Does application of mechanical stress lead to a more mature phenotype of stem cell derived cardiomyocytes?


Pediatric Cardiology, University of Cologne, Cologne, Germany (1), Institute for Neurophysiology, University of Cologne, Cologne, Germany (2), Department of Molecular and Cellular Sports Medicine, German Sport University, Cologne, Germany (3)

Introduction: Pluripotent stem cells can be differentiated to cardiomyocytes (ES-CM) and are therefore an attractive cell source for cell replacement strategies. However, current knowledge on the mechanisms of cell integration and processes of physiological reconstitution as well as mechanical and electrical coupling after transplantation into the host tissue is still fragmentary. One major obstacle for a successful integration is the immaturity of the transplanted cells; ES-CMs have an immature phenotype compared to native cardiomyocytes of comparable age. There is cumulating evidence reporting beneficial effects on differentiation processes by inducing mechanical stress on myocytes. Aim of this study was therefore to investigate whether applied mechanical stress during differentiation will result in more mature phenotype of ES-CMs. Methods: ES-CM clusters were generated from transgenic ES cells expressing puromycin resistance and enhanced green fluorescent protein (GFP) cassettes under control of a cardiac-specific promoter a-myosin heavy chain. Confluent ES cells were trypsinized and resuspended in the differential medium and maintained on a shaker for 2 days initiating the differentiation to cardiomyocytes. Mechanical stress application was performed by a shockwave inducer (swiss dolorcast). Shock waves were applied at day 12, 14 and 16 of differentiation with a frequency of 2Hz at 1.2 bar for a duration of 1000 impulses. Cell survival was assessed by propium iodid staining. Size and percentage of cardiomyocytes (GFP+ cells) was measured by FACS analysis. Beating rate of ES-CM clusters were recorded before and after treatment. Results: Treatment of ES-CM clusters resulted in a higher yield of cardiomyocytes (90.5%+/-5.86, n=16) compared to untreated ES-CM clusters (79.48%+/-6.98, n=13). Treatment was not associated with a higher percentage of cell death. Beating rates did not differ between the groups. FACS analysis showed that the treatment groups consists of bigger cardiomyocytes with a denser granulation. Conclusion: Our results indicate that shockwave application results in a higher yield of cardiomyocytes and that the mechanical is not associated with a higher cell death. Graphical display of the FACS analysis suggests that mechanical stress leads to bigger cardiomyocytes with more pronounced granulations. This could indicate a more mature phenotype, nevertheless additional experiments are required to confirm a more pronounced maturity.