A multiplexed microfluidic and microscopy study of vasodilation signaling pathways following microbubble and ultrasound therapy

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February 4th, 2021

Séminaire virtuel 2021

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Disclosures

- N/A

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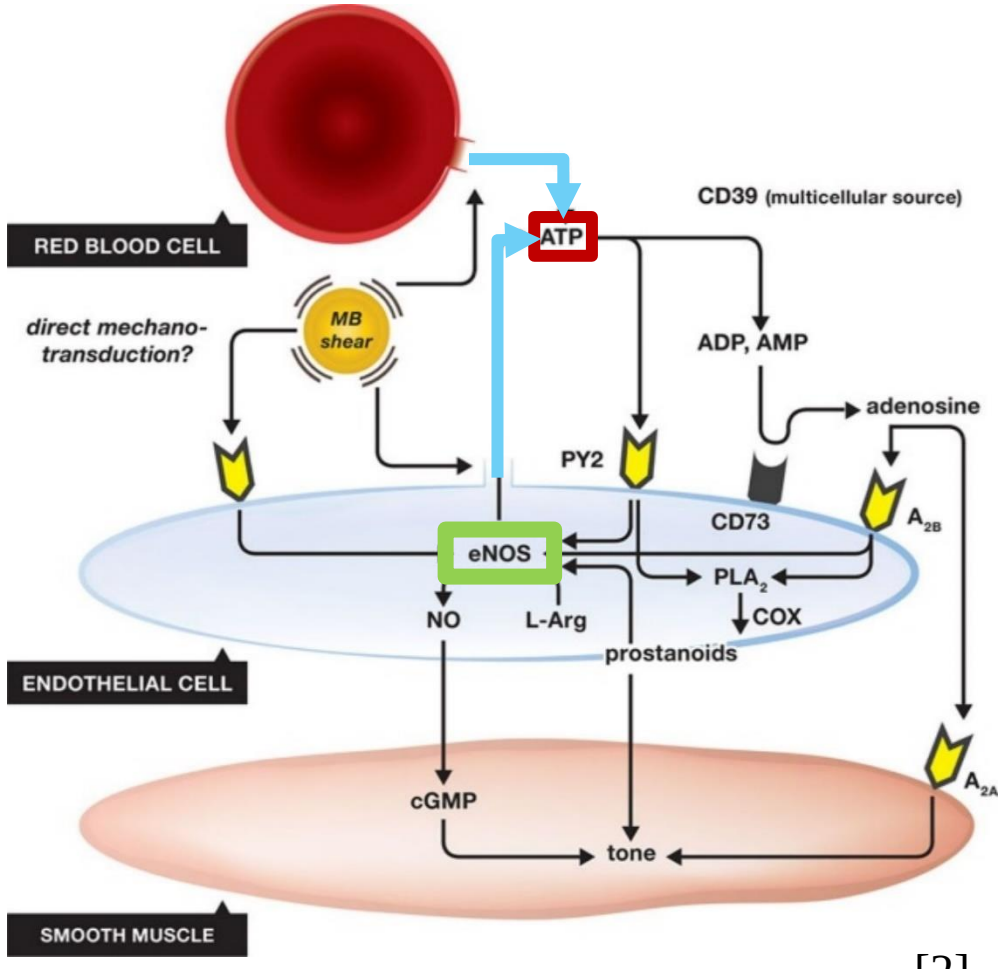
Introduction :

It was previously shown that microbubble (MB) oscillations in an ultrasound (US) field can cause vasodilation in muscle [1-2] which we propose to leverage as a targeted provascular therapy approach to reduce hypoxia in solid tumors before radiotherapy.

One key signaling pathway is ATP, which, when released in the extracellular space, can phosphorylate eNOS through the P2Y receptor. However, very little is known about ATP release kinetics following MB cell interactions. In this study, we used microfluidic chips to study ATP signaling and cell viability following MB+US therapy.

The use of microfluidics will allow to study MB/cell interactions in flowing conditions, which more realistically mimic physiological conditions



[2]

[1]. Yu, F. T., Chen, X., Straub, A. C., & Pacella, J. J. (2017). The role of nitric oxide during sonoreperfusion of microvascular obstruction. Theranostics, 7(14), 3527.

[2]. Belcik, J. T., Davidson, B. P., Xie, A., Wu, M. D., Yadava, M., Qi, Y., ... & Harmann, L. (2017). Augmentation of muscle blood flow by ultrasound cavitation is mediated by ATP and purinergic signaling. Circulation, 135(13), 1240-1252.

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Methods :

Ultrasound (US) and Microbubble (MB) conditions

Pressure Variation

- $1x10^7$ MB/mL
- Single Pulse
- 1000 cycles
- [200, 300, 500] kPa
- 4T1 cells

Cycles Variation

- $1x10^7$ MB/mL
- Single Pulse
- [10, 100, 1000] cycles
- 300 kPa
- 4T1 cells

To assess the cellular response to US and MB therapy, cell viability and ATP release were quantified. The effects of both US pressure and number of cycles of the US pulse were tested using the conditions above.

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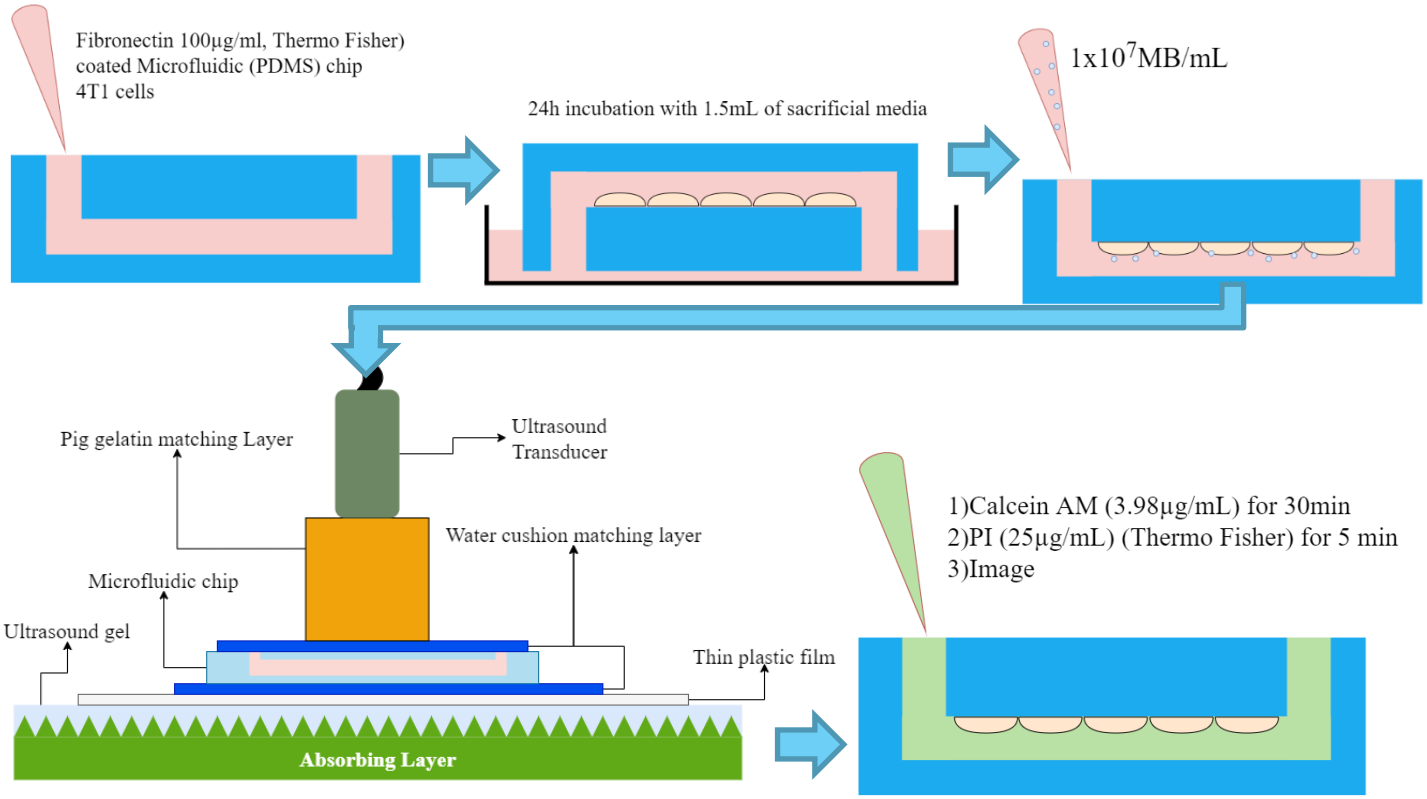
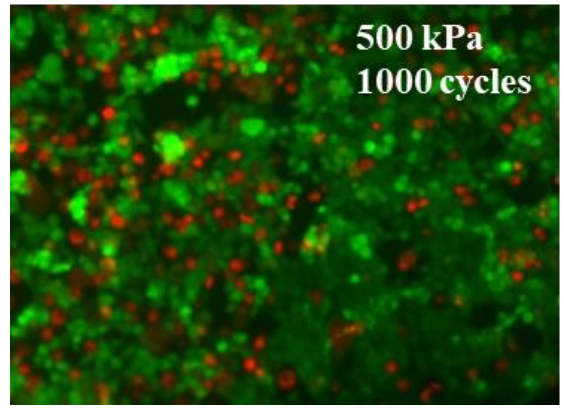
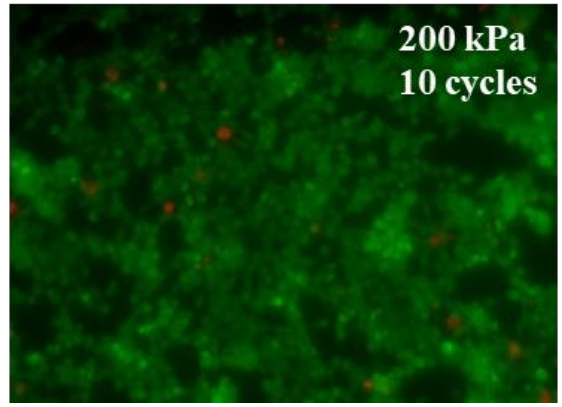
Methods :

Cell Viability



Example of typical live/death staining





Cell viability was imaged using Calcein AM (green) to stain live cells and PI (red) to stain dead cells after US and MB therapy. % Dead cells was quantified with an automated MATLAB script that uses the thresholded areas of the fluorescent images.

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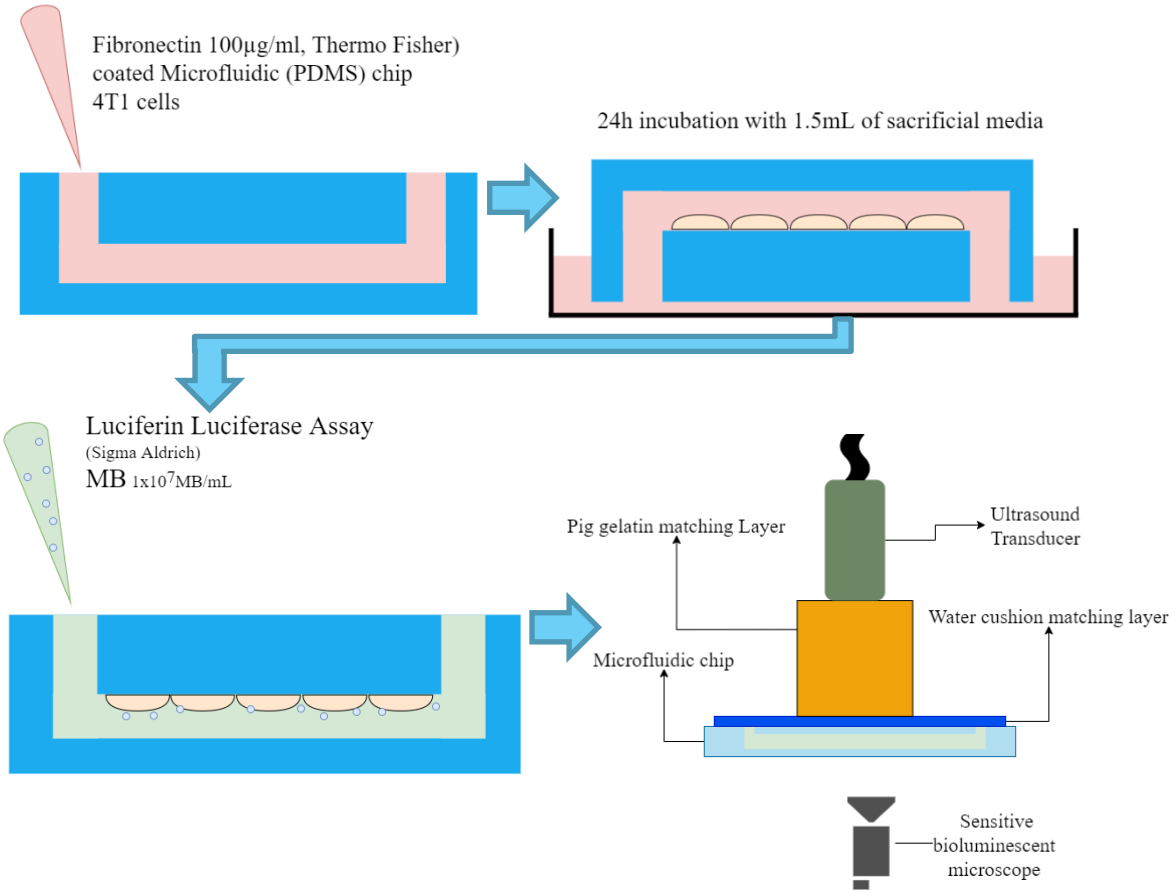
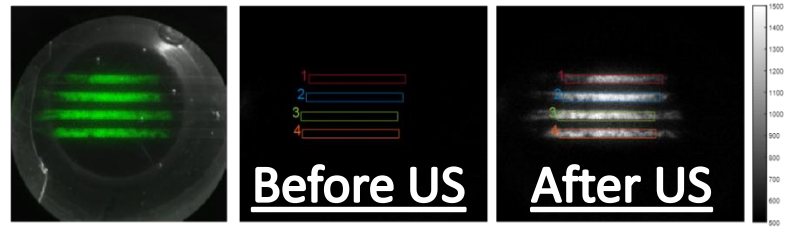
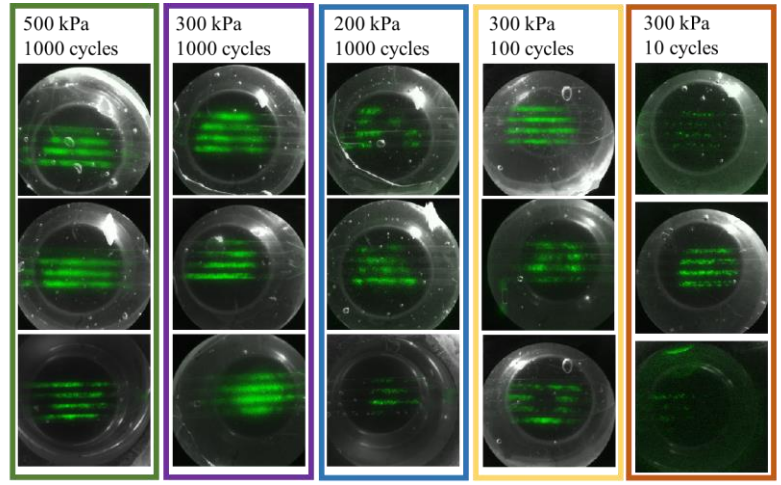
Methods :

ATP Imaging



Typical ATP Images before and after US





ATP release was imaged using a luciferin and luciferase assay and a sensitive bioluminescent camera (Evolve 512, Photometrics) underneath the chip and the US probe. ATP release was quantified by integrating the time course signal inside four rectangular ROIs of equal area.

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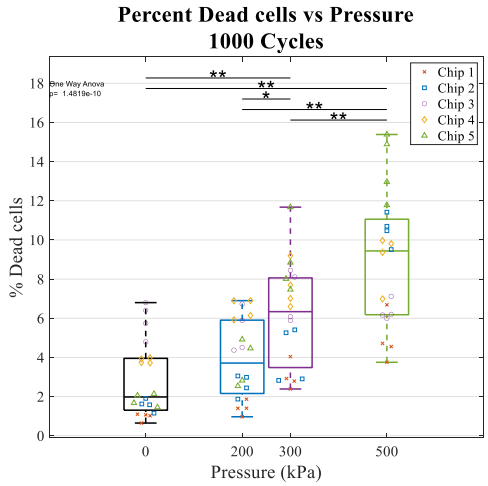
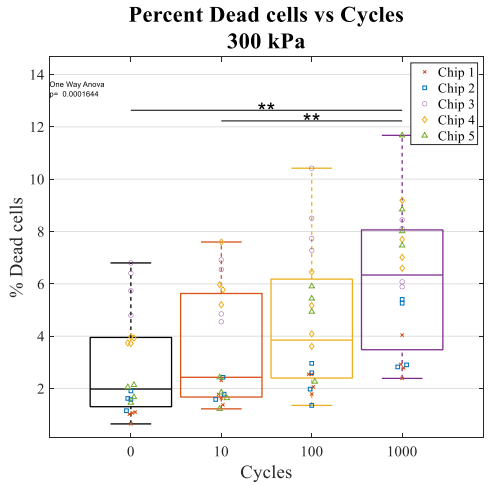
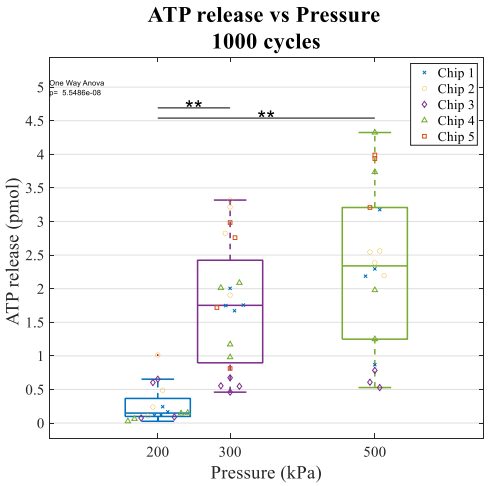
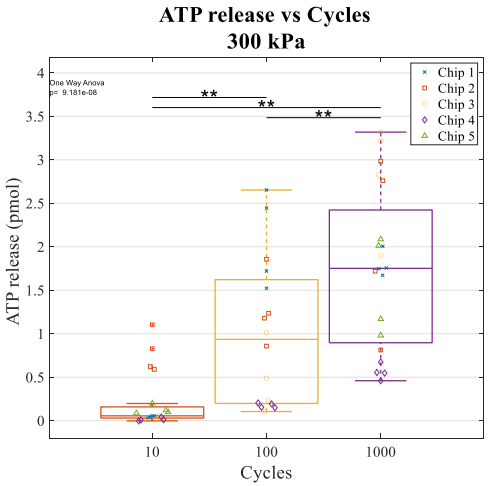
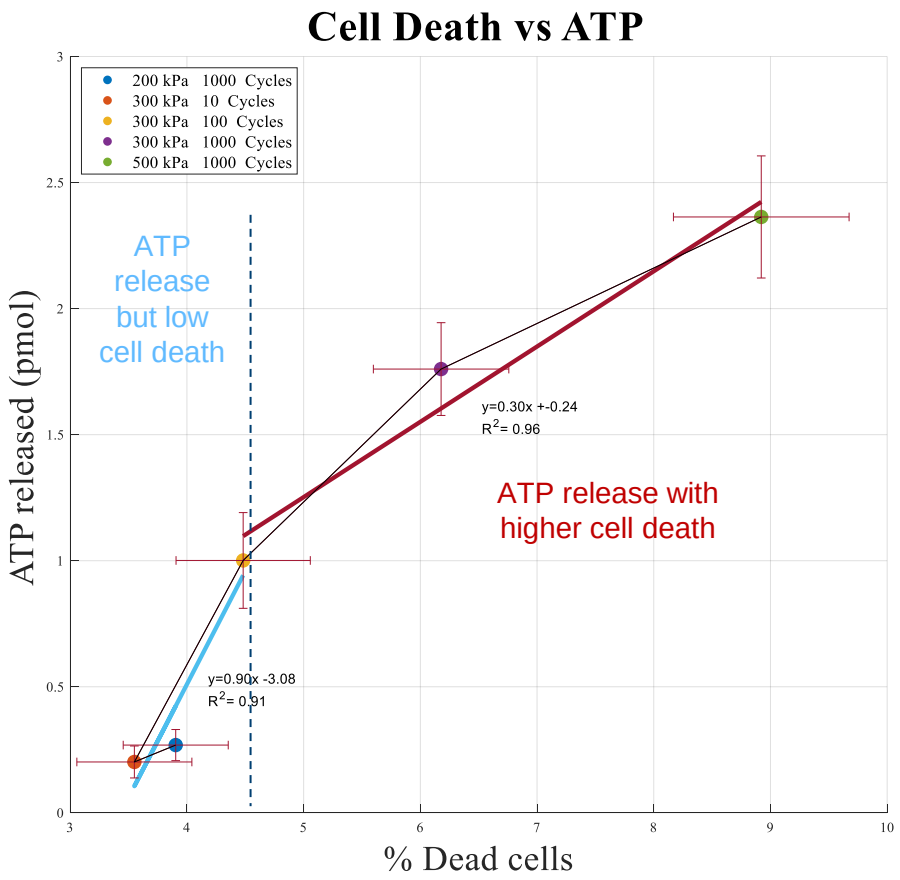
Results











Pressure and number of cycles affected ATP release and cell viability (One way Anova $p<0.001$). When comparing the results at 300kPa 100 cycles and at 300kPa 10 cycles there was a significant increase in ATP release but no significant increase in cell death (Multiple comparisons Tukey test $p<0.01$).

ATP release followed two regimes: with lower energy pulses, ATP release increased sharply with a very small increase in cell death; conversely, with higher energy pulses, ATP release continued to increase with cell death.

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Conclusions

Our results support that we can quantify ATP and that different mechanisms of ATP release can likely be triggered by MB+US therapy

This work will help create better US pulses to sensitize tumors before radiotherapy.

With flow, MB replenishment and repeated US pulsing will be possible, thus allowing to more closely mimic in vivo conditions and better understand the mechanisms of MB+US mediated ATP release.

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