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ENGINEERING

Identifying Extracellular Matrix Protein Turnover Rates for Tissue Engineers

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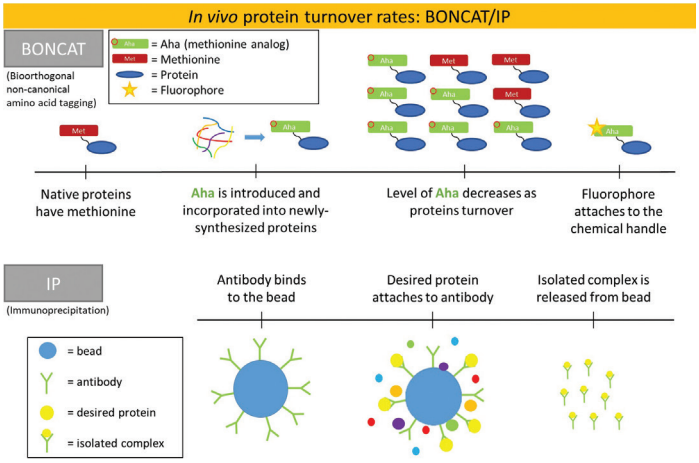
Tissue engineers create extracellular matrix (ECM) grafts to promote regeneration of damaged tissue. To recreate lost tissue, grafts should resemble the composition of developmental stages that support growth rather than adult tissue where it is designed to simply maintain homeostasis. Currently, there is a lack of knowledge of the influence of the ECM during development to help guide the design of grafts to fully restore the functionality of damaged tissue. It is necessary to identify developmental growth-factor turnover rates and the rate a molecule is synthesized and depleted to understand the influence of the ECM on tissue formation. However, this form of detailed molecular analysis has not been performed due to a lack of techniques available. The goal of this research is to establish a method that quantifies ECM and growth-factor turnover rates as a function of development.



A schematic detailing the protocol for the established BONCAT/IP method of identifying turnover rates of ECM proteins and associated proteins (i.e. growth factors). BONCAT tags newly synthesized proteins with a fluorophore while IP enriches for specific protein.

The method combines biorthogonal non-canonical amino acid tagging (BONCAT) and immunoprecipitation (IP). BONCAT labels and visualizes newly synthesized proteins using a methionine analog that contains a chemical handle, azidohomoalanine (Aha), and a fluorescent tag. Afterwards, IP uses antibody-antigen interactions to isolate a desired protein. We hypothesize that using these techniques in tandem at different developmental stages will determine the turnover rates of any protein of interest. Specifically, the intensity of fluorescence will decrease over time as the newly synthesized proteins turn over.

Proof-of-concept studies were done *in vitro*, where the BONCAT/IP method labeled and isolated newly-synthesized, fluorescently tagged actin from C2C12 mouse myoblast cells. The IP procedure was then adapted for the successful isolation of TGF-β1, a critical growth factor during development, from embryonic mice. Future studies will involve image analysis to quantify turnover based on the intensity of fluorescence. Furthermore, these established methods will be used to study turnover rates of different ECM proteins during development, shaping the future of ECM-based grafts.

Research advisor Sarah Calve writes: "Alita started working in the lab as a sophomore and I am excited for her to continue this project as a 5th-year master's student. Her project has the potential to identify the in vivo dynamics of protein turnover, which will provide important design parameters for regenerative therapies."

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