MODELLING PANCREATIC CANCER THROUGH PANCREATIC EXOCRINE ORGANOIDS USING PancreaCult™ SERUM-FREE MEDIUM

Charis Segeritz-Walko[1], Yvonne Luu[1], Riya Sharma[1], John Stingl[1], Michael J. Riedel[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Sharon A. Louis[1]

[1]STEMCELL Technologies Inc., Vancouver, BC, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

Introduction

Despite the prevalence and urgency of pancreatic cancer research throughout the previous decades, a method for the long-term in vitro maintenance of pancreatic exocrine tissue as 3-dimensional (3D) organoids has been described only recently[1]. Pancreatic exocrine organoids are composed of a polarised monolayer epithelium that retains many of the features of in vivo exocrine pancreatic tissue, and thus can serve as a physiological model system to address diverse research questions related to pancreatic development, stem cell biology, secretory function, and disease modelling. We have developed PancreaCult™ Organoid Growth Medium (Mouse), a defined cell culture medium for the initiation and long-term maintenance of pancreatic exocrine organoids. To establish these cultures, resected mouse pancreatic tissue was enzymatically dissociated to liberate ductal fragments that contain the putative stem cell niche. When embedded into Corning® Matrigel® domes and cultured in PancreaCult™, these ductal fragments formed spherical organoids within 1 week (n = 59 mice). Established organoids were passaged weekly at an average split ratio of 1:25 and maintained in continuous culture for > 1 year. Expanded organoids were also cryopreserved using CryoStor® CS10 for long-term storage. Pancreatic exocrine organoids cultured in PancreaCult™ are composed of cells expressing genes specific for pancreatic stem cells (Lgr5), progenitor cells (Pdx1, Sox9), and ductal cells (Car2, Muc1, Krt19, Cftr). Additionally, we observed that primary and metastatic tumor cells isolated from Kras$^{+/LSL\text{-}G12D}$; Trp53$^{+/LSL\text{-}R172H}$; Pdx-Cre mice and cultured in PancreaCult™ generate tumour organoids that recapitulate the features of the original tumour, thus providing a model system to study ductal pancreatic carcinoma[2]. Due to the robust growth of pancreatic exocrine organoids in PancreaCult™ and their close resemblance to the in vivo pancreatic epithelium, this organoid technology can complement or replace other experimental methodologies for studying the exocrine pancreas and could reduce or even eliminate the need for animal experimentation.

Protocol

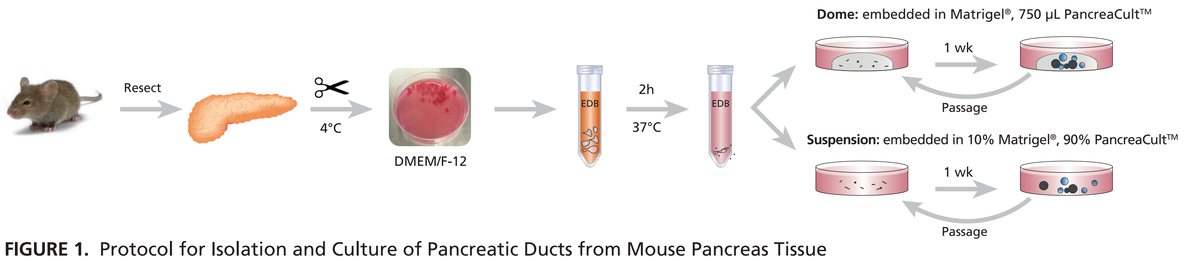


FIGURE 1. Protocol for Isolation and Culture of Pancreatic Ducts from Mouse Pancreas Tissue
Mouse pancreas tissue was resected, minced and digested with enzymatic digestion buffer (EDB) for 2 hours at 37°C. Digested pancreatic ducts were pelleted and embedded in Matrigel® by either plating 30 µL domes at the centre of a pre-warmed 24-well plate or mixing pelleted ducts with 10% Matrigel and cooled 90% PancreaCult™. The domes were solidified at 37°C for 10 minutes and subsequently flooded with 750 µL of PancreaCult™, while cooled suspension cultures were gradually warmed to 37°C on an orbital shaker at 80 rpm. Once pancreatic organoid cultures have been established from primary mouse tissue after 5 - 7 days of culture in PancreaCult™, organoids can be passaged by mechanical trituration into organoid fragments and plated in desired densities using an average split ratio of 1:16 or fragment counts.

Results

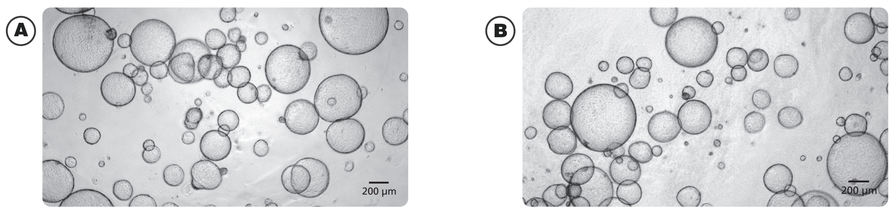


FIGURE 2. Organoids Grown in PancreaCult™ Organoid Growth Medium (Mouse)
Pancreatic exocrine organoids are observed within one week when cultured in **(A)** Corning® Matrigel® domes or **(B)** suspension with dilute Matrigel®. Organoids were imaged during passage 2, on day 4.

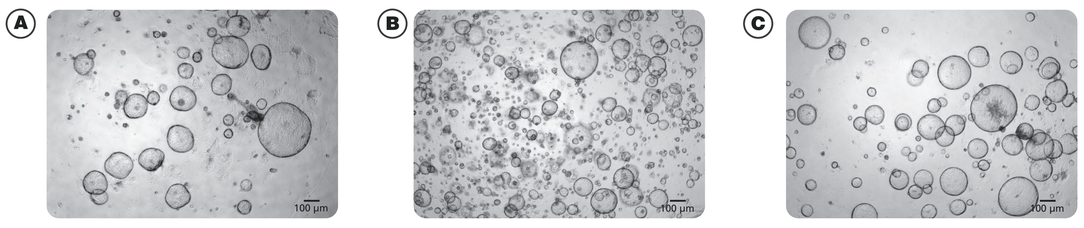


FIGURE 3. Mouse Pancreatic Organoids can be Initiated from a Variety of Starting Materials
PancreaCult™ Organoid Growth Medium (Mouse) enables the initiation of pancreatic exocrine organoids from **(A)** duct fragments, **(B)** single cells and **(C)** cryopreserved organoid fragments. All organoids were grown in Matrigel® domes. Organoids were imaged on day 4 or day 5 of primary culture (duct fragments and single cells, respectively) or day 3 of the first passage post-thaw (cryopreserved organoids).

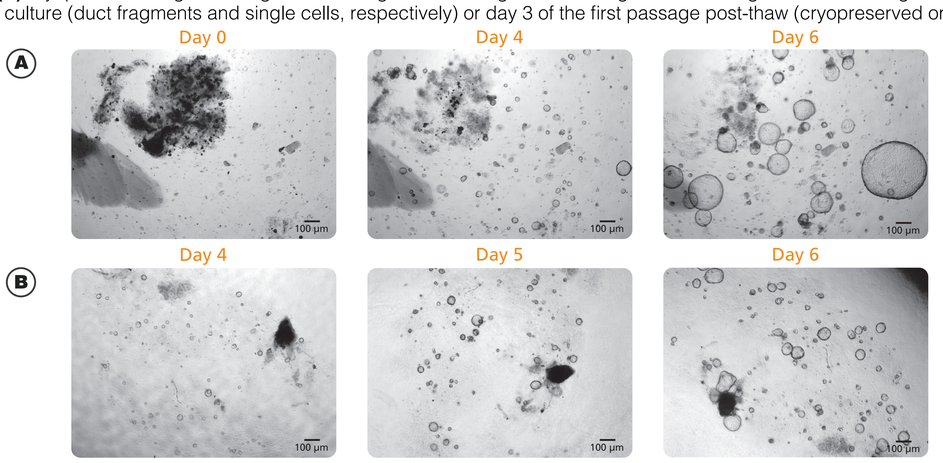
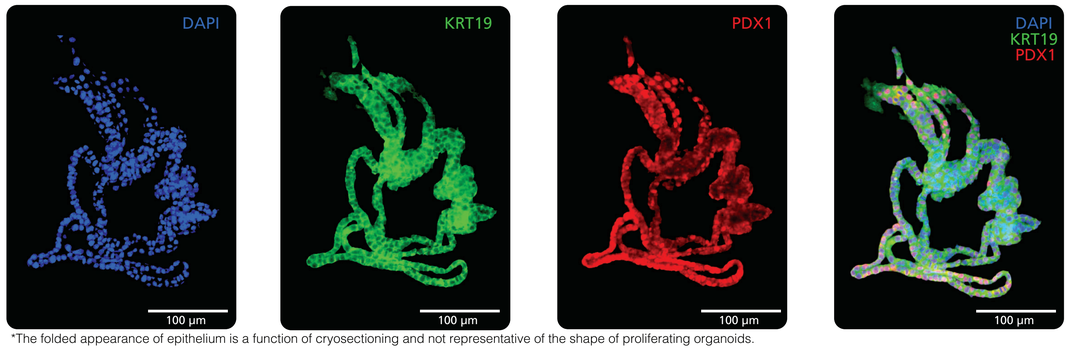


FIGURE 4. Pancreatic Organoids can be Grown in Matrigel® Domes or in Suspension with Dilute Matrigel®
Organoids cultured using PancreaCult™ Organoid Growth Medium (Mouse) from freshly isolated pancreatic tissue fragments and plated in **(A)** Matrigel® domes or **(B)** as a dilute suspension with dilute Matrigel®. Organoids grown in either culture condition are typically ready for passage within 3 - 6 days.



*The folded appearance of epithelium is a function of cryosectioning and not representative of the shape of proliferating organoids.

FIGURE 5. Pancreatic Exocrine Organoids Display Markers of Pancreatic Progenitor and Ductal Cells
Pancreatic exocrine organoids grown in PancreaCult™ and stained for nuclei (DAPI, blue), ductal marker KRT19 (green) and pancreatic progenitor marker PDX1 (red). Organoids were imaged during passage 12 on day 5.

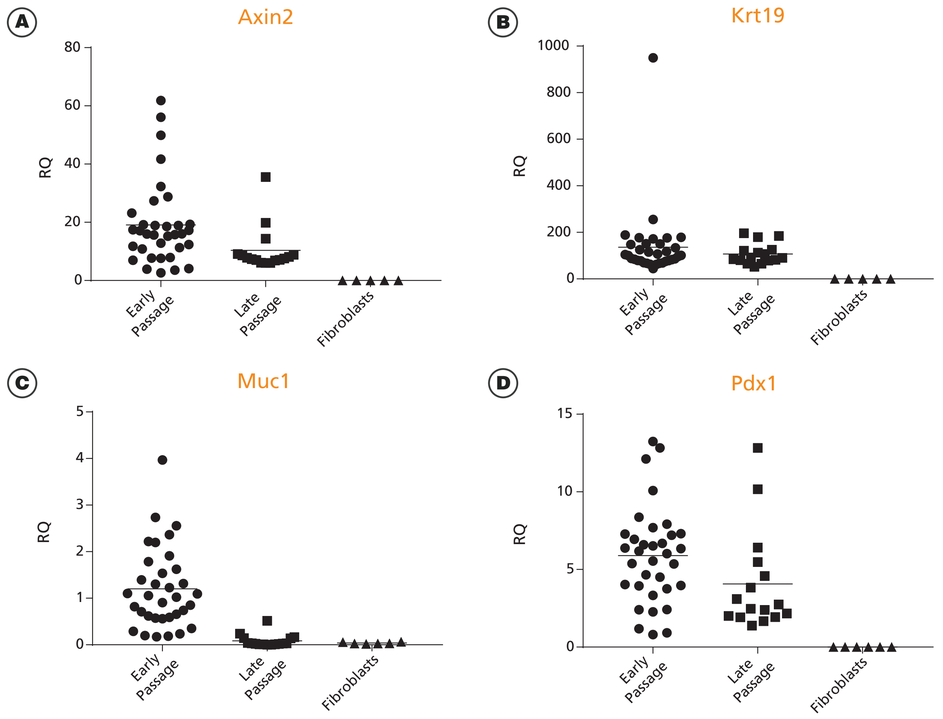


FIGURE 6. Cells Within Pancreatic Exocrine Organoids Retain Pancreatic Marker Expression During Passaging
Cells within pancreatic organoids express stem cell markers and those typical of the pancreatic exocrine system, including **(A)** Axin2, **(B)** Krt19, **(C)** Muc1, and **(D)** Pdx1. Relative quantification (RQ) of each marker is reported relative to the 18S and TBP housekeeping ge nes and normalized to C57/Bl6 pancreatic tissue. Marker expression was assayed during early passages (passage 1 - 5) and late passages (passage 6 - 10).

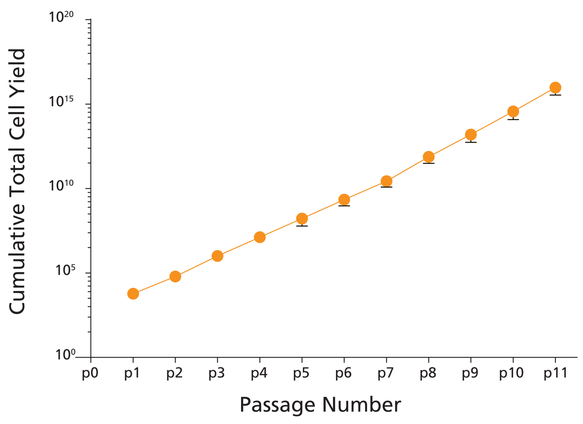


FIGURE 7. Expansion of Organoids Grown in PancreaCult™ Organoid Growth Medium (Mouse)
Organoids cultured with PancreaCult™ Organoid Growth Medium (Mouse) show efficient growth over multiple passages. Cultures were split with an average split ratio of 1:16 at each passage (n = 12).

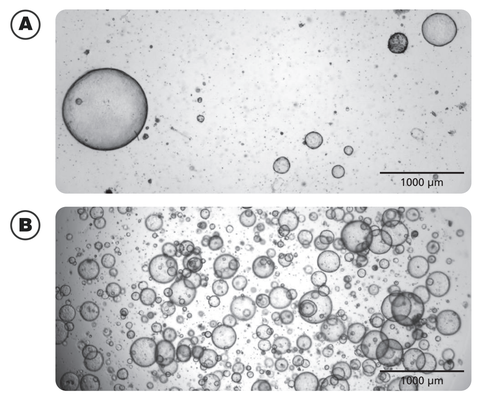


FIGURE 8. Pancreatic Exocrine Organoids Provide a Model for Pancreatic Carcinomas
PancreaCult™ Organoid Growth Medium (Mouse) supports the growth of organoids from pancreatic carcinomas. Pancreatic ducts were isolated from KPC mice (Kras$^{+/LSL\text{-}G12D}$; Trp53$^{+/LSL\text{-}R172H}$; Pdx1-Cre) and cultured in PancreaCult™ Organoid Growth Medium (Mouse). Organoids were imaged on **(A)** day 4 of primary culture and **(B)** day 3 after the first passage. An activated KRAS genotype was retained in organoids during culture. Data used with permission from Dr. David Tuveson.

Summary

- Pancreatic progenitor-derived organoids can be generated from pancreatic ducts, organoid fragments, and single cells in PancreaCult™ Organoid Growth Medium
- Pancreatic organoids can be expanded and maintained in PancreaCult™ Organoid Growth Medium while embedded in Matrigel®
- Pancreatic progenitor organoids can be maintained for over 50 passages (> 1 year) and used to model pancreatic cancer
- Pancreatic exocrine cells within organoids cultured in PancreaCult™ Organoid Growth Medium express ductal and pancreatic exocrine markers detected by qPCR and immunocytochemistry

References: (1) Huch M, et al. (2013) EMBO J. 32(20): 2708–21.2 (2) Boj SF, et al. (2015). Cell. 160(1-2): 324–38.