Precision cut liver slices: an *ex vivo* model for anti-fibrotic drug screening

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Highlights

Precision cut liver slices (PCLS), an *ex vivo* culture model, represent a highly relevant drug screening platform for studying the efficacy of anti-fibrotic therapeutic agents, given their complete reconstitution of the native tissue microenvironment. By coupling normal or diseased human tissue or animal models of disease with endpoints ranging from gene expression to biochemical analyses and high-content imaging-based analyses, precision cut tissue assays represent a powerful, modular approach to pre-clinical drug discovery.

Introduction

Non-alcoholic fatty liver disease (NAFLD) affects 30-40% of adults in the United States. In approximately 20% of NAFLD patients, fatty liver progresses to non-alcoholic steatohepatitis (NASH), which is characterized by inflammation and, ultimately, hepatocellular damage. Throughout this progression, inflammatory cytokine and growth factor production (i.e. TGFβ-1 or PDGF-BB) drive the activation of hepatic stellate cells, which in turn deposit extracellular matrix (ECM; i.e. collagen, fibronectin, elastin). As the balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) is disrupted, remodeling of such ECM is dysregulated, such that the liver parenchyma is progressively replaced by scar tissue, ultimately resulting in cirrhosis or liver cancer.

Since most individuals are asymptomatic until late fibrotic or cirrhotic stages of the disease, much of the drug-development effort in the space has focused on ameliorating fibrosis, though no successful therapeutics have been approved by the FDA to date. Since most therapeutic failures have come to light in later stage clinical trials, many scientists in the pre-clinical space have begun re-evaluating in vitro models in hopes of minimizing costs and improving the efficiency of drug development in this therapeutic area. While the limitations of traditional 2D cell culture approaches have been well recognized, even more innovative cell culture models such as 3D co-culture models or organ-on-a-chip models have fallen short of recapitulating the complexity of *in vivo* fibrotic tissues.

In this application note, the development and validation of a precision cut liver slice (PCLS) model to fill this need is discussed. Thin tissue slices (5 mm diameter, ~250 µm thick disks) are generated from fresh liver tissue, enabling the maintenance of cellular heterogeneity and tissue architecture seen in the in vivo microenvironment. PCLS can be generated from diseased human or animal tissues or alternatively from normal tissues stimulated *ex vivo* to induce disease state. Combined with histology, imaging, gene expression, and protein secretion analysis, this platform is ideally suited for reducing the number of animal studies and bridging the translational gap between animal and human studies in the development of anti-fibrotic therapeutics.

Methods

Precision-cut liver slices

Prior to receipt, tissues were flushed with preservative or PBS. Once received, liver lobes were dissected, macrosectioned, cored (5 mm), and microsectioned to achieve 250 µm thick PCLS. Slices were placed on permeable culture supports in supplemented Williams E Medium with or without test articles (ALK5 inhibitor, A 83-01, ApexBio cat # A3133 or Imatinib, Sigma cat # SML1027). All plates were maintained in a standard tissue culture incubator (37°C, 5% CO_2) with agitation.

ELISA, RNA isolation and qPCR analysis

PCLS culture supernatant was collected to evaluate IL-6 secretion. The ELISA was performed according to manufacturer's instructions.

PCLS were snap frozen and homogenized using a tissue homogenizer prior to RNA isolation. Total RNA isolation was done by using QIAGEN RNeasy micro kit according to the manufacturer's protocol, followed by cDNA synthesis using Verso cDNA synthesis kit. Transcript levels

were measured by quantitative real-time PCR (qRT-PCR) using QuantStudio 3 instrument and PowerTrack SYBR Green Mix (Thermofisher). The expression level was normalized to that of RPL19 housekeeping gene in the same sample.

Immunolabeling, clearing, and high content imaging of PCLS

PCLS were fixed, permeabilized, and blocked prior to incubation with a primary antibody cocktail overnight at 4°C. PCLS were then washed and labeled with secondary antibodies and DAPI, washed again, and then taken through a methanol gradient to remove as much water as possible. Methanol was exchanged for Visikol HISTO-1, which preceded transfer of slices to a glass bottom plate for exchange into Visikol HISTO-2 prior to imaging using a Molecular Devices ImageXpress high content confocal imager.

Results and Discussion

Morphology of PCLS in *ex vivo* culture

PCLS cellular morphology was studied using H&E staining. At day 0, F3 human PCLS displayed classical diseased liver features with presence of lymphocytes, indicating inflammation in the liver as well as macrovesicular steatosis. These features were retained in the human PCLS after *ex vivo* culture up to 72 hours (Figure 1A). Whole mount PCLS staining revealed abundant vimentin-positive stellate cells, mostly located adjacent to collagen network (Figure 1B).

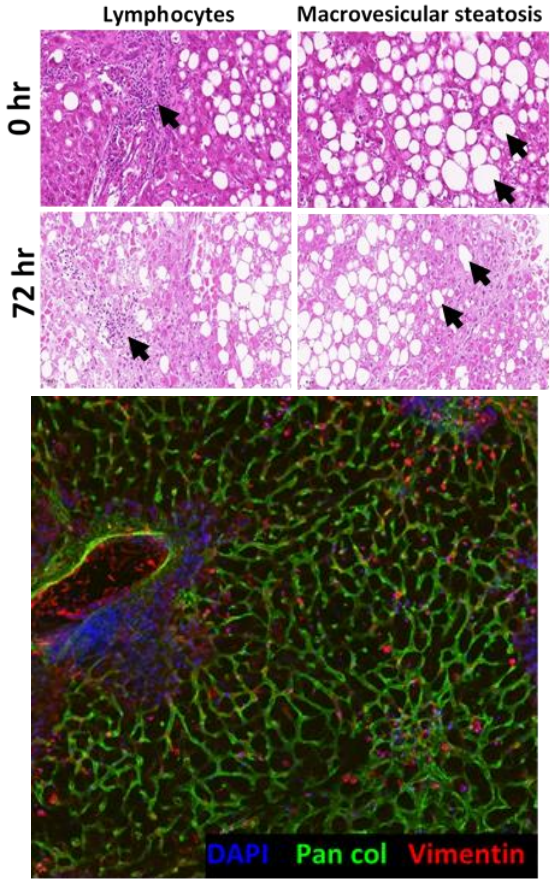

Figure 1. (A) H&E staining of untreated F3 human PCLS at 0 and 72 hours in *ex vivo* culture. (B) Whole mount labeling of F3 human PCLS.

Gene expression and protein secretion as key endpoints in evaluating anti-fibrotic agents in diseased human and mouse PCLS

After validating the maintenance of overall tissue architecture in culture, the ability to evaluate the effects of antifibrotic therapeutics was next validated, beginning with gene expression as a primary endpoint. In F3 human PCLS treated with an ALK5 inhibitor (ALK5i), collagen I alpha I (COL1a1) and tissue inhibitor of metalloproteinase 1 (TIMP1) were decreased relative to vehicle treatment after 72 h (Figure 2A). Similarly, both Imatinib and ALK5 inhibitor treatment decreased the expression of COL1a1 and platelet-derived growth factor receptor beta (PDGFRβ) in a CCl4-induced model of liver fibrosis (Figure 2B). However, it should be noted that in this model, the AILK5 inhibitor had a greater effect than imatinib at a matched dose (Figure 2B).

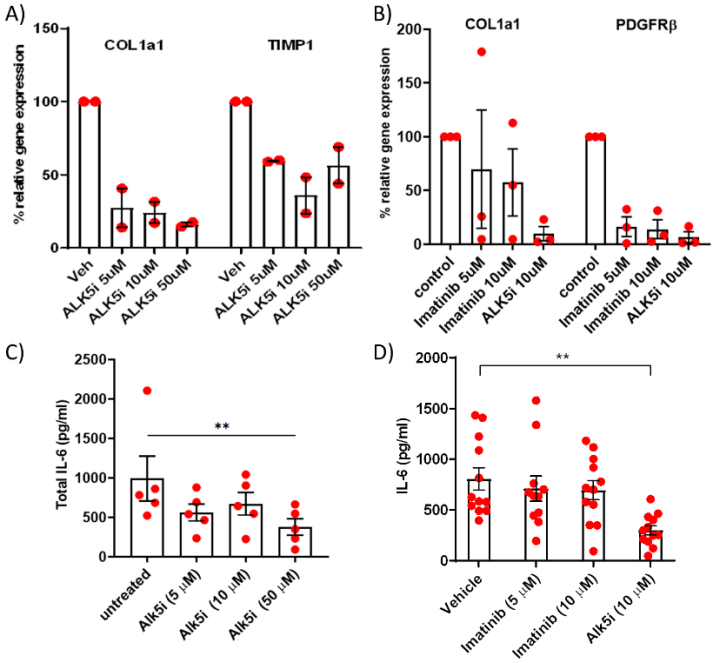

Figure 2. (A) Transcript levels of COL1a1 and TIMP1 in F3 human PCLS. (B) Transcript levels of COL1a1 and PDGFRβ in PCLS from CCl4-induced liver fibrosis mouse model. (C) IL-6 secretion in F3 human PCLS and (D) mouse CCl4 PCLS. ALK5 inhibition reduced the secretion of IL-6 significantly in both human and mouse PCLS after 72 hours in culture. In all graphs, data represent mean ± SEM, * indicates $p<0.05$ in a two-way ANOVA with Dunnett's correction for multiple comparisons in a post-hoc analysis.

The ability to use cell culture supernatant from the culture of PCLS to measure secreted products as an alternative endpoint for evaluating protein production in response to therapeutic treatment was next evaluated. ELISA analysis revealed that the secretion of the inflammatory cytokine, IL-6 was significantly reduced by ALK5 inhibition in both human and mouse diseased PCLS models. These data suggest that ELISA evaluation of secreted products in cell culture supernatant can be an effective means to evaluating

therapeutic treatment. This is especially important in that assays conducted on culture supernatants enable higher-plexing of multiple endpoints, since they do not require tissue sacrifice for conducting the assay.

Quantitative image analysis as an alternative endpoint in evaluating anti-fibrotic efficacy in diseased human and mouse PCLS

As an alternative endpoint to the more traditional means of evaluating gene expression and protein secretion, the ability to quantitatively evaluate therapeutic efficacy using immunolabeling, 3D confocal imaging, and advanced image analysis was next validated. Quantification of the area fraction of both PDGFRβ and αSMA in PCLS generated from a CCl4 mouse model of fibrosis revealed that imatinib reduced the expression of both of these markers of stellate cell activation, especially at higher doses (Figure 3).

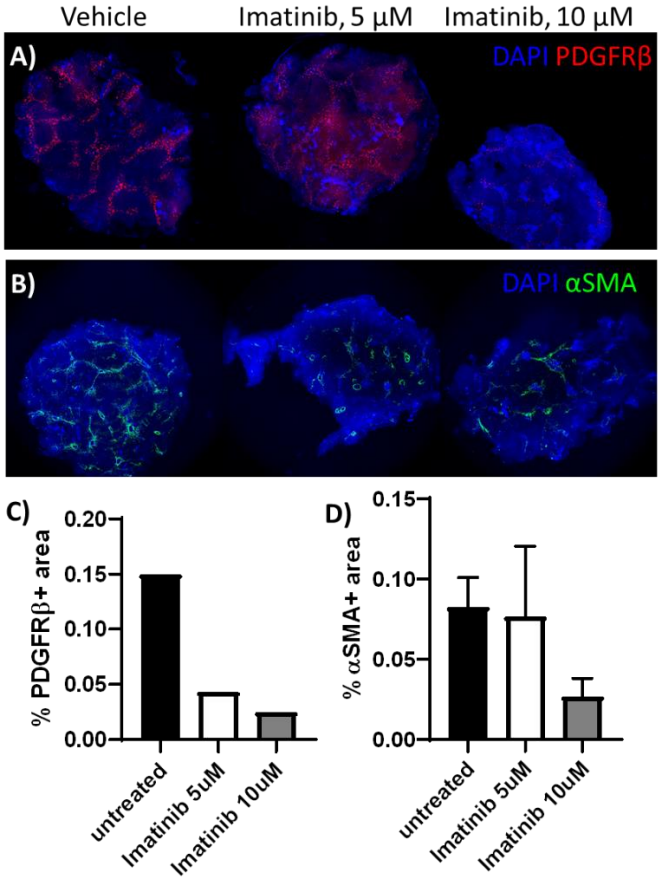

Figure 3. (A-B) Representative confocal whole mount images showing labeling of PDGFRβ (A) and αSMA (B) in PCLS from CCl_4-induced liver fibrosis in a mouse model. (C-D) Quantification of label-positive area, normalized to total slice area; data represent mean ± SEM.

Conclusion

Altogether, these data demonstrate the utility of precision cut liver slices as a platform for anti-fibrotic drug screening. While the data presented herein were generated using PCLS from diseased human donor samples or a diseased mouse model, the platform is highly modular in that normal tissue can be used to generate slices that are stimulated to induce disease state ex vivo. Additionally, other tissues can similarly be implemented in a precision cut assay to answer questions in fields ranging from toxicology to lung fibrosis to immune cell infiltration in tumor biopsies. Regardless of application, the power of this platform lies in its unmatched in vivo relevancy compared to existing pre-clinical drug discovery platforms.

References

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