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Research Week 2024

An engineered model to elucidate molecular clutch mechanisms of mechanotransduction during bone nanoscale mineralization

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Keywords

Tissue Engineering, Bone Regeneration, Mineralization, Mechanotransduction, Collagen Hydrogel

Abstract

Introduction

Ossification is a developmental process where bone is created in appositional layers through the gradual calcification of the native extracellular matrix (ECM). This evolution is orchestrated by biological processes that allow for mesenchymal condensation of stem cells and their subsequent differentiation into bone-forming cells. While bone mechanobiology has been studied in skeletal development and fracture healing, the interplay between mechanics and biology during the transition of calcifying osteoid to mature bone remains poorly understood. Our objective is to replicate the critical changes in the ECM during bone formation to study the step-by-step process of bone regeneration *in vitro*, as current models are limited. Ultimately, we hope to advance the fundamental understanding of the mechanobiology behind bone formation, contributing to future research on regenerative therapies.

Methods

Here, we employ a materials engineering strategy to recreate the densification and mineralization of bone tissue in a controllable manner. Collagen hydrogels were fabricated in four ways: 1) soft collagen, 2) high density fibrillar collagen (HDFC), 3) partially mineralized HDFC and 4) fully mineralized HDFC. Bone-marrow derived human mesenchymal stem/stromal cells were seeded on the surface of the hydrogels.

Results and Conclusions

Immunofluorescent staining of YAP/RhoA and FAK/paxillin at 2 and 24 hours respectively indicated higher expression in dense and calcified samples evidencing high stiffness sensing, formation of adhesion complexes, and engagement of the molecular clutch under these conditions. Additionally, there was an increased nuclear expression of RUNX2 at 24 hours in dense and calcified samples, signifying a greater differentiation into bone under these conditions.

In summary, we successfully replicated the stepwise changes in the ECM that occur during bone formation, creating a bone-like environment to mimic the ossification process. The findings of this work contribute to understanding the molecular clutch of stem cells during the complex process of bone regeneration.