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

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Results: A uniform monolayer developed using 25k-40k cells/well iPSC-CMs in the presence of increasing densities of HEK293 cells. Contractility recordings from Cor.4U iPSC-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 ± 135ms vs 292 ± 26ms, respectively, n=8 vs 0.01), respectively. Earlier and later culture times showed no significant difference in spontaneous contractile activity up to day 12. In contrast, Pluricyte HEK293s 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 iPSC-CMs but different sources of iPSC-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.

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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 efficiency of improving myocardial function appears labile under standard in vitro conditions. Slices involved during cell therapy is required but a suitable representative in vitro model is lacking. Organotypic heart slices are multisecular 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 3D culture. At this time point the myocardial slices showed a significant degree of functional deterioration. Slic contractility decreased to 13% by day 6 and this was due to myocytes desynchronization 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.

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Enhancement of the paracrine potential of human adipose derived stem cells when cultured as spheroids
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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 source 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.

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Abstracts

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-dimensional (3-D) structure (i.e. as a spherical body (SB)). In vivo, the aim is to compare the anti-inflammatory potential of these two cell structures in periortis 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 spherical 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 CD105, CD44 and CD90. ASCs 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: 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 3D culture. At this time point the myocardial slices showed a significant degree of functional deterioration. Slic contractility decreased to 13% by day 6 and this was due to myocytes desynchronization 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.