Investigating the osteogenic potential of decellularized extracellular matrices derived from different tissues of origin

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Introduction: Natural extracellular matrix (ECM) materials contain an inherent microstructural and biochemical complexity that can modulate cell behaviors and tissue remodeling. This complexity is difficult to replicate in synthetic scaffolds, thus decelluarized ECM matrices are recognized as an attractive tool in regenerative medicine. Different types of tissue have different properties. Thus it has been shown that decellularized material generated from different types of tissue elicit unique cellular responses in vitro (1-2). Here we have fabricated decellularized ECM spot arrays from several tissues of origin and investigated their effect on osteogenesis.

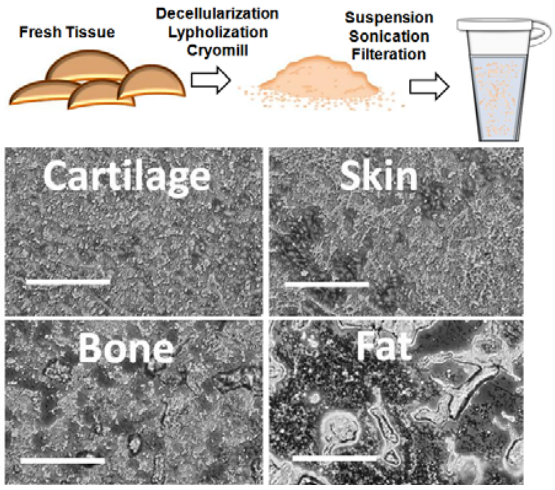

Fig. 1: *(Top): Schematic showing tissue processing. (Bottom): Light microscope pictures showing the morphology of different types of processed tissue particles. Scale bar = 100um*

Material/Methods: Porcine tissue was harvested and decellularized with (1) 3% peracetic acid (2) 1% Triton X-100, and (3) 200 U/ml DNAse. Decellularized tissue was washed thoroughly, lyophilized, particularized in a cyromill, suspended in water, sonicated, and balanced to the desired concentration (Fig 1). Tissue particles were spotted onto glass cover slips and transferred to a second glass coverslip coated in PEG. Human adipose-derived stem cells (hASCs) were isolated and characterized as previously described (3) and seeded on ECM spotted arrays in growth media. Cultures were switched to differentiation media at 1 day and cultured up to 2 weeks.

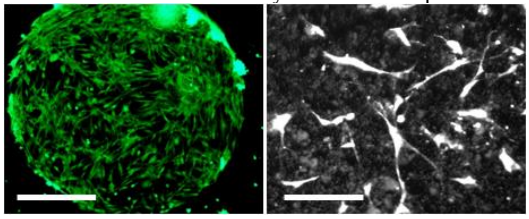

Fig. 2: *hASC cells cultured on bone ECM spots stained with calcien AM. Scale bar: Right = 1mm, Left = 100um*

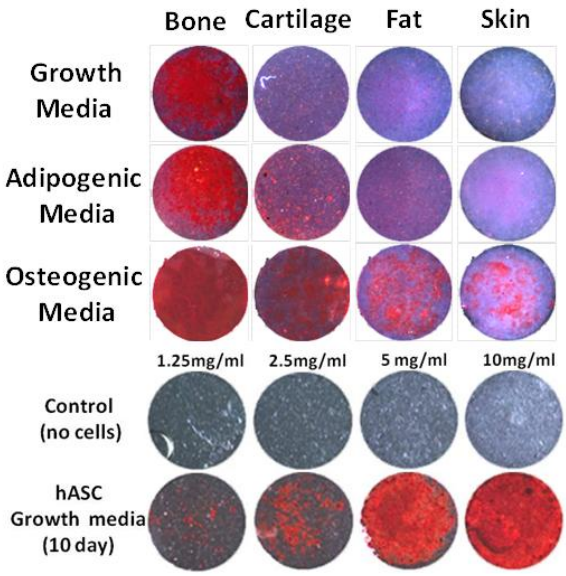

Fig. 3: *(Top) Alizarin red staining for calcified matrix deposition on various ECM spots after 2 week culture in growth or differentiation media. (Bottom): Alizarin red staining on bone ECM spots, from various concentration suspensions, cultured with hASC cells in growth media for 10 days.*

Results/Discussion: Positive staining with calcien AM confirms hASC cell viability and spreading on ECM spots (Fig 2). Cell attachment was confined to the ECM spots due to the PEG surface present in between ECM materials. Alizarin red staining for calcified matrix deposition demonstrates the ability of different types of ECM to stimulate or disrupt osteogenic differentiation of hASC cells. After two weeks in culture hASCs cultured on bone ECM showed strong bone differentiation in all media types, while hASCs cultured on fat and skin ECM showed limited staining even in osteogenic media (Fig 3). Bone ECM induced a dose response on osteogenic differentiation of hASCs with increasing alizarin red staining as mass of ECM per spot was increased (Fig 3).

Conclusions: Novel processing and fabrication techniques provided an effective platform for comparing the effects of several different ECM materials on the osteogenic differentiation of hASC cells. Results demonstrate the capacity of tissue specific ECM to variably promote or impede osteogenic differentiation of hASC cells and highlights the potential of ECM materials in regenerative medicine applications

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References:

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