CULTURE AND DIFFERENTIATION OF MOUSE HEPATIC ORGANOIDS USING SERUM-FREE HepatiCult™ ORGANOID GROWTH MEDIUM

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Introduction

Liver organoids are miniature three-dimensional (3D) cell culture systems for studying liver cell biology. Liver organoids retain many features of in vivo hepatocytes, including a polarized epithelium, and represent a more physiological system than conventional two-dimensional cell culture for studying hepatic development, regeneration, detoxification, metabolism, and disease. We have developed a novel, serum-free HepatiCult™ Organoid Growth Medium (HepatiCult™ OGM) and protocols for establishing and expanding hepatic progenitor derived organoids derived from mouse liver tissue. Mouse livers were enzymatically treated to isolate the putative liver stem cell niche contained in hepatic ducts. The ducts were then further dissociated into single cells to derive clonal organoids. Liver organoids formed within 4 - 7 days from hepatic ducts or single cells that were embedded in Corning® Matrigel® and cultured in HepatiCult™ (n = 148 mice). The organoids were passaged every 5 - 7 days at split ratios between 1:10 and 1:30 and could be maintained in culture for > 2 years, indicating the presence of self-renewing hepatic stem cells. Cells within the organoids expressed genetic markers representative of hepatic stem and progenitor cells (Prom1, Axin2, Sox9, Cd44), ductal cells (Krt19, Hnf1b), and hepatocytes (Hnf4a, Afp), and were primed for downstream differentiation into mature functional hepatocytes. Differentiation was easily induced using a published protocol[1]. Production of the organoids could be scaled up by culturing them on an orbital shaker in a dilute suspension of Matrigel® in HepatiCult™. Methods were also established to cryopreserve the organoids for long-term storage. Our results demonstrate that HepatiCult™ promotes the establishment, expansion, long-term propagation, and banking of mouse hepatic organoids that maintain their capacity for differentiation.

Protocol

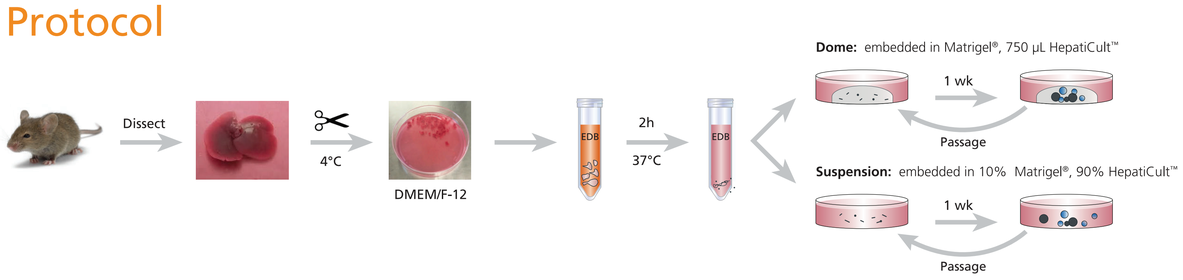

FIGURE 1. Protocol for Isolation and Culture of Hepatic Ducts from Mouse Liver Tissue
Mouse liver tissue was dissected, minced and digested with enzymatic digestion buffer (EDB) for 2 hours at 37°C. Digested hepatic ducts were pelleted and embedded in Matrigel® by either plating 30 µL domes at the centre of a pre-warmed 24-well plate or mixing pelleted ducts with 10% Matrigel and cooled 90% HepatiCult™. The domes were solidified at 37°C for 10 minutes and subsequently flooded with 750 µL of HepatiCult™, while cooled suspension cultures were gradually warmed to 37°C on an orbital shaker at 80 rpm. Once hepatic organoid cultures have been established from primary mouse tissue after 7 days of culture in HepatiCult™, organoids can be passaged by mechanical trituration into organoid fragments and plated in desired densities using an average split ratio of 1:25 or fragment counts.

Results

FIGURE 2. Hepatic Ducts Embedded in Matrigel® and Cultured in HepatiCult™ OGM Yield Budding Organoids over the Course of 7 Days
Hepatic ducts first thicken and form organoid buds at the ductal ends before buds also form at the ductal sides. Single organoids may also be generated from small hepatic fragments that have been generated during digestion.

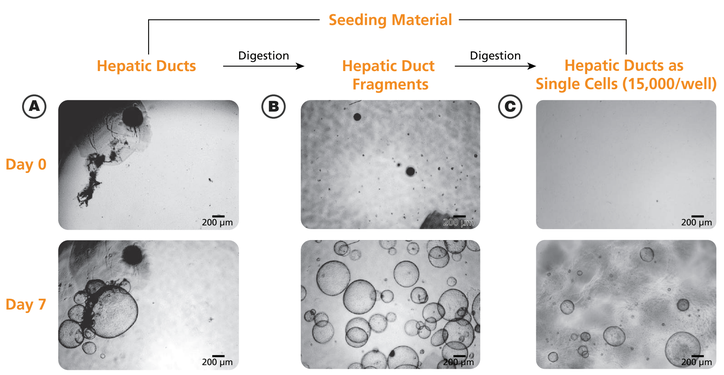

FIGURE 3. Hepatic Organoids Expand from Hepatic Ducts, Hepatic Duct Fragments, and Single Cells
Hepatic ducts isolated from mouse liver tissue may be plated as **(A)** whole ducts or may undergo an additional cell dissociation step, yielding **(B)** hepatic duct fragments or **(C)** quantifiable single cells (in suspension). The yield of hepatic organoids from whole ducts and duct fragments exceeds that of embedded single cells.

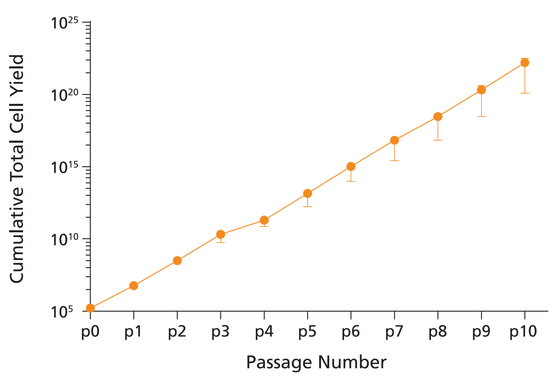

FIGURE 4. Hepatic Organoids Can be Maintained Long-Term and Scaled Up when Cultured in HepatiCult™ OGM
Organoids cultured with HepatiCult™ show efficient growth expansion over multiple passages. Cultures were split every 5 - 7 days with an average split ratio of 1:25 at each passage (n = 10 mice).

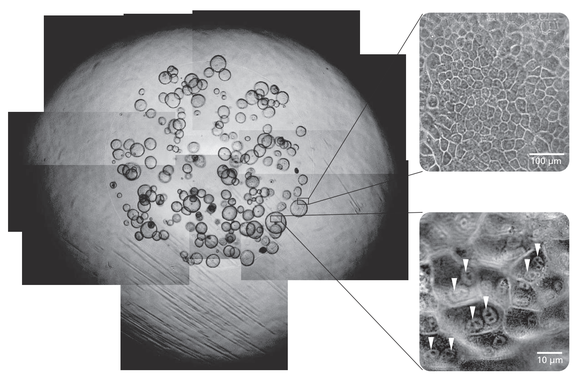

FIGURE 5. Hepatic Progenitor-Derived Organoids Exhibit Characteristic Hepatocyte-Like Morphologies
Established organoids display the typical polygonal morphologies of hepatic cells, tight junctions, and bi-nucleation. Hepatic progenitor-derived organoids are shown at passage 20.

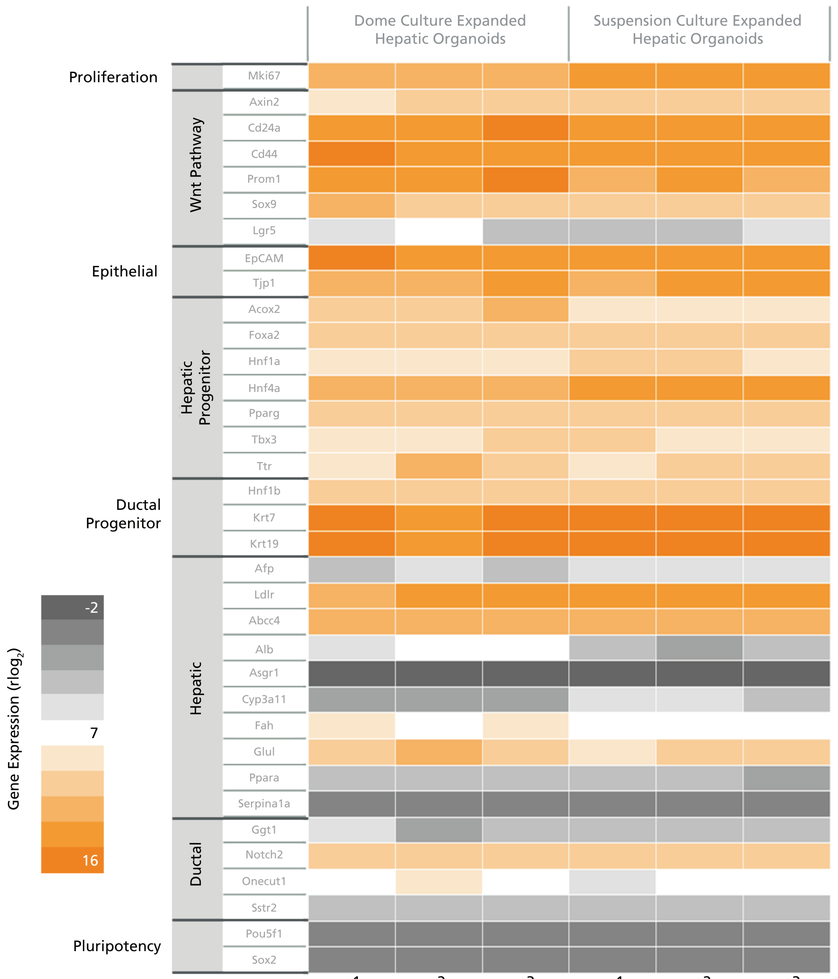

FIGURE 6. Gene Expression Profile of Hepatic Progenitor-Derived Organoids Cultured in HepatiCult™ OGM
Analysis of marker expression by RNA-seq shows organoids cultured in HepatiCult™ in either Matrigel® domes or in a dilute Matrigel® suspension express markers associated with hepatic stem and progenitor cells. The organoids also express low levels of genes associated with mature hepatic cell types, including cholangiocytes and hepatocytes. Columns represent biological replicates at passages ranging from passage 1 - 40.

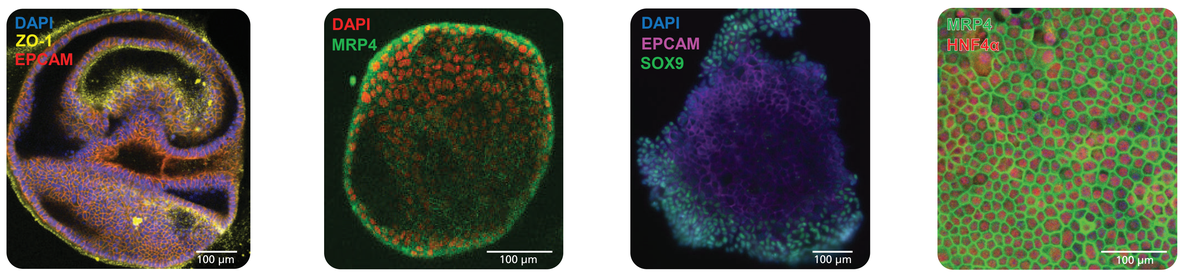

FIGURE 7. Immunocytochemistry Analysis of Hepatic Progenitor-Derived Organoids Cultured in HepatiCult™ OGM
Representative hepatic organoids emerging from seeded ducts stained positive for epithelial transmembrane protein EpCAM, tight-junction protein ZO-1, multidrug resistance protein MRP4, progenitor marker SOX9, and hepatic transcription factor HNF4α.

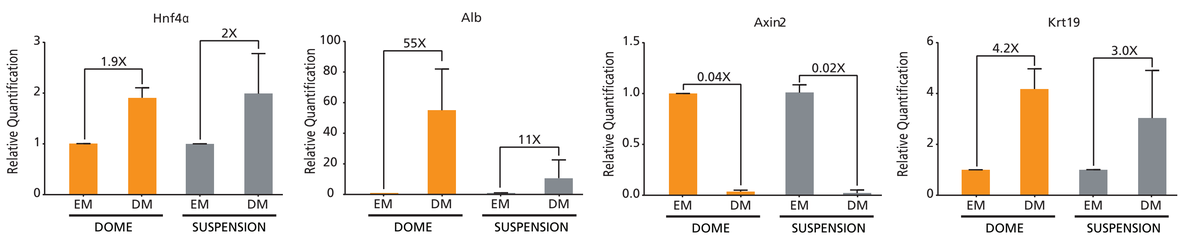

FIGURE 8. Differentiation of Hepatic Progenitor Organoids
Hepatic progenitor derived organoids grown in HepatiCult™ OGM can be differentiated to resemble more mature cell types when switched to a differentiation medium (DM). After switching to a published differentiation medium, hepatic organoids cultured in dome or suspension cultures show the upregulation of mature hepatic markers including Hnf4α and Alb, downregulation of hepatic stem cell marker Axin2, and upregulation of ductal marker Krt19.

Summary

- Hepatic progenitor-derived organoids can be generated from hepatic ducts, organoid fragments, and single cells in HepatiCult™ Organoid Growth Medium
- Liver organoids can be maintained and expanded in HepatiCult™ Organoid Growth Medium while embedded in Matrigel®
- Hepatic progenitor organoids can be maintained for over 50 passages (> 1 year)
- Hepatic progenitor cells within organoids cultured in HepatiCult™ Organoid Growth Medium express ductal and hepatic progenitor markers detected by qPCR and immunocytochemistry

References: (1) Huch M et al. (2013). Nature 494: 247–50.