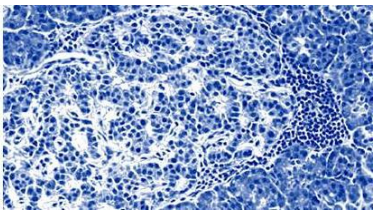
nPOD
Network for Pancreatic Organ Donors with Diabetes



Developing an assay platform to evaluate reprogramming of beta-like cells in gastrointestinal tissues from non-diabetic and type 1 diabetic donors

AUTHORS

Carmen Y. Lam[1], Irina Kusmartseva[2], Atkinson Mark[2], Hua V. Lin[1]
[1]Forkhead BioTherapeutics, [2]nPOD

PURPOSE

Gut organoids (gutoids) are 3-dimensional in vitro structures that have substantial anatomical and functional similarities to the human gut. It was previously shown that FOXO1 inhibition in human gutoids derived from induced pluripotent stem cells (iPSCs) promotes generation of glucose-responsive insulin-positive (beta-like) cells. However, the iPSC differentiation protocol is extremely time-consuming and therefore unsuitable for drug discovery purposes. We propose to use gutoids derived from primary gut tissues collected from deceased non-diabetic controls and Type 1 diabetic subjects to establish an assay platform to study the reprogramming of gut endocrine progenitor cells into beta-like cells. While human gutoids are routinely cultured from intestinal crypts isolated from fresh gut biopsies that are processed within 24 hours, a protocol for culturing gutoids from deceased donors for more than 24 hours is not established.

METHODS

The time from x-clamp to tissue processing and crypt isolation, shipping media, and organoid culturing media were explored to obtain the optimum condition for developing human gutoid cultures.

SUMMARY OF RESULTS

We successfully derived and maintained enteroids and colonoids from the small intestine and colon tissues from 3 out of 4 deceased donors withdrawn from life support for as long as 48 hours. Cultured organoids were subsequently used for studying enteroendocrine cell differentiation, gene expression, and drug screening.

CONCLUSIONS

Gutoids from deceased donors' gut tissues have been established as a reproducible and readily available platform and is an invaluable translational tool that bridges the gap between conventional two-dimensional cell line culture and in vivo models.