**Responses to Editorial and Peer Review Comments**

**Editorial comments:**  
The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. **The updated manuscript (55106\_R0\_061616.docx)** is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. **Please download the .docx file and use this updated version for any future revisions**.  
  
Changes to be made by the Author(s):  
  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Reply:

We want to thank the editor for giving us this opportunity. We have proofread the manuscript, and corrected spelling and grammar errors.  
  
2. Please abbreviate all journal titles.  
Reply:

We have abbreviated all journal tiles.

3. Please include volume, issue numbers, and DOIs for all references.  
Reply:

These are included for all references now.

4. Please define all abbreviations before use.  
Reply:

We have defined all abbreviations before use by adding brackets with abbreviations enclosed after the full expression.

5. Formatting:  
-Please include spaces between numbers and units.  
-All figure legends should have a title and a brief description. For Figure 3, all panels should be described.  
-References – Please abbreviate journal titles.  
Reply:

We have taken care of these formatting issues. All panels are now described in the figure legend of Figure 3.

6. Grammar:  
-Please copyedit the manuscript for numerous grammatical errors throughout. Such editing is required prior to acceptance. A few examples are listed below.  
-Line 137: “yet small enough to avoid individual cells to adhere on it”  
-1.1.3.2. “Set up the photolithography recipe in hard exposure mode with 9 s UV exposure. Moves directly to: “After UV exposure, bake the slide at 95 °C for 1min, and then let it cool down to room temperature.” This is confusing, “set up” sounds like the action is preparing for, not carrying out the UV exposure. Please re-word here, and in 1.2.2.2  
-1.1.5.2 – “Clean the slide in the reactive”  
-1.1.5.6 – “Bake dry the slide before clean it’  
-The discussion requires extensive editing for minor errors in grammar, particularly in article usage (a, an, the).

Reply:

We want to thank the editor for pointing out these errors. We have corrected these grammatical errors accordingly in the manuscript.  
  
7. Visualization: Protocol is discontinuous. Please highlight a continuous protocol (that is, all steps in a section required for success of the protocol) are highlighted so that we can form a linear narrative. For instance, 1.2.2.2 and 1.2.2.5 should also be highlighted.

Reply:

Thank the editor for finding this issue. A more continuous protocol is now highlighted. For example, 1.2.2.2, 1.2.2.5, 1.2.3.1, and 1.3.1 are highlighted now.  
  
8. Additional detail is required:  
-1.1.2 – What speed/time is used for spin coating?  
-1.1.5.2 – How is this performed? Please provide stepwise detail.  
-1.1.5.3 – Please provide a citation or detail.  
-2.2 – Please provide a citation.  
Reply:

-1.1.2 -The speed/time for spin-coating is described in 1.1.2.2;

-1.1.5.2 –The slide is cleaned with plasma from the RIE machine, the parameter settings are already described.

-1.1.5.3 –A related citation is now added for this step.

-2.2-A citation is now provided.

9. Please remove all commercial branding:  
-4.3.4 – Opti-MEM  
-Please remove trademark symbols from the materials table.

Reply:

Opti-MEM has been removed from 3.4.1 and 3.4.3.

Trademark symbols are now removed from the materials table.  
  
**Reviewers' comments:**  
**Reviewer #1:**  
*Manuscript Summary:*  
This is a nice list of steps needed to conduct the experiments. Not sure how many people have 2 high-speed cameras and the bio and laser tools, yet it should help someone with the equipment and expertise to repeat the experiments. The experiments have been published extensively in a number of journals. Thus they are sound.  
  
*Major Concerns:*  
Very expensive experiment  
Response:

We thank the reviewer for the supportive comments on the experimental protocols. The experimental system was set up initially to collect sufficient data to establish the correlation between bubble dynamics/jetting flow and cell deformation/bioeffects. The most expensive component of the system is the ultra high-speed camera; other expensive components such as the pulsed Nd: YAG lasers may be replaced by pulsed LEDs in the future since only about 10 μJ pulse energy is needed to generate a bubble in our system. Similarly, once the methodology is established, simple optical techniques (e.g., a linear array of photodiodes) can be used to measure maximum bubble size in real time, thus potentially eliminating the need for an ultra high-speed camera. It is our ultimate goal to simplify the experimental system after the fundamental studies are completed.

*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A  
  
**Reviewer #2:**  
*Manuscript Summary:*  
In this study, cavitation bubble(s)-cell interaction and resultant bioeffects were investigated using a microfluidic system. The flow is characterized with PIV technique. The authors presented an interesting study and provided details on their experimental setup and procedure. They successfully combined fluid mechanics with biophysics, which constitutes the strength of the study. It also seems that the experiments were carefully performed and necessary discussion on the results is present. This study will make a fine contribution to the literature. However, there are some issues the authors should address.

Response:

Thank you very much for your supportive and encouraging comments.  
  
*Major Concerns:*  
1-Introduction: It is short for a journal article. It should be extended with recent biotechnology studies related to cavitation such as   
Hydrodynamic cavitation kills prostate cells and ablates benign prostatic hyperplasia tissue,  
Z Itah, et al., Experimental Biology and Medicine, 2013.  
Bubbly cavitating flow generation and investigation of its erosional nature for biomedical applications  
A Kosar et al., IEEE Transactions on Biomedical Engineering 58 (5), 1337-1346, 2011.

Response:

We have expanded the introduction with citation of these studies.

2-Were the cells tested under non-cavitating conditions? It would be nice to filter out shear effects due to liquid flow with control experiments.

Response:

We have tested the cells with liquid flow induced by single cavitation at 20 µm standoff distance. The cell viability is 100%, with much weaker deformation and poration. Previously, we also tried liquid flow on cells without cavitation bubble. It turns out that liquid flow with no cavitation is even weaker than single bubble, which is similar to the reference[1](#_ENREF_1) mentioned above.

3-It will be nice to include non-dimensional parameters such as Reynolds number, cavitation number, bubble to channel diameter ratio to represent the results. Thus, they could be converted to universal results.

Response:

The cavitation number is not applicable here because of the nature of laser-induced cavitation. Particularly, the water temperature is not constant in the laser focus that breaks the balance between the kinetic and potential energy in liquid.

Bubble to channel diameter ratio: bubble diameter/channel widith=(50±2)/800=0.625±0.0025;

We have added a table listing the standoff distance, Reynolds number and averaged shear stress into the manuscript.

4-What are the uncertainties in the experimental parameters? They should be included.  
Response:

The statistic error is ±1/√N, N is the number of cells tested;

Bubble size 50±2 µm;

Standoff distance (SD): ±0.5 µm.

These information is now included into the manuscript.

5-The authors mentioned necrosis and apoptosis. How about autophagy? Does it play a role in this study?

Response:  
Autophagy is related to the physiological process in the body that deals with destruction of cells. We are most interested in repairable membrane poration, which has potential for cavitation-targeted drug delivery, and mechanical stretch-induced calcium response that may be used for stimulating cell/tissue growth. Therefore, autophagy is beyond the scope of this study.

6-Cell-PIV particle bead interactions: Were such interactions present? If yes, how did the authors filter out these effects in their results?

Response:

The PIV beads are different from the RGD beads for cell membrane deformation. They are polystyrene beads without any surface functionalization, thus not able to attach to the cell membrane by integrin binding as the RGD beads. Besides, our PIV studies are performed without cells, thus PIV beads are not interacting with cells. We have added more description of the beads in the manuscript to distinguish these issues.

7-What are the stresses on the emerging bubbles and impact pressure upon bubble collapse? This info is necessary to make the results complete.

Response:

The estimated maximum shear stress in our setup is about 1 kPa, which is within the range of previously reported critical shear stress of 0.1 kPa for cell detachment[2](#_ENREF_2) and water-hammer pressure of 3 kPa for jet impact induced membrane rupture[3](#_ENREF_3).

8-It will be nice to summarize the ranges for experimental parameters such as cavitation number, Reynolds number, diameter ratio, stresses in a separate table.

Response:

This comment is similar to comment #3. The cavitation number is not applicable to our study. We have listed other parameters (Reynolds number, diameter ratio, stresses) in a separate table.

9-More discussion about flow physics is necessary and should be linked to experimental results. The authors can borrow some from the studies on micro scale cavitating flows.  
Response:

We have local stresses in our systems, and it is different with the global stresses from the studies on micro scale cavitating flows[1](#_ENREF_1),[4](#_ENREF_4).

The focus of this manuscript is the experimental protocols and representative results. More details about the flow physics and the correlation to experimental results can be found in our previous publications[3](#_ENREF_3),[5](#_ENREF_5).

*Minor Concerns:*  
1-3.2.5-3.2.6: The equations should be formatted so that they would become clearer.

Response:

There is a display issue with the previous submission. We have taken care to make sure the equations are correctly formatted.

2-The quality of Fig. 5 should be improved.

3-More info about repeatability of experiments should be included.

4-It would be nice to include error bars in the figures.

Response to comments 2-4:

We have added error bars to Fig. 5d and specified the number of cells treated in Fig. 5c to improve the figure quality and clarify the repeatability of the experiments. For more details about the error analysis of the area strains calculation, please refer to our previous publication[5](#_ENREF_5).

*Additional Comments to Authors:*  
N/A

References

1 Kosar, A., Sesen, M., Oral, O., Itah, Z. & Gozuacik, D. Bubbly cavitating flow generation and investigation of its erosional nature for biomedical applications. *IEEE Trans Biomed Eng.* **58** (5), 1337-1346, doi:10.1109/TBME.2011.2107322, (2011).

2 Ohl, C. D. & Ikink, R. Shock-wave-induced jetting of micron-size bubbles. *Phys Rev Lett.* **90** (21), 214502, doi:10.1103/PhysRevLett.90.214502, (2003).

3 Sankin, G. N., Yuan, F. & Zhong, P. Pulsating tandem microbubble for localized and directional single-cell membrane poration. *Physical Review Letters.* **105** (7), 078101, doi:10.1103/PhysRevLett.105.078101, (2010).

4 Itah, Z. *et al.* Hydrodynamic cavitation kills prostate cells and ablates benign prostatic hyperplasia tissue. *Exp Biol Med (Maywood).* **238** (11), 1242-1250, doi:10.1177/1535370213503273, (2013).

5 Yuan, F., Yang, C. & Zhong, P. Cell membrane deformation and bioeffects produced by tandem bubble-induced jetting flow. *Proceedings of the National Academy of Sciences.* **112** (51), E7039-E7047, doi:10.1073/pnas.1518679112, (2015).