Characterization of chick embryo neural tube material properties using atomic force microscopy

Joel A. Lefever, Ioannis Binopoulos*, Priyanka Ghosh*, and Ashok Ramasubramanian
*Denotes equal contribution.

1 Introduction

During early development, mammalian and avian embryos undergo flexion and torsion (FT), starting in the cranial region. Improper FT can lead to congenital defects. Research indicates that the developing brain tube plays a major role in the progression of FT; however, the material properties of the embryonic brain tube are poorly understood. Using the chick embryo as the experimental model, we characterized the material properties of the developing neural tube with atomic force microscopy (AFM)-based indentation.

2 Methods

Fertilized white Leghorn chick eggs were incubated to reach Hamburger and Hamilton (HH) developmental stages 10 or 11. The embryo is separated from the yolk and sandwiched between a filter paper and a coverslip inside a Petri dish filled with saline. The vitelline membrane covering the dorsal side of the embryo is removed to allow direct access to the neural tube. Indentations were performed using an AFM (MFP-3D, Asylum) with a 10 μm diameter colloidal probe tip (NanoAndMore). We characterized regional stiffness for the forebrain (F), midbrain (M), and the hindbrain (H), (Figure 1A; HH stage 11-12; scale bar approximate). In our procedure, the stiffness is extracted from the retraction portion of the force curve (Figure 1B).

3 Results

Our preliminary experiments reveal a greater stiffness measurement (Figure 1C; 12 embryos total) than in previous studies [1]. This is likely due to the encephalic fluid pressure, which was not relieved in our experiments but was relieved in the prior study. This effect is partially mitigated by our smaller diameter probe which results in a reduction of contact area and a decrease in the measured stiffness. Compression of the soft matter beneath the embryo likely results in an underestimate of the embryo stiffness, but this effect is likely to be approximately equal in each region of the neural tube, so comparisons may still be made between different locations. Finite element modeling corroborates the present results and enables a more direct comparison with the indentation geometry used in prior research (results not shown).

4 Discussion

The contact area is comparable to the size of embryonic cells, which are on the order of 3-9 μm [2]. Therefore, it is likely that the present experiments measure a combination of cell-level and tissue-level properties. Our work indicates that AFM-based nanoindentation is a viable method for characterizing embryonic material properties with high spatial resolution.

5 Acknowledgements

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6 References

[1] Xu, G et al., J Biomech Eng, 132:011005, 2010.

[2] Garcia, K et al., J Mech Behav Biomed Mater, 65:383-397, 2017.