# Systematic Review Protocol for Animal Intervention Studies

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<table>
<thead>
<tr>
<th>Item #</th>
<th>Section/Subsection/Item</th>
<th>Description</th>
<th>Check for approval</th>
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</thead>
<tbody>
<tr>
<td>A. General</td>
<td>Title of the review</td>
<td>Influence of antimicrobial photodynamic therapy on alveolar bone loss: a systematic review and meta-analysis of animal studies</td>
<td>✓</td>
</tr>
<tr>
<td>2. Authors (names, affiliations, contributions)</td>
<td>Marta Aparecida Alberton Nuernberg(^a) Daniela Maria Janjacomo Miessi(^a) Camila Ayumi Ivanaga, Mariane Bocalon Olivo(^b), Edilson Ervolino(^b), Valdir Gouveia Garcia(^b), Mark Wainwright(^c), Leticia Helena Theodoro(^a). (^a) UNESP, São Paulo State University, Group of Study and Research in Lasers in Dentistry, School of Dentistry, Araçatuba, SP, Brazil (^b) Latin American Institute of Dental Research and Education (ILAPEO), Curitiba, PR, Brazil. (^c) School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>3. Other contributors (names, affiliations, contributions)</td>
<td>Marta Aparecida Alberton Nuernberg: <a href="mailto:marta.nuernberg@hotmail.com">marta.nuernberg@hotmail.com</a> Daniela Maria Janjacomo Miessi: <a href="mailto:danieljanjacomo@hotmail.com">danieljanjacomo@hotmail.com</a> Camila Ayumi Ivanaga: <a href="mailto:camilaivanaga@gmail.com">camilaivanaga@gmail.com</a> Mariane Bocalon Olivo: <a href="mailto:marianebocalon.56@hotmail.com">marianebocalon.56@hotmail.com</a> Edilson Ervolino: <a href="mailto:eervolino@foa.unesp.br">eervolino@foa.unesp.br</a> Valdir Gouveia Garcia: <a href="mailto:vg.garcia@uol.com.br">vg.garcia@uol.com.br</a> Mark Wainwright: <a href="mailto:mark_wainwright@hotmail.com">mark_wainwright@hotmail.com</a> Leticia Helena Theodoro: <a href="mailto:letheodoro@foa.unesp.br">letheodoro@foa.unesp.br</a></td>
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<tr>
<td>4. Contact person + e-mail address</td>
<td>None.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>5. Funding sources/sponsors</td>
<td>None.</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>6. Conflicts of interest</td>
<td>No conflicts of interests.</td>
<td>✓</td>
<td></td>
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<tr>
<td>7. Date and location of protocol registration</td>
<td>-</td>
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<td>8. Registration number (if applicable)</td>
<td>-</td>
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<td>9. Stage of review at time of registration</td>
<td>-</td>
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<td>B. Objectives</td>
<td>Background</td>
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<td>10. What is already known about this disease/model/intervention? Why is it important to do this review?</td>
<td>Some meta-analysis and systematic reviews have evaluated the adjuvant use of antimicrobial photodynamic therapy (aPDT) in the periodontal treatment of patients with chronic periodontitis [37, 38, 41], aggressive periodontitis [39], patients with systemic impairment (diabetes) and in the treatment of residual pockets [40]. However, the results of experimental animal studies have not been evaluated and discussed to compare the</td>
<td>✓</td>
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</table>
parameters of aPDT regarding its real effectiveness in the control of alveolar bone loss. Therefore, the purpose of the present study was to assess the effects of aPDT as an adjunctive therapy to SRP compared to SRP alone on alveolar bone loss in experimental periodontitis in rats, through a systemic review and meta-analysis.

### Research question

11. Specify the disease/health problem of interest
   - Experimental periodontitis

12. Specify the population/species studied
   - Model of experimental periodontitis in Wistar rats

13. Specify the intervention/exposure
   - Treatment with aPDT as an adjunct to scaling and root planning (SRP)

14. Specify the control population
   - Treatment with SRP alone followed irrigation with saline solution

15. Specify the outcome measures
   - Alveolar bone loss in furcation region

16. State your research question (based on items 11-15)
   - "What are the effects of aPDT as an adjunctive therapy to SRP compared to SRP alone on alveolar bone loss in experimental periodontitis in rats?"

### C. Methods

#### Search and study identification

17. Identify literature databases to search (e.g. Pubmed, Embase, Web of science)
   - MEDLINE via PubMed
   - SCOPUS
   - EMBASE
   - Other, namely:

18. Define electronic search strategies (e.g. use the step by step search guide\(^{15}\) and animal search filters\(^{20, 21}\))
   - When available, please add a supplementary file containing your search strategy: [Supplemental file 1]

19. Identify other sources for study identification
   - Reference lists of included studies
   - Reference lists of relevant reviews
   - Conference proceedings, namely:
   - Contacting authors/organisations, namely:
   - Other, namely: ProQuest Dissertations and Theses

20. Define search strategy for these other sources
   - Supplemental file 1

#### Study selection

21. Define screening phases (e.g. pre-screening based on title/abstract, full text screening, both)
   - 1) screening based on title and abstract
   - 2) full-text screening of the eligible articles

22. Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved
   - Each phase: Two blind reviewers (M.A.A.N. and D.M.J.M) independently will assessed each article. Differences will be solved through discussion or by consulting a third investigator (L.H.T).

*Define all inclusion and exclusion criteria based on:
|   | Type of study (design) | Inclusion criteria: animal studies
Exclusion criteria: review papers, clinical trial, case reports, letters to the editor, commentaries, interviews, updates, in vitro studies. | ✓ |
|---|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
|   | Type of animals/population (e.g. age, gender, disease model) | Inclusion criteria: studies with a model of experimental periodontitis in Wistar rats induced by a cotton ligature placed in a sub marginal position for 7 days.
Exclusion criteria: studies with other protocols for induction of experimental periodontitis (without ligature), studies with model of experimental periodontitis induced by a cotton ligature placed in a sub marginal position for more than 7 days; studies with protocol of ligature permanence. | ✓ |
|   | Type of intervention (e.g. dosage, timing, frequency) | Inclusion criteria: studies which compared conservative SRP to the use of aPDT (with laser) as an adjunctive therapy to SRP in experimental periodontitis in rats.
Exclusion criteria: studies which assessed the use of aPDT as monotherapy. | ✓ |
|   | Outcome measures | Inclusion criteria: studies which assessed the alveolar bone loss in furcation region by histometric analyses.
Exclusion criteria: studies which assessed the alveolar crestal bone loss by histometric analyses, studies without histometric analyses. | ✓ |
|   | Language restrictions | Inclusion criteria: all publication dates.
Exclusion criteria: none. | ✓ |
|   | Publication date restrictions | Inclusion criteria: all publication dates.
Exclusion criteria: none. | ✓ |
|   | Other | NA | ✓ |
|   | Sort and prioritize your exclusion criteria per selection phase | **Selection phase title and abstract screening:**
1. Review papers, letters to the editor, commentaries, interviews, updates
2. Human (clinical trial, case reports)
3. No full paper (abstract, comment)
4. Data published in duplicate
5. Not in vivo (e.g. ex vivo/in vitro/in silico)
6. No comparison SRP/ aPDT + SRP
7. Protocols for induction of experimental periodontitis without ligature
8. studies with model of experimental periodontitis induced by a cotton ligature placed in a sub marginal position for more than 7 days; studies with protocol of ligature permanence
9. aPDT as monotherapy
10. No histometric analyses
11. Assessed the alveolar crestal bone loss by histometric analyses

**Additional criteria for full text screening:**
12. No comparison SRP/ aPDT + SRP
13. aPDT as monotherapy
14. Protocols for induction of experimental periodontitis | ✓ |
without ligature

15. studies with model of experimental periodontitis induced by a cotton ligature placed in a sub marginal position for more than 7 days; studies with ligatures permanence protocol.

16. No histometric analyses

17. Assessed the alveolar crestal bone loss by histometric analyses

| Study characteristics to be extracted (for assessment of external validity, reporting quality) | Authors and year | ✓
| Study design characteristics (e.g. experimental groups, number of animals) | Number of animals, details about experimental groups, experimental periods (time-points) | ✓
| Animal model characteristics (e.g. species, gender, disease induction) | Animal species, gender, age, weigh, systemic condition | ✓
| Intervention characteristics (e.g. intervention, timing, duration) | Parameters used in aPDT therapy (laser types, wavelengths, spot size, power, energy density, total energy, type and concentration of photosensitizer, pre-irradiation time, irradiation time and laser irradiation method). | ✓
| Outcome measures | Processing for microscopic analyses, and mean bone loss in mm² and standard deviation. | ✓
| Other (e.g. drop-outs) | None. | 

| Assessment risk of bias (internal validity) or study quality | 
| Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved | a) 2 reviewers. The criteria will be independently assessed by two reviewers (M.A.A.N and D.M.J.M), independently, by using collectively predefined assessment criteria. b) discrepancies will be resolved by a third reviewer (L.H.T.). | ✓
| Define criteria to assess (a) the internal validity of included studies (e.g. selection, performance, detection and attrition bias) and/or (b) other study quality measures (e.g. reporting quality, power) | By use of SYRCLE’s Risk of Bias tool | ✓
| Collection of outcome data | 
| For each outcome measure, define the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement) | Alveolar bone loss in furcation area by the mean of bone loss in mm² and standard deviation. | ✓
| Methods for data extraction/retrieval (e.g. first extraction from graphs using a digital screen ruler, then contacting authors) | The outcome data of alveolar bone loss will be extracted from text and tables, and the corresponding authors will be contacted for missing data in the paper. | ✓
| Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved | a) Two reviewers (M.A.A.N and E.E ) will extract all data b) discrepancies will be resolved by a third reviewer (L.H.T) | ✓
| Data analysis/synthesis | 
| Specify (per outcome measure) how you are planning to combine/compare parameters used in aPDT will be combine in tables for | | ✓
<table>
<thead>
<tr>
<th>The data (e.g. descriptive summary, meta-analysis)</th>
<th>Descriptive analysis. Meta-analysis with subgroup analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>43. Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed</td>
<td>No restrictions in terms of heterogeneity will be applied, instead, sources of heterogeneity will be investigated through subgroup analysis.</td>
</tr>
<tr>
<td>If a meta-analysis seems feasible/sensible, specify (for each outcome measure):</td>
<td></td>
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<tr>
<td>44. The effect measure to be used (e.g. mean difference, standardized mean difference, risk ratio, odds ratio)</td>
<td>Mean difference (MD) with the corresponding 95% confidence intervals</td>
</tr>
<tr>
<td>45. The statistical model of analysis (e.g. random or fixed effects model)</td>
<td>Random-effects model.</td>
</tr>
<tr>
<td>46. The statistical methods to assess heterogeneity (e.g. I², Q)</td>
<td>I² statistic</td>
</tr>
<tr>
<td>47. Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)</td>
<td>Systemic condition (systemically healthy animals and systemically modified animals) Experimental periods postoperative</td>
</tr>
<tr>
<td>48. Any sensitivity analyses you propose to perform</td>
<td>Study quality</td>
</tr>
<tr>
<td>49. Other details meta-analysis (e.g. correction for multiple testing, correction for multiple use of control group)</td>
<td>If studies report data for a series of time points, we will pool the data of different time points to correct for repeated measurements of dependent variables</td>
</tr>
<tr>
<td>50. The method for assessment of publication bias</td>
<td>-</td>
</tr>
</tbody>
</table>

Final approval by (names, affiliations): Marta Aparecida Alberton Nuernberg\textsuperscript{a} 
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Edilson Ervolino\textsuperscript{a} 
Valdir Gouveia Garcia\textsuperscript{a,b} 
Mark Wainwright\textsuperscript{c} 
Letícia Helena Theodoro\textsuperscript{a} 

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\textsuperscript{c} School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK. 

Date: 16/10/2018