Mechanosensitivity of cardiomyocyte progenitor cells: the strain response in 2D and 3D environments

Results: A uniform monolayer developed using 25k-40kcells/well hiPSC-CMs in the presence of increasing densities of HEK293 cells. Contractility recordings from Cor.4U hiPSC-CMs showed that from day 3 onwards all cultures were spontaneously active. Higher densities of Ik1-expressing HEK293 (1:10) lead to an increase in interval time between beats of approximately 60% on day 9 (1972 ± 592 vs 1213 ± 114ms, n=8 $p<0.05$). Time for relaxation was also significantly prolonged in 1:10 and 1:30 compared with control on day 9, 283% and 128% (875 ± 265 and 522 ± 133ms vs 229 ± 26ms, respectively, n=8 $p<0.01$), respectively. Earlier and later culture times showed no significant difference in spontaneous contractile activity up day 12. In contrast, Pluricyte hiPSC-CMs were initially quiescent, becoming spontaneous at approximately day 4. Co-culture ratios of 1:10 and 1:30 did not show any spontaneous activity up to day 11.
Conclusions: Co-culturing with Ik1-expressing HEK293 may provide a method of adding Ik1 conductance to a network of hiPSC-CMs but different sources of hiPSC-CMs respond differently. With Cor.4U cells higher densities of HEK293, such as 1:10, lead to a slowing of the spontaneous rate and slowing of relaxation time suggesting effects on the electrophysiology of the co-culture. Pluricyte cells responded differently suggesting a higher sensitivity to co-culture with Ik1 expressing HEK293 cells.

519

Cell therapy of the heart studied using adult myocardial slices in vitro

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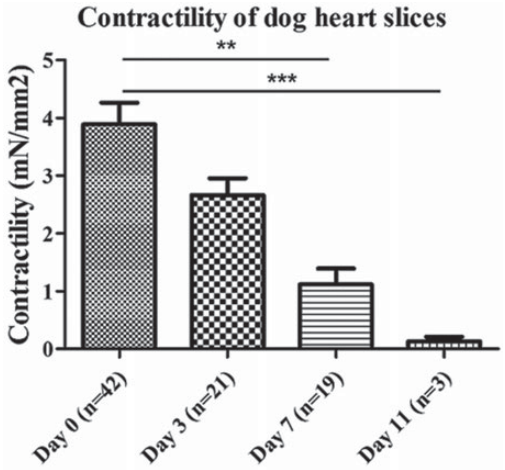
Introduction: Cardiac cell therapy is the introduction of stem cells in the heart to repair/replace damaged myocardium. In vivo studies have revealed that this therapy can induce arrhythmias, and the efficacy in improving myocardial function appears limited. A better understanding of the mechanisms involved during cell therapy is required but a suitable representative in vitro model is lacking. Organotypic heart slices are multicellular preparations with preserved structural, biochemical and electrophysiological properties.
Purpose: Here we use heart slices to study the mechanisms of functional integration, proliferation and direct/indirect effects on recipient myocardium of transplanted cells.
Methods: In this study human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were cultured in vitro on 300μm thick vibratome-cut slices prepared from adult dog left ventricular tissue. Viability and functionality were assessed by force measurement, histology and immunohistochemistry. Calcium transients were recorded by optical mapping.
Results: iPSC-CMs attached to the slices, within 24 hours formed electrical connection with the other grafted cells and beat spontaneously. Their beating activity however could not trigger the activation of the recipient tissue. Some cells, after 3 days in culture, could be paced with field stimulation at 1Hz and contracted synchronously with the slice. When point stimulation was applied on a distant region of the slice, while the slice contracted, the signal did not propagate to the iPSC-CMs, suggesting a lack of coupling with the recipient tissue. After 9 days in culture some iPSC-CM started to integrate and aligned with the slices myocytes, but others did not and spread into a separate layer as with 2D culture. At this time point however, myocardial slices showed a significant degree of functional deterioration. Slice contractility decreased to 23% by day 6 and this was due to myocytes dedifferentiation and cell death.
Conclusions: Vibratome-cut slices are a viable platform to study cell therapy, particularly in the first few hours. Culture conditions need to be improved to better preserve myocardial slice structure and functionality for long term studies.



Contractility of dog slices

520

Enhancement of the paracrine potential of human adipose derived stem cells when cultured as spheroid bodies

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Background: Ischemic heart disease remains a leading cause of mortality and morbidity worldwide. Cardiac cell therapy (CCT) is a promising therapeutic strategy to help in cardiac repair. Multiple cells have been proposed as candidates in CCT. Adipose tissue constitutes an important and accessible reservoir for the stem cells. Both preclinical and clinical data have shown that adipose derived stem cells (ASCs) could improve cardiac function and volumes, mostly through a paracrine mechanism.
Study Aim: The objective of our in vitro study is to characterize and compare the secretion profile as well as the survival of ASCs, when cultured under standard conditions (i.e. as a monolayer (ML)) versus in a three-dimension (3-D) structure (i.e. as a spheroid body (SB)). In vivo, the aim is to compare the anti-inflammatory potential of these two cell structures in peritonitis in a rat model.
Methods: Human ASCs (hASCs) were expanded in standard culture conditions in a monolayer form. ASCs were characterized according to both surface markers expression (assessed by immunofluorescence) and their ability to maintain multilineage differentiation. Alternatively, ASCs were also cultured as 3-D structure as spheroid bodies (SBs), by using the hanging drop technique. Luminex and ELISA assays were conducted to quantify key immunomodulators and angiogenic mediators to compare the two study groups. Western blots were used to study proteins involved in apoptosis.
Results: hASCs expressed similar surface markers as those documented for bone marrow MSCs including CD44, CD105 and CD90. Their ability to differentiate into adipogenic, chondrogenic and osteogenic lineage were unaltered. Paracrine activity of hASCs was enhanced when cultured as hASC-SBs. SBs secreted higher levels of immunomodulatory cytokines such as MCP-1, IL-6, IL-8 and IL-10, in a time dependent manner. Similarly, they exhibited greater pro-angiogenic potential as VEGF levels were increased also compared to hASC-MLs. Activation of caspase 3, shown by its cleaved form, was described in MLs under basic culture conditions and in response to TNFa stimulation, whereas cleaved caspase 3 was undetected in SB structures. Also, inflammation was reduced in both hASC-ML and hASC-SB treated groups of rats with induced peritonitis compared to the untreated group, with a slight efficacy of SBs over MLs.
Conclusion: hASC represent a promising cell source for stem cell therapy. Their paracrine, therapeutic potential can be optimized. Our findings clearly showed that hASCs cultured as 3D structures (i.e. as SBs) exhibit an improvement in both anti-inflammatory and angiogenic properties associated with resistance to apoptosis. Spheroid body formation thus represents an effective alternative to enhance the therapeutic potential of hASCs.

521

Mechanosensitivity of cardiomyocyte progenitor cells: the strain response in 2D and 3D environments

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Purpose: Cardiomyocytes progenitor cells (CMPCs) are a candidate cell source for cardiac regenerative therapy. To assess their full potential for cardiac regeneration, it is essential to know if and how CMPCs sense and respond to the three-dimensional (3D) environment and mechanical stimuli provided by the beating heart. Therefore, we study the response to cyclic strain of undifferentiated and predifferentiated human CMPCs in a 2D environment, as well as how CMPCs respond to unidirectionally constrained versus stress-free (unconstrained) 3D environments. The latter responses were studied using a hydrogel system that allows for interaction of the cells with a simulated 'host' tissue.
Methods: To test mechanosensitivity of CMPCs in 2D and 3D environments, the response of L9TB CMPCs to uniaxial (cyclic) strain (10% with 0.5 Hz) was investigated. To represent the 3D environment, undifferentiated CMPCs were cultured in unidirectionally constrained and stress-free collagen/Matrigel hydrogels, where the constrainment provides a static strain to the cells. The cellular mechanoresponse to the applied (cyclic) strain was quantified by cellular re-orientation away from the strain direction (strain avoidance). Next to cellular re-orientation, the effect of strain on cell differentiation was analyzed.
Results: We observe that while undifferentiated cells maintain their original orientation, upon early cardiomyogenic differentiation (predifferentiated) CMPCs exhibit a distinct strain avoidance response during 48hrs of cyclic straining in a 2D environment. In 3D unidirectionally constrained hydrogels, undifferentiated CMPCs retain their cardiomyogenic stem cell profile. CMPCs cultured in 3D collagen/Matrigel hydrogels respond to static mechanical strains as expected by cell alignment.
Conclusions: Our results suggest that CMPCs respond to the presence of mechanical stimuli, in this research mimicked by the application of uniaxial (cyclic) strain in 2D and 3D environments, suggesting that CMPCs are indeed mechanosensitive. Although in 2D environments, mechanosensitivity of the CMPCs is dependent on their differentiation status. Our findings provide the first understanding of the ability of human CMPCs to sense mechanical stimuli, which is the first initial step in mechanotransduction. Mechanotransduction is essential for optimal recruitment, migration, and mechanical integration of progenitor cells into the injured myocardium. Therefore, the presented results can contribute to enhance efficacy of current treatments of cardiac disease, as well as to develop novel endogenous regeneration strategies.

522

The effect of the vascular-like network on the maturation of the human induced pluripotent stem cell derived cardiomyocytes.

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Introduction: Stem cells and specifically induced pluripotent stem cell derived cardiomyocytes (iPS-CM) provide unique alternative to model human cardiomyocyte differentiation, the function of the cardiac cells and the pathology of severe cardiac diseases. However, the differentiated iPS-CMs are classified to resemble embryonal or fetal like cardiomyocytes due to their gene expression pattern, size, shape and mononuclear nature. Moreover, the lack of t-tubule network has been suggested to be a reason for the slow excitation-contraction coupling and calcium handling. The culture platforms that orientate the cardiac cells could have a positive effect on the overall maturation state of the differentiated cardiomyocytes, e.g. patterned biomaterials could induce the orientation and furthermore the more mature phenotype of iPS-CMs.
Methods: We have utilized natural topography provided by the network formed by endothelial cells and fibroblasts in the maturation of iPS-CMs. The iPS-CMs have been differentiated and the beating differentiated cells have been plated to the aforementioned platform and the maturation state of the