



## Mechanical analysis of chorion softening in pre-hatching stages of Zebrafish embryos

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### Abstract

During early development, the chorion envelope of zebrafish embryo undergoes a thinning process called "chorion softening," which has so far only been characterized chemically. In this study, a micromechanical force sensing system was used to characterize and quantitate mechanical modifications of the zebrafish embryo chorion during early development. Quantitative relationships between applied forces and chorion structural deformations were established at various embryonic stages. The measured penetration force into the chorion at the blastula stage was 1.3 fold greater than those at the pre-hatching stage. Furthermore, chorion elastic modulus values were determined by using a biomembrane elastic model. The elastic modulus of the chorion at the blastula stage was 1.66-fold greater than that at the pre-hatching stage. The experimental results quantitatively describe "chorion softening," which is most likely due to proteolytic activities at the pre-hatching stage. Gradual chorion softening during embryonic development was also artificially achieved by treating blastula chorion with pronase, a proteolytic enzyme. The force required to penetrate the pronase-treated

chorion were similar to those at the pre-hatching stage. This similarity suggests that "chorion softening" may be induced by the release of protease from the embryos, and the chemical nature of the process involves proteolytic fragmentation of ZP2 protein.

Keywords : *zebrafish, chorion softening, cellular force sensing, elastic modulus proteolytic activities, hatching, protease, peptide fragmentation*