Cellular and Angiogenesis Therapy for Congenital Heart Disease

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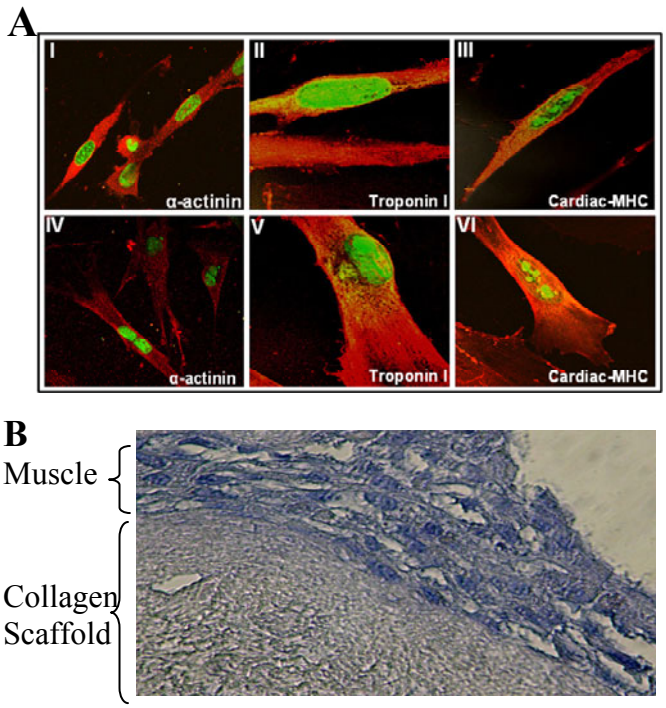
Statement of Purpose: Cell therapy has been proposed as a means to promote the regeneration of damaged heart muscle. Amniotic fluid derived stem cells (AFSC) present a novel source of multipotent stem cells derived from primitive fetal cells present in human and mouse. These cells are easy to culture and can be induced to differentiate *in vitro* into a wide variety of cell types, including all three embryonic germ layers. The aim of our study is to investigate whether multipotent stem cells isolated from amniotic fluid can be used for regenerative medicine applications. We characterized these cells for their stem-cell properties. We have induced these cells to differentiate into cardiomyocytes and have demonstrated the expression of cardiac specific marker as well as tissue formation in vitro. We hypothesize that AFSC can be used for cardiac regeneration.

Methods: Mouse amniotic fluid was collected from 11.5 days pregnant female C57BL/6 mice aging from 4 to 6 weeks. We immuno-isolated subpopulations based on expression of the stem cell markers. The isolated cells were induced into different cell lineages using specific differentiation conditions. We utilized treatment with 5-Aza-deoxycytidine (5-AzaC) and retinoic acid (RA) to induce differentiation of mAFS cells along the cardiomyocyte lineage. mAFS cell-derived cardiomyocytes were seeded on bioscaffolds and preconditioned in a bioreactor system with cyclic strain stimulation mimicking the wall motion of the native heart muscle. Functional assessment was carried out in organ baths. Cell survival and cardiac tissue formation were determined.

Results / Discussion: Multipotent stem cells were successfully isolated from mouse amniotic fluid and were positive for OCT4, Thy-1 and Alkaline Phosphatase, indicating their stem cell origin. The cell-type-specific inductions resulted in mAFS cells differentiating into muscle, endothelial, bone and fat. mAFS cell cultured in cardiomyocyte medium changed phenotype within 2 weeks. Immunohistochemical analysis revealed the expression of α-actinin, cardiac myosin heavy chain and cardiac troponin I. RT-PCR analysis showed expression of transcription factors important in ventricular cardiomyocyte development, such as GATA 4, myocyte enhancing factor (MEF) 2C and MEF 2D. AFS cell-derived cardiomyocytes preconditioned in the bioreactor showed muscle tissue organization and contraction.



A: Cardiomyogenic Differentiation. These cells stained positive for cardiac muscle markers, such as α-actinin, Troponin I and Cardiac MHC. **B**: α-actinin staining of bioengineered cardiac-like muscle after 8 days in bioreactor.

Conclusions: In this study we have shown that mAFS cells can serve as a source of multipotent stem cells. We demonstrated that mAFS cells can be differentiated into cardiomyogenic lineage cells and can be further induced to generate cardiac muscle-like tissue when exposed to cyclic strain. The cells may be further genetically modified to express angiogenic growth factors that would enhance cardiac tissue perfusion and regeneration. We believe that this represents a promising approach of cells for regenerative therapies in a variety of chronic and acute cardiac diseases with a deficiency in functional myocardium.