Elastin Secretion from Stem Cells Seeded in Bio-scaffold vs. Synthetic Scaffold Under Dynamic Flow Culture

CV-PEUTICS Cardiovascular Therapeutics Lab

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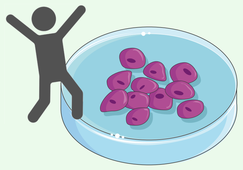
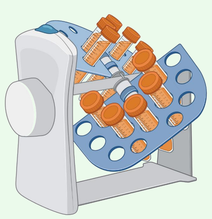
Cardiovascular Matrix Remodeling Laboratory

METHODS



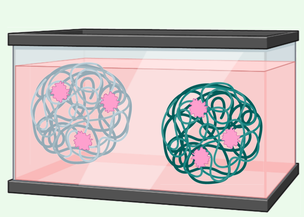
Human bone marrow stem cells (RoosterBio, Frederick, MD)

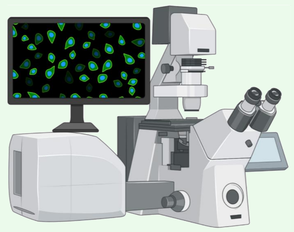
Seeded at 2 million cells per 2.5 cm^2 in 1.5 cm x 1 cm PSIS bio-scaffolds and PGA-PLLA synthetic scaffolds using Dulbecco's Modified Eagle Medium (DMEM) at 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (P/S), 82 µg/mL L-ascorbic acid 2-phosphate (AA2P), and 2 ng/mL basic fibroblast growth factor (bFGF)



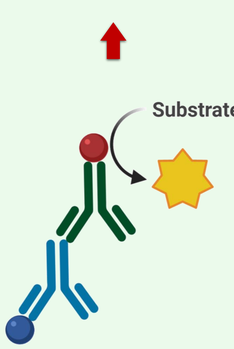
Seeded scaffold strips were placed in rotisserie culture for 8 days

The specimens were then conditioned in a bioreactor under a physiologically relevant oscillatory flow environment with shear stress of 3 dyne/cm^2 for an additional 14 days. Static culture of both scaffolds for 22 days were used as control group.



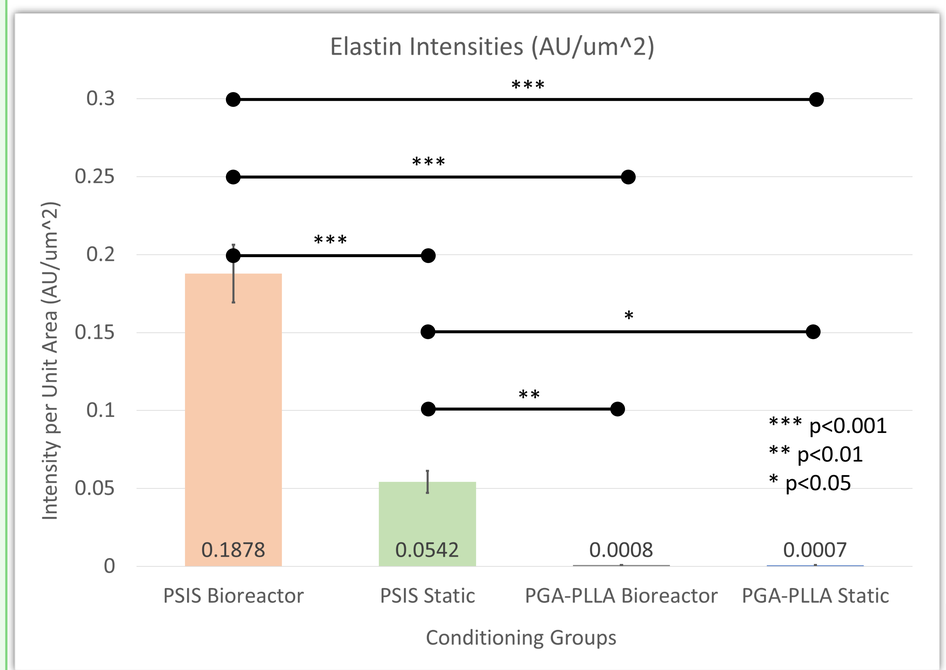


Stained samples were imaged using a confocal microscope and the images were quantified using heatmaps (MATLAB MathWorks, Natick, MA)



Samples were then fixed in 10% formalin at 4°C overnight, embedded in optimal cutting temperature (OCT) compound, and sectioned at 16 µm using a cryostat. Sectioned samples were subsequently stained with elastin mouse monoclonal primary antibody (Novus Biologicals, Littleton, CO), followed by goat anti-mouse polyclonal secondary antibody (Thermo Fisher, Waltham, MA) and 4',6-diamidino-2-phenylindole (DAPI).

Bio-scaffold facilitates **higher production of elastin from seeded stem cells** compared to synthetic scaffold, particularly under dynamic oscillatory flow conditions



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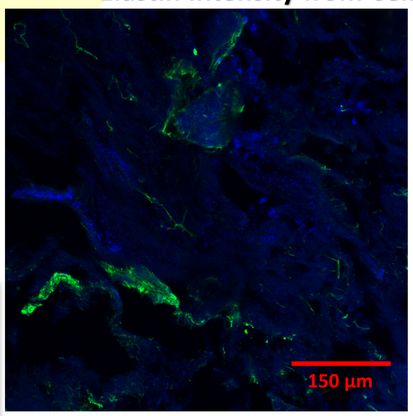
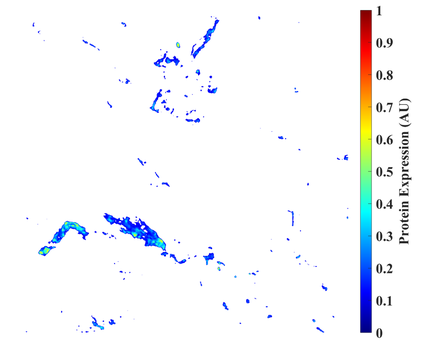
INTRODUCTION

Elastin is an important component of extracellular matrix in cardiovascular tissue regeneration. The objective of this study is to determine whether porcine small intestinal submucosa (PSIS) bio-scaffolds can better promote this tissue regeneration from bone marrow stem cells compared to polyglycolic acid poly L-lactic acid (PGA-PLLA) synthetic scaffolds under physiologically-relevant oscillatory flow environments.

RESULTS

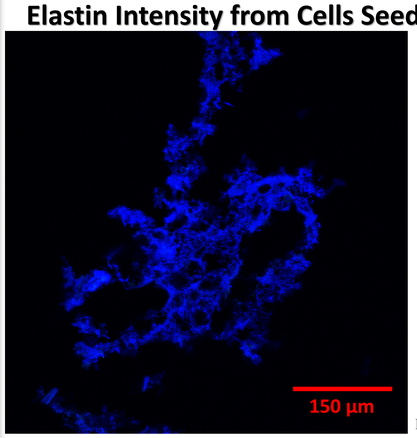
Elastin Intensity from Cells Seeded in PSIS Bio-scaffold

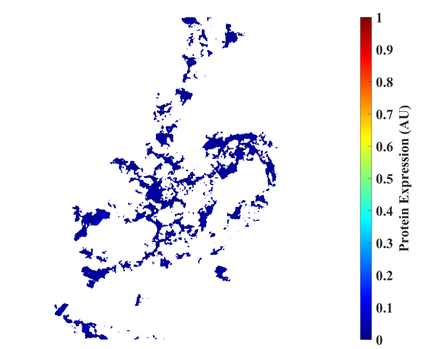




Elastin Intensity Per Unit Area: 0.2064 +/- 0.1051 AU/um^2

Elastin Intensity from Cells Seeded in PGA-PLLA Synthetic Scaffold





Elastin Intensity Per Unit Area: 0.0007 +/- 0.0003 AU / um^2

CONCLUSION & DISCUSSION

Stem cells seeded in PSIS bio-scaffolds facilitate higher production of elastin, particularly under oscillatory flow mechanical conditions compared to PGA-PLLA synthetic scaffolds. Bio-scaffolds extracellular components, with flow stimulation will allow bone marrow stem cells to communicate and secrete engineered matrix components, such as elastin that will be useful for enhancing cardiovascular regeneration.