Internship Proposal

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Project Title:
Engineering a 3D Annulus Fibrosus model to investigate glucose-dependent ECM Remodeling

Level:

Master Student

Project Summary:

The annulus fibrosus (AF), which provides structural integrity to the intervertebral (IVD) through its highly organized collagen-rich matrix, undergoes substantial biochemical and structural changes during degeneration (1). In particular, changes in IVD microenvironment, including altered nutrient availability, may affect cellular metabolism and consequently influence ECM synthesis and remodeling (2).

Three-dimensional tissue-engineered models based on decellularized extracellular matrices provide an opportunity to investigate these processes in a biomimetic environment while maintaining key features of the native tissue (2,3). In this project, an optimized decellularized AF matrix (dAFM) will be used as a bioengineered platform to investigate how glucose availability influences AF cell function and ECM remodeling. Human AF cells will be incorporated into the 3D matrix and cultured under controlled glucose conditions. Cellular activity and ECM deposition will be monitored over time using complementary metabolic, biochemical and imaging-based approaches.

The project will establish a controlled 3D experimental platform for studying the relationship between the cellular microenvironment and matrix remodeling in the AF. Importantly, the generated model and optimized experimental conditions will provide the basis for future integration of advanced dynamic monitoring approaches, including bioluminescence techniques.

Work to be developed by the student:

Task 1: Establishment of the cellularized dAFM model

Human AF cells will be expanded and characterized using established laboratory procedures. Cells will subsequently be seeded onto the optimized dAFM using the laboratory's existing protocol.

Cell distribution, morphology, viability and metabolic activity will be assessed to determine the most reproducible culture conditions. Appropriate culture duration and sampling timepoints will be established for subsequent glucose experiments.

Task 2: Glucose-controlled 3D culture

Cellularized dAFMs will be cultured under defined glucose conditions, including a control condition, reduced and increased glucose conditions. Experiments will be performed over multiple timepoints to capture the temporal response of the cells and matrix. Cell viability and metabolic activity will be monitored, complemented where appropriate by analysis of glucose consumption and metabolic products in the culture medium.

Task 3: Analysis of ECM remodeling

ECM remodeling will be evaluated using complementary approaches, including: quantitative assessment of collagen/ECM deposition; immunostaining and fluorescence imaging of selected ECM components; biochemical quantification of collagen or related matrix components where technically feasible; image-based (e.g. bioluminescence) quantification of ECM deposition and spatial distribution; analysis of temporal changes in matrix production under different glucose conditions.

References:

[1]: Lyons, G., et al., Biochim Biophys Acta, 1981

[2]: Castro, AL., et al., Acta Biomat., 2025

[3]: Fiordialisi, MF., et al., Biomaterial Advances, 2022

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