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STRUCTURAL MATURITY OF INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES DEPENDS ON GROWING ENVIRONMENT

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Cardiovascular disease is the leading cause of death in US. In recent years, induced pluripotent stem cell (iPSC) derived cardiomyocytes (CM) have emerged as a useful model to study cardiovascular disease. However, its use is limited by their inability to develop a mature phenotype. It is known that the iPSC to CM differentiation process is influenced by the cell environment. CMs can be grown in a 2D environment (using a standard tissue culture dish) or in a 3D environment using an extracellular matrix (ECM) obtained by slicing a decellularized porcine heart. We propose that the presence of a collagen 3D structure could be important to improve the iPSC-CM structural maturity, since it provides an environment that mimics natural environment of cell development.

To test our hypothesis, we generated human iPSC from blood samples and differentiated them into cardiomyocytes in either a 3D ECM or 2D monolayer. In some 2D monolayer samples, we added hydrogel (collagen solution) to the well to separate the structural effect from the presence of collagen. Once the samples were beating, we fixed them in 4% paraformaldehyde (PFA) and performed a standard immunostaining protocol using antibodies against cardiac troponin T (a cardiomyocyte marker) and vimentin (a marker for fibroblast). The samples were scanned using either a Leica or Zeiss Airyscan Confocal Microscope and processed using Fiji and Imaris Software.

We found that the iPSC-CM differentiated in the 3D ECM formed longitudinal fibers adjacent to strands of fibroblast. In contrast, when the cells were grown in 2D monolayer, with or without the presence of hydrogel, they formed cell clusters with no apparent orientation.

We concluded that iPSC-CMs differentiated in a 3D environment (ECM) show a more mature structural development than those differentiated in a standard tissue culture plate (2D), and that the improved maturation cannot be explain by the presence of collagen alone. Future studies involve using a combined approach to adding hydrogel to the ECM structure before seeding the cells.