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IN VIVO TISSUE-LEVEL THRESHOLDS FOR SPINAL CORD INJURY

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INTRODUCTION

The Impactor weight drop model is the gold standard technique for studying spinal cord injury (SCI) and has been extremely well characterized biologically, physiologically, and functionally [1], but very little is understood about the underlying biomechanics. We have conducted an experimental study to examine the severity and extent of microvascular injury following SCI in the rat immediately following graded mechanical trauma produced using the Impactor weight drop technique. We specifically examine vascular injury, which is a common primary and secondary product of spinal cord trauma [1]. We are developing a computational simulation of the weight drop model that will be used to identify the anatomical and biophysical characteristics of SCI, including thresholds for injury to the blood-spinal cord barrier. Herein we report on the results of our in vivo experiments and preliminary comparisons to our FEM.

METHODS

Impactor Weight Drop

SCI was induced at the T9-T10 vertebral level using the Impactor to drop a 10-gram weight 12.5, 25, and 50mm. Ten minutes prior to injury, animals were given an i.v. bolus of tracer solution comprising a low molecular weight fluorescent marker (Alexa 568-labeled hydrazide – 730 Da) and a serum protein marker (Alexa 488-labeled bovine serum albumin – 70 kDa) at a final concentration of 1mg/kg of each tracer. Five minutes following injury, animals were euthanized and perfused transcardially (Rutgers IACUC #02-015). Microvascular hemorrhage was then visualized by staining longitudinal sections for erythrocytes (Alexa Fluor 647 secondary antibody). The extravasated volume of each species was determined by thresholding individual images, summing the injured areas, and multiplying by the linear distance between images.

Finite Element Model

Mesh generation – An anatomically accurate mesh that includes the gray and white matter geometry was generated from MR images of rat spinal cord explants. A freshly excised, intact spinal column was placed in a 15ml conical tube with saline. The tube was inserted into a custom-built solenoid MR coil and the contents imaged with spin-echo magnetization in a 4T magnet. The cord was imaged over ~3cm in length. Coronal images were segmented into gray and white matter and were blended into continuous surfaces with ProEngineer. The surfaces were converted to solids, portioned into uniform shapes, and meshed with 8 node hexahedron elements with Abaqus CAE. The vertebrae were simulated by expanding the surface of the white matter 4% and meshing with quadrilateral shell elements.

Material properties – The non-linear elastic properties of the model were approximated from Bilston and Thibault [2], who defined a one-term Ogden hyperelastic strain energy density function for the human spinal cord in tension. The shear modulus was assumed to be 10MPa, alpha was set at 0.01, and Poisson's ratio was set at 0.45. The model was assumed to be homogeneous. The viscoelastic properties were determined from compression tests on spinal cord explants. Fresh excised spinal cords (n=8) were subjected to stress-relaxation tests in uniaxial compression, in which samples were compressed 40% in 10msec and then held for 10sec. The first 200msec of the normalized relaxation portion was fit to a 2-term Prony series exponential decay.

Weight drop_simulation – Impactor experiments were simulated using Abaqus Explicit by placing an analytical rigid surface in direct contact with the exposed surface of the spinal cord and prescribing the displacement of the Impactor to match experiments (approximately 1.5-2.5mm displacement in <5msec).

The results from the animal model were quantitatively compared to finite element model with a logit analysis. Horizontal sections of the FEM mesh were extracted from the 3D model and superposed on the binary images from the appropriate anatomical section from the experimental analysis of microvessel injury for each severity marker (molecule, protein, cell). Elements were graded as either injured (1) or uninjured (0). Peak values of maximum principal strain were identified from the simulations. For each element, the injury status (1 or 0) was plotted against the peak values, and logistic regression was performed to identify the dependence of injury to strain. The predictive ability of strain was then evaluated with receiver operating curves (ROCs) [3].

RESULTS

.esion Volume Analysis

The volume of extravasation significantly increased with increasing drop height and with decreasing species size (ANOVA, $P>0.05$)

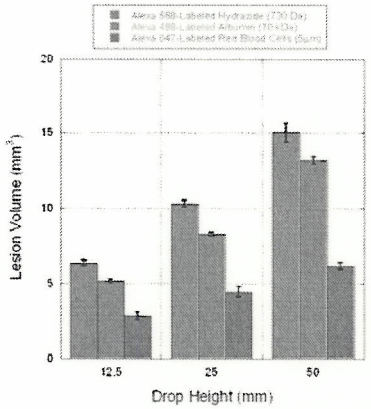

Figure 1 – Lesion Volume Data for weight drop experiments.

Finite Element Model

Coronal MR images of the rat spinal cord (Fig.2) were segmented into gray and white matter and used to generate a finite element mesh (Fig 3). The mesh includes a finer mesh seed in the impacted region.

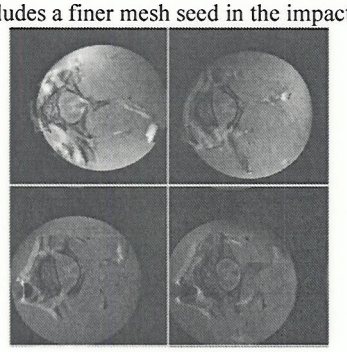

Figure 2 – MRI of the rat spinal cord

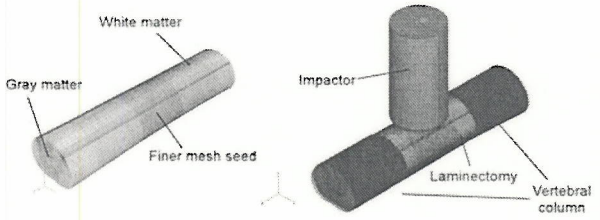

Figure 3 – FEM of the Impactor Weight Drop Model

In this preliminary model, the spinal cord was assumed to be homogeneous. Viscoelastic testing indicated that 40% +/- 10% of the stress dissipated at a time constant of 4.4 +/- 3.0 msec, and an additional 27% +/- 16% dissipated at a time constant of 150 +/- 160 msec. The increased error in the second time constant is due to truncating the data at 200msec. Since the primary effects of the impact occur over <10msec, the second time constant does not contribute significantly.

We have quantitatively compared the results from our experimental animal model to the preliminary output of the finite element analysis with logit analysis (Fig 4). (A) A slice of the FEM mesh is superposed over the image of injury at the appropriate level. (B) Elements are graded as injured (blue) or uninjured (red). (C) The peak value of max principal strain is identified and (D) regressed against injury status with a logit analysis. The quality of the regression is seen in ROCs in Fig 5. Strain was a good predictor of hydrazide (red) and albumin (green) extravasation, but not as good for RBC's (purple), as determined by the area under the curve.

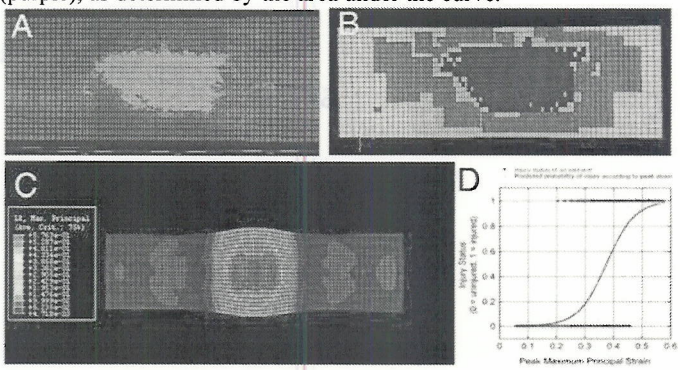

Figure 4 – Injury maps and logit analysis

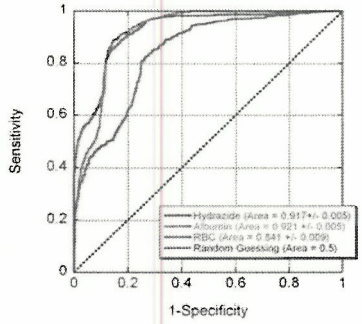

Figure 5 – ROC analysis

SUMMARY

Though still very preliminary, these results indicate that strain is a good predictor of blood-spinal cord barrier injury (albumin and hydrazide extravasation), but not as good for microvasular hemorrhage (RBC's). We are currently determining the material properties of rat spinal cord gray and white matter in vivo with an inverse engineering approach from MR images. After these properties are imported into our model, we will have a better sense of the stress that is generated in the spinal cord, and we can repeat the statistical analysis to determine the parameter that best predicts injury. This parameter(s) will then be the target for designing rational means of SCI prevention. Sponsored by the CDC (R49CCR 221744-01) and a fellowship from the NJ Commission on Spinal Cord Research.

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