Letters From Our Readers

To: Editor, *The Angle Orthodontist*

Re: In vitro biocompatibility of nickel-titanium esthetic orthodontic archwires by Rongo Roberto, Rosa Valletta, Rosaria Bucci, et al. *The Angle Orthodontist*; 2016;86:789–795.

I would like to congratulate the authors on the theme of their study because it is current and important to take into account the health concerns of patients. I have some questions to clarify the methodology used in the study.

According to the parameters of ISO10993 which regulates in vitro cytotoxicity tests, negative and positive controls shall be included in each assay because they are important for the control of the means in relation to the maximum and minimum response. It does not appear that these two control groups were part of the methodology so how do you think this might affect interpretation of the results?

Thank you for your positive comments.

In the article, the 100% of cell viability was calculated, for each experiment, by using the optical density of the cells exposed to the blank, namely the culture medium without archwire samples stored in the same conditions as the extracts. We also had as negative control cells exposed to fresh culture medium, and as positive control cells exposed to 10 mM $NiCl_2$, but we did not use their optical density to evaluate the cytotoxicity of tested materials.

Additionally, the paper does not specify the length of the wires used. This is important because wire length and thickness must be evaluated in the calculation of the area of contact of the samples with cells in the cytotoxicity assay.

Instead of the surface/volume ratio, we used a standard mass/volume ratio of 0.1 g/ml. It is important to clarify that, as for other studies, we used the ISO norm as a guideline, but we adapted it to the objective of the research, e.g. the extracts were obtained after aging periods of 1, 7, 14 and 30 days, that are not suggested by ISO10993.

Thank you for publishing this interesting paper.

Thank you for your relevant comments.

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