Author's response to reviews

Title: Interaction of titanium, zirconia and lithium disilicate with peri-implant soft tissue: study protocol for a randomized controlled trial

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Version: 2
Date: 12 August 2015

Author's response to reviews: see over
Dear Prof. Altman, Prof. Furberg and Prof. Grimshaw,

we have revised our paper "Interaction of titanium, zirconia and lithium disilicate with peri-implant soft tissue: study protocol for a randomized controlled trial" (former title: “Dental materials' interaction with peri-implant soft tissue: study protocol for a prospective, randomized clinical pilot-trial”) according to the Editorial requests and the Reviewer's comments. We were able to address all issues raised (see below). The main improvements to the manuscript are the addition of many technical details and an extensively revised Introduction and Discussion. Independent of reviewers requests, we updated the literature and trial status.

We have uploaded a copy with tracked changes (main manuscript) and a clear copy of the revised manuscript (additional material files).

We hope that the revised version of our paper will be acceptable for publication in Trials.

Sincerely yours,

Katharina Kuhn

Editorial requests:
1. Please ensure the title conforms to journal style for study protocol articles. The title should follow the format ?___________: study protocol for a randomized controlled trial.? Please note that the title in the submission system should match that of your manuscript.

We adjusted the title accordingly and according to a request of the second reviewer (“Interaction of titanium, zirconia and lithium disilicate with peri-implant soft tissue: study protocol for a randomized controlled trial”) both in the manuscript and in the submission system.

2. Please include the email addresses of all authors on the title page.

We included the email addresses of all authors on the title page.
3. Please include the date of registration with the trial registration number at the end of the Abstract.

We included the date of registration with the trial registration number at the end of the Abstract.

4. Please include a statement in your Methods section explaining that you obtained informed consent from each participant.

Under the subheading “Settings and locations where the data is collected” we added this information. Besides, we already mentioned “Twenty-four patients are intended to participate in the study after giving informed consent.” under the subheading “Trial design”.

Reviewer 1:
Reviewer's report
Title: Dental materials' interaction with peri-implant soft tissue: study protocol for a prospective, randomized clinical pilot-trial
Version:1 Date:16 June 2015
Reviewer: Zeeshan Sheikh
Reviewer's report:
Minor Essential Revisions THERE ARE SOME MINOR ENGLISH LANGUAGE ISSUES… THESE ARE JUST A FEW. PLEASE CORRECT THEM.
Page 2, bottom – extend should be extent
We deleted the sentence “The study is funded to a major extend by Ivoclar Vivadent AG (Schaan, Liechtenstein)” from the abstract, as it is part of the subheading “Acknowledgments”. We corrected the mistake under the subheading “Acknowledgments”.

Page 4- materialS.
We corrected it.

Page 5- Ulm University not The Ulm University
We corrected it.
Reviewer 2:

Reviewer's report
Title:Dental materials' interaction with peri-implant soft tissue: study protocol for a prospective, randomized clinical pilot-trial
Version:1 Date:17 June 2015
Reviewer:Rubens Albuquerque

Reviewer's report:
General comments:
The study protocol (...). Overall, the proposal is clearly described and feasible.
Specific comments:
Minor Essential Revisions:
1. Despite the protocol being clear, it would benefit from a grammar revision. *An Expert English Editing Service edited the manuscript linguistically before resubmitting it.*

2. The main issue with this protocol is related to its crossover design. While there is clear potential for carry over, period and time effects, no explanation has been presented on how these problems are going to be dealt with. Moreover, while statistical methods exist to minimize these problems a sample size of 4 patients per group may not render satisfactory results and so the hypothesis might not be adequately tested. The use of washout periods should be considered and some sort of sample size calculation or power test could perhaps be performed based on similar outcomes of previous investigations as an attempt to minimize the negative impact of those factors. Another possibility for consideration is the withdrawal of one of the three test materials from the investigation.
This is a reasonable criticism. We have discussed the same issues with our biometricians when we designed the study. During the establishment of the study design, there have been no clinical results, on which a biometrical sample size estimation could have been based as stated under the subheading “Sample size”. Using a study with a design not adequately comparable to ours for a sample size calculation/power test could be a confounder instead of being clarifying. Consequently, we decided with our biometricians on applying an explorative data analysis. A power analyses as well as sample size estimation will be done for similar clinical studies in the future as stated under the subheading “Statistical methods”. We agreed on the cross-over design to enhance the collected information from the pilot study which is already very time- and cost-consuming.

During the design of the study, we have considered washout periods between the change of 2 abutments. However, we decided against due to feasibility reasons. Washout periods may be used between the different abutment materials to exclude an influence of the preceding abutment material on the tissues. Washout periods may be performed either by wearing a “neutral” abutment for a specific time between the tested abutments or by permitting tissues to heal without an abutment and punch biopsy again before inserting the next abutment. Following discussion with biometricians, the additional punch biopsies and time on top of an already prolonged procedure were deemed ethically unjustifiable. In addition, the newly formed cells and tissue in direct contact with each abutment are most likely to be affected and are circularly removed by the stamping press. However, an influence of the preceding abutment material on the biomolecular and immunohistochemical findings of the next abutment material cannot be fully excluded. To the author’s knowledge, there is no study which has tested this aspect yet. If our biometricians detect clear evidence of previous abutment influence (worst-case scenario) only the first abutment material (n=8 for each abutment material) shall be used for analysis. We have added this methodological aspect under the subheading “Discussion”. We didn’t renounce one of the three test materials as both ceramics-zirconia and lithium disilicate- have become clinically highly relevant. Titanium serves as gold standard. We added this methodological specification under the subheading “Discussion”. Thus, we decided for all three materials in combination with the cross-
over design to enhance the collected information from the pilot study which is already very time- and cost-consuming as mentioned above.

3. On the methodology, the authors should give further explanation on how the supragingival plaque will be removed (p. 8, l. 17), what temperature the paper strips will be frozen at (p. 9, l. 9), how the PICF extracts will be analyzed for biomolecular detection of specific markers (p. 9, l. 15), and provide more details on the immunohistochemical/morphometric analyses. Lack of this information could preclude the replication of the work or comparison with related analyses.

*The supragingival plaque is carefully removed using cotton balls.*

*The paper strips are frozen immediately after sampling at -80°C.*

*MMP-8 is analyzed with the Fluorokin MAP Multiplex Human MMP Panel (R & D Systems GmbH, Wiesbaden, Deutschland) in the Luminex 200 System (Bio-Rad Laboratories, Inc. CA, USA). The MMP-8 activity is detected by means of gelatine-zymography. IL-1β is measured by the Bio-Plex Cytokine Assay Kit (Bio-Rad Laboratories Inc., Hercules, CA, USA) in combination with the Luminex 200 System. PMN-Elastase is measured by means of the Human Elastase ELISA Kit (HyCult Biotechnology, Uden, Netherlands) and MRP8/14 is measured with the MRP8/14 ELISA kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland). The optical density for both kits is measured at 450 nm. The sensitivities are as follows: MMP-8, 8.9 pg/ml; IL-1β, 0.2 pg/ml; PMN-Elastase 0.4 ng/ml; MRP8/14, < 0.4 µg/ml.*

*We added these informations under the specific subheadings and added more details about the immunohistochemical/morphometric analyses under the subheading “Immunohistochemical analyses”.*

4. In addition, there seems to be conflict between the number of PICF samples reported to be collected from the 24 participants (page 11, line 5; 24 samples), and the weekly PICF sampling described on page 8, line 21, considering that the patients will wear each abutment for one month (p. 8, l. 25). Please, revise.

*The weekly PICF sampling for one month per each abutment material (thus: 4 times for each abutment material) takes place both buccally and lingually as described under the subheading “Interventions”. For the 3 abutment materials, this procedure results in 24 PICF samples for each patient. We clarified this aspect under the subheading “Sample size”.*
5. The 2nd phrase on page 13, “Due to the huge amount...” is either not clear or out of context.

We deleted “Due to the huge amount of biomarkers” and wrote “Because of the huge number of biomarkers available, the present study has only IL-1β in common with the other clinical study.” instead.

Discretionary Revisions

1. The title could be more specific since “dental materials” implies a very wide range of materials while only three of them will be investigated.

We adjusted the title accordingly and according to the Editorial requests (“Interaction of titanium, zirconia and lithium disilicate with peri-implant soft tissue: study protocol for a randomized controlled trial”).

2. The Background section is interesting but relatively short. Since no results are presented, the content of Discussion could be moved to that section as a complement of the literature review. I see no need for a Discussion topic.

We have moved several paragraphs from the Discussion section to the Background section. However, due to the issues raised by the methods, which we find necessary to address, we still see a need for a Discussion section.

3. The 2nd paragraph of page 10 is repetitive and should be removed.

We have removed this repetitive paragraph.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer 3:
Reviewer's report
Title: Dental materials' interaction with peri-implant soft tissue: study protocol for a prospective, randomized clinical pilot-trial

Version: 1 Date: 1 July 2015
Reviewer: Leslie Laing
Reviewer's report:

Minor Essential Revisions:
• This is a very pertinent study and a timely one at that.
  (No change requested.)

• The study design will adequately test the hypothesis.
  (No change requested.)

• In addition to different abutment materials, patients involved with the study will have to wait an extra 2 months prior to receiving their final crown.
  (No change requested.)

• On what basis will the final abutment type be chosen? Perhaps patients should be asked if they preferred one type of material over another, and if so, why.

  After the completion of sampling for the biomolecular and immunohistochemical analyses, the study is finished and the patient receives a screw-retained lithium disilicate crown. We added this information under the subheading „Interventions“. Thus, no abutment is used for the final restoration (no cemented crown but screw-retained crown).

  Thanks for your suggestion to ask the patients if and why they preferred one type of material over another. We haven’t investigated this patient-related factor so far, but it seems interesting too.

• Will patients be compensated for having to wait an extra 2 months prior to receiving their final crown?

  The patients are financially compensated for the additional appointments and the extra time prior to receiving their final crown. Besides an accident insurance is provided for the way to the study appointment and back home. Do you want us to add this information to the manuscript?
• Although mention of why the 4 biomolecular markers were chosen is indicated in the discussion, it would be useful to also have this written in the Introduction/Background. More details as to why each marker was chosen and what relevance each one has to the study would be useful. 

We added information about the biomolecular markers to the Introduction/Background section and gave more details as to why the markers were chosen and their relevance.

• Since the tissue biopsies will be taken from the same site, will there be any differences in cell type in the original biopsy material, versus one taken from a later, healed site?

Different cell types are possible. This will be detected with the aid of the histological analyses. The original biopsy material (punch biopsy from second-stage surgery) serves as histological control specimen as it has not been in contact with an abutment material so far. It will be stained immunohistochemically to detect the 4 biomarkers too. We added this point to the Discussion section.

• When referring to the location of something intra-orally, please use “lingual” rather than “oral”.

We replaced “oral” by “lingual”.

• Sufficient details are provided at this stage to allow replication of the work or comparison with related analyses.

(No change requested.)

However, we added some more information about the biomolecular and immunohistochemical analyses as requested by the 2nd reviewer.

• The planned statistical analysis appears to be appropriate.

(No change requested.)

• The figure and table presented appear to be genuine.

(No change requested.)

• The writing overall is acceptable. There are a few sentences where the
grammar could be improved but that should come in the final draft of the paper.

An Expert English Editing Service edited the manuscript linguistically before resubmitting it.

Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Needs some language corrections before being published

An Expert English Editing Service edited the manuscript linguistically before resubmitting it.

Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests.