Synthesizing Stable Matrices from the HS-5 Bone Marrow Stromal Cell Line

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Background/Objective:

Our lab is focused on investigating the physical and biochemical mechanisms through which the bone marrow extracellular matrix (ECM) regulates musculoskeletal and hematopoietic homeostasis. Previously, we showed that primary cells from young and aging bone produce matrices which recapitulate aging-related remodeling of the bone marrow microenvironment. Among these changes is a significant depletion of CCN matricellular proteins. However, isolating the impact of CCNs from the variety of aging-associated changes, requires producing ECMs from immortalized bone marrow stromal cell lines, in which individual CCNs may be deleted without disturbing other matrix components. However, matrices produced by immortalized stromal lines are highly susceptible to damage by decellularization.

Methods:

A 9-day matrix production protocol was performed with HS-5 bone marrow stromal cells maintained on a variety of commercially-available substrates/surfaces. Briefly, the procedure consisted of ascorbic acid induction on day 5 and decellularization on day 8, followed by ECM collection and analysis/imaging with Coomassie Blue stain. Substrates evaluated included tissue culture plastic (TCP), Cell-Tak, Collagen, PDL, CellBind, Silane, Supra, UpCell; with and without fibronectin coating.

Results:

While HS-5 cells readily produced matrix on several substrates, plates coated with Collagen I, further supplemented with fibronectin, most consistently supported the preparation of a well-anchored matrix, which remained intact following decellularization.

Conclusion and Potential Impact:

Producing stable ECMs from HS-5 bone stromal cells will allow our lab to specifically modify and study proteins of interest, including CCN1 and CCN2, which have been suggested to play important roles related to the aging-associated remodeling of the bone marrow niche. When coated with fibronectin, UpCell (Corning) supported stable matrix anchoring, while also allowing for detachment from the culture surface, suggesting potential applications in manufacturing of orthobiologic implants.