An *in vitro* Magnetic Twisting Cytometry Model for Studying the Role of Specific Cell Adhesion Molecules in Traumatic Brain Injury

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I. INTRODUCTION

Forces directed through cell adhesion molecules (CAMs), such as integrins, regulate multiple physiological processes [1] and have been recently implicated in both normal brain function [2] and in Traumatic Brain Injury (TBI) [3]. Current *in vitro* models of TBI typically lack the precision required to study the potential role of mechanosensitive CAMs in TBI biomechanics. Here we present a novel adaptation of Magnetic Twisting Cytometry (MTC) [4] designed to determine if cell-level forces, associated with TBI induced tissue deformation, directed through specific mechanosensitive CAMs contribute to the initiation of cellular injury.

II. METHODS

Small (5 µm) ferromagnetic microbeads were coated with adhesive proteins and bound to neurons. Fibronectin (FN) coated beads bind specific CAMs, such as integrins, whereas Poly-l-lysine (PLL) coated beads bind non-specifically to the cell membrane. Custom built electromagnetic magnetizing (not shown) and twisting (Fig 1A) coils generate magnetic fields (B Field) that align the magnetic moments (M) of all beads and then rapidly twist them resulting in shear stress at the bead-CAM adhesion. The twisting coil (Fig 1B) is incorporated into a microscope stage to facilitate live cell imaging and injury identification.

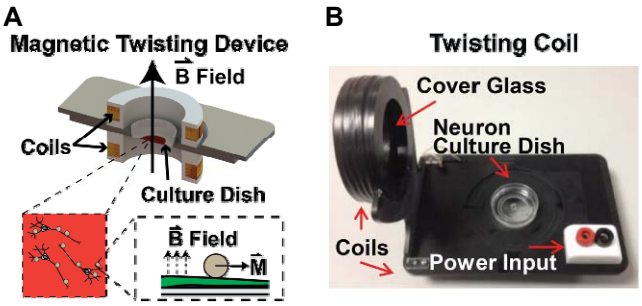

Fig 1: The MTC device uses electromagnetic coils to twist small ferromagnetic beads bound to neurons to direct injury inducing forces through specific CAMs.

III. INITIAL FINDINGS

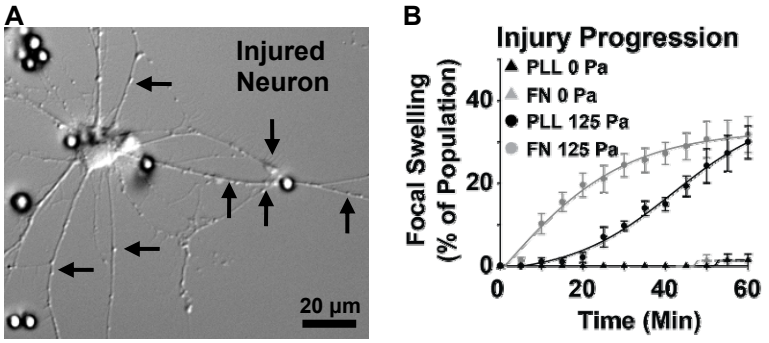

Fig 2: A single, rapid twist induces the formation of neurite focal swellings (arrows), an indicator of injury, and a bead coating dependent injury response.

Injured neurons were identified by the appearance of neurite focal swellings (Fig 2A). The percentage of injured neurons following a single twist, resulting in 125 Pa of shear stress for 50 ms, was greater than control (0 Pa) for both FN and PLL coated beads (Fig 2B). The temporal progression of injury percentage was dependent upon bead coating, with FN coated beads inducing higher levels of injury at earlier time points compared to PLL coated beads.

IV. DISCUSSION

The initial findings suggest that the adhesion molecules through which forces are directed may influence the extent of axonal injury. This is significant because the tendency of previous *in vitro* TBI models to focus on the magnitude or rate of mechanical loading may have neglected the contribution of specific mechanosensitive cellular components that transmit and bear loads at the cellular level. Although tissue deformation *in vivo* will direct forces through both specific and non-specific cellular linkages, mechanosensitive CAMs and their associated signaling pathways may provide novel injury mechanisms to explore in the context of TBI.

V. REFERENCES

1. Alenghat et al., Science Sig, 2002. 2. Tyler, Nat Rev Neuro, 2012. 3. Hemphill et al., PLoS One, 2011. 4. Wang et al., Science, 1993.

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