Author's response to reviews

Title: Palatal mucosa derived fibroblasts present an adaptative behavior regarding cytokine secretion when grafted on the gingival margin

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Author's response to reviews:

Bauru, January 31st, 2014

Executive Editor: Christopher Morrey

Dear Editor,

Enclosed please find a revised version of our manuscript: “Palatal mucosa derived fibroblasts present an adaptive behavior regarding cytokine secretion when grafted on the gingival margin” that we are resubmitting after the minor changes for publication in BMC Oral Health.

We appreciate the careful review of our manuscript. After carefully considering the reviewers’ suggestions during the revision process, we have now added further information, according to the required minor revisions. We believe the revised manuscript is substantially improved. As you will see, all the changes we performed in the revised text are written in blue, for Reviewer #1, and red, for Reviewer #2.

Thank you for the opportunity to revise our manuscript. We hope that you and the two reviewers will now find it acceptable for publication in BMC Oral Health.

We look forward to receiving your final decision.

Yours sincerely,

Carlos F. Santos
Corresponding Author
Response to Reviewer #1

Thank you for your careful review of our manuscript. We have revised the manuscript along the lines suggested by you. The minor changes we made to satisfy your observations are written in blue.

Reviewer #1

Reviewer Comments to Author:

1. “In each figure legend (at least figure 3), can authors show full name of PMF and GMF?”

Response: We accepted your recommendation. It is now included, in figures 3-6, the full name of PMF and GMF, written as the following: “A-D: primary cell cultures were obtained from periodontal tissues from three healthy subjects and, after the fourth passage, were stimulated with LPS from P. gingivalis and from E. coli in a concentration of 1 µg/mL. Culture medium without stimuli was used as the control. ELISA was performed for the quantification of the cytokines on the cell culture supernatants. Representative figure of the average of three independent experiments, n=3. *P<0.05 was considered significantly different. PMF = palatine mucosa fibroblasts. GMF = marginal gingival fibroblasts.”

Reviewer Comments to Author:

2. “In figure 3-6, can authors show average plus SD or SE? (Additionally, average is derived from 3 independent experiments or 2-3 wells from ELISA wells?)”

Response: All the figures were made using the average of three independent experiments (n=3).

Response to Reviewer #2

Thank you for your careful review of our manuscript. We have revised the manuscript along the lines suggested by you. The minor changes we made to satisfy your observations are written in red.

Reviewer #2

Reviewer Comments to Author:

Abstract:

1. The brief background of the study could be revised, as such readers can understand the aim of study without difficulty. Similarly, the Methods and Results could have been presented more clearly. The control group should be indicated.

Response: Thank you for your suggestions. We revised the background and added information regarding Methods and Results in order to make it easier to understand and satisfy your recommendation: “Considering that grafted gingival tissue might have to be adapted to the receptor area and that fibroblasts have the ability to respond to bacterial stimuli through the release of various cytokines, this study investigated whether fibroblasts from the palatal mucosa behave differently when grafted on the gingival margin regarding cytokine secretion.”
Methods: Biopsies from the palatal mucosa were collected at the time of free gingival graft surgery, and after four months re-collection was performed upon surgery for root coverage. Fibroblasts were isolated by the explant technique, cultured and stimulated with Porphyromonas gingivalis (Pg) and Escherichia coli (Ec) LPS for 24 or 48 hours for comparative evaluation of the secretion of cytokines and chemokines, such as IL-6, IL-8/CXCL8, MIP-1#/CCL3, TGF-#, VEGF and CXCL16. Unstimulated cells were used as the control group. Cells were tested for viability through MTT assay, and secretion of cytokines and chemokines was evaluated in the cell supernatants by Enzyme-Linked Immunosorbert Assay (ELISA). Results: Fibroblasts from the palatal mucosa maintained the same secretion pattern of IL-6 when grafted on the gingival margin. On the contrary, fibroblasts from the marginal gingival graft showed increased secretion of IL-8/CXCL8 even in the absence of stimulation. Interestingly, MIP-1#/CCL3 secretion by fibroblasts from the marginal gingival graft was significantly increased after 48 h of stimulation with Pg LPS and after 24 h with Ec LPS. Only fibroblasts from the marginal gingival graft showed secretion of TGF-#. VEGF and CXCL16 secretion were not detected by both subsets of fibroblasts.

Introduction:
2. The background and hypothesis of the study as well as the relevant question posed should be better defined and presented.

Response: We rewrote part of the background and the hypothesis of the study in the last paragraph of introduction in order to address this recommendation: “Gingival tissue exposure to bacterial plaque can result in tissue inflammation with clinical signs of color change, size, shape, consistency and bleeding with the possibility of alveolar bone loss due to periodontal disease progression [1]. The cumulative effect and repetitive pathological events to gingival tissue can lead to the occurrence and progression of gingival recession, especially in cases with narrow band or absence of attached gingiva [2-4]. […]. Grafted gingival tissue might have to be adapted to the receptor area and there is a large body of evidence that gingival fibroblasts are able to react to several stimuli through cytokines and chemokines release, which in turn play an important role in inflammatory response. Therefore, this study aimed to investigate whether the secretion of cytokines and chemokines including repair mediators by human gingival fibroblasts would be modulated when cultured from the palatal mucosa onto the gingival marginal area in a free gingival graft procedure.”

Materials and Methods:
3. … ‘three healthy individuals’… It means systemically and periodontally healthy? In this case, a brief presentation of these subjects may be required.

Response: Correct, the donors were systemically and periodontally healthy. A brief presentation about them was included in the text: “After approval by the Institutional Review Board of the Bauru School of Dentistry, University of São Paulo, #055/2011, three systemically and periodontally healthy individuals (28, 38 and 50 years-old, 3 females) were selected in Periodontics Clinics, all of
whom needed root coverage in areas with scarce keratinized mucosa. They had no signs of gingival inflammation, no bleeding on probing or critical probing depth. All individuals were submitted to anamnesis, periodontal clinical examination and radiographic exams. The inclusion criteria were individuals with deficient keratinized mucosa, both in quantity and quality, without systemic complications that could contraindicate the surgical procedures, and that provided written informed consent to participate in the study.”

4. The overall study design could have been presented briefly.
Response: We believe that the study design is presented and divided in each subheading of the Material and Methods section.

5. Some details of Pg LPS and E coli LPS should be given.
Response: Thank you for your suggestion. Both PgLPS and EcLPS were purchased from Invivogen (San Diego, CA, USA). We included this information in the text in order to emphasize that both of them were commercially available and ultrapure LPS: “The cells were seeded in 24 well plates at a density of 2x10⁴ cells/well and incubated at 37°C in a humidified atmosphere of 5% CO₂ during 18 hours in DMEM 1% FBS. After overnight adhesion, DMEM containing 1 µg/mL of Pg LPS (Invivogen, San Diego, CA, USA) or Ec LPS (Invivogen) was added to the wells for 24 or 48h. Non-stimulated cells were used as controls. Both Pg LPS and Ec LPS were ultrapure and purchased from Invivogen.”

6. Dose-dependent and time dependent assays could have been undertaken. In addition, the mRNAs of the cytokines investigated should have been measured as well.
Response: We consider this a very interesting point. However, dose-dependent and time dependent assays were not performed due to the variety of previous works that have already shown the profile of the studied cytokines’ secretion by marginal gingival fibroblasts in a concentration-dependent manner and over various time points. We elected a specific concentration of LPS in only one time point because of the evidence of previous works by our group (Morandini et al., 2010) and by others (Koka et al., 1997; Ekhlassi et al., 2008; Herath et al., 2011). In addition, we highlighted this point in the discussion. Regarding mRNA expression analysis, we agree this would be very interesting to be done, but due to financial restrictions and because mRNA transcripts might not always be translated to protein due to post-transcriptional modifications, we decided by protein measurement.

Results:
7. GMF control shows a higher basal expression of IL-6, IL-8 and TGF-beta than PMF is well noted. While it is difficult to appreciate that E coli LPS could not upregulate their expression (Figs. 3, 4 and 6). It seems that PMFs are unable to react to the Pg and E coli LPS stimulation. It should be interrupted with caution.
Response: We recognize that GMF presented a higher basal expression of IL-6,
IL-8 and TGF-beta when compared with PMF. Possibly, it might had happened due to a higher state of activation of these marginal cells, which could be explained by the continuous stimulus provided by the receptor area/marginal sulcus, discussed throughout the manuscript. However, it does not mean that GMF are unable to react to Pg and E.coli LPS stimulation but only that it is in a different state of basal activation compared with PMF.

Discussion:

8. The major limitations of the study could be elaborated. The conclusion could be questioned due to the controversial data presented.

Response: We agreed with your recommendation and the lack of mRNA measurement is now included in the discussion as a possible limitation. It is written as the following: We recognize that it would have been valuable to investigate mRNA expression in order to better understand whether transcript levels would be different between them. This could give us some information about possible post transcriptional modifications that can alter production of cytokin