Collection, Culture, and Analysis of Precision-Cut Liver Slices.

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Introduction

Precision-cut liver slices (PCLS) are a powerful tool for the study of liver physiology, chronic liver disease, and toxicology. PCLS capture all resident liver cell populations and the extracellular matrix, preserving the cell-cell and cell-matrix interactions that are lost in in vitro cell culture models. They also significantly reduce the number of animals required for experiments, as dozens of slices can be collected from each animal. These factors are especially impactful for the study of liver fibrosis and cirrhosis, where the interactions between cells and the matrix are fundamental to the pathophysiology and animal models require months of preparation. PCLS can also be collected from human liver biopsies, facilitating the translation of preclinical research to clinical impact.

Procedures

PCLS are collected using a vibratome (7000smz-2, Campden Instruments Ltd). Liver samples are mounted to the stage with cyanoacrylate glue, the cutting tray is filled with chilled sterile Krebs-Henseleit buffer, and the PCLS are cut at 50Hz frequency, 2.5mm amplitude, 0.15mm/s advance speed, 250µm slice thickness. The collected slices can then be trimmed with a biopsy punch to ensures all PCLS are uniform in size and to remove any tissue that may have come into contact with the glue.

Tissue oxygenation is a crucial aspect of PCLS survival in culture. The traditional method to address this involves culturing the PCLS under hyperoxic conditions (95% oxygen)[1]. More recently an alternative method has been used where the PCLS are cultured in permeable inserts so that they sit near the media-air interface[2]. This approach allows the use of standard cell culture incubators and provides a tissue oxygen concentration closer to the *in vivo* environment[3]. Incubating PCLS on a rocking platform and supplementing culture media with dexamethasone and insulin-transferrin-selenium-ethanolamine (ITS-X) help maintain the PCLS closer to fresh tissue[4]. Viability of PCLS remains stable for up to 4 days in culture, though significant changes in expression of some key genes can be seen after 2 days[4]. There are reports of maintenance of PCLS in culture for up to 15 days[2]. An appropriate culture length for PCLS depends on the hypothesis under investigation.

During the culture period, media can be collected to assay concentrations of soluble biomarkers like albumin or inflammatory cytokines. For imaging studies, PCLS can be used with *in situ* confocal imaging, formalin-fixed paraffin-embedded protocols, or cryosectioning. RNA can also be collected for gene expression analysis, though an isolation protocol

designed for small tissue samples is required to obtain high yield and purity[4].

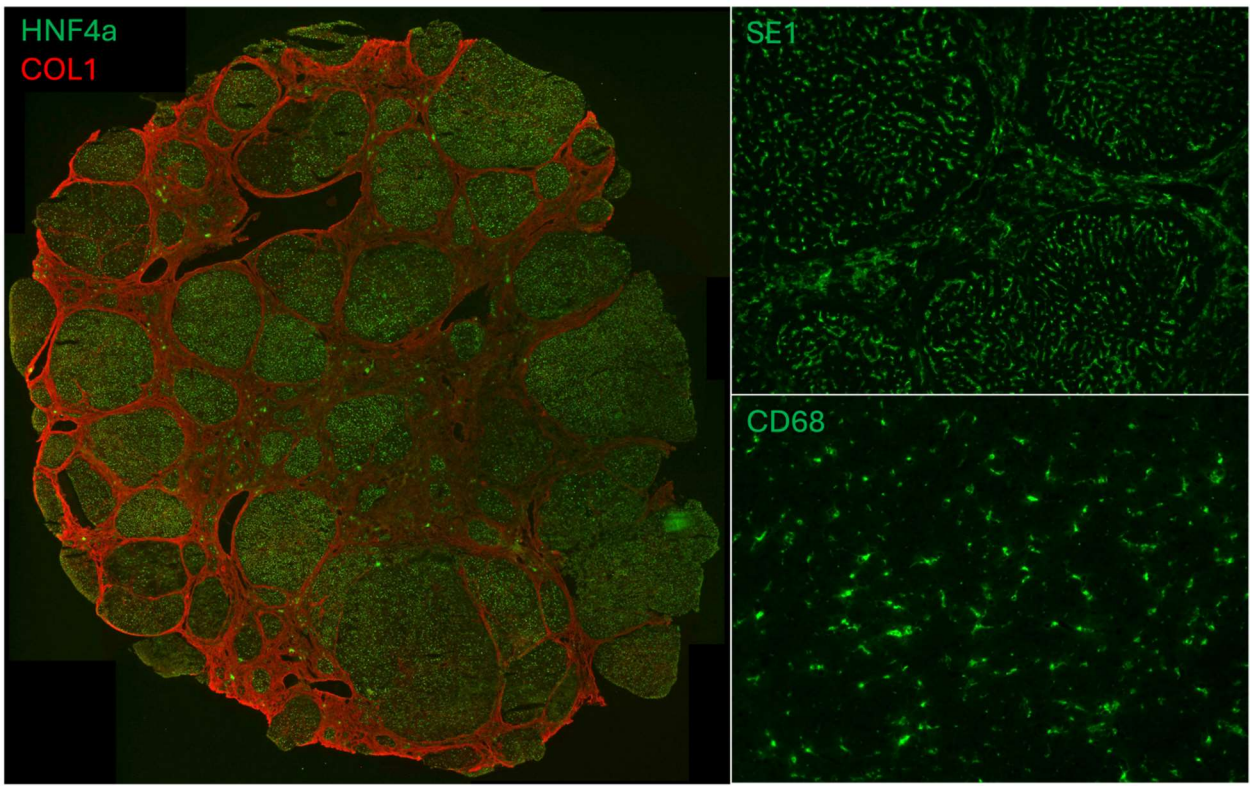

Fig.1. Immunofluorescence staining of PCLS collected from cirrhotic rats. HNF4a – hepatocyte marker. SE1 – liver sinusoidal endothelial cell marker. CD68 – macrophage marker.

Specific examples

We have used PCLS to study the effects of the antifibrotic drug erlotinib[5]. We compared the effects of this drug between PCLS, cell culture, and *in vivo* models of multiple chronic liver disease etiologies.

We are currently using PCLS to study the role of cytoskeletal signal transduction in liver cirrhosis. We collected PCLS from healthy and cirrhotic rats, then treated them with either a drug that disrupts actin filaments or an siRNA targeting a key mechanosensitive transcription factor. This study aims to investigate how cells in the liver sense their environment and the effect that has in chronic liver disease.

References

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