated with the study, whether or not considered intervention-related.

Serious adverse event means any adverse event that results in the following outcomes: death, a life-threatening adverse event, inpatient hospitalization, a persistent or significant incapacity, substantial disruption of the ability to conduct normal life functions or congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

12.2. CLASSIFICATION OF ADVERSE EVENTS

Each adverse event that occurs during the study will be classified by the investigator(s) in terms of severity, relation to the studied device and expectedness. The classification of an adverse event may be updated as new relevant information, that may have an influence on the classification, becomes available.

Severity: adverse event's severity will be classified in accordance with the following:

- **Mild –** events that require minimal or no treatment and do not interfere with the participant's daily activities.

- **Moderate –** events that result in a low-moderate level of inconvenience and may cause some interference with functioning.

- **Severe –** events that interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating, however, the term "severe adverse event" does not necessarily equate to "serious adverse event".

Relation to studied device: adverse event's relation to the studied device will be classified in accordance with the following:

- **Definitely related -** this classification will be applied if there is clear evidence to suggest that the adverse event is related to treatment with the studied device, and other possible contributing factors can be ruled out. For this classification to be applicable, the adverse event, including an abnormal laboratory test result, should be an event that occurred within a reasonable time following treatment with the studied device and the event cannot be explained by other factors such as concurrent disease or other drugs, chemicals, treatments or lifestyle.

- **Probably related -** this classification will be applied if there is evidence to suggest that the adverse event is related to treatment with the studied device, and the influence of other factors is unlikely. For this classification to be applied, the adverse event, including an abnormal laboratory test result, should be an event that occurred within a reasonable time following treatment with the studied device, and the event is unlikely to be attributed to other factors such as concurrent disease, drugs, chemicals, treatments or lifestyle.

- **Possibly related -** this classification will be applied if there is some evidence to suggest that the adverse event is related to treatment with the studied device but it's possible that other factors have caused or contributed to the event, such as concurrent disease, drugs, chemicals, treatments or lifestyle.

- **Unlikely to be related -** this classification will be applied if it is deemed improbable that the adverse event is related to treatment with the studied device (e.g., the event did not occur within a reasonable time following treatment), or in cases which other factors, such as concurrent disease, drugs, chemicals, treatments or lifestyle provide plausible explanations for the event.

- **Not related -** this classification will be applied if the adverse event is deemed to be completely independent of treatment with the studied device, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the investigator.

Expectedness: an adverse event will be considered unexpected if the nature, severity or frequency of the event is not consistent with the risk information previously described for the studied device.

12.3. DOCUMENTATION OF ADVERSE EVENTS

All adverse events will be documented by study investigators or study staff on the participant's Case Report Form. Information to be collected includes event description, time of onset, treatment given, clinician's assessment of severity, relationship to studied device

(assessed only by those with the training and authority to make a diagnosis), whether the event is expected and time of resolution/stabilization of the event. All adverse events occurring during the course of the study will be documented appropriately regardless of relatedness to the studied device. All adverse events will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an adverse event. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an adverse event.

12.4. REPORTING OF ADVERSE EVENTS

Investigators will notify the sponsor of any unexpected or serious adverse events, whether related or not related to the studied device. If the adverse event is reportable, the manufacturer will report the event in accordance with applicable regulations.

13. PREPARATION AND STORAGE

13.1. STORAGE AND HANDLING

The Pocket-X Gel will be stored in accordance with manufacturer instructions between 20°C - 25°C, away from direct heat and sunlight.

13.2. PREPARATION

Prior to treatment, the device will be prepared for application according to manufacturer instructions:
–the Pocket-X Gel pre-filled syringe will be removed from its packaging, its cover will be removed, and one of the 2 tips provided with the device will be attached to the syringe.

14. REGULATORY AND ETHICAL CONSIDERATIONS

14.1. INFORMED CONSENT

A signed informed consent form must be obtained from each participant prior to enrollment. The informed consent will provide details of the study, the studied device and the intervention and procedures the participant will undergo as part of the study.

Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participant will sign the informed consent document prior to initiation of any study procedure. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time.

The informed consent will be approved by the study's ethics committee prior to initiation of participants screening.

14.2. STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the investigators will promptly inform study participants, the ethics committee and sponsor and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that is not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor and ethics committee.

14.3. PARTICIPANTS CONFIDENTIALITY

Participants confidentiality and privacy will be strictly held in trust by the participating investigators, their staff, and the study's sponsor.

All study procedures will be conducted in as private setting as possible.

The study participants identifying information (i.e. full name and/or ID number) and contact information will be securely stored at the clinical site for internal use during the study.

The participants study data will be available to the sponsor for the purpose of monitoring, statistical analysis, safety evaluation, scientific reporting and regulatory obligations. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number and the participant's initials.

15. STATISTICAL ANALYSIS

15.1. SAMPLE SIZE CONSIDERATION

The rationale for sample size was based on demonstrating a difference in change in pocket depth between the tested segments and the control, reflecting effect size of 0.5 with 80% power and 5% significance level.

Effect size is defined as the difference in change between the treatments adjusted to standard deviation, and calculated as follows:

(Change from baseline in Test segments - Change from baseline in Control segments) / Common standard deviation of the change.

Sample size Justification *

A sample size of 27 will have 80% power to detect an effect size of 0.5 using a Paired t-test with a 0.05 one-sided significance level.

Assuming ~20% dropout rate, 34 subjects will be recruited, in order to have 27 participants who complete the study.

Reference:

O'Brien, R.G., Muller, K.E. Applied Analysis of Variance in Behavioral Science Marcel Dekker, New York (1993) Chapter 8 pp. 297-344

* Additional sample size justification presents the same sample size and effect size, but specifies the expected changes within each treatment and the common standard deviation:

A sample size of 27 will have 80% power to detect a difference in means of -0.5 (e.g. a First condition mean, μ_1, of 2.0 and a Second condition mean, μ_2, of 2.5), assuming a standard deviation of differences of 1.0, using a paired T-test with a 0.05 one-sided significance level.

The calculations are summarized in the table below:

	1	2
Test significance level, α	0.05	0.05
1 or 2 sided test?	1	1
First condition mean, μ_1	2.0	
Second condition mean, μ_2	2.5	
Mean difference, $\mu_d = \mu_1 - \mu_2$	-0.5	
Standard deviation of differences, σ_d	1.0	
Effect size, $\delta = \lvert\mu_d\rvert / \sigma_d$	0.5	0.5
Power (%)	80	80
N	27	27

15.2. STATISTICAL METHODS

Study Population:

Intent-to-treat (ITT) population – all patients enrolled to the study who received at least one treatment.

Per Protocol (PP) population - all patients in the ITT population who completed the study according to the protocol and with no major protocol violations.

General

All measured variables and derived parameters will be listed individually and, if appropriate, tabulated by descriptive statistics. For categorical variables, summary tables will be provided giving sample size, absolute and relative frequency and 95% CI (Confidence Interval) for proportions by study arm. For continuous variables, summary tables will be provided giving sample size, arithmetic mean, standard deviation, median, minimum and maximum and 95% CI (Confidence Interval) by study arm for means of variables.

All tests will be two-tailed, and a p-value of 5% or less will be considered statistically significant.

The data will be analyzed using the SAS ® version 9.3 (SAS Institute, Cary North Carolina).

Periodontal pockets treated with Pocket-X Gel will be analyzed vs.control pockets.

Efficacy assessment

The following efficacy parameters will be summarized in appropriate tables by treatment and visit. Changes from baseline will be summarized as well.

- Pocket probing depth
- Bleeding index
- Recession level
- Clinical attachment level
- Mobility grade
- Furcation
- Specific periodontal bacteria quantification [optional]

The Paired T-test or Signed rank test for two means (paired observations) (as is appropriate) will be applied for analyzing the calculated changes by time point within each treatment.

The Paired T-test or Signed rank test for two means (paired observations) (as is appropriate) will be applied for analyzing the difference in the above measurements and changes between the treatments.

Responder will be defined based on improvement of gingival healing and natural sealing of periodontal pockets. Chi-square test or Fisher's Exact test (as is appropriate) will be applied for comparing the proportion of responders between the tested and the control segments.

The efficacy results will be compared also to literature data, if available. The two-sample T-test or Non-parametric Wilcoxon-Mann-Whitney Rank sum test for independent samples (as is appropriate) will be applied for comparative analysis.

Safety assessment

Safety assessments will be based on Adverse Events (AEs) and oral inspections recorded at each visit.

All AEs will be coded using coding dictionaries MedDRA (most updated version) and will be

summarized by system organ class (SOC) and preferred term (PT).

AEs will be listed individually including SOC, PT, seriousness of event, onset and resolution dates, duration, severity, relationship to the study treatment, action taken and outcome.

Frequency AEs and treatment-related adverse events will be summarized in tables by SOC, PT and treatment and by seriousness.

In case that the adverse event is localized, frequency of local AEs will be compared between the segment(s) treated with Pocket-X Geland the untreated segments of the same patient using Chi-square test or Fisher's Exact test (as is appropriate).

Adverse events will also be compared to adverse events reported during scaling and root planing studies, based on available literature. Chi-square test or Fisher's Exact test (as is appropriate) will be used for comparison in incidence of events.

16. RESPONSIBILITIES

16.1. INVESTIGATORS RESPONSIBILITY

Study Investigators will be responsible for the following:

- Ensuring that the investigation is conducted in accordance with the study protocol and applicable regulations of the local regulatory authority.
- Obtaining an informed consent from every participant.
- Reporting unexpected or serious adverse events to the sponsor.
- Supervising the use of the device. The study investigators shall permit the device to be used only by themselves or qualified dentists.
- Maintaining study records.
- Providing the sponsor with study records required for monitoring, statistical analysis, safety evaluation, scientific reporting and regulatory obligations.

16.2. SPONSOR'S RESPONSIBILITY

The study's sponsor will be responsible for the following:

- Selecting qualified investigators.
- Providing the investigators with the studied device, after they have undergone all necessary quality testing and product release procedures.
- Providing the investigators with information required for properly conducting the study.

- Reporting of serious adverse events that occur during the study to regulatory authorities in accordance with applicable regulations.

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