

# Hygiene protocols for the treatment of denture-related stomatitis: local and systemic parameters analysis - a randomized, double-blind trial protocol.

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**Study protocol**

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# Abstract

**Background.** The Denture-related stomatitis (DS) is a chronic multifactorial inflammation, strongly related to the presence of the biofilm that is the complex structure formed by microorganism held together by a mucus-like a matrix of carbohydrate that adheres to different surfaces, including denture surface. DS has recently been correlated with deleterious cardiovascular alterations. The potential effect of hygiene protocols in the control of DS and Randomized clinical trials that address this oral condition with cardiovascular complications are necessary for clinical decision-making. **Material/design.** A clinical trial, randomized, double-blind, of parallel groups, will be conducted in Brazil and the sample will be composed of 100 patients without teeth in both arches, users of at least maxillary complete dentures, diagnosed with DS that will be allocated in groups (n=25), according to the different hygiene protocols: (1) brushing of the palate and immersion of the prosthesis in 0.25% sodium hypochlorite solution (positive control); (2) brushing of the palate and immersion of the prosthesis in 0.15% Triclosan solution; (3) brushing of the palate and immersion of the prostheses in lactose monohydrate; (4) brushing the palate with citric acid and immersing the prosthesis in lactose monohydrate. The response variables will be: heart rate variability and alteration of blood pressure (systemic level), remission of Denture-related Stomatitis, removal of biofilm, reduction of microbial load (CFU), mouth and prosthesis odor level, expression of MUC1, proinflammatory cytokines, C-reactive protein level - CRP, viscosity, pH and salivary flow (locally); qualitative analysis patient-centred will also be held. Measurements will be performed in the Baseline and ten days after the interventions. The results obtained will be submitted to the pertinent statistical analysis with a level of significance of 0.05. **Discussion.** This study will provide a guideline of clinical practice regarding the use of hygiene protocols in the treatment of oral diseases (DS) mediated by biofilm. Also, it may provide evidence of correlation of oral manifestation with cardiac risk.

## Background

The elderly population continues to expand, and today, there are about 810 million people 60 and older in the world. By 2050, this number can reach two billion (22% of the global population). Intrinsic and extrinsic factors may promote decline in oral health, leading to tooth loss [1]; and edentulism or complete tooth loss would be the final consequence of oral disease [2].

Complete dentures are a widely used option in the rehabilitation of the stomatognathic system [3] and may be associated with Denture-related stomatitis (DS). *Candida albicans*, a common microorganism of the microflora of the oral cavity of humans, is often found in the biofilm of total dentures [4, 5]. However, in the presence of dentures and favorable conditions, such as biofilm, low salivary pH, regular sugar consumption and alterations of the local immune system (reduction of the activity of salivary antimicrobial enzymes, increase of transformer growth factor  $\beta$  and levels of nitric oxide), *C. albicans* becomes an opportunistic pathogen that leads to DS [6], as well as may trigger halitosis [7].

DS is the most commonly found oral manifestation, and the main indicator of poor oral health, among the edentulous population, affecting one in three individuals using removable dentures [8]. It is a chronic

multifactorial inflammation associated with the continuous use of maladaptive prostheses, hyposalivation, and poor hygiene; being considered one of the main factors responsible for the evolution of inflammation due to the prevalence of *Candida* spp. [2, 9, 10, 11, 12].

This inflammation can affect the individuals' quality of life since the clinical signs include erythema and edema of the palate mucosa, combined in some situations with subjective symptoms, such as dysgeusia (change in taste sensation) and burning sensation [8, 9, 13, 14]. However, in some patients, non-specificity of symptoms makes this disease often undiagnosed and untreated for long periods [15]. Furthermore, although considered to have a little overall impact in terms of mortality/morbidity, early diagnosis and correct treatment may avoid potentiation of the immune response at other sites and/or systemic consequence [1, 16, 17].

Local inflammation associated, or not, with a biofilm that is the complex structure formed by microorganism held together by a mucus-like matrix of carbohydrate that adheres to different surfaces, including denture surface [10], may trigger activation of monocytes and T cells, with overproduction of cytokines, such as interleukin (IL) -6, interferon  $\gamma$ , C-reactive protein (CRP) [17], tumor necrosis factor (TNF) - $\alpha$  and other proinflammatory cytokines; subsequently leading to atherosclerosis and hypertension, with increased cardiovascular risk [17, 18].

The relationship among stomatitis, *Candida albicans* infection, and systemic inflammatory response is recent and has not yet been clarified. Maciag et al. [16] evaluated, through the analysis of peripheral blood immune cell activation, whether the antifungal treatment of local inflammation, caused by DS, would influence the systemic immune response [16]. The authors did not find evidence of response complex immune mechanisms involved in the defense against oral fungal infection though, verified a possible systemic inflammatory response to the topical application of nystatin, a macrolide polyene antifungal [17, 18]. Although transient and not intense, this effect should be considered a clinically important finding; since patients with DS are generally elderly, and as such, more susceptible to changes in the immune function. Since IL-1 $\beta$  is a proinflammatory cytokine in this susceptible population; even a level low production, this non-physiological outcome can influence the risk of inflammatory diseases, such as atherosclerosis or rheumatoid arthritis.

Osmenda et al. [17] evaluated the clinical relationship between DS treatment and endothelial dysfunction, since local inflammation in the oral cavity may cause the production of anti and pro-inflammatory cytokines, triggering systemic inflammation and activation of an immune response. The results indicated that DS treatment improved the endothelial function, whose deterioration is known to precede the development of serious cardiovascular disorders, such as atherosclerosis and hypertension.

The evaluation of the pre-disposition of the individual with local inflammation to develop systemic diseases can be assessed through the detection of salivary mucins, which play an important role in the protection of buccal mucosa against mechanical and microbial aggressions, and the residual saliva presenting higher amounts of mucins compared to total saliva. Recently, the correlation between salivary glycoprotein expression (MUC1, MUC5B, and MUC7) and buccal candidiasis has been suggested. These

glycoproteins are responsible for the lubrication and protection of the oral tissues, as well as; they can act in the modulation of the response of microorganisms [19, 20].

Glycosylated transmembrane mucins, such as MUC1, are known as the second line of defense, possibly acting as sensors for any disturbance in the environment, signaling this information into the cell [20, 21], and interacting with local bacteria [22], acting as a barrier for opportunistic infections, against several bacterial strains. At this time, the microorganisms of the dental biofilm are disseminated into the systemic circulation, through the invasion of the gingival tissue throughout the ulcerated epithelium [23]. Also, several proinflammatory cytokines, produced by local inflammation, can reach the systemic circulation [24]; thus, justifying the correlation of stomatitis with systemic diseases.

Heart Rate Variability (HRV) is one of the reliable and non-invasive approaches to evaluate the autonomic control of the cardiovascular system in healthy individuals as well as patients with the cardiovascular disorder [25]. Although the correlation of the sympathovagal modulation is not a consensus on chronic inflammatory processes, the vagus nerve may be correlated with inflammation using two pathways. The first one, through the activation of the pituitary-hypothalamic-adrenal axis, resulting in systemic the secretion of cortisol, which reduces the inflammation. The second one, through the vagal and sympathetic branches that reach the spleen, reflecting cholinergic and then noradrenergic signals, triggering splenic T-cells via adrenergic receptors. These memory T cells will secrete the vagal neurotransmitter - acetylcholine - responsible for the innate immune response, which binds to the alpha-7 nicotinic acetylcholine receptor (nAChR) in monocytes, resulting in the inhibition of the synthesis of inflammatory cytokines [26]. Together, these two pathways constitute the vagal anti-inflammatory reflex [27].

Scientific evidence of the correlation among the biofilm, DS, cardiovascular disease, and adequate denture-related stomatitis treatment, may contribute to the establishment of a protocol for the prevention and treatment of local inflammation; to be applied to primary care, which can significantly impact the costs of public health and quality of life of patients. To our knowledge, this is the first proposal of a clinical, controlled, randomized, and double-blind study that proposes to correlate the treatment of DS, through hygiene and brushing solutions, with analysis of local and systemic inflammatory responses and cardiovascular impairment.

## **Study hypothesis**

The primary null hypothesis of the trial is that there is no difference between the protocols for the prevention and treatment of local and systemic inflammatory responses and cardiovascular risk.

## **Methods**

### **Study setting**

A randomized, controlled, double-blind, clinical trial with parallel groups named according to each hygiene protocol will be performed (figure 1). Figure 2 shows the study timeline, according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) diagram. Additional file 1 presents the SPIRIT checklist.

The sample will be of convenience, composed of patients who will attend routinely in the discipline of Complete Denture, at the School of Dentistry of Ribeirão Preto - University of São Paulo (FORP / USP).

## **Eligibility criteria.**

### *Inclusion criteria*

(1) Patients may be of both sexes; (2) must present good general health status; (3) patients without teeth in both arches, users of upper and lower conventional complete prostheses, or, necessarily, users of complete upper prosthesis (although edentulous mandibular) in good condition [28]; (4) prosthesis made from thermally polymerized acrylic resin and acrylic teeth; (5) presenting DS between types IB, II or III, according to the Newton Modified Classification [10]; and (6) their prostheses should present biofilm with a score equal to, or greater than 1, according to Additive Index Exclusion criteria.

Will be excluded: those patients who (1) present their prostheses with adaptation problems, with failures, repairs, or fractures; (2) allergy to any of the products studied; (3) Severe/serious illness that requires frequent hospitalization; (4) systemic conditions favorable to the development of *Candida* spp.; (5) use of antibiotics, anti-inflammatories, or antifungal agents in the last four weeks prior to the study; (6) other lesions on the oral mucosa; (7) practice of palatal mucous brushing already present during recruitment; (8) and replacement of prostheses in use during the experimental period.

## **Planned Interventions**

All patients will receive verbal and written instructions according to the hygiene protocols and they should do to brushing with soft toothbrush and water during 2 minutes the palate region, once a day (Toothbrush CS 5460C Adulto Ultra Macia, Curaprox, Curaden Swiss do Brasil Imp. Exp. LTDA, São Caetano do Sul, São Paulo, Brasil). To immerse their dentures in the specific product, once a day, according to the time proposed by the manufacturer, as well as to perform the brushing of the prosthesis for 2 minutes with a specific brush (Prosthesis Brush BDC150/152/153, Curaprox, Curaden Swiss do Brasil Imp. Exp. LTDA, São Caetano do Sul, São Paulo, Brasil) and neutral soap, 3 times a day. In addition, all patients will be instructed to remove the prosthesis during the night period and leave them in a container with clean water; and at morning, rinsing the prosthesis under running water, before inserting them into the oral cavity. Neutral soap and the solutions will be available to participants in identical dosing vials and sufficient quantity for continuous use for ten days; for greater control and monitoring of the hygiene

protocol. Citric acid in the form of an effervescent tablet will be removed from the package and placed in neutral packaging.

The parallel groups according to different hygiene protocols are Parallel groups will consist of 25 participants, according to the hygiene protocol:

G1 – brushing the palate with a soft brush and prosthesis immersion in 0.25% sodium hypochlorite solution (control group);

G2 - brushing the palate with a soft brush and prosthesis immersion in 0.15% triclosan solution;

G3- brushing the palate with a soft brush and prosthesis immersion in citric acid (Nitradine, Bonyf AG, Liechtenstein, Swiss); and

G4- prosthesis immersion and brushing of the palate with citric acid and soft brush (Nitradine, Bonyf AG, Liechtenstein, Swiss).

The risk of adverse effects is low, although the use of palatal brushing may promote trauma in case of excessive force. If the patient has a complaint about the products under investigation or adverse events, they may interrupt the treatment at any time, and notifying us of what has happened. To verify if the patient is performing the protocol and minimizing any risks, there will be a consultation five days after the beginning of the treatment, for clarification of doubts and follow-up. We expect good adherence levels due to the provision of an effective treatment modality as a major benefit combined with the need routinely for clinical attend at School of Dentistry of Ribeirão Preto, that the provisions post-trial care will be made. We will also remind participants a few days before each follow-up appointment by telephone.

The patients will be instructed to avoid the consumption of beverages or foods that alter the metabolism, such as coffee, soda, alcohol, chocolate, as well as physical exercises, 24 hours before the tests.

### **Randomization, allocation, and blinding**

The study will be double-blind, and for this, each researcher (R) will have an assignment during the experiment: Participants will be distributed into groups taking into account a random numerical sequence (on a 1:1:1:1 ratio), generated by a computer; the numbers of identification of the patients will be placed into an envelope by a blinded researcher, not involved in the clinical steps, which also will perform step the preparation of the products (R1). Another researcher will be responsible for opening the envelope at the moment of the delivery of the appropriate product to the patients, according to the hygiene protocols, and will perform the examination of patients and the collection of samples (R2). The R3 will perform the distribution of the protocols and the orientation of the participant, and finally R4: perform statistical analysis of data (blind). The researchers involved in the clinical steps (R2 and R3) and patients will not be

blinded because of the nature of the intervention. Patients' allocated interventions will not be revealed until the statistical analysis is completed.

## **Study outcomes**

### ***Primary: Denture-related stomatitis remission***

To evaluate the effect of the hygiene protocols on DS remission, the participants will be examined under the Baseline condition and also ten days after starting their specific of the use of the protocol. To quantify the inflammation, standardized photographs of the palate will be obtained (Digital Camera, Canon EOS, Canon EF 100 mm / 2: 8 Macro Lens and Canon ML3 Circular Flash), with the focus centered on the median raphe region. The images will be transferred to the computer and two blinded, previously trained researcher, will assign scores according to the classification of Kabawat et al. [10].

### ***Secondary***

#### ***Systemic evaluation***

The indirect measurement of the patients' blood pressure will be performed by the oscillatory sphygmomanometer method using an automated device (HEM7130, Omron Healthcare Brasil, São Paulo, SP); at which, 2 to 3 measurements with a 5-minute interval will be performed, recording the systolic (maximum) and diastolic (minimum) blood pressure values. The technique for obtaining and classifying the individuals will follow the categorization proposed by the American Heart Association, (2019).

Patients will be referred for continuous electrocardiographic monitoring with the Einthoven's II lead, combined with the monitoring of the respiratory rate with an elastic strap holding a stretch sensor around the thorax. The electrocardiogram and the respiratory sensor signal shall be filtered (100 Hz to 0.5 kHz), amplified (BioAmp ADInstruments, Bella Vista Australia), digitalized (PowerLab 2/20 ADInstruments Bella Vista Australia) and sampled (1000 Hz), continuously, using an IBM / PC. The files with the electrocardiogram recordings will be processed using a computer program (ECG Module for LabChart, ADInstruments, Bella Vista, Australia), which identifies the QRS complex of the electrocardiogram and calculates the duration of successive intervals between R waves (RR interval or cardiac interval). This processing will allow the generation of time series, beat-to-beat, from the cardiac interval values.

From these recordings, it will be performed the analysis of the cardiac interval variability (spectral analysis). The series with RR interval values will be re-sampled at 3 Hz by cubic interpolation, to regularize the interval between beats. The series with interpolated RR interval values will be divided into segments with 512 values each, with a 50% overlap. The stationarity of each segment will be examined visually, and those with artifacts, or transients, will be excluded. Each segment will have its spectrum

calculated by the Fast Fourier Transform (FFT), after Hanning windowing. The RR range spectra will be integrated into low (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.50 Hz) bands. The relative power (normalized units) of the RR interval spectra, in each frequency band, as well as the ratio of the LF and HF (LF / HF) powers thereof, will be determined.

Symbolic Analysis searches for patterns of changes between successive cardiac interval value, classify these changes and quantifies their occurrence. Sequences of 3 symbols will then be analyzed and classified into four different families, according to the number of variations found. The frequency of occurrence of each pattern will be analyzed, and indicated, as 0V%, 1V%, and, 2V%. The frequency of variations of type 0V (sympathetic) and 2V (vagal) are of interest as indicators of cardiocirculatory autonomic modulation.

The Sampling Entropy (SampEn) will be calculated from the IC series with the help of the JBioS software. The  $n$  practical terms, SampEn quantifies the (logarithmic) probability that near-size patterns  $m$  will continue to  $m + 1$ . In other words, of the size patterns  $m$  that are similar, SampEn indicates which percentage of these will remain similar for  $m + 1$ , that is, when an extra point is considered. High probability of the patterns continuing close indicates regularity, yielding low values of entropy. Cardiac variability and blood pressure will be recorded in the control period and after the treatment of DS through hygiene protocols. Thus, each will be the control of himself.

### *Local parameters*

To verify the biofilm removal of the inner surface of the upper prosthesis, the technique described will be performed according to Badaró et al. [11], that from the biofilm evidence, the prostheses will be photographed in standardized positions. The areas of biofilm and the surface of the prosthesis will be calculated using software and will be applied in a formula to identify the amount of total area of the biofilm, before and after treatment.

The microbial load of the prostheses and the palate will be evaluated. The biofilm collection of these sites will be followed according to the protocol recommended by Kabawat et al. [10] and de Souza et al. [29]. Serial dilutions will then be obtained which will be seeded in Petri dishes, with culture medium specific for the growth of *Staphylococcus* spp. (Mannitol Salt Agar, Kasvi Imp. e Dist. de Prod. para Laboratórios Ltda, Curitiba, Brasil), Gram-negative bacteria (MacConkey Agar, Himedia Laboratórios PVI Ltd, Mumbai, Índia), *Candida* spp. (CHROMagar™ *Candida*, Becton Dickinson, Paris, França) and *Streptococcus mutans* (SB20 Modified Agar with Casitone, Himedia Laboratories PVI Ltd, Mumbai, Índia), and incubated in a microbiological stove (De Leo Equipamentos Laboratoriais, Porto Alegre, RS, Brasil) at 37 ° C for 48 hours. The cultivation of *S. mutans* will be in an environment of microaerophilic in jar of anaerobiosis (Permutation, Curitiba, PR, Brasil). After the incubation period, the CFU count will be performed to quantify the microbial load. The biological specimens present in biofilm collected will be stored at -

80°C for future analysis, if utilized; we will formally communicate any amendments to the protocol during the trial.

The Breath Alert™ portable device (Tanita Corporation®-Japan), used according to the manufacturer's instructions, will measure the odor of the cavity with and without the denture. The odor level will be given using by score, presented in the apparatus, with values that can vary from 1 to 4. Thus, the classification of the odor is classified according to the scores [30] 1) Odorless, normal; 2) Mild, normal odor; 3) Moderate, bad breath - perceptible; 4) Strong odor, noticeable. The evaluation will be performed with the participant without the prostheses in position, and then with the prostheses seated in the oral cavity. Thus the odor related to the prosthesis will be calculated based on the difference between the odor of the cavity with the prosthesis and odor of the cavity without the prosthesis.

Saliva samples will be collected to evaluate salivary parameters. The non-stimulated total saliva will be collected for 10 min by the method of spitting, which will be subjected to viscosity analysis and pH measurement. The calibration of pH will be performed in a pHmetro (PHTEK, Curitiba, Paraná, Brazil) after calibration of the equipment. The kinematic viscosity analysis of saliva will be measured using glass viscometer, and the liquid viscosity coefficient will be calculated according to Shekhar et al. [31]. The total stimulated saliva will be collected for 5 min using the habitual chewing of 1g of gum base [19], from which the calculation of saliva volume will be obtained to evaluate the salivary flow. Subsequently, saliva samples will be centrifuged at 10,000 xg, for 15 min, at 4°C, to remove cellular debris. Aliquots of supernatant will be stored at - 80°C for the analysis. The precipitates will be evaluated by ELISA [22] in identification and quantification of MUC1 expression. The absorbance at 405 nm will be measured after 30-45 min in an ELISA reader.

As a control, wells without saliva will be used. The assay for saliva will be performed in triplicate, and the results will be presented as the mean difference between optical density (OD) readings in experimental and control wells. Salivary concentrations of cytokines (IL-6 and TNF- $\alpha$ ) will be measured using enzyme-linked immunosorbent assay kits (Enzyme Linked Immunosorbent Assay – ELISA) Multiplex Human Cytokine Magnetic Bead Kit (Millipore, United States), according to the manufacturer's instructions [32]. The determination of C-reactive protein in saliva will be performed using CRP ELISA kits (Salimetrics Europe Ltda.) [33]. Both ELISA methodologies will be performed in duplicates on two standard 96-well microplates, according to the protocol provided by the respective suppliers.

### *Characterization of the sample*

Socio-demographic characteristics of the participants of the study will be collected during the first consultation through medical and dental history reports. Information will be collected such as edentulism time, age of prostheses in use, drug profile, hygiene habits (use of oral antiseptics or chemical hygiene of prostheses, frequency of hygiene of the prostheses), continuous nocturnal use, and smoking.

The Quality of Life associated with Oral Health will be evaluated by applying the Oral Health Impact Profile questionnaire, specific for edentated patients (OHIP-EDENT), validated for the Brazilian population [34]. The questionnaire presents 19 questions for four domains: "complaints related to chewing", "psychological discomfort and incapacity", "social incapacity", and "pain and mouth discomfort". Participants will be asked to answer the questions according to their feelings according to one of the following: 'never', 'sometimes', or 'almost always'.

Patient satisfaction will be assessed by the frequency of specific symptoms, such as local pain, burning sensation, bad breath, and buccal dryness. The responses will be collected based on a 100 mm visual analog scale (VAS), which will provide parameters for assessing heterogeneity of the eligible sample in the Baseline. They will also be asked to give an open-label response to other sensory side effects [10, 29]. The Quality of Life associated with Oral Health and patient satisfaction will be collected in baseline, 10 and 30 days after treatment with hygiene protocols (figure 1).

During clinical examination, the conditions of the prostheses in use, such as stability and retention according to Anastassiadou et al. [28], as well as biofilm deposits and visible debris, will be observed [11]. Data on the shape and resilience of mucosa both arches ridge will also be collected. The data on the quality of life - related to oral health, quality of the prostheses and general satisfaction will be collected for the characterization of the sample in the baseline [29].

### **Sample size estimation**

Sample size for the quantitative outcomes was determined based on the primary outcome of this study (Denture-related stomatitis remission). According to previous trial [11], considered the standard deviations 2.19 (1-saline group) and 1.79 (sodium hypochlorite group 2), a 95% confidence interval (bilateral), and a detectable difference of at least 2 logs. Based on a power of 80%, this study clinical requires at least 21 participants. An additional 20% will be added to the planned sample to compensate for possible dropouts, thus resulting in a total of 25 participants.

### **Statistical analysis**

Data entry and analysis will be conducted in a blinded fashion. The data collected for the groups, prior to adherence to the hygiene protocols, such as age, gender, and grade of schooling, will be compared to ascertain the initial similarity. The effect of groups on primary and secondary outcomes will be assessed. When applicable (ex. OHIP-EDENT), pre-treatment values will be applied as a co-variable in the statistical model. The significance level of the tests will be 0.05. Noncompliant participants will be followed up, such as those requiring interruption of one of the hygiene protocols, and to evaluate the significance of protocol deviations. In other words, the statistical analysis will consider the participants according to the

treatment received (per protocol), as well as according to the planned treatment (intention-to-treat - ITT); and the results will be confronted.

The data will be analyzed for homogeneity; in the case of non-normal distribution, the Brunner Langer nonparametric test will be used. For the categorical variables (questionnaire) it will be applied the Friedman test to compare the different times, and the Kruskal-Wallis test to compare the groups. A correlation test will be performed between the quality of life indices and the quantitative variables (Pearson correlation coefficient). The tests used for subgroup analysis will be Tukey with Bonferroni adjustment. For Kruskal-Wallis the post-test will be the Dunn test.

A flowchart of the participants will be prepared for a detailed explanation of the characteristics of the sample and the quantification of quitters and missing participants. This part will provide the number of individuals examined, and reasons for exclusion, as well as the recruited, treatment-allocated participants who complete the trial and analyze it at the end. The flowchart will provide the reasons for any deviation from the protocol.

### **Data management, monitoring, and auditing**

A data monitoring committee composed of an independent researcher will check collected data regularly. This researcher shall have no relationship with the trial sponsors. Moreover, the Institutional Board at Sao Paulo University may conduct an independent audit at any time. Our study will end when reach their sample size goal, or when they reach their scheduled date of closure.

### **Ethical Considerations and Dissemination**

This study protocol was approved by the Research Ethics Committee of the School of Dentistry of Ribeirão Preto (CAAE 93712418.1.0000.5419), and Registered on November 9, 2018, on the ReBec platform <http://www.ensaiosclinicos.gov.br/rg/?q=RBR-4hhwjb>, and will be reported in compliance with the CONSORT statement.

Eligible candidates will be invited to participate in the study and given sufficient time to read the informed consent and ask any questions pertaining to their participation. After signing the consent form (Additional file 2), the participants will be formally enrolled in the study and baseline assessment will be conducted. As a consent clause, we will grant the individuals the right to withdraw from the study at any time. All documents relating to the participants, such as terms of consent and clinical data, will be kept in a locked cabinet to guarantee their confidentiality.

On the consent form, participants will be asked if they agree to use of their data should they choose to withdraw from the trial. Participants will also be asked for permission for the research team to share

relevant data with people from the School of Dentistry of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil taking part in the research or from regulatory authorities, where relevant. This trial does involve collecting biological specimens for storage.

Electronic data handled by the researchers will contain numerical codes in place of the names. Any changes to the protocol will be conducted after the opinion of the Research Ethics Committee and development agencies. Also, the authors will disclose the results of this proposal, regardless of the findings. The results of the RCT will be presented at major scientific conferences, including the International Association for Dental Research (IADR) General Session; and will be disseminated in a peer-reviewed journal.

## **Discussion**

This RCT will provide a guideline of clinical practice regarding the use of hygiene protocols in the treatment of oral diseases mediated by biofilm (DS). Also, it may provide evidence of correlation of oral manifestation with cardiac risk.

The DS has been recently associated with systemic implications (variations in blood pressure and endothelial dysfunction) that precede the development of serious cardiovascular disorders, such as atherosclerosis and hypertension, which are changes in general health with high mortality / morbidity rates [16-18]. However, the mechanisms that correlate oral inflammation and cardiovascular effects are not yet fully described, but one of the most important hypotheses is the pre-activation of the immune system [17].

The information from this study will also improve clinical decision-making and potentially protect edentate patients from harm caused by ineffective treatment, as well as, the incorporation of hygiene protocols for oral tissues and prostheses can achieve favorable results associated with low costs and minimal adverse effects, besides possibly avoid the involvement of opportunistic diseases, which may lead to an decreased risk of cardiac diseases. The resulting will be published information will provide evidence for the development of clinical recommendations for DS, which will not only be used for publication in indexed journals but also for public health information services.

Thus, we predict a favorable impact on public health, while the results will provide the basis for future investigations of the characterization of possible changes in HRV, correlated or not, with changes in blood pressure, salivary pro-inflammatory markers and salivary parameters with DS. Given the vast prevalence of denture stomatitis in this elderly population, such knowledge may be crucial for effective control as well as for the detection of cardiovascular disease risk.

## **Trial status**

First version (01) of the study protocol. Date: July 22, 2019.

Second version (02) of the study protocol. Date: October 17, 2019.

Recruitment of patients:

Initial date: 2018-09-03

This trial is currently recruiting patients.

## Abbreviations

CONSORT: Consolidated Standards of Reporting Trials; OHIP-EDENT: Oral Health Impact Profile for edentulous people; RCT: Randomized clinical trial; SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials; VAS: Visual analog scale, CFU: Colony forming unit; DS: Denture stomatitis; ITT: Intention to treat; CRP: C-reactive protein level; MUC: Transmembrane mucin; ELISA: Enzyme Linked Immunosorbent Assay; OD: optical density.

## Declarations

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### **Authors' contributions**

All the authors contributed to the elaboration of the project. ABR<sup>a</sup>, CBA and CHS-L idealized and contributed intensely in the development of the study; RFJ, HCS, EW and HFOP analyzed and verified study design; EW, ABR<sup>a</sup>, ABR<sup>b</sup> and LEVS elaborated and thoroughly reviewed the manuscript; VCO, FLB and CVF supported the laboratory methodologies. The authors ABR<sup>a</sup>, CHS-L and HCS were responsible for the search for funding to this research. EW will be to PCR analysis of specimens biological.

### **Ethics approval**

This study has been approved by the ethics committee of the School of Dentistry of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil (register CAAE number 93712418.1.0000.5419).

### **Consent for publication**

Not applicable since there are no identifying images or other personal or clinical details of participants presented. Informed consent materials are available from the corresponding author.

### **Competing interests**

The authors declare that they have no competing interests.

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## Additional Files

Additional file 1: The Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) checklist for this trial.

Additional file 2: Model consent form.

## Figures

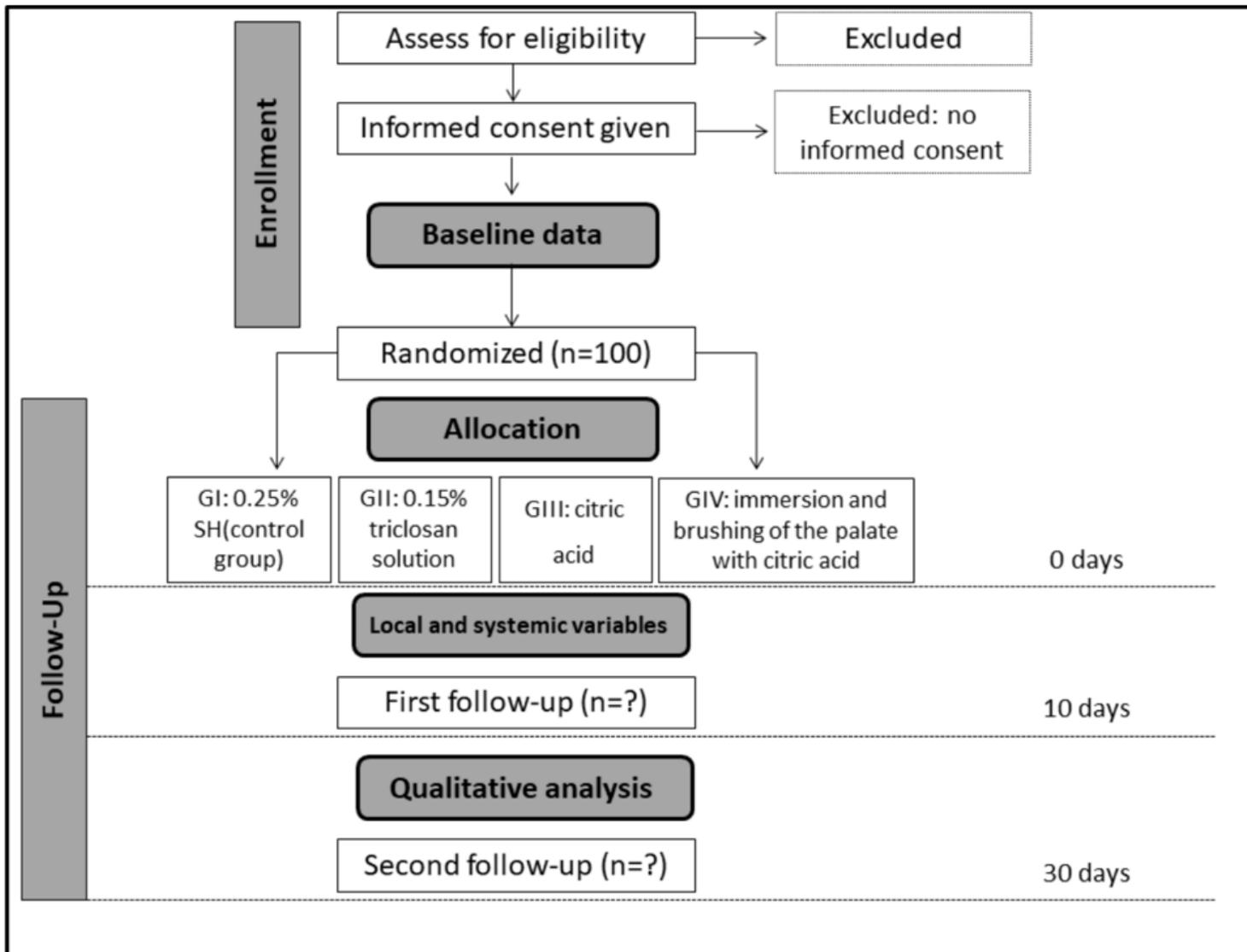


Figure 1

Flowchart of the RCT (adapted from the CONSORT statement). For each follow-up, numbers of withdrawn and lost participants will be reported with reasons.

STUDY PERIOD							
	Enrolment	Preparatory stage	Allocation	Post-allocation			Close-out
TIMEPOINT**	-t <sub>2</sub>	- 1 week (-t <sub>1</sub> )	0	5 days	10 days	30 days	24 months
<b>ENROLMENT:</b>							
Eligibility screen	X						
Informed consent	X						
Allocation			X				
<b>INTERVENTIONS:</b>							
Sodium hypochlorite immersion			←————→				
Triclosan solution immersion			←————→				
Citric acid immersion			←————→				
Citric acid immersion and brushing			←————→				
<b>ASSESSMENTS:</b>							
Socio-demographic and medical variables		X					
Oral health and denture-associated variables		X					
Denture-related stomatitis remission			X		X		
Systemic evaluation			X		X		
Local parameters			X		X		
Patients satisfaction and OHIP-EDENT		X				X	
Data analysis						X	X
Knowledge dissemination						X	X

Figure 2

Study schedule: enrolment, allocation, baseline, interventions and post-intervention assessments

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TCLEModelconsentformenglish.pdf](#)
- [Ethicsformhygieneprotocols.pdf](#)
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