A. INTRODUCTION

I. Abstract

The compartments of cell organization can frequently be associated to their adhesive and elastic properties. In this study, we investigate the bio-mechanical properties of zebrafish embryos through damping oscillations. We construct a homemade electromagnetic oscillator coupled to an optical lever to enhance the viscoelastic responses. This device allows us to extract the viscous damping coefficient and elasticity of cells in various stages of embryonic development, through nonlinear damping processes. The strain of the cell in each developing period can be obtained by measuring the alternations in the amplitudes of the damped oscillations. The elasticity of the zebrafish embryo in the blastula period is significantly lower than those of other periods, reflecting the reduction in the buffer resistance of the outer shell. A temporal exponent that indicates the growth rate of cell development can also be extracted from the nonlinear damping profile. This proper time of embryonic development is found to be noticeably different in each stage, which can be coupled to the changes in cell compartments. Modifications of the fluid compartments of the outer shell by emerging the embryo into solutions of slightly different pH values would significantly alter the oscillation profile. Surprisingly, the viscosity of the shell is extremely sensitive to the environment experienced.

II. Motivation

We learned in biology class that a cell’s function is greatly affected by its shape, and the shape is decided by the cell’s elasticity. This physical characteristic is a result of cytoskeleton movement and inner cellular structure. However, cytoskeleton and subtle structural changes can only be observed under expensive equipment such as Scanning Electron Microscope (SEM). Therefore, we wish to develop an easier experimental method that enables us to observe the subtle changes in biological structure.

III. Research Goals

The project aims to understand the relationship between physical characteristics and structural changes in biological cell. Furthermore, a new experimental method which is low-cost and capable of extracting precise data for biophysics research is established.
B. MATERIALS AND METHODS

I. Apparatus: Electromagnetic Oscillator

The Electromagnetic Oscillator is designed to apply appropriate magnitude of force on the zebrafish embryo and amplify the slight deformation when exerting force on the embryo.

There are four forces in this mechanical system: gravity, electromagnetic induction, cell stress and cell adhesion. The apparatus is able to tell the measurement of cell elasticity and cell adhesion.

(i) Induction Coil

When an electric current is passed though the induction coil, due to the magnetic effect in current, the strong magnet fixed at one end of the apparatus will be pulled by the coil and further exert a force on the testing body put onto the sample stage.

Different magnitude of force is exerted to the testing body by adjusting the value of the electric current input. Also, the tip of the probe is specially designed to make sure that the force is exerted equally to the testing body, in this case, the zebrafish embryo.

(ii) Oscillation

The current passed through the induction coil initiate the oscillation. This oscillation is caused by gravity and the inducted magnetic field. Figure 4 is a typical oscillation curve obtained from the oscillation of pure apparatus without any testing body on the sample stage.

The oscillation curves give details on the mechanical system. If a material is put on the sample stage, the oscillation curve changes. By analyzing the change, we can understand the physical properties of this material.
(iii) Optical Lever

Optical lever is a device used to amplify small angular displacement in thermal expansion experiment.

A beam of laser light is directed to the mirror of the apparatus, and reflects on the screen. Once the apparatus oscillates, the reflecting light-spot will move along with the oscillation. Using trigonometry and geometry theory, we can easily calculate the slight deformation of the embryo.

(iv) Momentum Transfer

Using the piezoelectric force sensor, the instant force applied to the testing body is extracted. Figure 6 illustrates the magnitude of force at the first time of contact during the oscillation. The integration of the data point represents the momentum transfer in each contact. As shown in figure 7, there is also an exponential decrement in the momentum of each contact which proves that there is a damping system coming from the induction.
II. Physical Parameters

The motion of the probe can be described by the following equation of motion:

$$M \ddot{X} = -kX - b\dot{X} + f_c(t, \Delta d)$$

The first and second terms in this equation are the general term for a damped oscillation. As for the cell function, $f_c$, it represents a complicated force function from the cell. However, in the project, the damping coefficient, $b$, is not a constant. In addition, the complicated cell function is unclear. Therefore, we add another temporal exponent into a general damping expression and obtained the following expression which can more precisely describe the motion of the probe:

$$X(t) = A \exp(-\alpha t^\beta) \sin(\omega t + \phi)$$

The oscillation curve resembles the pattern of a sine wave which is indicates in the sine term in our expression. However, due to damping phenomena, the amplitude decreases as in function of time. Therefore an exponent term is added into the expression.

Parameter $\alpha$ is the damping constant of the testing materials. This constant comes from the elasticity of the material. The materials exerted a stress force on the probe of our oscillator. As defined in damping equation, the magnitude of this force is related to the speed of the probe. In our project, the inner structure of the embryo mainly contributes to the value of this constant.

Furthermore, instead of a symmetric pattern for a typical damped oscillation, the oscillation curves obtained shows asymmetry. This asymmetric pattern suggests that there is another force in the mechanical system other then the defined stress force, and the two forces together caused this nonlinear damping. The additional force is defined as $\beta$ in our equation.

This additional force only exists when the probe makes contact with the testing material. The value of $\beta$ tells the direction of this force. If the motion of the probe is retarded by drag forces, it would give a $\beta$ value smaller than 1. On the other hand, if the motion is advanced by repulsive forces, $\beta$ would be greater than 1.

Fig.8. Physical meaning of nonlinear temporal exponent.
Viscosity

In order to understand the role of $\alpha$ and $\beta$ in viscosity, we performed our experiment on the glycerol. The viscosity of testing body, such as zebrafish embryo, can be estimated using the result of the experiment.

III. Zebrafish Embryo

Zebrafish is a common laboratory animal with adequate information in biology. The embryonic development takes about two to three days, dividing into seven broad periods: Zygote Period, Cleavage Period, Blastula Period, Gastrula Period, Segmentation Period, Pharyngula Period, and Hatching Period. The biological structure of the embryo is fully understood, and we can further refer our data to the embryonic development.

The project focuses on the first three periods: Zygote Period, Cleavage Period and Blastula Period.

In Zygote (0-75 mins) Period, the molecules in the yolk are moving toward the animal pole of the embryo to prepare for the cell division. The appearance of the first cleavage marks the end of this period. Only one cell is seen in this period.

As for Cleavage Period (75-130 mins), the embryo mainly goes through its cell mitosis process. The newly divided cells are in a regular orientation, and ultimately, become a compacted structure named Morula. The appearance of this structure marks the start of the Blastula Period.

The cellular differentiation starts in Blastula Period (130-315 mins). The newly divided cells first move toward the animal pole and start to consume nutrient from its vegetal pole. Then, the blastomere goes through epiboly process, the formation of the three germ layers.
C. Results and Discussion

I. Oscillation Curves

The damped oscillation can be described as a forced oscillation with a parameter $\beta$ in addition to the damping constant $\alpha$.

Two regimes are identified in the oscillation curve as shown in figure 11: an induction damped regime at the lower portion and a stress damped regime at the upper portion. The tip of the probe contacts the embryo during the stress damped regime and a dragging force is exerted to the probe.

In the induction damped regime, forces contribute to the oscillation curves are: gravity, damping force from cell stress, and friction. On the other hand, in the stress damped regime, the amplitude of the curve is slightly bigger than the one in the induction damped regime, and thus suggests an adhesive force in this regime.

Respectively, the two parameters used to describe the oscillation reveals the physical characteristics of the system. $\alpha$ describes the damped phenomena and $\beta$ marks the nonlinearity. The two values are used to calculate cell stress and the interaction force.

II. Nonlinearity

There are two damping system in the mechanical system, one from the oscillator and the other from the zebrafish embryo. Due to the different time scale of the two systems, $\beta$ is added into the expression to mark the nonlinearity.

Figure 12 compares the endpoints from the stress damped and induction damped regimes. The asymmetric curves prove that other then the stress force from cell deformation, another force is exerted to the probe.

A $\alpha$ value and a $\beta$ value are given to each of the curves. $\alpha_s$ and $\beta_s$ are identified according to the endpoints in the stress damped regime, whereas $\alpha_i$ and $\beta_i$ are given to the induction damped regime.

The differences between the $\alpha$ and $\beta$ values reveal the mechanical system. The difference between two $\alpha$ values is responsible for the deforming force while the difference between two $\beta$ values is used to calculate the force from cell reaction.
As shown in figure 12, the amplitude of the stress damped regime decreases at a slower rate than in the induction damped regime. It was expected that the two amplitudes will correspond to each other. However, the data plot reveals that there is an adhesive force against the dragging force from the electromagnetic induction. This adhesive force delays the movement of the apparatus. Furthermore, one can conclude that when \(0<\beta<1\), a force against the dragging force is applied on the probe while when \(\beta>1\), a force in the same direction as the dragging force is exerted and advance the movement of the probe.

**D. APPLICATIONS**

The experimental method proposed in this project has a wide range of application. It enables us to observe subtle changes in biomechanical structure by looking into its mechanical system. The biological characteristics which were only visible under the scanning electron microscope (SEM) are represented by \(\alpha\) and \(\beta\) values which are measured by the electromagnetic oscillator. To explain how to apply this method into biological experiments, we conducted the following experiment:

**I. Mechanical hysteresis**

The compressing curve indicates the amount of deformation in the zebrafish embryo, and the releasing curve shows that the cell doesn’t maintain the same shape once the pressure is released.

The differences in the displacement of the compressing curve and the releasing curve presents a mechanical hysteresis in the deformation of the zebrafish embryo.

The hysteresis phenomenon comes from the viscoelasticity of the embryo. It suggests the energy lost in the motion.

![Fig.12. Direct comparisons of the endpoints in the stress damped, and induction damped regimes.](image)

![Fig.13. Mechanical hysteresis curve observed in the shaping epilayer stage](image)
II. Cell Stress

The same magnitude of force is exerted on the embryo throughout the course of development. The oscillation curves shows how embryos from different stages of development response to the applied stress.

The variation of $\alpha_s$ is shown in figure 14.

In single cell stage, the damping constant weakens reflecting that the cell structure becomes tender. Furthermore, the embryo mainly goes through cell division in the first two stages, single cell and multiple cell stage. Therefore, the damping constants in the two stages are quite similar.

The dramatic rise in the later stage, shaping epilayer stage, marks the beginning of cell migration. The newly divided cells from the previous stages start to move from the vegetal pole to the animal pole of the embryo. This migration process causes the nonuniform local mass distribution in the embryo. Furthermore, the structure of the embryo is comparatively harder in this period.

III. Self-protection

In nature, all organisms have their own way to protect themselves. Even just a single cell has its own protecting system. As mentioned before, the nonlinear temporal exponent reflects how the embryo reacts to an external force, either a drag force or a repulsive force. This force is used to protect the embryo from an external force applied on it.

In single cell stage, the temporal exponent gradually increases and reaches unity which...
demonstrates that the retarded drag forces are weakening.

Later on, the exponent continues increasing and become larger than one indicates that the reaction force is now repulsive.

The self-protection responses of the zebrafish embryo are observed within the three earlier stages. The data shows that the embryo will generate a resisting reaction to protect itself from external force.

IV. Effects of pH pollution

A living cell is very sensitive to environmental variation. Three hours after fertilization, the embryos are put into solutions with different pH values for two hours to simulate the effect of acid rain.

Figure 16 is the amplitude graph of the embryo submerged into solution of different acid level. The embryos in this project are kept in its natural environment with a pH value of 7.4. For the variation, PBS buffer is used to control the pH value.

From the figure, amplitudes of embryos submerged into solution ranging from pH7.4 (natural environment) to pH7.0 decreases at a similar rate. However, the oscillation curves alter when the pH value is smaller than 6.8.

Base on direct observation, the acid solution slows down the development of the embryo. Also the H\(^+\) and H\(_3\)O\(^+\) ions in the solution open up the porin on the cell membrane which allows ions to diffuse into the embryo.

Other interesting applications are: (a) effects from thermal pollution, (b) detecting the early mutant signal of the cells by looking into its physical properties, and (c) understanding the evolutionary proof of close species by looking into their embryo.

E. CONCLUSION

Cell elasticity and adhesion of zebrafish embryo have been measured using a home-made electromagnetic oscillator. Damping constant \(\alpha\), temporal exponent \(\beta\), and angular frequency \(\omega\) can be used to study the deforming force, adhesion, and elasticity of the cell.

The existence of cell adhesion is clearly demonstrated as the mechanical hysteresis curves appear in the damping routes.
Self-protection of zebrafish embryo is observed, as it generates resistive reactions to the external pressure throughout the development.

A slight change in the pH environment can significantly affect the embryo development.

F. REFERENCES


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