**P164. Tissue Engineering Of Stentless Pulmonary Valve Using Mesenchymal Stem Cell-endothelial Progenitor Cell Co-culture On A Fibronectin-coated Poly-4-hydroxybutyrate biodegradable Scaffold**

Eva Maria Delmo Walter[1,2]; Virna Liza Sales[1]; David Martin[3]; Sirisha Emani[1]; Moritz Wyler von Ballmoos[1]; Grace Kim[4]; Roland Hetzer[2]; John Mayer[1]
*[1]Childrens Hospital Boston/Harvard Medical School, Boston, United States; [2]Deutsches Herzzentrum Berlin, Berlin, Germany; [3]Tepha Inc, Cambridge, United States; [4]Harvard University, Cambridge, United States*

**OBJECTIVES:** We evaluated the effects of in vitro culture conditions, type of stem cells, cell concentration in co-culture, and mechanical loading on the retention of mechanical properties of the microbial polymer poly-4-hydroxybutyrate (P4HB) scaffold.

**METHODS:** Four varying thicknesses (100u, 220u, 440u and 660u) of melt blown mesh P4HB were studied to determine the best construct for pulmonary leaflets and its sewing ring. Mesenchymal stem cells (MSC) and endothelial progenitor cells (EPC), alone and in varying combinations, were seeded onto fibronectin-coated P4HB scaffolds. A 30-day dynamic rotational seeding protocol was followed. All studies were performed in a simulated physiological environment at 37 C.

**RESULTS:** Histology and scanning election microscopy of fibronectin-coated P4HB scaffolds of 100 u and 220 u thickness showed enhanced tissue formation with co-culture of MSC (40%) and EPC (60%) and increased cellular density that deposited extracellular matrix proteins filling the pores and interconnecting fibers. Immunoflourescence studies showed that they expressed alpha-SMA, alpha tubulin, CD 29, CD 31 and VWF. Mechanical tests showed increased tensile strength over strain.

**CONCLUSIONS:** Sequentially seeded MSC-EPC on a 40:60 cell concentration were capable of producing enhanced tissue formation and the highest cellularity within the luminal surface and enhanced cellular ingrowth into the " interstitial" layer of the P4HB scaffold of 220µ and 100u thickness. Hence, P4HB of 200u thickness is used to create pulmonary valve leaflet while the 100µ film covered with 220u melt blown mesh P4HB thickness, is suitable for annular sewing ring of the stentless pulmonary valve, of which the in vivo study is on-going.