

CHANGE OF CONSTITUTIVE MATERIAL PROPERTIES IN ORGANOTYPIC BRAIN CULTURES IN VITRO

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INTRODUCTION

Over the past decade, organotypic brain slice cultures emerged that allow preservation of the *in vivo* heterogeneous cell population and cytoarchitecture in a controlled culture environment over a prolonged period of time. Most recently, organotypic brain slice cultures have been employed for *in vitro* modeling of traumatic brain injury to study long term injury cascades and potential therapeutic interventions (Miesch et al., 2001, Morrison et al., 1998). Such injury models induced a mechanical insult to organotypic brain cultures to determine the consequential biological injury cascade. To characterize the mechanical insult on the tissue level, accurate knowledge of the viscoelastic constitutive properties of brain tissue is essential. While numerous studies have investigated material properties of fresh brain tissue, no study to date has determined the material properties of organotypic brain cultures, and their change over time *in vitro*.

In this study, we used a parallel plate measuring system (Bohline CS Rheometer, Cranbury, NJ) to investigate the shear material properties of organotypic mouse brain tissues up to six days in culture. We hypothesized that the material properties of organotypic mouse brain tissues change over time of culture.

METHODS

Organotypic Culture: Whole brains (excluding the cerebellum) were removed from 8 days old Swiss mice pups after sacrifice with the use of halothane. The brains were placed in sterile, chilled dissecting media and cut coronally to 400 μ m with a vibratome (Leica VT 1000). Each slice was then transferred on a cell culture membrane (Millipore, Millicell-CM) containing neuro-basal nutrient medium. Slices were maintained in an incubator at 37°C with a 5% CO₂ enriched atmosphere.

Oscillatory frequency sweep tests: For dynamic shear tests, 32 brain slices were randomly selected and tested at day 0 (n=6), and after 1 day (n=7), 2 days (n=8), 3 days (n=6), and 6 days (n=5) in culture. For each test, a brain slice sample was placed in between a 6mm diameter cylindrical spindle and a 50mm diameter base plate of the rheometer (Fig. 1). The Millipore substrate was securely attached to the based plate of the rheometer with super glue. All brain slices were subjected to a prescribed oscillatory frequency sweep between 0.1 Hz and 1 Hz, with a target strain of 1%. The strain applied was defined as the plate displacement at the edge of the sample divided by the sample height. The sample height was adjusted by the distance between the spindle and base plate to fit sample thickness. To prevent slip between the spindle and the brain slice surface, a

sandpaper disk of 6mm diameter was glued to the spindle. During each test, a chamber encapsulated the brain culture to minimize potential for dehydration.

Three repeatability frequency sweep tests were performed on each specimen to ensure result consistency, but only measurements from each first test were used for result compilation. The complex shear modulus

(G^*) was calculated from $G^* = \sqrt{G'^2 + G''^2}$, where G' represents the elastic modulus and G'' represents the viscous modulus.

Statistical analyses were performed using a student's t-test with a 95% confidence interval.

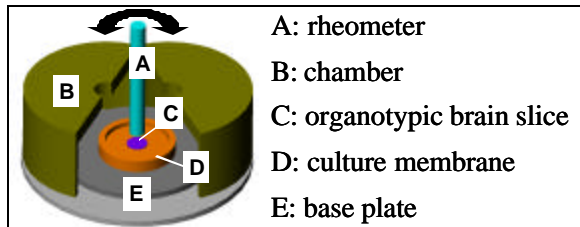


Fig.1: Experimental setup for frequency sweep tests.

RESULTS

Complex shear moduli (G^*) from *day 0* through *day 6* in culture are depicted in Figure 2.

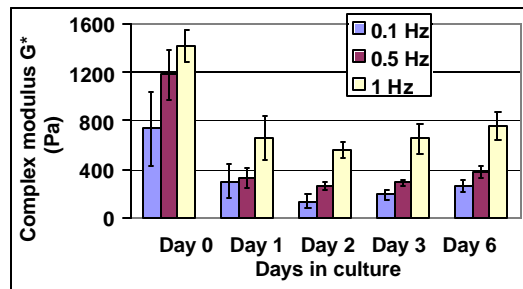


Fig. 2: Complex shear modulus (G^*) of organotypic brain cultures at 0.1, 0.5 and 1 Hz frequency versus days in culture.

For all days in culture, brain samples became stiffer with increasing frequency. Acute brain slices (*day 0*) demonstrated the highest shear stiffness and the shear

modulus reached 1416 ± 150 Pa at a frequency of 1 Hz. At *day 1*, the average values of complex shear moduli at 0.1 Hz, 0.5 Hz and 1 Hz dropped significantly ($p < 0.005$) by 61%. The least shear stiffness was observed at *day 2* in culture. From *day 3* to *day 6 in vitro*, the shear stiffness gradually increased. However, this increase was not statistically significant.

DISCUSSION

The observed complex shear moduli of acute mouse brain slices is comparable to that of porcine brain (608 Pa at 0.1 Hz, 715 Pa at 1 Hz, Brands et al., 1999) and bovine brain (2500 Pa at 0.1 Hz and 3162 Pa at 1 Hz, Bilston et al., 2001). Variations among shear modulus reports may be due to differences in specimen species, age, and interface conditions during shear testing.

This study documented for the first time changes in shear modulus over time *in vitro*. The decrease in shear modulus may be attributed, in part, to an increase in water content as brain slices observed maximum swelling at day two *in vitro* (Sommers et al., 2002).

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