Inhibiting Myosin Motor Protein Activity Reversibly Softens Human Cortical Organoids

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I. INTRODUCTION

Accurate prediction of traumatic brain injury (TBI) risk during head impact depends on understanding the biomechanical properties of human brain tissue. We recently showed that inhibiting myosin motor proteins reduced the apparent stiffness of human brain organoids [1]. This result suggests that a significant portion of the measured stiffness of these cultures is geometric stiffness, i.e. stiffness due to pre-existing tensile stress in the tissue. Myosin motor proteins use adenosine triphosphate (ATP) produced by cellular metabolism to generate tension. There are many disease states, including concussion, that impair cellular metabolism [2]. The possibility that metabolic crisis reduces brain tissue stiffness has important implications. It means that a stressed brain may deform more during a head impact than a healthy brain.

II. METHODS

We obtained human cortical organoids with a diameter of 400–500 µm from a commercial vendor (Stemonix Inc.). Organoids are not adherent, so we immobilised them for indentation testing by capturing them in a fine nylon mesh. We indented the cultures to a depth of 10 µm using a cantilever-based microindentation instrument (PIUMA, Optics11). The indentation motion profile consisted of a 1 s approach ramp, a 1 s hold, and a 1 s retraction ramp. The cantilever had a stiffness of 0.23 N/m and a spherical tip 23 µm in diameter. Then, we treated them with 10 µM blebbistatin for 1 hour at 37°C and repeated the indentation measurements. After that, we cultured the organoids at 37°C in blebbistatin-free media for 90 minutes and then indented them for the third time. We calculated the effective Young's modulus value from each indentation curve using a Hertz model of spherical indentation of an elastic half space, per Equation (1):

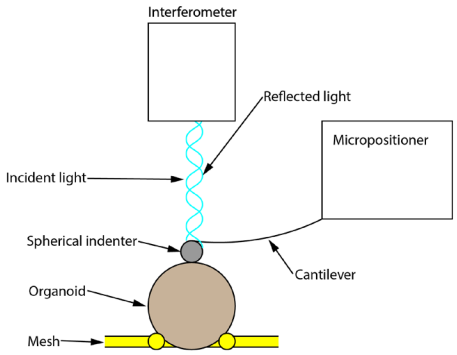

Fig. 1. Schematic of indentation apparatus.

$$F = \frac{4}{3} E_{eff} \sqrt{R_i}.h^{3/2} \quad (1)$$

where F is indentation force, E_{eff} is effective Young's modulus, R_i is indenter radius, and h is indentation depth. E_{eff} is $E/(1 - \nu^2)$ and ν is Poisson's ratio (we assumed the tissue was incompressible in this analysis).

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III. INITIAL FINDINGS

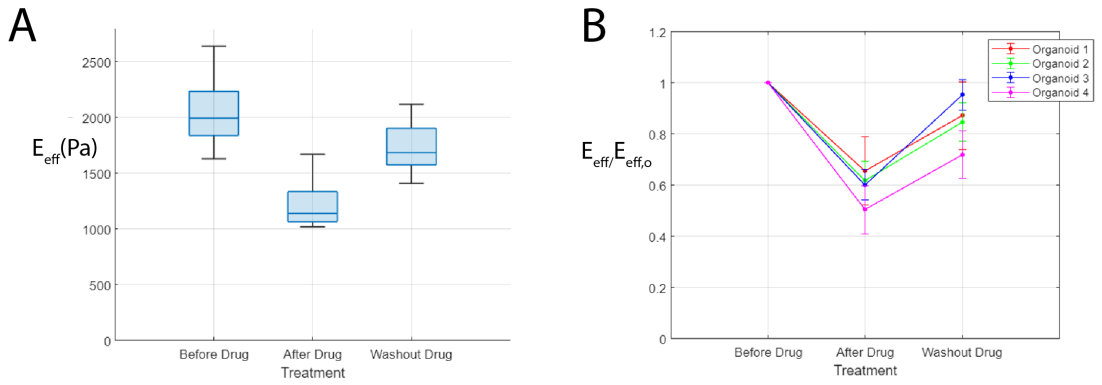

Fig. 2. The effect of blebbistatin treatment on organoid stiffness. A. Absolute values of E_{eff} (n = 20 indentations, N = 4 organoids, blue line = median, blue boxes = inter-quartile range, error bars = range). B. E_{eff} normalised to its initial value, $E_{eff,o}$ (error bars = standard deviation, n = 5 indentations per organoid at each treatment condition).

The stiffness of the organoids declined after treatment with blebbistatin. Approximately half of the lost stiffness was recovered when the drug was washed out (Fig. 2).

IV. DISCUSSION

These results show that the reduction in organoid stiffness after blebbistatin treatment is at least partially reversible. The period allowed for myosin motor proteins to recover their activity was short. It is possible further recovery would occur during a longer recovery period, but further testing will be required to confirm this hypothesis. The reversibility of the treatment has important consequences for how we interpret these data. The drug has not permanently changed the structural integrity of the tissue. On the contrary, the data suggest that the stiffness of the tissue tracks myosin motor protein activity over time. Therefore, restoring normal myosin motor protein activity may be a viable strategy for restoring the resilience of the brain to head impact.

The absolute values of pre-treatment, effective Young's modulus found in this study (median 1995 Pa, see Fig. 2A) are consistent with values reported in the literature, although those values vary over a wide range [3]. Nevertheless, there are important differences between organoids and mature human brain tissue. Organoids contain astrocytes and neurons but lack white matter, microglia and vasculature. Therefore, the relative trends in this dataset are more informative than the absolute values. Also, the mathematical model used to compute stiffness values relies on the assumption of small strains and linear elastic behavior. These are both gross approximations of the actual mechanics of brain tissue during traumatic brain injury events. Nevertheless, the correlation between myosin motor protein activity, metabolism and stiffness in human brain cells merits further investigation because it can help us understand how the brain's vulnerability to head impact evolves when the brain is not healthy.

V. REFERENCES

[1] Ryu, H., *et al*., *Adv Mater*, 2021.
[2] Champagne, A. A., *et al*., *Brain Imaging Behav*, 2021.
[3] Chatelin, S., *et al*., *Biorheology*, 2010.