Mechanism for microtsunami-induced intercellular mechanosignalling

To the Editor — In a recent Article in *Nature Photonics*, Compton and colleagues¹ presented a method for large-scale Ca^{2+} wave propagation through multiple living cells using a laser-generated microtsunami ($\mu\text{tsunami}$) initiated by a microcavitation bubble (μCB) for high-throughput screening (HTS) of molecules relative to cellular Ca^{2+} . However, the paper does not clearly explain the underlying biological mechanism of the $\mu\text{tsunami}$'s effect on living cells while discussing the application of the effect to HTS. Here we propose a possible explanation of the mechanism to further improve the method used by Compton *et al.* for screening many more molecules than 2-APB (the only molecule screened in the paper).

Based on the results in the paper, we propose the following mechanism for the $\mu\text{tsunami}$ -induced Ca^{2+} wave generation as depicted in Fig. 1a: the μCB solely induces the cell below the laser focus to activate intracellular Ca^{2+} generation and release Ca^{2+} signalling molecules (for example, ATP) which diffuse away and excite other cells to produce a propagating intercellular Ca^{2+} wave. This is supported by previous reports in which a long-range Ca^{2+} wave can be produced by irradiating a single cell with a focused femtosecond pulse laser^{2,3}. Here ATP molecules are released from the irradiated cell and diffuse to generate a Ca^{2+} wave that propagates over a distance of more than 200 μm (refs 2,4). The μCB may play a similar role to the femtosecond laser pulse for triggering the release of Ca^{2+} in the targeted cell, which then releases intercellular Ca^{2+} signalling molecules.

If this proposed mechanism is correct, the HTS method used by Compton *et al.* can be improved significantly as it can distinguish and screen other molecules in addition to 2-APB. There are a number of factors and receptors in the cell membrane that can mediate the release of intracellular Ca^{2+} , such as ATP receptors, growth factors, G protein-coupled receptors (for example, growth-regulating hormones) and survival factors. For example, assuming that the intercellular Ca^{2+} signalling molecule is ATP, blockers for the ATP receptor in the cell membrane⁵

Figure 1 | Microtsunami-induced Ca^{2+} wave generation. **a**, Possible mechanisms for microtsunami-induced Ca^{2+} wave generation. The Ca^{2+} wave may be generated by Ca^{2+} signalling molecules (i), mechanical stress by shear flow (ii) or by an acoustic wave (iii). **b**, Possible intracellular Ca^{2+} release pathways created by Ca^{2+} signalling molecules, which can activate several signalling pathways by different mechanisms.

(for example, the P2Y receptor) and for molecules in ATP signalling pathways⁶ can also be screened as shown in Fig. 1b. Furthermore, the μCB stimulation can be made more precise and safe to cells. The laser power can be reduced to a much lower level that is sufficient to excite the release of Ca^{2+} in just one or two nearby cells and therefore keep all cells viable, whereas in the paper by Compton and colleagues, most cells within the radius of 50 μm around the laser focus seem to undergo necrosis.

A few other possible, but less likely, mechanisms are as follows: a shear flow produced by the μCB provides mechanical stress onto every single cell to activate the release of Ca^{2+} within the cells; or an acoustic wave followed by the μCB provides mechanical stress onto every single cell to activate the release of Ca^{2+} within the cells. These cases are, however, improbable as the propagation speed of the Ca^{2+} wave ($4.5 \pm 0.3 \mu\text{m s}^{-1}$) reported in the paper is much lower than that of the shear flow ($\sim 1 \text{ m s}^{-1}$) and acoustic wave ($\sim 1 \text{ km s}^{-1}$) (refs 7,8) that would result in a simultaneous release of Ca^{2+} in all cells. □

References

1. Compton, J. L., Luo, J. C., Ma, H., Botvinick, E. & Venugopalan, V. *Nature Photon.* **8**, 710–715 (2014).
2. He, H. *et al. Appl. Phys. Lett.* **100**, 173704 (2012).
3. Liu, X., Lv, X., Zeng, S., Zhou, W. & Luo, Q. *Appl. Phys. Lett.* **94**, 061113 (2009).
4. Zhao, Y. *et al. J. Biomed. Opt.* **15**, 035001 (2010).
5. Bootman, M., Fearnley, C., Smyrniak, I., MacDonald, F. & Roderick, H. L. *J. Cell Sci.* **122**, 2337–2350 (2009).
6. Schwiebert, E. M. *Am. J. Physiol. Cell Physiol.* **279**, C281–C283 (2000).
7. Rau, K. R., Guerra, A., Vogel, A. & Venugopalan, V. *Appl. Phys. Lett.* **84**, 2940–2942 (2004).
8. Vogel, A., Noack, J., Hüttmann, G. & Paltauf, G. *Appl. Phys. B* **81**, 1015–1047 (2005).

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