Universit à Politecnica delle Marche

Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche
*(Direttore: Prof. Andrea Luigi Tranquilli)*

August 5, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4088-rev Orsini WJS 2013.doc).

**Title:** Cytotoxicity of a silorane-based dental composite on human gingival fibroblasts

**Author:** Giovanna Orsini, Alberto Catellani, Concetta Ferretti, Marco Gesi, Monica Mattioli-Belmonte, Angelo Putignano

**Name of Journal:** *World Journal of Stomatology*

**ESPS Manuscript NO:** 4088

The manuscript has been improved according to the suggestions of reviewers and all changes were highlighted "in red":
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
**(1). On 18-06-18 the referee wrote**: 1.For direct toxicity test, the sample preparation procedures (70% alcohol for 3 h, PBS 24 h, and DMEM+10% FBS 24h) may affect the results. The authors may try to prepare samples by only rinsing in medium/or PBS with antibiotics before seeding of cells. 2. For MTT assay, what kind of culture plate (24-well or 6-well plates or 6-cm dishes) were used> 3. Figure 1 and Figure 2 lacked of longitudinal axis units. The labeling of horizontal axis can be bigger. A asterisk was needed to label the data that showed significant difference relative to control. Whether the viability of cells exposed to polished samples (90 +/- 19%) showed significant difference to control? 4. In figure 2, why 3-weeks samples showed less toxicity than 1 week samples? 5. The authors considered and concluded that the materials are biocompatible. However, the results showed that the silorane-based material exhibited some toxicity. The conclusion should be more conservative.

RESPONSE: 1. This is the procedure commonly performed for biomaterial (eg. Polymers, composites, etc..) sterilization in *in vitro* studies. Ethanol 70% is used to disinfect, the subsequent washing in PBS and the incubation with complete medium (this means that the medium contains 10% FBS and 1% antibiotics) for at least 24h are sufficient to neutralize the alcohol remnant, which could be toxic (please see Gentile P et al. J Biomed Mater Res Part A 2012 DOI: 10.1002/jbm.a.34205; Ciardelli G et al. J Biomed Mater Res Part A 2010; 92A :137-151).
The above mentioned informations have been accordingly added in the Mat & Meth section.

2. 24-well plates were used for cultures. This detail has been added in Mat & Meth.
3. All the suggested changes have been performed and the new figures 1 and 2 accordingly modified. Indeed, different symbols for statistical significance were added.
4. A brief comment on the results of figure has been added in the Discussion section (in according with the paper by Sheridan et al).
5. We thank the Reviewer for this last comment. Indeed, there is some toxicity expressed by silorane-based materials, especially when we evaluate the 1 week data. Therefore, this fact has been reviewed in the discussion and the conclusion was made more conservative than the previous one. Indeed it now reads as follows: "In conclusion, although the poor chromatic availability of this material remains a big limit that restricts its use to posterior sectors, the silorane-based material can be considered an option to perform restorations when aesthetic demands are not the priority, such as the class II restorations [29]. The behavior of silorane-based materials seems to be comparable to the one observed for conventional composite material [30], thus decreasing the citotoxicity after long time exposure. Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry". References have been added.

**(2). On 26-06-26** the referee wrote: Thank you for your efforts to revise the manuscript. The introduction clearly summarizes the study's content. Generally the study is well written and shelf explanatory.

RESPONSE: We thank the reviewer

**(3). On 09-07** the Referee wrote: Dear authors, we acknowledge the submission of yours to WJS. Some sugestions are shown, and then your article may be appreciated for further evaluation according to the editor opinion. 1)Introduction is appropriate and objectives are clear and reached; 2) Methods are good and detailed. Although, some information about size and site of human biopsies would be of interest. Also, "sterelized"should be replaced by "desinfected" during the preparation of silorane samples with 70% alcohol solutions; 3) Statistical analysis could be added with detailed information about every hypothesis tested. Eg.: a) Teste cell viability according to samples surfaces (polished and non-polished). 4) Discussion: It would be of good value if some clinical applications of silorane-based products could be listed (last paragraph or in the Introduction section). 5) Conclusions about cell toxicity / viability may be misinterpreted. According to your results, some samples demonstrated rates of cell viability less than 75% (78% +/- 11% - i.e., rates of 67%), which means slightly citotoxity - ISO 10993-5. We recommned revison of clinical extrapolation conclusions.

RESPONSE: 1) we thank the reviewer. 2) Size of the biopsies have been added in the methods; the word "sterilized" has been changed with "disinfected" throughout the manuscript. 3) Statistical analysis has been improved providing the detailed explanation of the analyzed data. Indeed, the following sentence was added: "In detail, cell viability

was evaluated on fibroblasts: 1) directly cultured on polished samples (P), unpolished samples (UnP) and control (CTRL); 2) in contact with the eluates of P, UnP and CTRL samples at 1 week and at 3 weeks". 4) A brief sentence on clinical applications of silorane-resin composites for posteriors has been added in the Conclusion. 5) The final sentence of the conclusions about cell toxicity has been revised and changed, thus considering that there was some toxicity, which decreased after long exposure (3 weeks). Thus the last paragraph now reads as follows: "In conclusion, although the poor chromatic availability of this material remains a big limit that restricts its use to posterior sectors, the silorane-based material can be considered an option to perform restorations when aesthetic demands are not the priority, such as the class II restorations [29]. The behavior of silorane-based materials seems to be comparable to the one observed for conventional composite material [30], thus decreasing the citotoxicity after long time exposure. Further studies are still needed to characterize the biological response of these methacrylate-free composite formulations, in order to definitely demonstrate their safe use in restorative dentistry". Cited References have been added.

**(4)** References and typesetting were accordingly corrected

Thank you again for publishing our manuscript in the *World Journal of Stomatology*

Sincerely yours,

Giovanna Orsini

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