Reviewer’s report

Title: The impact of collagen membranes on 3D gingival fibroblast toroids

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Reviewer: Thomas E Lallier

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Review of OHE-D-18-00552

The impact of collagen membranes on 3D gingival fibroblast toroids

The author present evidence that gingival fibroblasts suspended in 3D collagen spheroids and toroids can survive for 3 days. The author conclude that these 3D toroid cell culture systems are suitable for use as a "simple assay to study attachment behavior to various biomaterials." While the authors findings are interesting, more detail is needed before it is ready for publication.

Concerns:

Sample size needs to be stated for all experiments.

What are the size of the toroids and spheroids? Diameter and thickness for the toroids. This is critical, since nutrients need to diffuse through the collagen gel in order to provide nutrients to the cells.

Are the toroids hollow in the center, or is there a thin membrane of collagen connecting the outer ring of collagen? The images in Figure 2A seem to indicate that they are disc on plastic, and the even on collagen membranes (Figure 2C) that the center is filled at 24 hours.

Resazurin assay measures metabolic activity. An independent measure of cell number is needed to determine metabolic activity per cell. Fewer cells have less combined metabolic activity.

State sample size and that the error bar represent standard deviation for all graphs.

Table 1: Why is attachment scored rather than presented as a percentage? Why was the sample size only 5?

Figure 4D: The metabolic activity is clearly declining. How was this not statistically significant? Is this decline on collagen membranes of any potential clinical importance?

Figure 4C: The cells are round. This does not indicate a healthy morphology for fibroblasts.
In monolayer cultures, the metabolic activity on collagen membranes declines from 6000 units to 2000 units (Figure 4D). In toroids (Figure 2B and 2D), the metabolic activity is never above 15000. Does this indicate that only a fraction of the cells are surviving in the toroids? Or is it that they have reduced metabolic activity that stabilizes at a reduced value due to poor diffusion of nutrients? Knowing cell numbers in all experiments could resolve many of these questions.

How long can cells survive within the toroids? Is 3 days the limit?

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

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