Temporal Regulation of GSK3/Wnt Signaling Molecules within a 3D Gelatin/chitosan Scaffold Promoting Cardiac Differentiation of Amniotic Fluid Derived Induced Pluripotent Stem Cells for Congenital Heart Defect Repair

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Statement of Purpose: Congenital heart defects are the most common type of birth defect and the leading cause of infant death. Current repair strategies involve surgical operations and the use of repair materials, such as autografts and homografts, which inevitability require repeat surgeries due to their inability to grow with the patient and a mechanical mismatch with native tissue. Congenital heart defects can be detected by prenatal ultrasound as early as the first trimester. The most severe defects, such as Tetralogy of Fallot and hypoplastic left heart syndrome, will require immediate surgical intervention at birth. This time between diagnosis and surgery can effectively be used to engineer functioning cardiac tissue. The goal of this study is to create an autologous, implantable cardiac patch that can promote the differentiation of induced human amniotic fluid derived stem cells. The patch consists of gelatin/chitosan based hydrogel, which contains small molecules inhibiting the GSK3 and Wnt signaling pathways at different time points promoting cardiac differentiation.

Methods: Cell source: By adapting previous cardiac differentiation of iPSC by Lian et. al. (2012), amniotic fluid derived induced pluripotent stem cells were first cultured in 2D to determine ideal seeding density and inhibitor concentrations for cardiac differentiation. Gelatin/chitosan scaffold: Cell viability in a 3D gelatin/chitosan was assessed by live/dead assay with neonatal rat ventricular myocytes (NRVMs). The scaffolds were fabricated from a 1:1 ratio of gelatin and chitosan and emulsified together through sonication. CHIR99021 and IWP2 were added to separate gelatin/chitosan solutions. Three layers of hydrogel were pipetted into Teflon molds, with the first and third layers containing CHIR99021 and the middle layer containing IWP2. The molds were left at room temperature for 2 hours to form a hydrogel, frozen on dry ice and lyophilized. The scaffold is then seeded with amniotic fluid derived induced pluripotent stem cells and cultured in suspension with RPMI + B27 media for 14 days. The cells were analyzed for different cardiac cell markers at days 1, 5, and 14.

Results: Cellular studies showed that induced amniotic fluid derived stem cells were capable of differentiating into beating cardiac cells. A seeding density of 3750 cells/mm^2 along with 12uM CHIR99021 and 5uM IWP2 at days 0 and 3, respectively, were determined to produce the greatest differentiation efficiency in 2D. Differentiated iPSC colony morphology and beating frequency are shown in Figure 1. Figure 2 shows cell viability within the center of a 3D gelatin/chitosan hydrogel. There was minimal cell death after 10 days in culture.

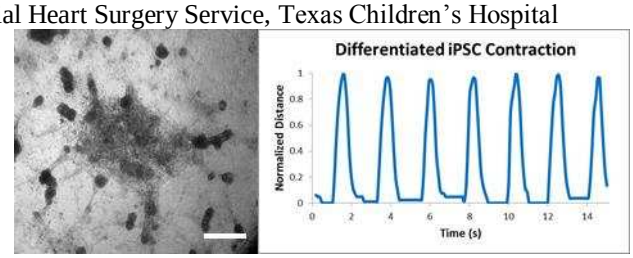

Figure 1: (Left) Differentiated iPSC colonies 13 days after the start of differentiation. Scale bar 150um. (Right) Contractions of colonies measured as relative distance over time.

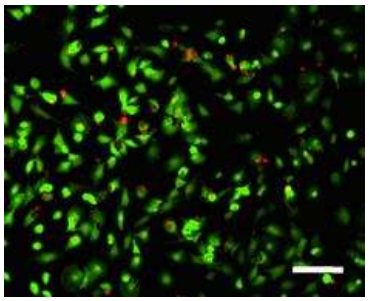

Figure 2: Live/dead assay of 3D gelatin/chitosan scaffold seeded with NRVMs 10 days in culture. Scale bar 50um.

Conclusions: The current study shows the potential for a completely autologous cardiac tissue patch for the treatment of congenital heart defects. Amniotic fluid derived stem cells can be readily isolated in utero at the time of diagnosis, then induced and differentiated into a beating cardiac lineage. This construct can be grown into functional, beating cardiac tissue which can be implanted directly into the patient at the time of surgical intervention. Continuing studies into small molecule release kinetics are planned. These studies will be conducted on a complete construct by high-performance liquid chromatography, incorporating CHIR99021 and IWP2 encapsulated in PLGA-mPEG nanoparticles. By encapsulating the small molecule inhibitors and combining them within the gelatin/chitosan scaffold, promotion to cardiac differentiation can be contained within this biomaterial construct. From these results, different nanoparticle co-polymer ratios can be investigated to achieve release kinetics promoting higher cardiac differentiation efficiency. Other future studies involve cell electrophysiology analysis through patch clamping, as well as action potential velocity and direction studies through the detection of calcium-sensitive dyes.

References: (Lian X. PNAS. 2010;109.27:E1848-E1857.)