Digital Object Identifier: 10.1109/ULTSYM.2008.0241

Nano-interrogation of a lipid shelled microbubble.

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I. INTRODUCTION

We have shown that the Atomic Force Microscope (AFM) can be used to study morphology, mechanical and adhesion properties of MBs [1][2]. The first study of the viscoelastic property of a MB used biSphere® (Point Biomedical Corp, San Carlos, CA, USA), that are considered to have a relatively hard shell. The high reproducibility of data acquisition as well as the impressive force resolution of the AFM allowed to detect a strong dependence of the shell stiffness to MB size. Shelled oscillations of these MBs, however, seem not to give strong echoes as a large number of MB provide a free bubble oscillation of gas escaped from the shell [3]. In other words the measured mechanical property with AFM here may not be useful for theoretical modelling of the MB behaviour. On the other hand lipid MB are known to provide shelled oscillations but the viscoelastic properties of such MBs have not been determined and are the subject of a model debate [4]. Here we will present measurements of the lipid shelled BR14 (Bracco Research SA, Geneva, Switzerland) and attempt to extract a figure for its static shell elasticity.

II. MATERIALS AND METHODS

The methodology is similar to previous work [1][2]. Briefly the MB solution was dissolved 100 times in volume with saline. The solution was brought in contact with Poly-L-lysine coated Petri dish for 20 minutes in order to allow a significant number to adhere to its surface. AFM force-distance (f-d) with working distance 1 μm, was used to acquire force-separation curves. The separation distance essentially becomes microbubble deformation distance at the point of contact. The tipless cantilevers CSC-12 (MikroMash, Talinn, Esthonia) with nominal spring constants ranging from 0.01-0.07 N/m were used as they produced best results. Cantilevers above 0.1 N/m were also tested to observe MB response.

As previously described the deflection of the AFM cantilever during compression of the MB with that of a hard surface, provided the stiffness of the microbubbles. Measurements were performed for a range of MB sizes.

III. RESULTS

A group of typical force-separation curves from an MB is shown in Fig. 1. The decompression (not shown in the figure) confirmed that the elastic deformation and microbubbles recovered the original shape. The measurements were reproducible especially when the maximum force applied onto the MBs was kept below 10nN. The measurements were therefore restricted to a maximum cantilever deflection of 200nm or lower if the higher stiffness cantilevers were used. Cantilevers with nominal spring constants above 0.2 N/m but generally induced permanent microbubble damage.

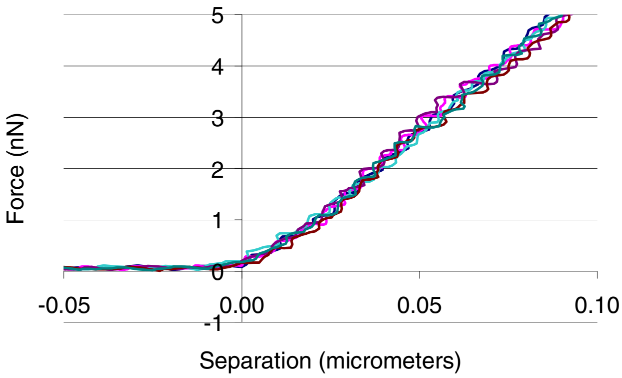

Figure 1. Typical group of 6 force vs separation (MB deformation) curves onto a BR14 MB with 3.16±0.05 μm diameter. The stiffness of the MB was calculated to be 0.0565±0.0026 N/m. The standard deviation was less than 5% demonstrating the high reproducibility of the measurement.

Results with the stiffness of 30 MBs vs MB diameter are displayed in Fig. 2. As can be seen from the figure there was not dependence of the stiffness to MB size. The mean stiffness was 0.054±0.030 N/m. Outside the range of one uncertainty 7 out of 30 MBs were found.

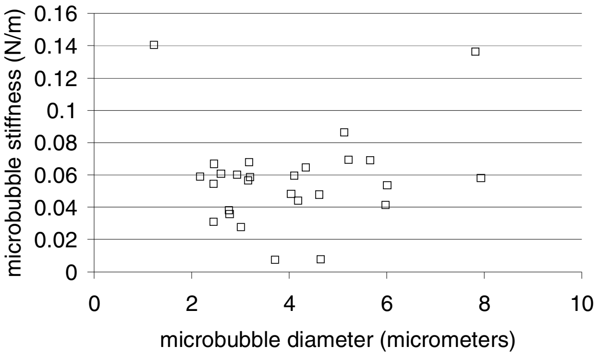

Figure 2. MB stiffness vs MB diameter. No correlation between these two parameters was found.

Using a simple model that converts the MB stiffness to shell Young modulus [5] we observe that it ranged between 0.5 and 4.0 GPa providing a loose correlation with size (r=0.55). In order to establish the relationship between shell stiffness and MB size more measurements are required. The above results are not in agreement with the MB models where the fitted MB elasticity was found in the order of MPa [6]. It is important to stress here that the MB shell is 5 nm and very soft, so it is likely that a model that extracts the shell property is required to incorporate the gas property. Future work will focus in building an appropriate structural model.

IV. CONCLUSIONS

Accurate stiffness measurements on the lipid MB BR14 are possible with Atomic Force Microscopy. No dependence of stiffness to MB size was found, but further measurements are required to assess this. The MB shell Young modulus extracted with a simple model disagreed with other findings, and a new structural model is required to test the contribution of the gas in the MB stiffness.

V. REFERENCES

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Acknowledgement: The work was funded by the British Heart Foundation, UK (fellowship grant FS/07/052)