TISSUE REPAIR

Cholangiocyte organoids can repair bile ducts after transplantation in the human liver

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Organoid technology holds great promise for regenerative medicine but has not yet been applied to humans. We address this challenge using cholangiocyte organoids in the context of cholangiopathies, which represent a key reason for liver transplantation. Using single-cell RNA sequencing, we show that primary human cholangiocytes display transcriptional diversity that is lost in organoid culture. However, cholangiocyte organoids remain plastic and resume their in vivo signatures when transplanted back in the biliary tree. We then utilize a model of cell engraftment in human livers undergoing ex vivo normothermic perfusion to demonstrate that this property allows extrahepatic organoids to repair human intrahepatic ducts after transplantation. Our results provide proof of principle that cholangiocyte organoids can be used to repair human biliary epithelium.

Organoids have a particular potential for tissue repair as they retain key functions and characteristics of their tissue of origin. Nevertheless, their ability to repair native epithelia and restore their complexity after transplantation has not yet been established in humans, and organoid engraftment and survival in vivo has only been demonstrated in a limited number of animal studies (*1*).

The bile duct epithelium presents an archetypal and clinically important system for addressing this challenge and for developing proof-of-concept studies in humans for multiple reasons. Disorders of the biliary system, which transfers bile from the liver to the duodenum, account for 70% of pediatric and up to a third of adult liver transplantation (*2*), creating a pressing need for therapeutic alternatives, such as cell-based therapy. Furthermore, organoids suitable for regenerative medicine applications can be easily derived from biliary epithelial cells, known as cholangiocytes (*3*). Finally, the bile ducts also recapitulate the epithelial diversity found in other hollow-lumen organs (*4*). In particular, different regions along the biliary tree display distinct transcriptional profiles and functional properties, such as the chemical modification of bile (*5*, *6*) as well as variation in disease susceptibility between the intrahepatic ducts, extrahepatic ducts, and the gallbladder. Nevertheless, the impact of this regional variation on the characteristics and regenerative potential of the organoids derived from different regions of the biliary tree remains to be characterized.

To address these questions and demonstrate the value of organoids for regenerative medicine in humans, we first characterize cholangiocyte diversity in vivo using single-cell transcriptomics and confirm that different regions of the human biliary tree contain cells with distinct transcriptional profiles. We then show that cholangiocytes lose these differences in organoid culture and become interchangeable but that their regional identity can be restored in vitro by environmental stimuli. We subsequently use a biliary injury mouse model and a newly developed model for cell transplantation in human organs undergoing ex vivo normothermic perfusion (NMP) to prove that this plasticity allows cholangiocytes from one region to repair a different region of the biliary tree, thereby paving the way for cell-based therapy using organoids.

To characterize the cellular composition of the human biliary epithelium, cholangiocytes from different regions [intrahepatic bile ducts (IHD): 5 patients, 587 cells; common bile duct (CBD): 3 patients, 3006 cells; and gallbladder (GB): 3 patients, 3702 cells] were isolated using magnetic bead sorting, and their transcriptome was determined using droplet encapsulation single-cell RNA sequencing (scRNA-seq) (Fig. 1, A and B, and fig. S1, A to C). The isolated cells expressed key cholangiocyte markers, including KRT7, KRT19, SOX9, and GGT (fig. S2A). The transcriptomes of all three biliary cell populations shared a core transcriptional profile, illustrated by their proximity in uniform manifold approximation and projection (UMAP) space and high connectivity in partition-based graph abstraction (PAGA) analysis when compared with different liver cell types, such as stellate cells and liver sinusoidal endothelial cells (LSECs) (fig. S2, B to E). However, more detailed analysis after subclustering the cholangiocytes revealed nonoverlapping expression modules of the three populations (Fig. 1B), which suggests that, despite their similarities, cholangiocytes from different regions exhibit distinct gene expression signatures (*6*).

Differentially expressed gene (DEG) analysis (data S1) identified known region-specific markers, including aquaporins (*7*), mucins (*8*), *FGF19* (*9*), and *SOX17* (*10*) in the extrahepatic biliary tree; *JAG1* (*11*), *TACSTD2* (*12*), and YAP target genes in intrahepatic cholangiocytes (*13*, *14*); as well as previously unidentified markers including *DCDC2*, *TFF1-3*, and *SLC15A1* (Fig. 1, C and D, and fig. S3, A to D). Most of these genes correspond to functional markers such as bile acid receptors or channels modifying bile composition (fig. S3C). Thus, the transcriptional divergence among cholangiocytes from different regions could reflect adaptation to their microenvironment, such as the variation in

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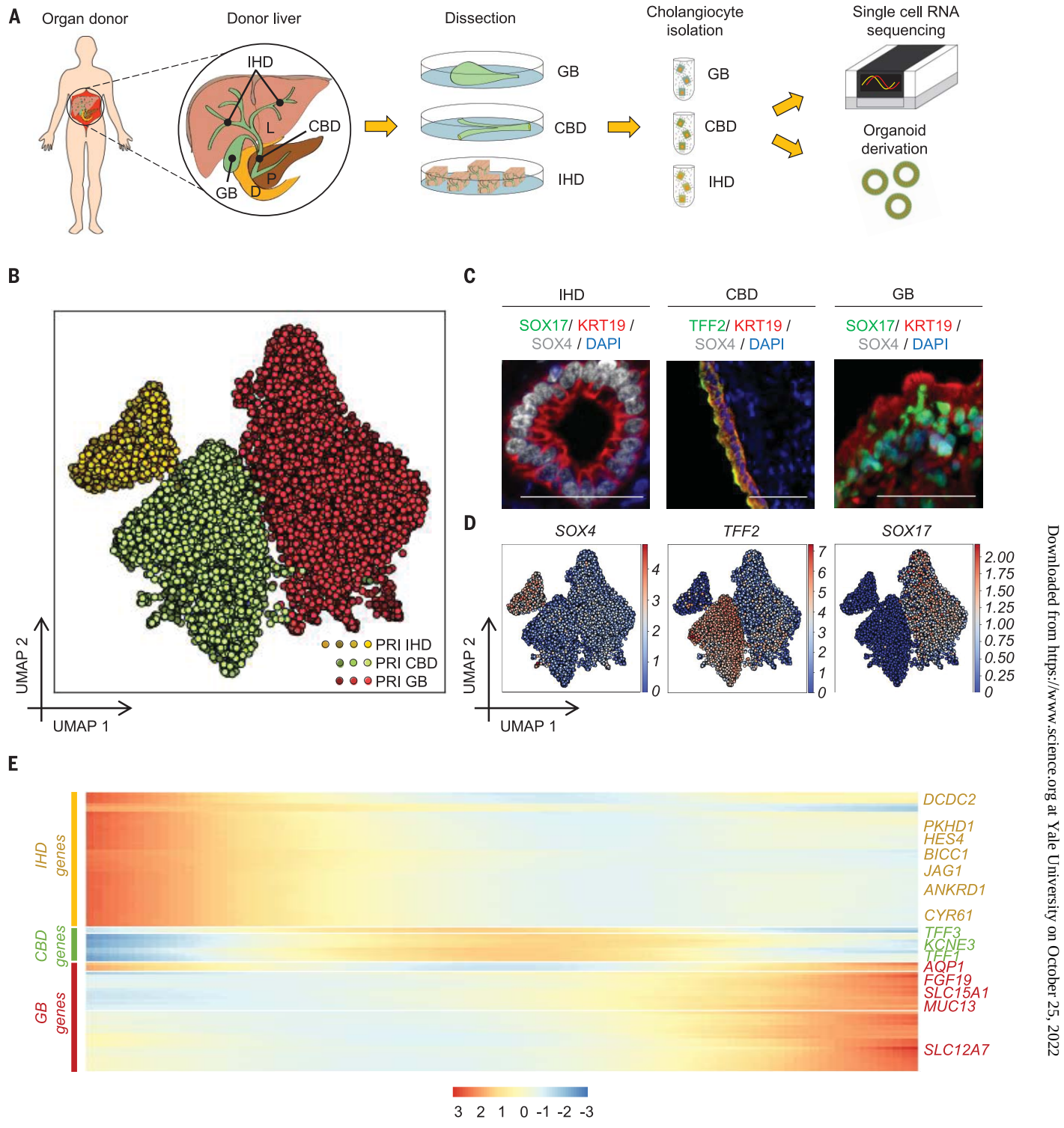


Fig. 1. Transcriptional profiling of primary cholangiocytes. (**A**) Schematic representation of the methodology used for scRNA-seq. (**B**) UMAP plot (7295 primary cells, n = 10 individuals) illustrating the distinct primary cholangiocyte populations in different regions of the biliary tree. (**C** and **D**) Immunofluorescence images (C) and UMAP representations (D) of normalized gene expression of primary cholangiocytes illustrating the differential expression of representative region markers. Scale bars, 50 μm. (**E**) Heatmap of top 100 DEGs in pseudotime (data S2) demonstrating a gradual transition in the transcriptional profile of cholangiocytes between different regions of the biliary tree. PRI, primary; IHD, intrahepatic ducts; CBD, common bile duct; GB, gallbladder; P, pancreas; D, duodenum; L, liver; DAPI, 4′,6-diamidino-2-phenylindole.

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bile properties along the biliary tree (*15*). Accordingly, cholangiocytes from anatomically adjacent and therefore environmentally similar regions (e.g., IHD and CBD versus GB) displayed higher transcriptional similarity. This was illustrated by PAGA analysis (fig. S3, E and F) in agreement with results from diffusion pseudotime and single-cell consensus clustering (SC3) analyses (figs. S4 and S5). These results point toward a progressive change in the expression of region-specific markers (Fig. 1E and data S2) and a gradual transition in the transcriptional signature of cholangiocytes from adjacent regions (fig. S4, A to C) rather than the existence of distinct subpopulations (Fig. 1E and figs. S4 and S5). This gradient in gene expression likely indicates adjustment of the cells to environmental conditions, such as the gradual change in bile composition from the intrahepatic ducts to the gallbladder. Our results demonstrate that the human biliary epithelium is composed of cholangiocytes that display a gradual shift in their transcriptional profile along the biliary tree, which is likely imposed by region-specific microenvironments.

We subsequently used this single-cell map of the human biliary tree as a framework to characterize cholangiocyte organoids. To this end, a fraction of the primary cholangiocytes isolated for scRNA-seq from each region (IHD, CDB, and GB) were propagated as organoids using our established conditions (*3*, *16*). The resulting organoids expressed cholangiocyte markers (KRT7, KRT19, SOX9, HNF1B, and CFTR; fig. S6, A and B) and displayed comparable functionality (ALP, GGT activity; fig. S6, C and D) and similar expansion potential regardless of their region of origin (fig. S6E). To further explore these similarities, we performed scRNA-seq on these organoids (two lines per region; GB, 5859 cells; CBD, 5321 cells; and IHD, 6641 cells) (fig. S1, A to C). Principal components analysis (PCA) and UMAP analyses demonstrated that organoids exhibited overlapping transcriptomic profiles (Fig. 2A and fig. S7, A to D), which indicates that cholangiocytes grown in vitro assume a similar

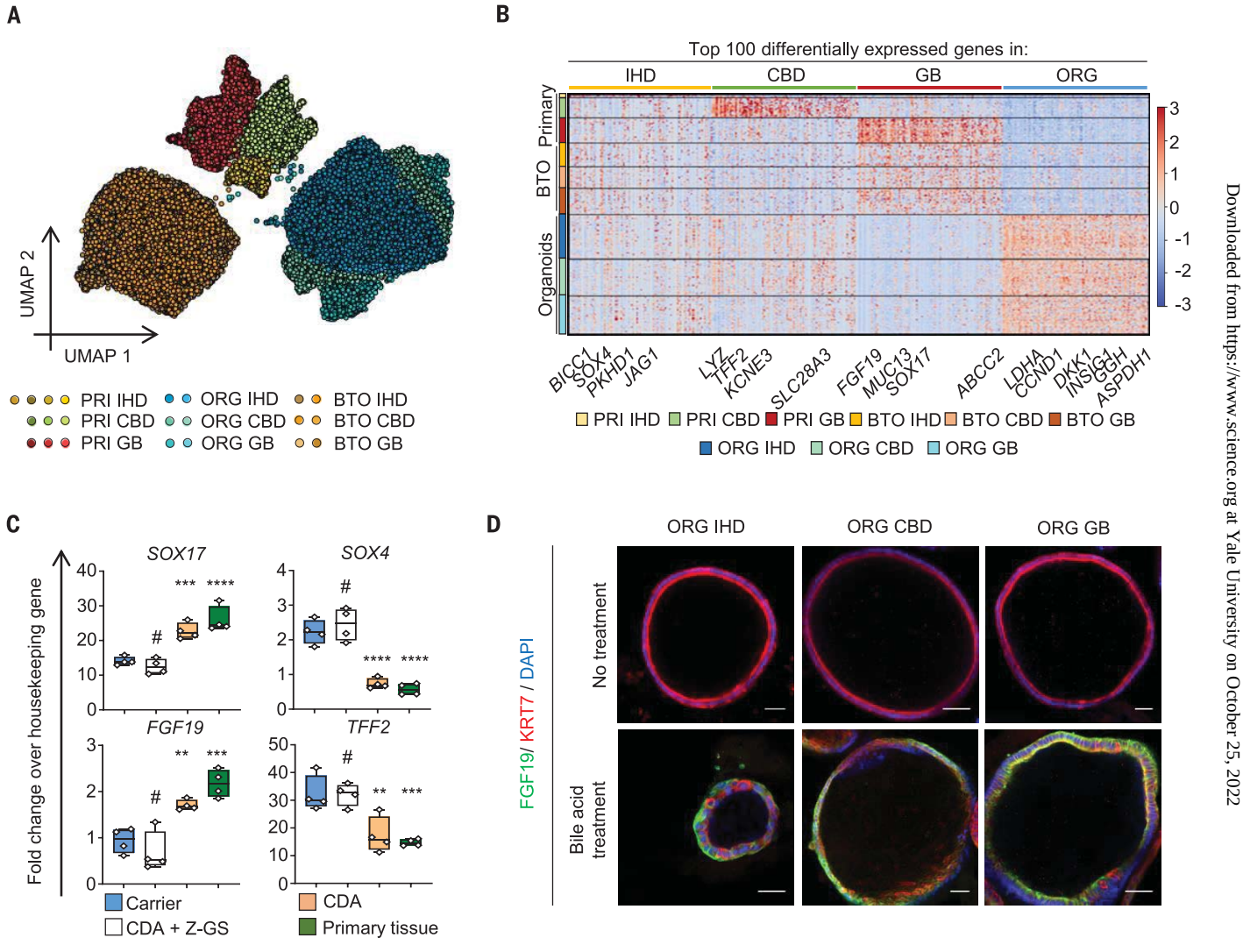


Fig. 2. Cholangiocyte organoid identity is controlled by niche stimuli. (**A**) UMAP (35,603 cells) of primary cholangiocytes and their corresponding organoids before and after gallbladder bile treatment showing the similarities between organoids from different regions and changes in their signature in response to bile. ORG, organoids; BTO, bile-treated organoids. (**B**) Heatmap of top 100 DEGs between primary regions, organoids, and BTOs (data S1 and S2) illustrating that organoids lose regional differences and up-regulate culture-related genes but reacquire gallbladder markers after bile treatment. (**C** and **D**) Quantitative real-time fluorescence polymerase chain reaction (QPCR) (C) [n = 4 samples per group; center line, median; box, interquartile range (IQR); whiskers, range; housekeeping gene, *HMBS*; $^{\#}P > 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$] and immunofluorescence (D) demonstrating the up-regulation of gallbladder markers and bile acid target genes after treatment with chenodeoxycholic acid (CDA) in the absence of the FXR inhibitor Z-GS. Z-GS, Z-guggulsterone. Scale bars, 50 μm.

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transcriptional signature independent of their region of origin. Notably, regressing cell cycle–related genes did not change these observations, which excluded the possibility that a common proliferation signature could mask the differences between organoids of different spatial origins (fig. S7, A to C and E). Furthermore, we did not detect any cells coexpressing known somatic stem cell markers (*LGR5*, *PROM1*, *TACSTD2*, or *NCAM*), which excluded the possibility that organoid similarities reflect a common progenitor or stem cell identity (fig. S7F).

We then compared organoids from different regions with primary cholangiocytes to explore whether these similarities corresponded to the loss of their original regional identity in vitro (Fig. 2A). Organoids and primary cells after cell cycle regression shared a core transcriptional profile that reflected their common cholangiocyte nature, which was illustrated by their proximity in UMAP space and high PAGA connectivity when compared with different liver cell types, such as stellate cells and LSECs (fig. S7, C and D). However, DEG analyses highlighted down-regulation of region-specific markers such as *SLC13A1* and *SLC26A3* (Fig. 2B and fig. S7G), whereas gene ontology (GO) and gene set enrichment analyses (GSEA) identified these DEGs as factors facilitating the adaptation of cholangiocytes to their respective microenvironments—e.g., bile acid versus culture medium–processing genes (fig. S8, A to C). Furthermore, we confirmed the up-regulation of YAP target genes (data S3) in organoids, in accordance with previous studies (*14*). Therefore, primary cholangiocytes, propagated as organoids, adapted to their new microenvironment by maintaining their core transcriptional signature while losing the expression of markers specific to their region of origin.

To explore the mechanisms controlling cholangiocyte identity, we decided to add bile to our culture conditions as the principal determinant of the cholangiocyte microenvironment. Different organoids (IHD, CBD, and GB) were treated with human gallbladder bile for 72 hours and then characterized using scRNA-seq (Fig. 2A and fig. S1, A to C) (GB, 3815 cells; CBD, 3224 cells; and IHD, 3653 cells). UMAP and PCA revealed that treated organoids assumed a distinct but overlapping gene expression profile (Fig. 2A and fig. S9A), confirming a shared capacity to adapt to exposure to bile. Notably, PAGA and DEG analyses showed that this transcriptional profile was shifted toward a gallbladder identity (Fig. 2B and fig. S9, B and C). To characterize the factors controlling this transition, we interrogated DEGs in bile-treated organoids. GO, GSEA, and UMAP analyses (fig. S9, D to F) confirmed the induction of region-specific markers (SOX17, MUC13, and FGF19; Fig. 2B and fig. S9F) and revealed up-regulation of bile acid receptor pathways and downstream targets (*NR1H4/FXR*, *NR1I2*, *NR0B2*, *SLC51A*, *FGF19*, *ABCA1*, and *PPARG*; Fig. 2B and fig. S9, D to F). Notably, these results were validated through activation and inhibition of the farnesoid X receptor (FXR) using chenodeoxycholic acid and Z-guggulsterone, respectively (Fig. 2, C and D), which confirms that, regardless of their origin, cholangiocytes grown in vitro can respond and adapt to environmental stimuli. Together, these results suggest that cholangiocyte organoids could assume different regional identities when instructed by the appropriate niche factors.

To validate cholangiocyte plasticity and explore its functional implications, we decided to assess whether organoids from one region of the biliary tree could repair a different region after transplantation. For this, we induced cholangiopathy in immunodeficient mice using 4,4′-methylenedianiline (MDA) (*17*) (Fig. 3, A and B, and figs. S10 and S11) and attempted to rescue the phenotype with intraductal delivery (*18*) of human gallbladder organoids expressing red fluorescent protein (RFP). Control animals that received carrier medium without cells lost weight (fig. S10A) and died within 3 weeks (Fig. 3B and table S1), developing cholestasis (fig. S10B) and cholangiopathy demonstrated by immunofluorescence (IF) (fig. S10C), histology (fig. S10D), and magnetic resonance cholangiopancreatography (MRCP) (Fig. 3C; fig. S11, A to C; and movies S1 to S6). By contrast, animals that received organoids were electively culled at the end of the experiment and survived for up to 3 months, with resolution of cholangiopathy and normal serum biochemistry (Fig. 3, B and C; fig. S10, A, B, and D; fig. S11, B and C; and movies S3, S4, and S6). The transplanted gallbladder cholangiocytes engrafted in different size intrahepatic ducts (Fig. 3D; fig. S12, A to C; and movies S7 to S9) corresponding to ~25 to 55% of the regenerated biliary epithelium (fig. S12C), and they assumed an intrahepatic identity by losing gallbladder markers (SOX17) and expressing intrahepatic markers (SOX4, DCDC2, and BICC1) (Fig. 3D and fig. S12, A and B). Core biliary markers (KRT7, KRT19, and CFTR) were also expressed (fig. S12A), whereas we observed YAP activation in both engrafted and native cells (fig. S12, B and E), in accordance with previous studies (*13*). Notably, we never observed expression of other hepatic lineage markers such as albumin, which indicates that cholangiocyte organoid plasticity is likely to be limited to their biliary lineage of origin (fig. S12A). Furthermore, the engrafted cells expressed proliferation markers (fig. S12, B and D) at similar levels to those observed in native mouse cholangiocytes. Abnormal growth or tumor formation was never noticed in any of the analyses performed (Fig. 3, C and D; fig. S10D; and fig. S12, A and B), including the T1-weighted body magnetic resonance imaging (MRI) performed at the end of the experiment (movies S1 and S3). Thus, organoid transplantation appears to provide the healthy cells required to repair the damaged epithelium and rescue acute injury.

To ensure that animal rescue and resolution of cholangiopathy was specific to cholangiocyte organoids, we repeated the experiment using mesenchymal stem cells (MSCs)—a different type of cell known to provide anti-inflammatory effects after transplantation through paracrine signaling (fig. S13, A to C). This experiment allowed us to explore whether organoids are essential for duct regeneration and animal rescue and whether some of the observed effects could be attributed to paracrine signals that are not specific to our cells. MSCs failed to engraft (fig. S13C) and rescue the transplanted animals, which exhibited no difference in survival compared with controls ($P > 0.05$; fig. S13A) and no resolution of cholestasis on serum biochemistry (fig. S13B). Consequently, cholangiopathy resolution is specific to the engraftment of cholangiocyte organoids, and, although additional therapeutic effects of our cells through growth factor and cytokine secretion cannot be completely excluded, these effects are specific to cholangiocyte organoids.

We then explored whether organoid culture is required to unlock the plasticity of the cells or whether this reflects an inherent property of primary cholangiocytes. To achieve this, we transplanted primary gallbladder cholangiocytes (fig. S13, A to C) directly after isolation without in vitro culture. Under these conditions, very few primary cholangiocytes engrafted in the mouse bile ducts (fig. S13C), most likely because of the cumulative stress of isolation and transplantation, and the cholangiocytes failed to rescue the animals or resolve cholestasis (fig. S13, A and B). Nonetheless, the engrafted cells expressed intrahepatic markers and lost expression of gallbladder markers (fig. S13C). Thus, cholangiocyte plasticity is not limited to organoids grown in vitro; however, organoid culture is necessary for the cholangiocytes to recover from the stress of isolation and to achieve the large-scale expansion that provides the cell numbers required for engraftment and biliary repair.

To ensure that our results are not specific to the intrahepatic compartment or gallbladder organoids, we used our established methodology (*3*) to transplant common bile duct–derived cholangiocyte organoids in the gallbladder of immunocompromised mice. The engrafted cells exhibited loss of common bile duct markers and up-regulation of gallbladder markers (fig. S14), which confirms that our previous findings apply to different compartments of the biliary tree and to organoids of different

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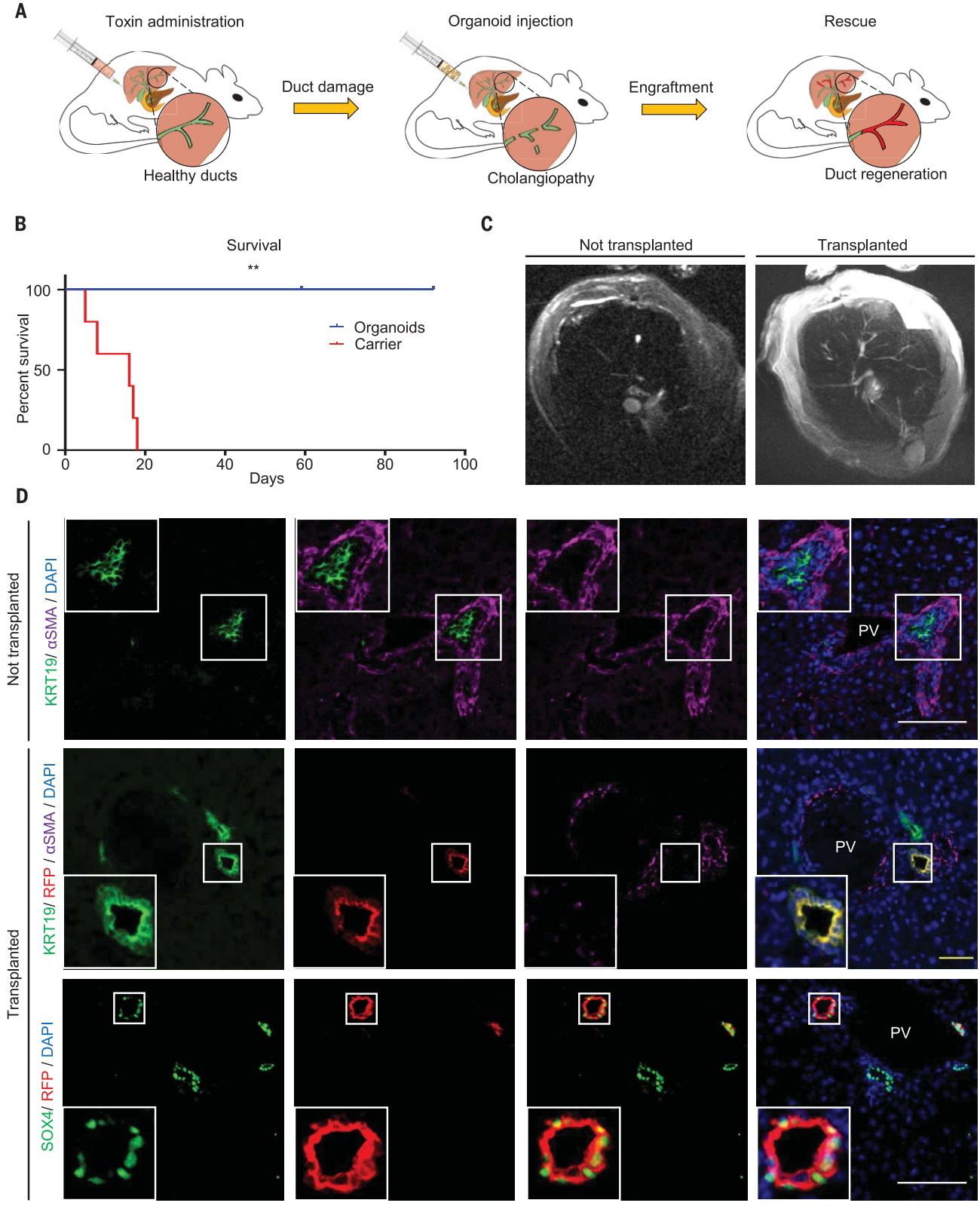


Fig. 3. Cholangiocyte organoids rescue cholangiopathy after transplantation and assume an identity corresponding to the site of engraftment. (**A**) Experimental outline schematic. (**B**) Kaplan-Meier curve (table S1: number of animals at risk) demonstrating animal rescue after gallbladder organoid injection. $^{**}P = 0.0018$; log-rank test. (**C**) MRCP demonstrating rescue of cholangiopathy after organoid injection. (**D**) Immunofluorescence demonstrating the engraftment of RFP-expressing gallbladder organoids in portal triads with the up-regulation of intrahepatic (SOX4) markers. Yellow scale bar, 50 μm; white scale bars, 100 μm. PV, portal vein.

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origin. Taken together, these results establish that cholangiocytes from different regions of the biliary tree are interchangeable, and they suggest that extrahepatic cells can be used to repair acute intrahepatic duct injury.

Cell transplantation experiments in mouse models are extremely useful but are not always predictive of therapeutic outcomes (*19*). Furthermore, the mouse liver microenvironment is different from that of humans, which raises the possibility that our results may not translate between species. To address these challenges, we developed a model for cell-based therapy in humans utilizing ex vivo organ perfusion (*20*). Ex vivo NMP was developed to improve organ preservation and reduce ischaemia-reperfusion injury by circulating warm oxygenated blood through liver grafts before transplantation. Notably, the biliary tree is particularly susceptible to ischaemia, which results in duct damage (*21*, *22*). Low bile pH (<7.5) during NMP is used as a predictor of this type of cholangiopathy (*23*).

To assess the therapeutic potential of our cells for repairing human bile ducts, RFP-expressing gallbladder organoids were injected in the intrahepatic ducts of deceased human transplant donor livers ($n = 3$) with a bile pH <7.5 at the start of the experiment, which signified ischemic duct injury. The organs were

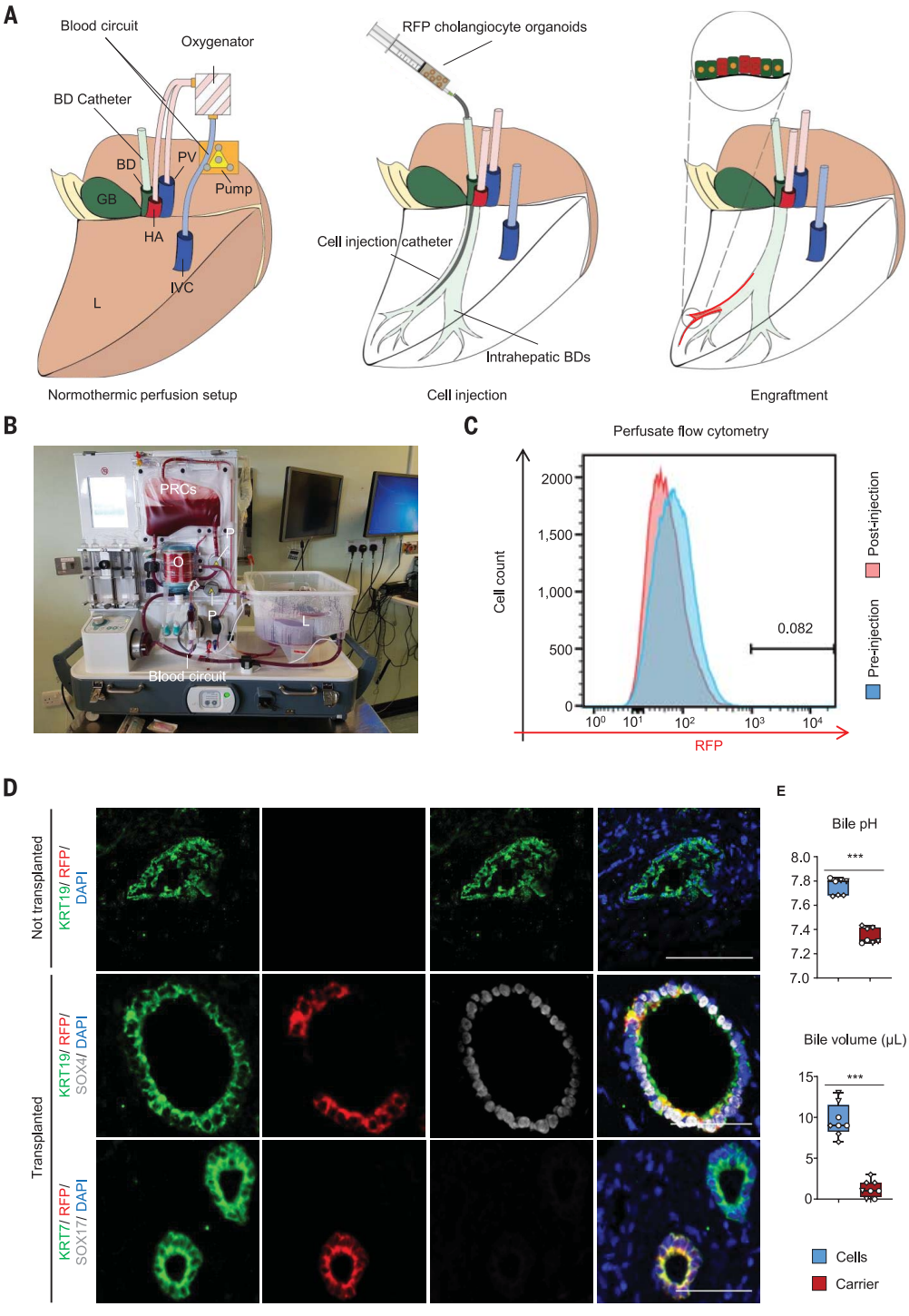


Fig. 4. Cholangiocyte organoids engraft in a human liver receiving NMP and improve bile properties. (**A** and **B**) Schematic representation of the technique for organoid injection (A) and photograph of the NMP circuit used (B). BD, bile duct; HA, hepatic artery; PV, portal vein; IVC, inferior vena cava; L, liver; P, pump; O, oxygenator; PRCs, packed red cells. (**C**) Flow cytometry revealing absence of RFP cells in the perfusate. (**D**) Immunofluorescence revealing the engraftment of RFP gallbladder organoids with upregulation of intrahepatic markers (SOX4) and loss of gallbladder markers (SOX17). Scale bars, 50 µm. (**E**) Organoid injection improves bile pH and choleresis. ***$P < 0.001$. $n = 3$ NMP livers. Each measurement is represented by a different data point, and each organ is represented by a different symbol.

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perfused with oxygenated blood and nutrients at normal body temperature (*20*) (Fig. 4, A and B, and fig. S15A) for up to 100 hours to maintain a near-physiological microenvironment. Notably, the organoids were delivered in a terminal branch of the intrahepatic ducts under fluoroscopic guidance to minimize the area of distribution of the cells and maximize cell density (fig. S15B). At the end of the experiment, ultrasound imaging revealed no evidence of duct dilatation or obstruction (fig. S15C), and RFP-expressing cells were not detected in the perfusate by flow cytometry, which confirms that the injected cells remained in the biliary compartment (Fig. 4C). More importantly, the transplanted organoids engrafted in the intrahepatic biliary tree (Fig. 4D and figs. S15D and S16A), with RFP cells regenerating ~40 to 85% of the injected ducts (fig. S16B) and expressing key biliary markers (KRT7, KRT19, CFTR, GGT). Furthermore, engrafted gallbladder organoids exhibited loss of gallbladder markers (SOX17) and up-regulation of intrahepatic markers (SOX4, BICC1, DCDC2) without differentiation to other hepatic lineages (Fig. 4D; fig. S15D; and fig. S16A). Thus, at the end of the experiment, the injected ducts consisted of a mixture of native and transplanted cholangiocytes (fig. S16, A and B), with multiple transition points between donor and recipient cells and no evidence of cholangiopathy (Fig. 4D, fig. S15D, and fig. S16A).

Conversely, control ducts not receiving cells demonstrated evidence of ischemic injury with loss of epithelial continuity and sloughing of cells in the duct lumen (Fig. 4D). We subsequently characterized the impact of engraftment on organ function. Physiologically, cholangiocytes modify the composition and pH of bile through water transfer and bicarbonate secretion (*6*). Therefore, we compared the bile from organoid-injected versus carrier-injected ducts. Accordingly, bile aspirated from ducts injected with cells exhibited higher pH and volumes (Fig. 4E), which confirms that transplanted cholangiocytes retain their function to modify bile composition. Together, these results provide the first proof of principle that perfused organs can be used to ascertain functional engraftment of human cells, and they validate our mouse data by showing that cholangiocytes are interchangeable for transplantation in human organs.

Our results show that the biliary epithelium is composed of cholangiocytes with diverse transcriptional profiles that are determined by their local environments. This diversity is lost in organoid culture because of the lack of niche stimuli. However, organoids can adapt appropriately to local environmental cues both in vitro and after transplantation, restore the expression of region-specific markers, and assume different regional identities. Thus, organoids from a single region could potentially repair the entirety of the biliary tree. This plasticity could have substantial implications for regenerative medicine. Although autologous cell-based therapy potentially avoids the need for immunosuppression, its application for primary organoids is limited by the impact of disease on the epithelium. However, cholangiopathies belong to a family of localizing diseases that predominantly affect specific regions of an organ (*24*). Consequently, our results provide proof of concept that cholangiocytes from spared regions, such as the gallbladder, could be used for autologous cell-based therapy to repair human intrahepatic bile ducts, which constitute the most common site of injury in cholangiopathies.

Our model for cell engraftment in human perfused organs paves the way for the use of ex vivo cell-based therapy to improve graft function before transplantation, which could ultimately increase the number of useable organs and reduce pressure on the transplant waiting list. In this context, quality-controlled and readily available allogeneic cholangiocyte organoids from a cell bank could be used routinely in the future to prevent ischemic cholangiopathy in organs at risk of biliary injury (e.g., low bile pH), because the organ recipients will receive immunosuppression as part of their standard care. Our results provide proof of principle for the transplantation of organoids in human organs, which could expedite regulatory approval and fast-track first-in-human clinical trials. Ultimately, the same approach could also be applied to a variety of ex vivo perfused organs and cell types to validate functional cell engraftment, demonstrate safety, improve cell transplantation technique and efficacy, and accelerate the clinical translation of newly developed cell-based therapies.

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ACKNOWLEDGMENTS

The authors would like to thank A. Petrunkina and the NIHR Cambridge BRC Cell Phenotyping Hub for their help with flow cytometry and cell sorting; K. Burling and the MRC MDU Mouse Biochemistry Laboratory [MRC_MC_UU_12012/5] for processing mouse serum samples; the Cambridge Biorepository for Translational Medicine for the provision of human tissue used in the study; P. Madrigal and C. Miau for bioinformatic support; C. Costa for his help with the NMP experiments; K. Reid and R. Clarke for their help with radiological imaging during the NMP experiments; and P. Humphreys, D. Clements, and S. McCallum for their help with confocal imaging. The monoclonal antibody TROMA-III developed by R. Kemler was obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology (Iowa City, IA). **Funding:** F.S. was supported by an NIHR Clinical Lectureship, the Academy of Medical Sciences Starter Grant for Clinical Lecturers, the Addenbrooke's Charitable Trust, and the Rosetrees Trust. T.B. was supported an EASL Juan Rodes fellowship. The L.V. laboratory is funded by the ERC advanced grant New-Chol, the Cambridge University Hospitals NIHR Biomedical Research Centre, and the core support grant from the Wellcome Trust and Medical Research Council of the Wellcome–MRC Cambridge Stem Cell Institute. L.G. and S.Si. were supported by a BHF Senior Research Fellowship (FS/18/46/33663). This research was supported by the NIHR Cambridge Biomedical Research Centre (BRC-1215-20014). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care. **Author contributions:** F.S. conceived and designed the study; performed experiments; acquired, interpreted, and analyzed the data; developed and validated the protocols described; performed bioinformatic analysis; generated the figures; and wrote and edited the manuscript. D.M. performed bioinformatics analysis. O.C.T. contributed to cell culture, harvesting and processing of tissue, scRNA-seq experiments, and animal experiments. T.E.B. contributed to animal experiments. S.Sa. performed the MRI experiments. E.M.G. performed the three-dimensional (3D) reconstruction of the mouse biliary tree from MRI images. E.M.G. and S.S.U. reviewed and reported the MRI images. T.B. contributed to tissue culture, QPCR, immunofluorescence, tissue clarification, and wholemount staining experiments. B.T.W. contributed to tissue dissociation and scRNA-seq experiments. J.G.-B. contributed to scRNA-seq experiments. K.M. contributed to primary tissue harvesting tissue. G.Ca. contributed to scRNA-seq experiments, flow cytometry, and magnetic-associated cell sorting. R.G. contributed to animal experiments, IF, and tissue histology. N.L.B. contributed to animal experiments. V.L.M. contributed to harvesting primary tissue. K.C., C.F., S.R., and L.S. contributed to ex vivo NMP experiments. J.B. contributed to imaging. L.G. contributed to tissue clarification and wholemount staining experiments as well as critical review of these data. D.O. and A.O. contributed to flow cytometry analyses and contributed to critical revision of the manuscript. P.H. contributed to 3D reconstruction and rendering of immunofluorescence images. S.E.B. contributed to experimental design and critical revision of the manuscript. G.Co. provided primary human samples. S.E.D. reviewed and reported the histology images and contributed to critical revision of the manuscript. I.A. contributed to bile aspiration using microdialysis catheters. A.J.B. and C.J.E.W. contributed to NMP experiments and critical revision of the manuscript. T.C.S. performed fluoroscopic cannulation of peripheral ducts in NMP experiments and contributed to critical revision of the manuscript. M.P.M., W.T.H.G., G.F.M., S.Si., S.A.T., P.G., and E.M. contributed to critical revision of the manuscript. K.S.-P. provided primary tissue, performed animal and ex vivo NMP experiments, contributed to the design and concept of the study, interpreted the data, and edited the manuscript. L.V. designed and conceived the study, interpreted the data, and wrote and edited the manuscript. All of the authors approved the manuscript. **Competing interests:** F.S., K.S.-P., and L.V. are founders and shareholders of BILITECH. L.V. is a founder and shareholder of DEFINIGEN. K.S.-P., F.S., and L.V. are inventors on the patent

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applications GB/19.96.17 and GBA 201709704 held by the University of Cambridge that cover the derivation and use of cholangiocyte organoids for regenerative medicine. The remaining authors have no competing interests to disclose. **Data and materials availability:** All data are available in the main text or the supplementary materials. scRNA-seq data are available on ArrayExpress (accession no. E-MTAB-8495). Cholangiocyte organoids described in this publication are available from University of Cambridge under a material transfer agreement with the university.

SUPPLEMENTARY MATERIALS

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10 November 2019; resubmitted 1 May 2020
Accepted 9 December 2020
10.1126/science.aaz6964

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Science

Cholangiocyte organoids can repair bile ducts after transplantation in the human liver

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Science, 371 (6531), • DOI: 10.1126/science.aaz6964

Organoids regenerate human bile ducts

Bile ducts carry bile from the liver and gall bladder to the small intestine, where it aids digestion. Cholangiocytes are epithelial cells that line bile ducts and modify bile as its transported through the biliary tree. Chronic liver diseases involving cholangiocytes account for a large fraction of liver failure and the need for liver transplantation. Because liver donors are in short supply, Sampaziotis *et al.* used organoid technology to develop a cell-based therapy using human tissue (see the Perspective by Kurial and Willenbring). Cholangiocyte organoids were transplanted into the intrahepatic ducts of deceased human donor livers undergoing ex vivo normothermic perfusion. The livers could be maintained for up to 100 hours, and the transplanted organoids engrafted, exhibited function, and could repair bile ducts.

Science, this issue p. 839; see also p. 786

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