Biomechanical initiation of neurodegenerative pathology in human astrocytes

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I. INTRODUCTION

Modern safety standards relating to traumatic brain injury (TBI) employ injury criteria that correlate head and brain kinematics with acute outcomes ranging from concussion [1] to skull fracture [2]. Chronic outcomes are of increasing concern given accumulating evidence that TBI increases the incidence of dementia [3]. However, correlating the impact kinematics with dementia symptoms that emerge decades later presents a daunting challenge. An alternative is to define mechanical tolerance criteria for acute pathologies that drive chronic neurodegeneration. Loss of spontaneous calcium dynamics in astrocytes may be such a pathology. Pre-clinical studies indicate an important role for astrocytes in the early stages of Alzheimer's disease (AD). Astrocytes regulate electrophysiological activity in neurons. When astrocyte activity declines, neuron activity increases, creating excitotoxic pathology that initiates AD [4]. To better understand this process, we cultured human-origin, spontaneously active astrocytes on a stretchable substrate and subjected them to strains and strain rates that typically occur in brain tissue during injurious head impacts. We demonstrated that *in vitro* trauma suppressed spontaneous activity in these astrocytes. This system provides a platform to explore and quantify the traumatic mechanical stimuli that initiate neurodegenerative pathology in vulnerable individuals and hence define relevant biomechanical tolerance criteria.

II. METHODS

Stretchable 96-well plates were manufactured by attaching sheets of taut, biocompatible, transparent polyurethane rubber to 96well plate bodies using biocompatible double-sided tape. Astrocytes were generated from human-induced pluripotent stem cells (hiPSCs) rapidly using lentiviral vectors that upregulated the transcription factors Sox9 and NFiB [5]. These cells were cultured on 6-well plates for seven days and then transferred to stretchable 96-well plates at 1500 cells/cm^2 and maintained until day 23. A custom-built, plate-stretching apparatus was used to push lubricated, Teflon-coated, aluminum indenters in and out of the underside of the cell culture membranes to depths of 1.5 mm or 2.5 mm in a period of 30 ms. The associated membrane strains have not yet been quantified, but prior publications provide context. A 2 mm indentation depth induced a biaxial, in-plane strain of 30% in this system [6]. In a study of cortical neurons derived from hiPSCs, the trauma phenotype emerged at approximately 20% strain and reached its maximal level at 40% strain [7]. These data points motivated the decision to centre the range of indentation depths used on a value of 2 mm. At either four hours or 24 hours after injury, the astrocytes were stained with 5 µM Calbryte 520 AM, a fluorescent calcium reporter, and imaged with an Olympus FV 3000 confocal microscope at 1 Hz for 150 s. In post-processing, pairs of consecutive images were compared with a difference operation using CellProfiler software to detect instances where the fluorescent intensity of a cell changed on a short time scale. The total number of pixels detected by this algorithm across the whole imaging time series was used as a quantitative index of activity. Separate populations of astrocytes were immunofluorescently stained for glial fibrillary acidic protein (GFAP) and S100B to confirm astrocytic differentiation.

III. INITIAL FINDINGS

Human-origin astrocytes attached well to a stretchable polyurethane substrate and survived for 16 days (Fig. 1A). These cultures stained uniformly for S100B while staining for GFAP was sporadic (Fig. 1B). Astrocyte cultures showed robust, spontaneous calcium dynamics indicative of electrophysiological activity after sham injury. These

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dynamics were almost entirely abolished by trauma at the four hour time point. Astrocyte activity remained suppressed at the 24 hour time point, although a trend towards recovery relative to the four hour time point occurred (Fig. 1 C, D). The four hour sham and four hour injury groups were more clearly separated in the 2.5 mm injury condition than in the 1.5 mm condition, but it remains to be seen if this is a real dose-response effect or a random event.

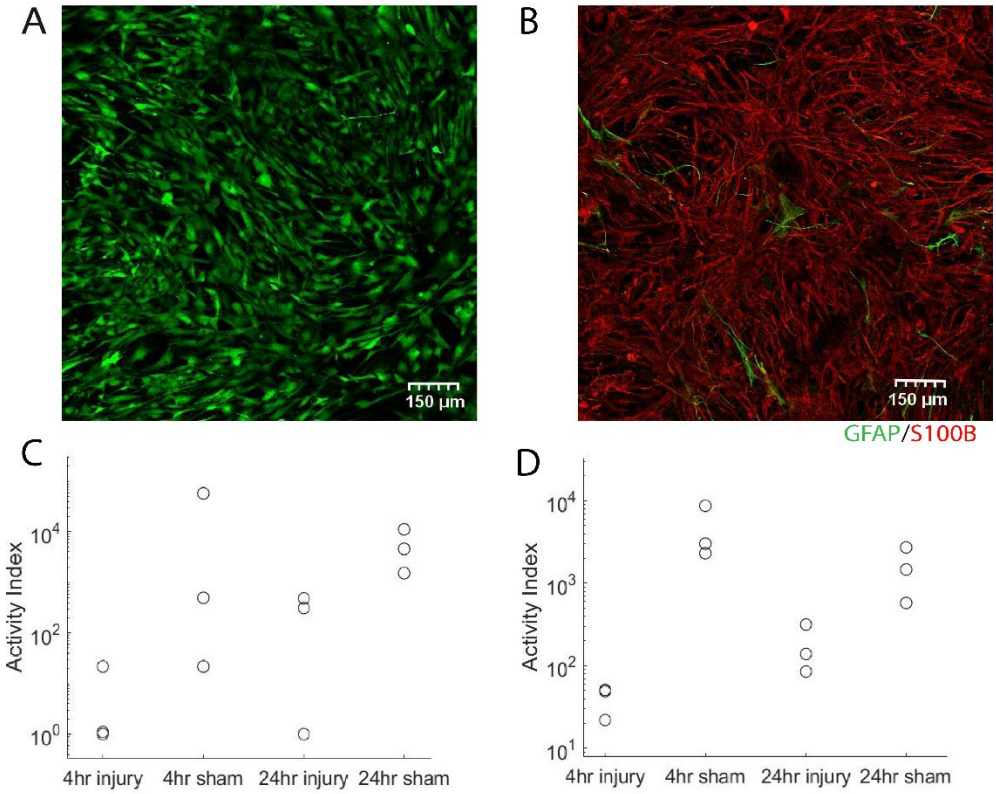

Fig. 1. (A) Astrocyte culture stained with CalBryte 520 AM. (B) Astrocytes stained positive for GFAP and S100B. (C) Calcium dynamics after 1.5 mm depth injury (n=3). (D) Calcium dynamics after 2.5 mm depth injury (n=3).

IV. DISCUSSION

These cells stained uniformly for S100B and sporadically for GFAP, suggesting that they are terminally differentiated astrocytes at an intermediate stage in their maturation (GFAP is a more mature marker than S100B), which is consistent with the number of days in culture. The fact that they have spontaneous, electrophysiological activity in the sham condition indicates that they are in good health and tolerating the unconventional rubber culture substrate well. The clear changes observed in the injury conditions suggest that this platform has potential to reveal the tolerance of this phenotype for mechanical deformation. In future experiments, we will investigate a wider range of strain amplitudes across a longer post-injury time course to identify a threshold value of strain that chronically suppresses astrocyte activity because chronic suppression is most likely to drive subsequent neurodegenerative pathology. This threshold may depend upon genetic factors. In this regard, it is worth noting that human astrocytes generated in this manner retain the genetic identity of the individual from whom they were derived, and that that genetic identity can be edited precisely. Therefore, this platform has the potential to not only quantify injury criteria for post-traumatic dementia pathology in humans but also to customise those criteria for different genetic subsets of the human population.

V. REFERENCES

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