Co-culture of hiPSC-CMs and ECs to mimic cardiac tissue

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INTRODUCTION

Human-induced pluripotent stem cells (hiPSCs) have revolutionized the world of basic and translational research. In particular, hiPSCs can be differentiated into cardiomyocytes (hiPSC-CMs), emerging as a reference system for cardiac studies due to their biological similarity to primary CMs (Gisone et al., 2022). However, compared to adult ventricular CMs, the main limitation of hiPSC-CMs is their phenotypic immaturity (Denning et al., 2016). To overcome this limitation and to reproduce the *in vivo* heart microenvironment better, researchers proposed the co-culture of hiPSC-CMs with other cardiac cells, such as endothelial cells (ECs) and fibroblasts (Giacomelli et al., 2020; Campostrini et al., 2021). Besides the influence of cell composition, it is also established that three-dimensional (3D) *in vitro* cell cultures can better mimic the tissue environment of cells in the organism, preserving cells' morphology, phenotype, and polarity, thus allowing for a better study of cells' physiological function and, in turn, obtaining a reliable tissue response (Gisone et al., 2022). This work presents the results of the characterization of hiPSC-CMs + Human Coronary Artery Endothelial Cells (HCAECs) co-culture in a 2D and 3D environment as a cardiac tissue replica for pathophysiological and/or toxicological studies.

EXPERIMENTAL

hiPSCs were cultured in Essential Flex basal medium. The differentiation of the hiPSCs into hiPSC-CMs, after 12 days of culture, was confirmed through immunofluorescence analysis (TNNT2 and NKX2.5 as cardiac cell markers) and Real-Time PCR of the TNNT2 cardiac gene. HCAECs were cultured in Endothelial Cell Basal medium-2 (EBM2). According to the literature on the heart cellular composition (Bai et al., 2018; Giacomelli et al., 2020; Campostrini et al., 2021), two different concentration ratios of cells were tested for co-cultures: 1) 90% hiPSC-CMs + 10% HCAECs; 2) 80% hiPSC-CMs + 20% HCAECs. In addition, 2 different culture media were tested: a) RPMI-1640 supplemented with 2% B27™ Plus Supplement; b) EBM2 + Vascular Endothelial Growth Factor human (VEGF). Cells we co-cultured for two weeks, and the viability (CellTiter-Blue® assay) and the cardiac genes' expression (RT-PCR of TNNT2, CX43, BNP, MYL2, NKX2.5, ACTN2) were evaluated. About the 3D microenvironment, cells were homogeneously dispersed into a Gelatin-Methacryloyl (GelMA, 100% degree of methacryloylation) hydrogel (added with catalytic amount of Lithium phenyl-2,4,6-trimethyl-benzoyl phosphinate as photoinitiator) and then seeded into the wells. Irradiation with UV light (40s, 365 nm, 10 mW/cm^2) was used to polymerize the gels. Cells were co-cultured for two weeks: the CellTiter-Blue® assay was performed to evaluate the viability of the cells.

RESULTS AND DISCUSSION

The immunofluorescence assay confirmed the efficiency of the differentiation protocol of hiPSCs in beating CMs. In addition, the analysis of the expression of the cardiac gene TNNT2 in both cell populations confirmed its presence only in CMs.
In 2D cell co-culture, the CellTiter-Blue® assay did not evidence a significant difference in the viability between the two cell ratios and media, with a trend of higher viability values in EBM2. The Real-Time PCR revealed a significantly higher expression of cardiac genes CX43, ACTN2, and BNP in the co-culture, particularly in the 80/20 ratio than in the hiPSC-CMs monoculture in the different microenvironments. The viability performed on the 3D co-culture models showed no significant differences.

CONCLUSION

The integration of endothelial cells, media culture supporting multi-cellular systems, and microenvironment for the two cell types provide evidence of the maturation process of hiPSC-CMs in terms of cardiac-specific markers (ACTN2, BNP) and intercellular communication (Cx43).

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