Using Human Cortical Organoids to Quantify How the Genome Influences Brain Mechanical Properties

Yasaman Samei[1], Paraskevi Papavasileiou[1], Ingrid Cheung[2], Colin K. Franz[2,3], John D. Finan[1*]

I. INTRODUCTION

Human cortical organoids are clusters of human brain cells derived from induced pluripotent stem cells. Organoids retain the genome of the donor from whom they were derived. These cultures can be traumatised *in vitro* to investigate how genetic factors affect the tolerance for trauma in human brain cells. Variation in trauma pathology across organoids from different donors may arise from differences in how much deformation the cells can tolerate or from differences in how much they deform under load i.e. their mechanical properties. Therefore, we measured the mechanical properties of organoids from different donors by microindentation.

II. METHODS

Experimental Testing

The size, elastic stiffness, and viscoelastic relaxation behaviour of the organoids were measured using a Chiaro microindenter (Optics11) mounted on a microscope. The organoid was immobilised by placing it in a shallow, conical recess drilled into the bottom of a 35 mm dish. It was indented using a cantilever with a 250 µm radius spherical tip and a stiffness of 0.5 N/m. For elastic measurements, it was indented at a speed of 90 µm/s until the load reached 0.3 µN and then unloaded at the same rate. For viscoelastic measurements, it was indented at 90 µm/s to a depth of 25 µm (inducing an equivalent strain of 0.064 [1] and an equivalent strain rate of 0.23 s^{-1}) and that indentation was sustained under closed loop feedback control for 180 seconds. After indentation, it was moved out of the recess and its height was measured as the difference between the contact positions for the top of the organoid and the bottom of the dish. Then, the organoid was imaged to quantify its horizontal dimensions.

Mathematical Modelling

The bright field image of the organoid was segmented and fit to an ellipse (all fitting was done in Matlab) to compute two horizontal semi-major axes, a and b. The height was halved to estimate the semi-major axis in the vertical plane, which was denoted c. The three-dimensional shape of the organoid was approximated as an ellipsoid with these semi-major axes and an effective radius was computed using Equation 1.

$$R_{organoid} = \sqrt[3]{abc} \tag{1}$$

For both elastic and viscoelastic tests, the contact point was identified using the manufacturer's software. For elastic tests, the Young's modulus, E, was computed by fitting the Hertz expression for contact between two spheres (Equation 2) to the force displacement data between the contact point and peak indentation depth.

$$F = \frac{4}{3}E^* R^{\frac{1}{2}} d^{\frac{3}{2}} \quad \text{where} \quad \frac{1}{R} = \frac{1}{R_{tip}} + \frac{1}{R_{organoid}} \quad \text{and} \frac{1}{E^*} = \frac{1-\nu_1^2}{E_1} + \frac{1-\nu_2^2}{E_2} \tag{2}$$

For viscoelastic tests, the relaxation function was assumed to take the form of a 3 term Prony series as shown in Equation 3. An expression for the force history was created by using a hereditary integral to convolve the experimental displacement history with the relaxation function in the Hertzian expression for indentation force, resulting in Equation 3, which was fit to the experimental force history to determine the coefficients of the relaxation function.

$$F(t) = \frac{16\sqrt{R}}{3}\int_{-\infty}^{t} G(t-s)\frac{\partial\left[d(s)^{\frac{3}{2}}\right]}{\partial s}ds \quad \text{where} \quad G(t) = G_\infty + G_1 e^{\frac{-t}{\tau_1}} + G_2 e^{\frac{-t}{\tau_2}} + G_3 e^{\frac{-t}{\tau_3}} \tag{3}$$

[1]Department of Mechanical and Industrial Engineering, University of Illinois at Chicago, Chicago, Illinois, USA.
[2]Biologics Laboratory, Shirley Ryan AbilityLab, Chicago, Illinois, USA. [3]Department of Physical Medicine and Rehabilitation, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA. *jdfinan@uic.edu

III. INITIAL FINDINGS

Three donors were included. They were designated WTC-11, BJF, and AN1 to protect the anonymity of the donors. The organoid radius did not depend on the donor, but the Young's modulus, E, did (see Fig. 1 A, B). Smaller organoids tended to be stiffer than larger organoids (see Fig. 1C). Multiple batches of organoids were produced from each donor and batch had a statistically significant influence on Young's modulus for all donors (see Fig. 1D-F). The relaxation functions were evaluated with times of 0.5, 5, or 180 seconds to compute time-dependent shear moduli, which were abbreviated as $G_{0.5}$, G_5, and G_{180}, respectively (see Fig. 1G). A repeated measures ANOVA followed by Bonferroni post-hoc testing revealed significant effects of time point and donor at $p < 0.05$. Within each donor, all the time points were significantly different. Within each time point, $G_{0.5}$ significantly differed between the BJF and WTC-11 lines, as did G_5, but G_{180} did not.

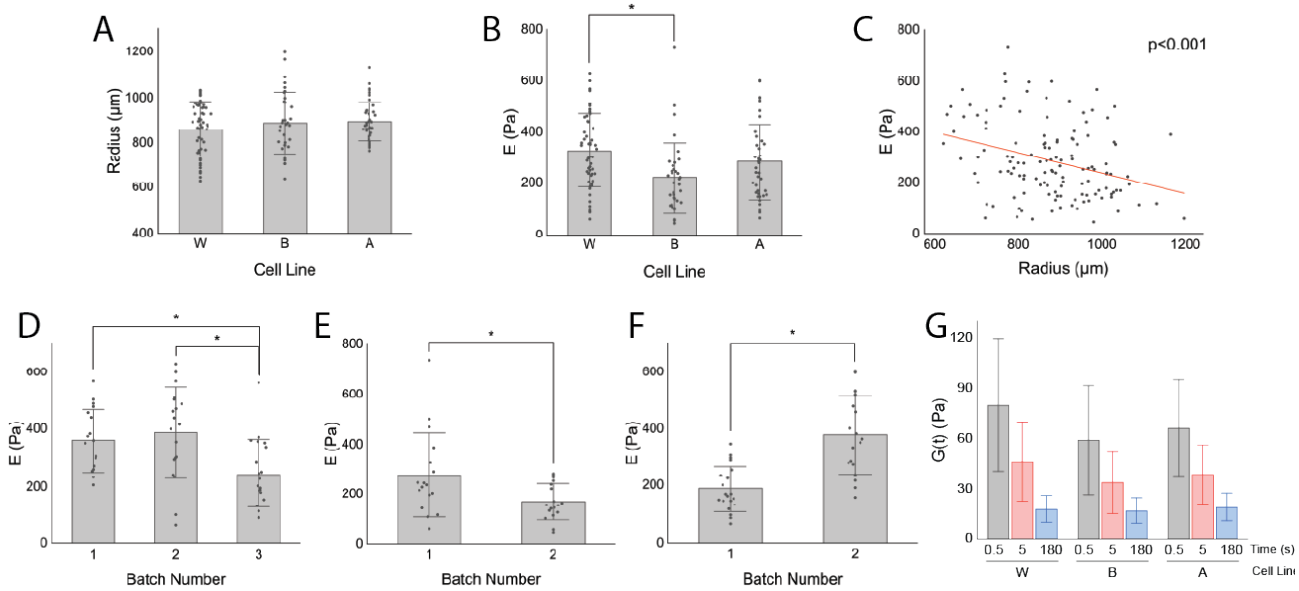

Fig. 1. Organoid size and mechanical properties. (A) Organoid size for various donors (W= WTC-11, B = BJF, A = AN1, n ≥ 17). (B) Organoid stiffness for various donors (n ≥ 17). (C) Dependence of stiffness on size (red line = linear regression with $p < 0.001$, $R^2 = 0.1$, n = 131). Young's modulus values across batches for (D) WTC-11, (E) BJF, and (F) AN1 donors. (G) Time-dependent moduli (error bars = standard deviations,* = $p < 0.05$ for ANOVA followed by Tukey's test).

IV. DISCUSSION

Relaxation function values for primary human cortical tissue vary from 50 to 5000 Pa at 0.5 seconds [1]. Our relaxation functions for organoids varied between 50 and 100 Pa at 0.5 seconds, putting them at the low end of this range. This fact may reflect the immaturity of the tissue because the cortex stiffens as it matures [1]. Mechanical properties also varied across donors, and across batches from a specific donor. Smaller organoids were stiffer. This trend may indicate that mature, post-mitotic cells are stiffer than immature, proliferating cells, which are likely more abundant in larger organoids. These results have important consequences for how we use organoids to understand patient-specific morbidity and other questions in neurotrauma. Currently, some cortical organoid models of trauma load the tissue [3] while others displace it [4]. Loading the tissue induces stiffness-dependent strains and strain rates while displacing the tissue induces stiffness-independent strains and strain rates (assuming the tissue is homogeneous). Therefore, tissue loading models will have an additional dimension of batch-to-batch variability compared to tissue displacing models.

V. REFERENCES

[1] B. S. Elkin et al., J Neurotrauma, 2011
[2] S. Chatelin *et al.*, Biorheology, 2010
[3] J. D. Lai *et al.*, Cell Stem Cell, 2024
[4] A. R. Shoemaker *et al.*, Dis Model Mech, 2021