Reviewer’s report

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

Version: 2 Date: 28 August 2015

Reviewer: Rubens Albuquerque

Reviewer’s report:

General comments:

Overall, most questions were properly addressed and the protocol has improved. However, there are a few minor issues that are commented below following the authors’ responses.

(Note: Supplementary file 1 could not be downloaded from the journal’s website. The copy of the main manuscript with tracked changes downloaded did not contain track-change marks.)

Specific comments:

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 gelatinezymography. 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 (Bu#hlmann 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”.
Reviewer’s reply: In relation to the “supragingival plaque” issue, the text now reads: “Before PICF sampling, any supragingival plaque is carefully removed with cotton balls to eliminate the risk of plaque contamination.” Please, remove the word “any” as supragingival plaque cannot be always completely removed by rubbing a cotton ball on an abutment surface.

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-1# in common with the other clinical study.” instead.

Reviewer’s reply: The problem with this phrase is the cause/effect relationship. Please, consider restructuring or removing it.