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Investigating the Mechanisms of Mitochondrial Transplant in Cardiac-on-a-Chip Models Spatiotemporal activation mapping and detection of conduction blocks in cardiac organoids using 3D shell microelectrode arrays (MEAs)

Acute myocardial infarction impacts 2.30% of adults over 20 years old in Canada. Restoring metabolic activity of injured and impaired organs via mitochondrial transplant is a potentially revolutionary approach to the treatment of human diseases. Mitochondrial transplant is being explored in ischemic-reperfusion injury (IRI) with ongoing trials in pediatric and adult patients. However, the unknown mechanism of action limits its clinical translation. Therefore, the objective of my research is to identify key cellular pathways involved in the therapeutic efficacy of mitochondrial transplant. I performed mitochondrial transplant of 3.0x106 DsRed2-tagged mitochondria in cardiac tissues composed of induced pluripotent stem cell-derived cardiomyocytes, endothelial cells, and stromal cells. Cardiac tissues were generated in a 3D fibrin hydrogel and allowed to mature for 1-2 weeks prior to mitochondrial transplant. IRI was modeled by exposing the cardiac tissues to 6hrs of ischemia in a hypoxic chamber, followed by 3hrs of reperfusion in normoxia. Mitochondrial transplants were performed at the start of the reperfusion period. Following transplant, tissues were evaluated for mitochondrial integration and oxygen consumption rate using immunofluorescent microscopy, flow cytometry, and Seahorse. We confirmed that DsRed2-tagged mitochondria were integrated in cardiac tissues with 27.4% DsRed2-positive cells at 24hrs post-mitochondrial transplant. Fluorescent microscopy revealed that modelled IRI increased the retention of DsRed2-tagged mitochondria in the cardiac tissue, which were visible in IRI tissues at 7 days post-mitochondrial transplant but not in control tissues. Furthermore, oxygen consumption rate significantly increased following mitochondrial transplant in control cardiac tissues that were matured for 2 weeks prior to transplant (FDR-adjusted p=0.028), while no increase was observed in tissues matured for 1 week (FDR-adjusted p=0.797). This effect was specific to the mitochondrial transplant as there was no change in oxygen consumption rate between controls tissues that were matured 1 or 2 weeks (FDR-adjusted p=0.5). Further experiments are needed to understand the effects of IRI on tissues that have been matured for longer durations, as well as the effect of metabolic maturation of tissues in more accurately representing in vivo cardiac tissue. Together, these

preliminary results from this study suggest that tissue injury enhances the uptake of donor mitochondria, potentially through endogenous tissue repair pathways. Understanding the mechanisms and pathways being altered by mitochondrial transplant is crucial for its clinical application