Reviewer’s report

Title: In-vitro sonothrombolysis using thick-shelled polymer microbubbles - A comparison with thin-shelled microbubbles

Version: 0 Date: 20 Jan 2020

Reviewer: Francesco Faita

Reviewer's report:

This study aimed to evaluate thrombolysis effect of ultrasound-stimulated thick-shelled microbubbles.

The topic is of interest; however, the paper has important some limitations (described below in Major issues section) that should be addressed before considering the paper for publication in Cardiovascular Ultrasound.

MAJOR ISSUES:

1) Phantom properties: authors cited another publication (ref. 23) about mechanical properties of the phantom. However, the process described by Fromageau et al. is quite different respect to the process of the present paper. Accordingly, it is not clear how authors evaluated properties of the phantom sed for experiments. Please clarify and comment.

2) Line 125: information about the material of the pore mesh should be added. In particular, it is not clear if pore mesh can interact with ultrasound beam thus having a protective (or deleterious) addictive effect on blood clots. Please comment and change the text accordingly. Finally, a picture of the pore mesh should be added with and without the blood clot.

3) Phantom: authors would like to mimic vessel properties. However, in a clinical scenario of thrombolysis, the vessel wall constitutes only a limited amount of tissue that should be crossed by ultrasound, while fat and muscle tissues are the most. Accordingly, the ideal phantom is a fat/muscle mimicking one.

4) Probe distance: the distance between the probe and phantom should be 15 mm instead of 10 mm in the case of high frequency probe according to figure 2. Please clarify. Furthermore, both distances seem to be unrealistic respect to clinical cases where the occluded vessel is deeper in the body, thus limiting the clinical usefulness.

5) Blood flow velocity is constant and quite far from the physiological blood flow velocity wave. This could be a limitation of the in vitro setup.
6) Lines 151-154: discarding of pressure measurements according to observations of leakage could introduce bias in measurements. This should be recognized as another limitation of the study.

7) Lines 179-182: saline with microbubbles at 2x10^6 MBs/ml was injected. However, this condition seems to be very different respect to the clinical scenario where a small amount of solution with 10^7-10^8 MBs/ml were injected in the circulation thus having a limited amount of microbubbles where the clot was formed. Please comment.

8) Figure 3: scale and focus position should be added.

9) Limitations in pulse customization of the Verasonics system were already recognized. However, the difference between protocol 1 and protocols 2-4 makes difficult to compare results. Please comment and strongly highlight this point in the limitations section.

10) Change in pressure over time was compared only by visual inspection. However, a more quantitative approach with an appropriate statistical test should be preferred.

11) Figure 4: mean pressure measurements over time exhibit quite variable variations around the means in the 5 protocols. Please comment.

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Please indicate the quality of language in the manuscript:

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