CASE STUDY

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# MASH modeling through innovative co-culture of iPSC-derived liver cell systems

Metabolic dysfunction-associated steatohepatitis (MASH) is a progressive form of fatty liver disease characterized by hepatocellular injury, inflammation, fibrosis, and is a leading cause of cirrhosis, liver failure, and transplantation. Traditional preclinical models often fail to fully capture the complexity of human liver pathology, limiting drug development and safety assessment. At pixlbio we have advanced this field with our pixHep (iPSC-derived hepatocytes) and pixStellate (iPSC-derived stellate cells) technology, enabling co-culture systems that closely mimic the cellular and molecular features of MASH, including lipid accumulation, inflammatory signaling, and fibrogenesis. This innovative human cell-based model provides a highly translational platform for disease modeling, predictive drug-induced liver injury (DILI) assessment, and comprehensive toxicity profiling, accelerating the development of safer and more effective therapies.

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## pixHep and pixStellate 2D co-culture

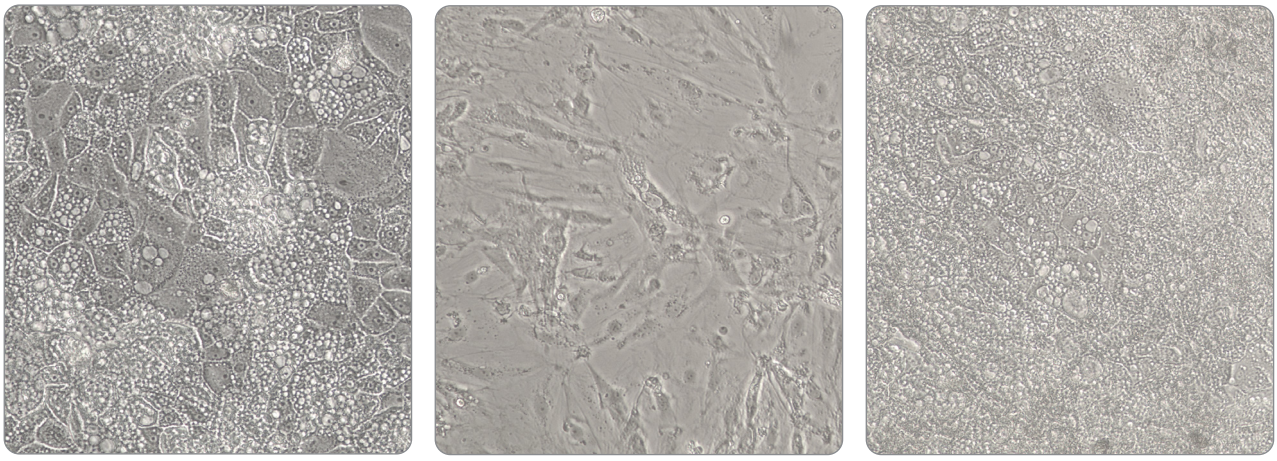

Figure 1: Representative brightfield pictures showing the morphology of pixlbio pixHep (left), pixStellate (middle), and pixHep/pixStellate co-cultures (right).

## pixHep and pixStellate express hepatocyte and stellate cell markers in co-culture

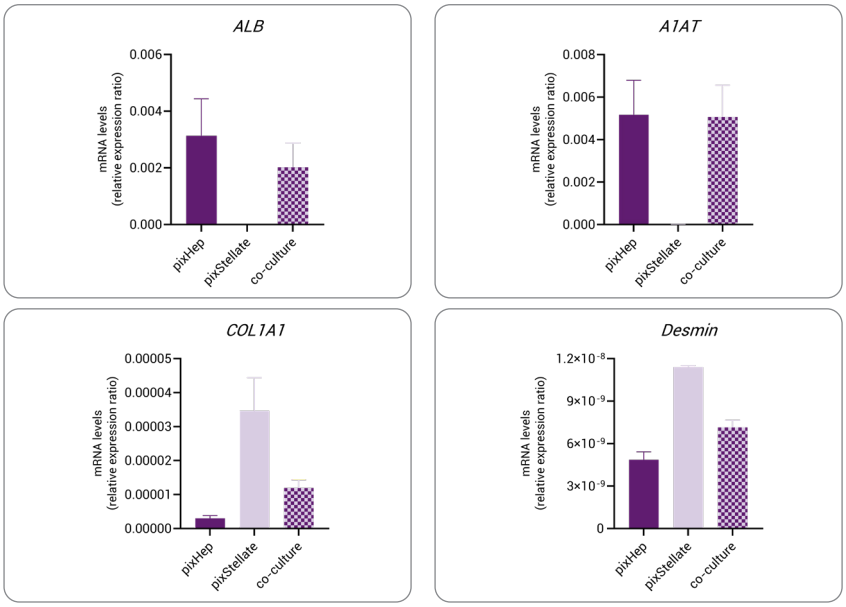


Figure 2: mRNA levels of the hepatocyte markers albumin (ALB) and alpha-1-antitrypsin (A1AT) and hepatic stellate cell markers collagen (COL1A1) and Desmin in pixHep, pixStellate, and pixHep/pixStellate co-cultures.

## Activated pixStellate drive hepatic steatosis in pixHep/pixStellate co-culture and this can be pharmacologically reversed

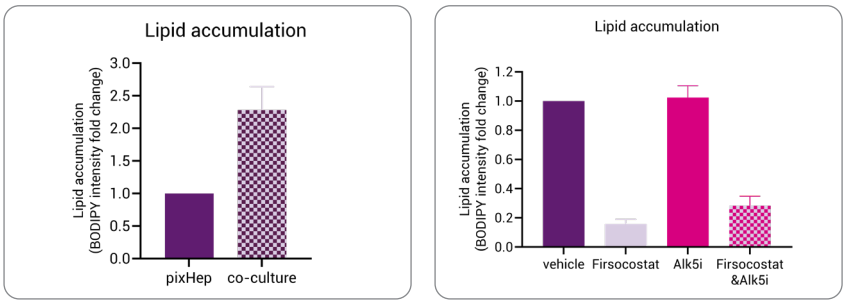


Figure 3: Quantification of lipid accumulation in pixHep and pixHep/pixStellate co-culture, as measured by BODIPY staining (left). B) Quantification of lipid accumulation in pixHep/pixStellate co-culture following treatment with either vehicle, Firsocostat (10 µM), Alk5i (5 µM), or combination of Firsocostat and Alk5i for 5 days, as measured by BODIPY staining. Data are presented as mean±SEM of n=2-3 independent experiments

## pixHep/pixStellate co-culture secrete collagen, and this can be pharmacologically reversed

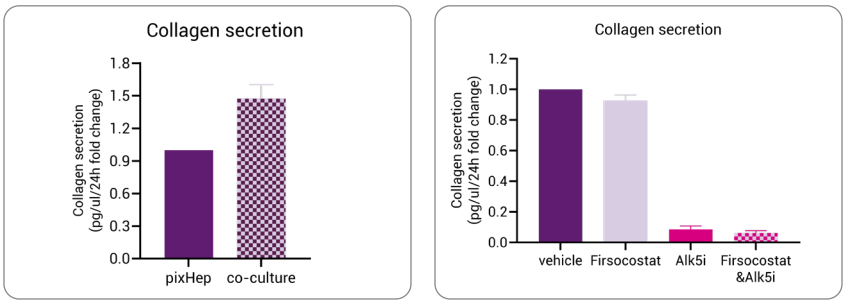


Figure 4: Collagen secretion in pixHep and pixHep/pixStellate co-culture, as measured by ELISA (left). Collagen secretion in pixHep/pixStellate co-culture (right).

## Fatty acid treatment drives steatosis and induces collagen secretion in pixHep/pixStellate co-culture, and this can be pharmacologically reversed

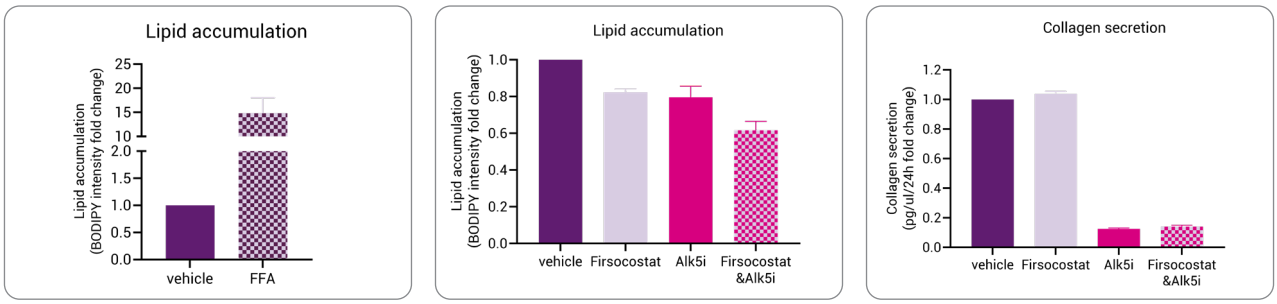


Figure 5: Quantification of lipid accumulation in pixHep/pixStellate co-culture pre- and post- free fatty acid (FFA) treatment, as measured by BODIPY staining (left). Quantification of lipid accumulation in FFA-treated pixHep/pixStellate co-cultures following treatment with either vehicle, Firsocostat (10μM), Alk5i (5 μM), or combination of Firsocostat and Alk5i for 5 days, as measured by BODIPY staining. Data are presented as mean±SEM of n=2 independent experiments (middle). Collagen secretion in pixHep/pixStellate co-culture following treatment with either vehicle, Firsocostat (10 μM), Alk5i (5 μM), or combination of Firsocostat and Alk5i for 5 days, as measured by ELISA. Data are presented as mean±SEM of n=2-3 independent experiments.

### Conclusions

These results demonstrate that co-cultures of pixHep and pixStellate effectively recapitulate key pathological features of MASH, including enhanced lipid accumulation and collagen secretion. The expression of canonical hepatocyte markers (ALB, A1AT) and stellate cell markers (COL1A1, Desmin) confirms the phenotypic stability and functional relevance of both cell types in mono- and co-culture systems. Co-cultures displayed markedly higher lipid accumulation and extracellular collagen deposition compared to hepatocyte monocultures, highlighting the importance of stellate cell-hepatocyte crosstalk in driving disease-associated steatosis and fibrosis. Furthermore, treatment with the ACC inhibitor Firsocostat and the TGFb receptor inhibitor Alk5i reduced both lipid accumulation and collagen secretion, particularly when used in combination, demonstrating the model's ability to capture drug responses relevant to MASH pathophysiology. Together, these findings establish the pixHep/pixStellate co-culture as a robust, mechanistically informative platform for modeling MASH progression and evaluating therapeutic interventions.

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