Reviewer’s report

Title: The study of calcified atherosclerotic arteries: an alternative to evaluate the composition of a problematic tissue reveals new insight.

Version: 0 Date: 07 Mar 2016

Reviewer: Daping Fan

Reviewer's report:

Fittipaldi and colleagues developed a new technique for the pathological examination of calcified artery tissues. They used the carnoy's solution to fix the tissue followed by a tissue digestion with type II collagenase in order to preserve the protein and nucleic acid for further analysis. They found that this technique preserved the cellular content of calcified tissue very well. This new technique may improve the analysis of calcified atherosclerotic arterial tissues. However, concerns are raised including:

1. Please list the sequences of the primers used in quantitative real-time PCR.

2. Figure 1, the author showed a standard section of calcified artery and collagenase spread tissues. Please also show a section after Carnoy’s solution fixation using the same artery tissue to compare with the standard section.

3. The author state that they performed von kossa staining for the confirmation of calcification, is there any difference of calcification staining between the new technique and standard procedure?

4. Figure 2, please use arrows to point to the SMCs. The structure of the SMCs seems not clear.

5. Table 4 showed that the mean ct values of the measured genes are above 30, indicating a very low RNA content in the tissues, can you use more tissue for the analysis so that the quantification may be more accurate?

6. Figure 3 C and D, please show images with lower magnification to indicate the location of the Ki-67 positive cells.
Minor points:

There are many grammatical, spelling and syntax errors throughout the text that need to be carefully corrected. For examples, uM should be µM; in the abstract, "information's" should be "information".

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

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

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
I am able to assess the statistics

Quality of written English

Please indicate the quality of language in the manuscript:
Needs some language corrections before being published

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