Trisomy 21 Alters Cardiomyocyte Response to Substrate Stiffness

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Introduction: Individuals with Down syndrome (DS), also known as trisomy 21, are 2000 times more likely to develop a congenital heart defect (CHD) than the typical population[1], where the septum between the upper and/or lower chambers fails to fully develop leaving a hole. Studies have shown that upregulation of two genes located on chromosome 21, type VI collagen (COLVI) and Down Syndrome Cell Adhesion Molecule (DSCAM) increase the risk of developing a septal defect. COLVI is a major component of endocardial cushions that give rise to cardiac septa[2] and overexpression of COLVI is known to directly increase production of hyaluronic acid[3], altering the composition and mechanics of the extracellular matrix. Therefore, I believe overexpression of COLVI and DSCAM stiffens the surrounding endocardial tissue, leading to altered mechanotransduction that predisposes the developing heart to a defect. **Methods**: Three pairs of patient-specific and age- and sex-matched DS and control induced pluripotent stem cells (iPSC) were differentiated into cardiomyocytes (iPSC-CM, characterization not shown) using a previously established small-molecule based monolayer protocol with minor changes[4]. Fetal-like in nature, iPSC-CM are the optimal tool for modelling heart development and response to mechanical stimulation. To study substrate stiffness-dependent responses of DS and control iPSC-CM, I cultured cells from both groups on norbornene-functionalized poly(ethylene glycol) (PEGNB; 8-arm, molecular weight 10 kg/mol (10kPEGNB), and molecular weight 5 kg/mol, (5kPEGNB)) photopolymerized hydrogels with tuned elastic moduli to vary resistance to cardiac cell contraction. The elastic moduli of PEGNB hydrogels were altered by varying weight percentage and molecular weight of PEGNB, and concentration of dithiothreitol (DTT, Sigma Aldrich) to recapitulate physiological stiffness of the fetal heart (1-5kPa), adult heart (10-15kPa) and adult pathological heart (>100kPa) in collaboration with Dr. Chelsea Magin's lab. Peptide sequences YIGSR and RGDS were covalently bound to the polymer chains to encourage cell attachment (Sigma Aldrich). Once seeded on hydrogels of each stiffness, cell proliferation will be measured using the Click-It™ EdU Cell Proliferation Kit (Thermo Fisher), changes in cell morphology will be observed using immunostaining with the cardiac troponin T antibody (cTnT, Thermo Fisher) and analyzed using ImageJ (NIH), and changes in mature cardiac gene expression will be analyzed using RT-qPCR (primers/assays from Thermo Fisher). **Results:** Data from PEGNB hydrogel preparation and parallel-plate rheological characterization showed my three formulations exhibited elastic moduli of 2.2 ± 0.9 kPa (fetal heart), 12.3 ± 1.3 kPa (adult heart), and 116.0 ± 4.4 kPa (adult pathological heart) ($n \geq 3$ replicates per group) (Fig. 1A). To encourage cell attachment to hydrogels and investigate the biochemical contributions to cardiomyocyte responses, binding ligands represented by the short peptide sequences YIGSR and RGDS were covalently attached to hydrogels to mimic integrin binding of type VI collagen ($\alpha_3\beta_1$), and fibronectin ($\alpha_5\beta_1$, a positive binding control), respectively. Photos of the hydrogels used for finalized, ongoing studies are seen in Fig. 1B-D. Control and DS iPSC-CM will be seeded onto PEGNB hydrogels to detect any changes in cardiac gene expression, cell morphology and proliferation (representative images with cardiac troponin T, cTnT, and proliferative marker, EdU Fig. 1E-G). In agreement with other studies, preliminary data from the Jacot lab found that control cell proliferation decreases on substrates of increasing stiffness (Fig. 1E-G), and studies using DS iPSC-CM are actively underway. Ultimately, these PEGNB hydrogels will serve as robust ECM mimics for measuring the altered cellular mechanotransductive response to substrate stiffness in Down syndrome.

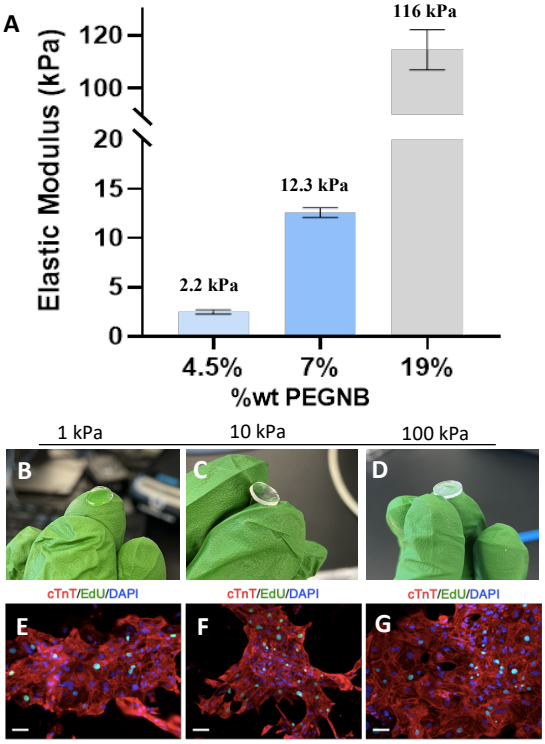

Fig. 1: Rheological Characterization and Application of PEGNB Hydrogels. (A) Elastic Moduli of PEGNB formulations (10kg/mol in blue, 5kg/mol in grey). (B-D) Images of each hydrogel. (E-G) Immunostaining of iPSC-CM plated on ~1kPa, ~10kPa, ~100kPa hydrogels, images courtesy of Jacot lab (cTnT: cardiac troponin T, red; EdU: proliferative marker, green).

References: (1) Freeman SB. Am J Med Genet. 1998;80:213-217. (2) Barlow GM. Genet in Med. 2001;3:91-101. (3) Karousou E. FEBS J. 2013;280:2418-2430. (4) Burridge PW. Nat Methods. 2014;11:885-860.