SB³C2021
Summer Biomechanics, Bioengineering and Biotransport Conference
June 14-18, Virtual

PROBING THE MECHANICAL PROPERTIES OF CHICK EMBRYOS USING ATOMIC FORCE MICROSCOPY

Joel A. Lefever (1), Jack L. Edson (1), Ashok Ramasubramanian (1)

(1) Department of Mechanical Engineering
Union College
Schenectady, NY 12308, USA

INTRODUCTION

We study early embryonic development in the chick embryo. Two morphogenetic changes occur between Hamburger & Hamilton (HH) Stages 11-15 [1]: the early embryo rotates, and a series of flexures form in the dorsal wall. The underlying forces causing these phenomena are not fully understood [2].

One hypothesis is that an asymmetry develops in the geometry or material properties of the neural tube, which drives flexure and torsion. Prior research has evaluated the mechanical properties of chicken embryos using flat indenters on sectioned embryonic neural tubes [3]. In order to probe the mechanical properties more thoroughly, it is necessary to perform indentation experiments using a technique which can precisely target various locations on the embryo with micron-level precision. In this paper, we discuss how atomic force microscopy (AFM) can be used to probe the material properties of the early neural tube (Figure 1A).

METHODS

Chicken embryos are harvested at Hamburger and Hamilton Stages 10-15 [1], separated from the yolk, and examined in a Petri dish containing phosphate-buffered saline. The sample is fully submerged to protect the tissue and to mitigate surface tension effects. A coverslip serves as a rigid substrate, and filter paper holds the embryo in place. An AFM probe with a 10 μm diameter colloidal tip (sQube) is used to indent the neural tube of the chicken embryo (Figure 1A) in the stages immediately prior to and following rotation. Building on prior research which performed indentation on sectioned embryos [3], the embryos here are tested intact to minimize invasive procedures; however, the vitelline membrane, which covers the dorsal side of the embryo, is removed in order to improve access to the neural tube. The embryos remain submerged during the indentation process. To avoid interference with the indentation measurements, the heart is removed as necessary. The AFM-based indentation technique provides the unprecedented ability to evaluate mechanical properties at a scale of roughly 5 μm, permitting these properties to be mapped over various locations on the embryo. Force-distance curves (Figure 1B) are extracted and various measurements of mechanical properties can be computed, including the stiffness of the neural tube tissue. The stiffness is evaluated as the slope of the retraction portion of the force-distance curve to eliminate plastic effects (arrow in Figure 1B).

RESULTS

Indentation depths are typically observed to be on the order of 2-5 μm, which is limited by the vertical range of travel of the AFM. This indentation depth corresponds with maximum loads on the order of 4-20 nN. The force curves shown in Figure 1B were taken on the neural tube and are typical of several experiments. For this particular embryo, they indicate a stiffness of 7.3 ± 0.2 nN/μm, which is comparable with prior research [3].

As shown in Figure 2, the measured stiffness varies moderately across a single embryo. Figure 2 presents the stiffness data for the 8 embryos investigated to date. On an embryo in which the vitelline membrane was not removed, the stiffness was found to be approximately one order of magnitude greater (data not shown). Using a glass coverslip as a substrate prevents rigid body translation and allows the stiffness of the sample to be evaluated directly after subtracting the compliance of the probe.

DISCUSSION

The greater stiffness observed in the present results (compared to previously-published results in [3]) likely occurs because, unlike the prior study, our procedure maintains an intact neural tube and its

associated pressure (estimated as 30 Pa [4]). This stiffening effect is only partially mitigated by the smaller indenter, which samples a smaller region of tissue and therefore encounters less resistance.

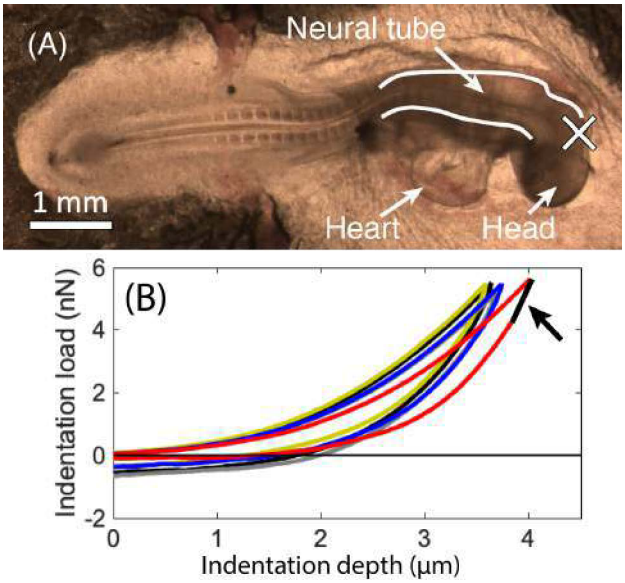

Figure 1: (A) A chicken embryo at HH Stage 14-15 as observed using optical microscopy prior to AFM-based indentation experiments. Major organs are indicated. (B) Five force-displacement curves resulting from a series of indentation experiments in the midbrain region using a colloidal AFM probe, for the embryo shown in (A). The indentations experiments shown in (B) were conducted at the location identified by the X in (A).

The variation in stiffness shown in Figure 2 is likely caused in part by differences in location, the exact stage of development that each embryo was in, and also physical differences between the embryos. Each box and whisker plot represents data from between 2 and 21 locations, as stated on the figure; at each of those locations between 3 and 19 successful indentations were performed. Each of the data points used in the box and whisker plot is an average from these indentations and the standard deviation within each such indentation set is generally small, as evidenced by the minimal scatter in each region in Figure 1(A).

A strong second-order mechanical response was observed in some indents, likely due to fluid flow in the Petri dish and inside the neural tube. This mechanical response affects the linear fit through which the stiffness is calculated; the outliers in Figure 2(B) correspond to the indents where a strong second-order mechanical response was observed.

The methodology described here allows for unprecedented control over the location of the indentation; however, it is not without limitations. The indentation experiment is susceptible to error due to the heartbeat, which cannot be stopped without an invasive operation to remove the heart (which was done for some of the data points) or by stopping the heart using chemicals which may also affect the material response (a chemical inhibitor of heartbeat was not used in this study). In the previous study with which we compare our data, the neural tube was sectioned and removed from the embryo, eliminating the heartbeat as a concern [3]. Experiments must be conducted in the period between heartbeats and it is not possible to confirm whether a heartbeat has occurred except by comparing several indents for uniformity.

Because the material is compliant and highly viscoelastic, it is challenging or impossible in some cases to exactly locate the position at which the indenter makes contact. Therefore, it is challenging to precisely evaluate the indentation depth to compare directly with [3]. We also note that our indenter is spherical, rather than the flat punch used previously [3], which alters the predicted mechanical response from linear to a power-law relationship.

In order to keep the embryos fresh, we attempt to do the indentation experiments as quickly as possible. However, some degradation is visible when the embryos are imaged after the experiments are completed. We notice no strong difference in the measured stiffness or indentation depth from the beginning to the end of the experiments, and therefore believe that this degradation has minimal effect on the measured properties.

The experimental methods described here can be extended to evaluate the mechanical properties across various locations on the embryo, and to determine whether locally stiffer or softer regions are observed. The results of this study will help to elucidate the fundamental mechanisms driving embryonic flexion and rotation.

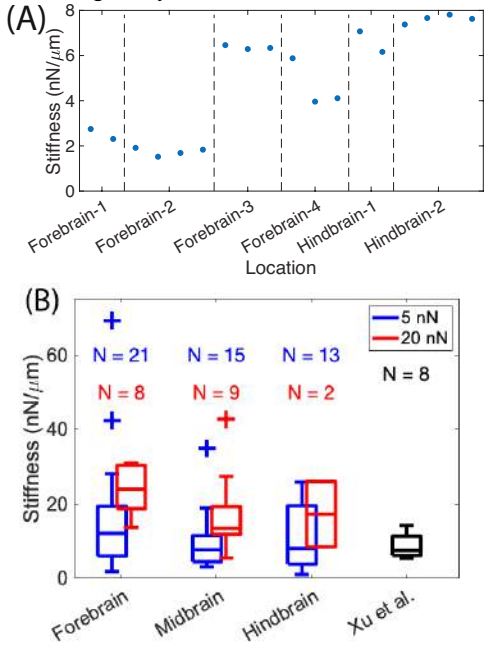

Figure 2: (A) A summary of the stiffnesses measured in six different locations on one embryo and (B) across a total of 68 locations on eight embryos, using AFM. The central mark represents the median, while the top and bottom of the boxes represent the 75th and 25th percentiles; the whiskers represent the range of the data, and crosses represent outliers. Each value of N gives the total number of locations on each section of the brain at which indentations were performed across all embryos. The total number of indentations is much higher. The indentation data in Xu et al. were collected using a custom microindentation device.

ACKNOWLEDGEMENTS

The authors acknowledge funding from the NSF (Award #1936733), and J.E. acknowledges a summer research fellowship and student research grant from Union College.

REFERENCES

[1] Hamburger, V & Hamilton, H L, J. Morph, 88:49-92, 1951.
[2] Ramasubramanian, A et al., J Biomech Eng., 141:1-12, 2019.
[3] Xu, G et al., J Biomech Eng, 132:011005, 2010.
[4] Jelínek, R & Pexiedner, T, Physiologia Bohemoslovaca, 17:297–305, 1968.