View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Apollo

Advances in the generation of bioengineered bile ducts

Alexander W. Justin[a], Kourosh Saeb-Parsy[b], Athina E. Markaki[a], Ludovic Vallier[c,d,e], and Fotios Sampaziotis[b,c,e,].

[a] Department of Engineering, University of Cambridge, Cambridge, UK.

[b] Department of Surgery, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, UK.

[c] Wellcome Trust–Medical Research Council Stem Cell Institute, Cambridge Stem Cell Institute, Anne McLaren Laboratory, University of Cambridge, Cambridge, UK.

[d] Wellcome Trust Sanger Institute, Hinxton, UK.

[e] Department of Hepatology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK.

Correspondence: Fotios Sampaziotis, Laboratory for Regenerative Medicine, West Forvie Building, Robinson Way, University of Cambridge. Cambridge CB2 0SZ, United Kingdom.
Telephone: +44 (0)1223 747489; E-mail: fs347@cam.ac.uk

Alexander W Justin, Department of Engineering, University of Cambridge, Trumpington Street, Cambridge CB2 1PZ, United Kingdom.
Telephone: +44 (0)7732 372611; Email: awj27@cam.ac.uk

Acknowledgements: AWJ gratefully acknowledges support from EPSRC (EP/R511675/1). LV has been supported by the ERC starting grant Relive-IMDs and the ERC advanced grant New-Chol. FS gratefully acknowledges support by the Cambridge Biomedical Research Centre, Addenbrooke's Charitable Trust (ACT), Sparks and the Medical Research Council (MRC).

Key Words

Bile Duct, Tissue Engineering, Bioengineering, Cholangiopathy

Abstract

The generation of bioengineered biliary tissue could contribute to the management of some of the most impactful cholangiopathies associated with liver transplantation, such as biliary atresia or ischemic cholangiopathy. Recent advances in tissue engineering and *in vitro* cholangiocyte culture have made the achievement of this goal possible. Here we provide an overview of these developments and review the progress towards the generation and transplantation of bioengineered bile ducts.

1. Introduction

Bile duct disease accounts for approximately one third of adult and 70% of pediatric liver transplants [1], [2]. Several of these disorders are anatomically diffuse and affect the small branches of the intrahepatic bile ducts that are not amenable to surgical replacement or reconstruction. However, some of the most impactful cholangiopathies that require, or are a consequence of, liver transplantation can be limited to the large ducts of the extrahepatic biliary tree. These include biliary atresia, which constitutes the leading cause for pediatric liver transplantation [1], [3] and dominant ischemic strictures, which after rejection, represent one of the most common causes of liver transplant failure [4]. Surgical replacement of the affected bile ducts is a potentially effective treatment, but is currently hampered by the lack of suitable healthy tissue. Portoenterostomy, which entails using a length of small intestine as a conduit to enable bile flow from the liver to the gut, can be used as an alternative treatment. Portoenterostomy, however, is associated with complications such as reflux cholangitis and stricture formation [5]. Furthermore, it is not curative for the majority of the patients with biliary atresia, who will proceed to need liver transplantation later in life [6].

Tissue engineering could address the lack of primary tissue by combining cells, materials and growth stimulating signals [7] to generate bioengineered bile ducts. Indeed, biliary tissue generated *in vitro* could provide a viable alternative for the management of common bile duct (CBD) disorders and potentially reduce the need for organ transplantation. However, despite the significant progress in tissue engineering for many other organs over the last decade, the generation of functional bile ducts *in vitro* has been hindered by a number of challenges. More specifically, until recently there was a lack of robust culture systems for growing biliary epithelial cells, which constitute the main functional cell population in the bile duct [8], [9]. Furthermore, the biliary system is very sensitive to ischemia necessitating the development of fully vascularized constructs to ensure adequate supply of nutrients and oxygen [4]. Over the last few years, there have been several breakthroughs in these fields leading to the first studies of functional bioengineered bile duct transplantation. Here we provide a summary of

these developments and review the progress towards the generation and transplantation of bioengineered bile ducts.

2. Cell types

2.1 Cholangiocytes

The primary function of the bile duct is unobstructed transport of bile, which is a toxic fluid, from the liver to the intestinal lumen [10], [11]. Cholangiocytes form an epithelial monolayer lining the lumen of the bile duct (Figure 1). They are responsible for providing a barrier against the toxic effects of bile on other cells within the duct, transferring water, electrolytes and bile acids and modifying the composition of bile [10]. Because this a pivotal role for the function and integrity of the biliary tree, cholangiocytes are crucial for the generation of bioengineered bile ducts.

Historically the biliary epithelium has proven difficult to access and *in vitro* propagation of cholangiocytes has remained challenging. To overcome these issues multiple groups have used human Induced Pluripotent Stem Cells (hIPSCs). hIPSCs can be easily derived from multiple readily-accessible tissues such as skin or peripheral blood lymphocytes and differentiated into almost any somatic cell type [12] including the biliary epithelium [13]–[17]. The resulting cholangiocytes express key biliary markers [13]–[16] and sustain functional properties of their *in vivo* counterparts [13]–[16]. Furthermore they can be genetically manipulated to generate patient lines in which genetic defects have been corrected [13], thereby providing a source of healthy autologous biliary epithelium. However, hIPSC-derived cholangiocytes also have limitations: The resulting cells are not fully mature but retain fetal characteristics [13]–[16] and successful orthotopic transplantation and repopulation of the biliary tree has not been demonstrated so far [13]–[16]. Moreover, the biliary epithelium generated corresponds to intrahepatic cholangiocytes [13]–[16] and generation of cholangiocytes lining the lumen of the extrahepatic bile ducts has not been reported.

To address these challenges, an organoid culture system has been developed to enable successful *in vitro* propagation of primary cholangiocytes derived from the CBD or the

gallbladder [18]. The resulting organoids sustain the function and genetic profile of extrahepatic cholangiocytes, survive *in vivo*, self-organize into tubular structures and can be used to reconstruct the biliary epithelium following transplantation [18]. However, some limitations still exist. In depth studies are required to elucidate the extent to which removing these cells from their niche and propagating them *in vitro* impacts on their properties. Furthermore, genetic modification of organoids is possible but remains more complicated compared to cells grown in monolayers. Finally, access is more limited compared to hIPSCs rendering the generation of autologous lines more difficult, although the use of gallbladder tissue, accessed by surgical cholecystectomy, could resolve some of these issues.

2.2 Other cell types

The biliary epithelium is supported by a layer of connective tissue [19], containing fibroblasts and elastic fibers (Figure 1). The distal third of the CBD is also surrounded by a sheet of smooth muscle [9] (Figure 1). Although these 'supportive' cell types do not demonstrate properties specific to the biliary tree, they are important for the structural integrity and nourishment of the bile duct and may contribute towards the mechanical properties for bio-engineered constructs.

3. Materials

The generation of bioengineered bile ducts requires incorporation of the cell types described above into suitable materials that can be fabricated into a tubular structure. In tissue engineering, a large variety of synthetic and biological materials can be used to produce scaffolds that are capable of maintaining a population of cells *in vitro* or *in vivo* [20]. The choice of appropriate material(s) for generating a suitable tubular matrix requires balancing a number of parameters. For a given tube radius and wall thickness, the elastic and plastic properties of the tube wall material are important for supporting the cells and maintaining an unobstructed lumen for drainage of bile. The biocompatibility of the scaffold is not only essential for the viability of the cells in the tube [21], but also to prevent, or at least minimize, the inflammatory response that is inherently associated with surgery and transplantation of tissues [22]. Control

of this inflammatory response is particularly critical for bile ducts, as it can otherwise lead to fibro-inflammatory strictures and ultimately occlusion of the lumen [23], [24]. Finally, a material which is bioresorbable is desirable since it permits eventual remodeling and replacement of the scaffold with native cells and tissue [25].

As expected, most materials fail to meet all the necessary requirements outlined above. Synthetic polymers, for example, whilst mechanically robust and easily processed into three-dimensional (3D) structures [26], often lack the necessary biocompatibility and resorbability [25]. Nonetheless, synthetic polymers are used to form uniaxial tubular structures (particularly in the field of vascular grafts) including materials such as polyglycolic acid (PGA), polylactic acid (PLA), polyurethane, poly(ε-caprolactone) (PCL) [27], expanded polytetrafluoroethylene (PTFE) [28], polyhydroxyalkanoates (PHA) and polyhydroxybutyrate (PHB) [29]. These synthetic polymer scaffolds are processed into a range of structures, demonstrate varying degrees of biocompatibility, and have degradation periods which can be tuned from a few weeks to years [29]. Biological materials, in comparison, are superior for cellular activity and their ability to be remodeled by cellular processes. However, their use can be constrained by their mechanical properties [26] and processing requirements (such as denaturation of proteins by high temperatures) [30], [31]. Some examples of tubular scaffolds fabricated from such biological polymers include collagen [18], collagen-agarose [32], and collagen membranes [33]. Hybrid scaffolds, such as tubes made from a polypropylene mesh and collagen sponge have also been produced [34].

4. Fabrication Techniques

There are a number of possible methods to fabricate the materials described earlier into bioengineered patent tubular bile ducts. These include rolling of a polymeric sheet, molding, 3D printing techniques, electrospinning, freeze-drying, and cellular self-assembly approaches. The simplest method for fabricating a uniaxial tube is to roll a polymeric sheet and suture along its length. While making large-scale tubes is relatively simple using this approach, use of suture material, even if absorbable, along the length of the native bile duct can lead to fibrosis

and strictures; portoenterostomy biliary reconstruction thus remains the current optimal treatment for iatrogenic bile duct injury [35][36].

Polymers can be molded to form a variety of structures, including tubes [37]. This method has the key advantage that, at least in the case of biological polymers, cells can be seeded into the precursor polymer solution providing a method for incorporating cells inside a dense scaffold [38], [39]. Alternatively, many polymers can be 3D printed, wherein a structure is constructed layer-by-layer, via a number of different techniques [40]–[42]. This enables complex architectures with very fine and reproducible features to be fabricated. However, we note that 3D printing includes a wide range of different approaches and there are particular challenges associated with each of these additive techniques [40], [41]. For example, extrusion-based 3D printing struggles in producing overhanging features and small-diameter tubes can collapse during fabrication [43].

Electrospinning operates by drawing out a narrow polymeric jet electrostatically [44], [45]. The solvent containing the polymer evaporates en route and a solid polymeric fiber is deposited on rotating collector, which can be used to form tubular structures consisting of a dense fibrous mesh [44]. This yields a mechanically robust tube, although the walls of the tube consist of a high density of polymer, making cellular infiltration difficult [45].

Freeze-drying involves freezing and dehydrating a polymer solution to produce a macro-porous sponge architecture [46][26][33]. This allows seeded cells to penetrate deep inside the scaffold and be maintained in a bioreactor, which is advantageous when forming structured tissue. However, the high porosity of the scaffold makes them difficult to use as a conduit for fluid such as bile. Indeed, in the context of biliary reconstruction, bile leakage through the wall of porous scaffolds leads to biliary peritonitis [18]. Nevertheless, culturing the scaffolds *in vitro* can allow cells to fill the pores within the matrix material to form a more robust and impermeable tubular structure [47]. One notable method involves rolling a polyester felt sheet into a tube between two concentric cylinders and filling the spacing with polyglycolic acid (PGA) or polyL-lactic acid (PLLA) [47]. Using freeze-drying techniques, the solvent is then removed and the scaffold seeded with cells, which can penetrate deep into the scaffold [47].

Finally, it is possible to produce tubular structures with self-assembled cell sheets, bioprinting, and other scaffold-free approaches in which cells are deposited directly to build a 3D structure [48], [49]. However, these generally produce structures that are mechanically very weak and require significant culture *in vitro* if to be used as a replacement conduit or vessel [26], [48]. Importantly, for most materials and fabrication processes described, cells cannot be loaded into the construct during the fabrication process since the processing environment is typically too harsh and results in loss of cell viability (e.g. due to toxic chemicals, organic solvents, acidity or high temperature). Cells are therefore usually loaded after processing of the scaffold is complete via *in vitro* culturing techniques, such as with a bioreactor system, or by surgically implanting the scaffold as an acellular construct and allowing for migration of native cells and blood vessels.

5. Vascular supply

Bile ducts are very sensitive to ischemia [19], [50]–[52]. Indeed, inadequate blood supply through branches of the hepatic artery and peribiliary vascular plexus results in ischemic cholangiopathy, which constitutes one of the most common complications following liver transplantation [4], [52]. Consequently, the supply of oxygen and nutrients through an adequately vascularized stroma is essential for the long term survival of bioengineered bile ducts.

However, the generation of vascular networks remains a key outstanding challenge in tissue engineering for the fabrication of thick tissue constructs and tissues with thickness greater than 400 μm require conduits for the delivery of metabolites and the removal of waste products [37]. Without such a system, highly populated cellularized constructs maintain inadequate metabolic activity, can form necrotic regions, and are limited in functionality.

To address this challenge, multiple vascularization methods have been devised and can be classified in two main categories: Cellular co-culture systems and vascular network formation by materials processing. While the generation of vascular networks is outside the scope of this review, the key approaches and challenges are summarized below.

In cellular co-culture systems, capillary networks are formed through seeding of endothelial and supporting cell types onto [53] or inside [54] a hydrogel construct. Prior to *in vivo* implantation, cells can be induced to form capillary-like structures [55] which, when the construct is surgically implanted, integrate into the native vasculature and can permit perfusion with blood [56]. A tissue construct that has been 'pre-vascularized' to form capillary-sized vessels is advantageous over an acellular construct, as the interconnecting network will rapidly integrate with the host vasculature and adapt dynamically to the metabolic requirements of the tissue. However, co-culture systems such as this require biologically-active polymers, such as collagen and fibrin, usually in low concentrations and with limited crosslinking, which limits the mechanical strength of the scaffold. Furthermore, incorporation of other signaling factors, such as VEGF and bFGF, may also be required in order to form vessels. This greatly limits the choice of materials that can be used for the replacement bile duct. Importantly, capillary networks cannot be surgically anastomosed to existing vessels and therefore require some time to form connections with the native vasculature.

Vascular network formation via processing of biomaterials is an active field of research [43] that utilizes a variety of methods including needle molding [57], soft lithography [58], and a range of 3D printing-based approaches [39], [59]–[61]. This potentially enables large cell populations to be maintained in a metabolically active state in the scaffold from the outset, aiding cellular remodeling of the scaffold and cell survival.

6. Advances in the generation of bioengineered bile ducts

The advances described above have set the foundation for the generation and transplantation of the first bioengineered bile ducts. So far, 3 different approaches for the development of engineered bile ducts have been described (Table 1); the generation of acellular tubular constructs, the generation of bioengineered tubes populated by bone marrow cells (BMCs), and the generation of functional bioengineered tubes populated by human cholangiocytes [18], [27], [32], [62]–[69].

6.1. Acellular constructs

The challenges in culturing cholangiocytes until recently have led to the use of multiple acellular constructs for bile duct repair or replacement [27], [32], [62]–[69]. More specifically, collagen, small intestinal submucosa, and human amnion combined with a polyglycolic acid (PGA) mesh have been used as bio-degradable scaffolds to successfully repair CBD wall defects [33], [65], [67], [68]. In all cases the scaffolds were completely absorbed and replaced by a healthy, epithelized and vascularized wall indistinguishable from the native CBD [33], [65], [67], [68]. These studies paved the way and provided invaluable information for the generation of tubular constructs; however the clinical applications of CBD patches remain somewhat limited mainly to the repair of iatrogenic CBD wall defects.

To address CBD strictures or obliterating disorders such as biliary atresia, acellular tubular constructs were developed using multiple materials [32], [33], [62], [63], [65]–[67], [69]. Some of the first studies used scaffolds based on human tissue such as amnion and vein grafts [66][67]. These initial attempts were complicated by bile leak and strictures [66], [67], failing to provide adequate CBD drainage in the absence of a biliary stent [66]. However, subsequent attempts using PGA, PCL and PLA, and collagen tubes coated with agarose gel in dogs, pig and guinea pig models have been more successful [27], [32], [62], [63]. These studies demonstrated re-absorption of the biodegradable scaffolds and replacement by a vascularized and epithelized CBD wall, almost indistinguishable from the animal's native CBD [66], [67]. However, these approaches also have limitations. The use of PGA scaffolds resulted in foreign body reaction early on, which was resolved by 8 months; while 55% of the animals were complicated by bile leak, cholangitis or biliary obstruction [63]. Cholangiography revealed CBD dilatation in the surviving animals but no stricture or abnormalities in liver function [63]. The use of collagen tubes resulted in epithelized tubes but with lower expression of biliary markers compared to the native CBD cholangiocytes [32], [62]. PCL/PLA tubes were associated with thickening of the neo-CBD connective tissue at 6 months [27]. Furthermore, the surgical approach used was the equivalent of a choledochoduodenostomy with the distal end of the PCL/PLA construct anastomosed to the duodenum rather than an end-to-end anastomosis to the distal CBD and the presence or absence of biliary tree dilatation was not assessed with

cholangiography [27]. Despite these limitations, the use of PCL/PLA is associated with the best outcomes described for an acellular construct. Finally, Polytetrafluoroethylene (PTFE) vascular grafts have also been used [69]; however, these resulted in asymptomatic bile duct dilatation, the animals were followed up for 8 days and no histological analyses of the grafts was performed [69].

6.2. BMC-populated constructs

Despite the promising results from the use of acellular tubes in animals, these constructs are not functional at the time of transplantation and it is possible that they might not be as readily populated by human cholangiocytes due to intra-species variation between pig and human. More importantly, all the studies using acellular constructs which were outlined in the previous section were performed in healthy animals. However, it is not clear if re-epithelization with native cholangiocytes can take place as rapidly or effectively following transplantation of an engineered conduit into a diseased/pathological niche. Consequently, while the incorporation of cholangiocytes into the scaffold may seem unnecessary in normal animals, this may not be the case in diseased states.

To address this challenge, PCL/PLA tubes seeded with Bone Marrow Cells (BMCs) were transplanted in pigs anticipating differentiation of the BMCs into cholangiocytes [27]. The BMC populated tubes were compared to acellular transplanted PCL/PLA constructs. However, no difference in animal survival, liver function or histology was observed between the two groups [27]; while survival and differentiation of BMCs into cholangiocytes was not demonstrated [27].

6.3. Constructs populated with human cholangiocytes

More recently, densified collagen tubes populated with primary extrahepatic cholangiocyte organoids (ECOs) were used to generate functional bio-engineered bile ducts *in vitro* exhibiting GGT and ALP activity [18]. These tubes were subsequently transplanted into immune compromised mice using end-to-end anastomosis and replaced the native CBD of these animals [18]. Following transplantation, the lumen of the constructs remained populated by human cholangiocytes retaining the expression of structural (CK7) and functional (CFTR) biliary markers, as well as GGT and ALP activity, while the patency of the biliary tree was

confirmed using Magnetic Resonance Cholangiopancreatography (MRCP) and cholangiogram [18]. Considered collectively, these studies suggest that the transplantation of cholangiocyte-populated tubular scaffolds could represent an ideal therapeutic approach for cholangiopathies characterized by defects in bile duct formation and regeneration, such as biliary atresia.

7. Future directions and conclusion

Despite significant recent advances in the field of bile duct engineering, several challenges remain. There is a need to address whether cholangiocytes are required for the generation of functional human bile duct constructs or if the use of acellular constructs to repair or replace diseased, damaged or absent bile ducts could have equally good outcomes through spontaneous cellularization and vascularization *in vivo*. However, due to intra-species variation and differences in the potential for biliary regeneration between healthy and disease state, it may be difficult to address this question definitely without robust human clinical trials or transplantation of acellular constructs in animal models of bile duct injury. An additional requirement and outstanding challenge for translation to human studies is the need to use Good-Manufacturing-Practice (GMP) materials and cells. Furthermore, the generation of human-sized cellularized bile ducts may require the development of pre-vascularized constructs to ensure delivery of oxygen and nutrients to the cholangiocytes and other cell populations. Finally an alternative to the generation of engineered tubular constructs, could entail repopulating decellularized bile ducts with human cholangiocytes and this approach has been used with very good results for the repopulation of decellularized human liver scaffolds [70], [71].

In conclusion, there is a pressing clinical need for the development of bio-engineered bile ducts and recent studies have demonstrated proof-of-principle for the feasibility of achieving this goal. Current advances in regenerative medicine, cell culture systems, materials, and fabrication methods provide a unique set of resources for overcoming the remaining challenges.

References

[1] K. F. Murray and R. L. Carithers, “AASLD practice guidelines: evaluation of the patient for liver transplantation,” *Hepatology*, vol. 41, no. 6, pp. 1407–1432, 2005.

[2] J. A. Leithead and J. W. Ferguson, “Chronic kidney disease after liver transplantation,” *J. Hepatol.*, vol. 62, no. 1, pp. 243–244, 2015.

[3] A. Asai, A. Miethke, and J. A. Bezerra, “Pathogenesis of biliary atresia: defining biology to understand clinical phenotypes,” *Nat. Rev. Gastroenterol. Hepatol.*, vol. 12, no. 6, pp. 342–352, 2015.

[4] A. I. Skaro, C. L. Jay, T. B. Baker, E. Wang, S. Pasricha, V. Lyuksemburg, J. A. Martin, J. M. Feinglass, L. B. Preczewski, and M. M. Abecassis, “The impact of ischemic cholangiopathy in liver transplantation using donors after cardiac death: the untold story,” *Surgery*, vol. 146, no. 4, pp. 543–553, 2009.

[5] R. M. Walsh, J. M. Henderson, D. P. Vogt, and N. Brown, “Long-term outcome of biliary reconstruction for bile duct injuries from laparoscopic cholecystectomies,” *Surgery*, vol. 142, no. 4, pp. 450–457, 2007.

[6] A. Gallo and C. O. Esquivel, “Current options for management of biliary atresia,” *Pediatr. Transplant.*, vol. 17, no. 2, pp. 95–98, 2013.

[7] B. P. Chan and K. W. Leong, “Scaffolding in tissue engineering: general approaches and tissue-specific considerations,” *Eur. spine J.*, vol. 17, no. 4, pp. 467–479, 2008.

[8] M. E. Sutton, S. Dries, M. H. Koster, T. Lisman, A. S. H. Gouw, and R. J. Porte, “Regeneration of human extrahepatic biliary epithelium: the peribiliary glands as progenitor cell compartment,” *Liver Int.*, vol. 32, no. 4, pp. 554–559, 2012.

[9] S.-M. Hong, G. H. Kang, H. Y. Lee, and J. Y. Ro, “Smooth muscle distribution in the extrahepatic bile duct: histologic and immunohistochemical studies of 122 cases,” *Am. J. Surg. Pathol.*, vol. 24, no. 5, pp. 660–667, 2000.

[10] X. Xia, H. Francis, S. Glaser, G. Alpini, and G. LeSage, “Bile acid interactions with cholangiocytes,” *World J. Gastroenterol. WJG*, vol. 12, no. 22, p. 3553, 2006.

[11] P. Fickert, A. Fuchsbichler, M. Wagner, G. Zollner, A. Kaser, H. Tilg, R. Krause, F.

Lammert, C. Langner, K. Zatloukal, and others, “Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice,” *Gastroenterology*, vol. 127, no. 1, pp. 261–274, 2004.

[12] F. Sampaziotis, C.-P. Segeritz, and L. Vallier, “Potential of human induced pluripotent stem cells in studies of liver disease,” *Hepatology*, vol. 62, no. 1, pp. 303–311, 2015.

[13] F. Sampaziotis, M. Cardoso de Brito, P. Madrigal, A. Bertero, K. Saeb-Parsy, F. a C. Soares, E. Schrumpf, E. Melum, T. H. Karlsen, J. A. Bradley, W. T. H. Gelson, S. Davies, A. Baker, A. Kaser, G. J. Alexander, N. R. F. Hannan, and L. Vallier, “Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation.,” *Nat. Biotechnol.*, vol. 33, no. 8, pp. 845–852, 2015.

[14] F. Sampaziotis, M. C. de Brito, I. Geti, A. Bertero, N. R. F. Hannan, and L. Vallier, “Directed differentiation of human induced pluripotent stem cells into functional cholangiocyte-like cells,” *Nat. Protoc.*, vol. 12, no. 4, p. 814, 2017.

[15] M. Ogawa, S. Ogawa, C. E. Bear, S. Ahmadi, S. Chin, B. Li, M. Grompe, G. Keller, B. M. Kamath, and A. Ghanekar, “Directed differentiation of cholangiocytes from human pluripotent stem cells,” *Nat. Biotechnol.*, vol. 33, no. 8, pp. 853–861, 2015.

[16] N. Dianat, H. Dubois-Pot-Schneider, C. Steichen, C. Desterke, P. Leclerc, A. Raveux, L. Combettes, A. Weber, A. Corlu, and A. Dubart-Kupperschmitt, “Generation of functional cholangiocyte-like cells from human pluripotent stem cells and HepaRG cells,” *Hepatology*, vol. 60, no. 2, pp. 700–714, 2014.

[17] J. H. Tabibian, C. E. Trussoni, S. P. O’Hara, P. L. Splinter, J. K. Heimbach, and N. F. LaRusso, “Characterization of cultured cholangiocytes isolated from livers of patients with primary sclerosing cholangitis,” *Lab. Invest.*, vol. 94, no. 10, p. 1126, 2014.

[18] F. Sampaziotis, A. W. Justin, O. C. Tysoe, S. Sawiak, E. M. Godfrey, S. S. Upponi, R. L. Gieseck, M. C. de Brito, N. L. Berntsen, M. J. Gómez-Vázquez, D. Ortmann, L. Yiangou, A. Ross, J. Bargehr, A. Bertero, M. C. F. Zonneveld, M. T. Pedersen, M. Pawlowski, L. Valestrand, P. Madrigal, N. Georgakopoulos, N. Pirmadjid, G. M. Skeldon, J. Casey, W. Shu, P. M. Materek, K. E. Snijders, S. E. Brown, C. A.

Rimland, I. Simonic, S. E. Davies, K. B. Jensen, M. Zilbauer, W. T. H. Gelson, G. J. Alexander, S. Sinha, N. R. F. Hannan, T. A. Wynn, T. H. Karlsen, E. Melum, A. E. Markaki, K. Saeb-Parsy, and L. Vallier, “Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids,” *Nat. Med.*, vol. 23, no. 8, pp. 954–963, Jul. 2017.

[19] M. Strazzabosco and L. Fabris, “Functional anatomy of normal bile ducts,” *Anat. Rec.*, vol. 291, no. 6, pp. 653–660, 2008.

[20] J. A. Hunt, R. Chen, T. van Veen, and N. Bryan, “Hydrogels for tissue engineering and regenerative medicine,” *J. Mater. Chem. B*, vol. 2, no. 33, pp. 5319–5338, 2014.

[21] B.-S. Kim and D. J. Mooney, “Development of biocompatible synthetic extracellular matrices for tissue engineering,” *Trends Biotechnol.*, vol. 16, no. 5, pp. 224–230, 1998.

[22] G. Chan and D. J. Mooney, “New materials for tissue engineering: towards greater control over the biological response,” *Trends Biotechnol.*, vol. 26, no. 7, pp. 382–392, 2008.

[23] A. D. Singhi and A. Slivka, “Evaluation of indeterminate biliary strictures: Is it time to FISH or cut bait?,” *Gastrointest. Endosc.*, vol. 83, no. 6, pp. 1236–1238, 2016.

[24] F. Sampaziotis, J. Elias, W. T. H. Gelson, A. E. Gimson, W. J. H. Griffiths, J. Woodward, M. Shariff, B. Macfarlane, A. King, G. Corbett, and others, “A retrospective study assessing fully covered metal stents as first-line management for malignant biliary strictures,” *Eur. J. Gastroenterol. Hepatol.*, vol. 27, no. 11, pp. 1347–1353, 2015.

[25] H.-Y. Cheung, K.-T. Lau, T.-P. Lu, and D. Hui, “A critical review on polymer-based bio-engineered materials for scaffold development,” *Compos. Part B Eng.*, vol. 38, no. 3, pp. 291–300, 2007.

[26] D. G. Seifu, A. Purnama, K. Mequanint, and D. Mantovani, “Small-diameter vascular tissue engineering,” *Nat. Rev. Cardiol.*, vol. 10, no. 7, pp. 410–421, 2013.

[27] M. Miyazawa, T. Torii, Y. Toshimitsu, K. Okada, I. Koyama, and Y. Ikada, “A Tissue-

Engineered Artificial Bile Duct Grown to Resemble The Native Bile Duct," *Am. J. Transplant.*, vol. 5, no. 6, pp. 1541–1547, 2005.

[28] D. S. Mendelowitz and J. M. Beal, "Expanded polytetrafluoroethylene in reconstruction of the canine biliary system," *Am. J. Surg.*, vol. 143, no. 2, pp. 221–224, 1982.

[29] Y. Naito, T. Shinoka, D. Duncan, N. Hibino, D. Solomon, M. Cleary, A. Rathore, C. Fein, S. Church, and C. Breuer, "Vascular tissue engineering: towards the next generation vascular grafts," *Adv. Drug Deliv. Rev.*, vol. 63, no. 4, pp. 312–323, 2011.

[30] S. Gorgieva and V. Kokol, "Collagen-vs. gelatine-based biomaterials and their biocompatibility: review and perspectives," in *Biomaterials applications for nanomedicine*, InTech, 2011.

[31] R. Parenteau-Bareil, R. Gauvin, and F. Berthod, "Collagen-based biomaterials for tissue engineering applications," *Materials (Basel).*, vol. 3, no. 3, pp. 1863–1887, 2010.

[32] A. J. P. Alonso, C. D. O. Rivas, I. M. Romero, F. J. C. Garcia, and P. T. Poyatos, "Tissue-engineering repair of extrahepatic bile ducts," *J. Surg. Res.*, vol. 179, no. 1, pp. 18–21, 2013.

[33] L. Tao, Q. Li, H. Ren, B. Chen, X. Hou, L. Mou, S. Zhou, J. Zhou, X. Sun, J. Dai, and others, "Repair of extrahepatic bile duct defect using a collagen patch in a Swine model," *Artif. Organs*, vol. 39, no. 4, pp. 352–360, 2015.

[34] S. Nakashima, T. Nakamura, K. Miyagawa, T. Yoshikawa, S. Kin, Y. Kuriu, Y. Nakase, C. Sakakura, E. Otsuji, A. Hagiwara, and others, "In situ tissue engineering of the bile duct using polypropylene mesh-collagen tubes.," *Int. J. Artif. Organs*, vol. 30, no. 1, pp. 75–85, 2007.

[35] J. K. Sicklick, M. S. Camp, K. D. Lillemoe, G. B. Melton, C. J. Yeo, K. A. Campbell, M. A. Talamini, H. A. Pitt, J. Coleman, P. A. Sauter, and others, "Surgical management of bile duct injuries sustained during laparoscopic cholecystectomy: perioperative results in 200 patients," *Ann. Surg.*, vol. 241, no. 5, p. 786, 2005.

[36] R. J. Moraca, F. T. Lee, J. A. Ryan, and L. W. Traverso, “Long-term biliary function after reconstruction of major bile duct injuries with hepaticoduodenostomy or hepaticojejunostomy,” *Arch. Surg.*, vol. 137, no. 8, pp. 889–894, 2002.

[37] F. A. Auger, L. Gibot, and D. Lacroix, “The pivotal role of vascularization in tissue engineering,” *Annu. Rev. Biomed. Eng.*, vol. 15, pp. 177–200, 2013.

[38] N. W. Choi, M. Cabodi, B. Held, J. P. Gleghorn, L. J. Bonassar, and A. D. Stroock, “Microfluidic scaffolds for tissue engineering,” *Nat. Mater.*, vol. 6, no. 11, p. 908, 2007.

[39] L. E. Bertassoni, M. Cecconi, V. Manoharan, M. Nikkhah, J. Hjortnaes, A. L. Cristino, G. Barabaschi, D. Demarchi, M. R. Dokmeci, Y. Yang, and others, “Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs,” *Lab Chip*, vol. 14, no. 13, pp. 2202–2211, 2014.

[40] J. W. Stansbury and M. J. Idacavage, “3D printing with polymers: Challenges among expanding options and opportunities,” *Dent. Mater.*, vol. 32, no. 1, pp. 54–64, 2016.

[41] N. C. Helena and M. W. Benjamin, “Recent advances in 3D printing of biomaterials,” *J. Biol. Eng.*, vol. 9, p. 1728, 2015.

[42] X. Li, R. Cui, L. Sun, K. E. Aifantis, Y. Fan, Q. Feng, F. Cui, and F. Watari, “3D-printed biopolymers for tissue engineering application,” *Int. J. Polym. Sci.*, vol. 2014, 2014.

[43] I. S. Kinstlinger and J. S. Miller, “3D-printed fluidic networks as vasculature for engineered tissue,” *Lab Chip*, vol. 16, no. 11, pp. 2025–2043, 2016.

[44] A. Hasan, A. Memic, N. Annabi, M. Hossain, A. Paul, M. R. Dokmeci, F. Dehghani, and A. Khademhosseini, “Electrospun scaffolds for tissue engineering of vascular grafts,” *Acta Biomater.*, vol. 10, no. 1, pp. 11–25, 2014.

[45] S. Agarwal, J. H. Wendorff, and A. Greiner, “Use of electrospinning technique for biomedical applications,” *Polymer (Guildf).*, vol. 49, no. 26, pp. 5603–5621, 2008.

[46] H.-W. Kang, Y. Tabata, and Y. Ikada, “Fabrication of porous gelatin scaffolds for tissue engineering,” *Biomaterials*, vol. 20, no. 14, pp. 1339–1344, 1999.

[47] J. D. Roh, G. N. Nelson, M. P. Brennan, T. L. Mirensky, T. Yi, T. F. Hazlett, G. Tellides, A. J. Sinusas, J. S. Pober, W. M. Saltzman, and others, “Small-diameter

biodegradable scaffolds for functional vascular tissue engineering in the mouse model," *Biomaterials*, vol. 29, no. 10, pp. 1454–1463, 2008.

[48] N. L'heureux, S. Pâquet, R. Labbé, L. Germain, and F. A. Auger, "A completely biological tissue-engineered human blood vessel," *FASEB J.*, vol. 12, no. 1, pp. 47–56, 1998.

[49] C. Norotte, F. S. Marga, L. E. Niklason, and G. Forgacs, "Scaffold-free vascular tissue engineering using bioprinting," *Biomaterials*, vol. 30, no. 30, pp. 5910–5917, 2009.

[50] C. M. Morell, L. Fabris, and M. Strazzabosco, "Vascular biology of the biliary epithelium," *J. Gastroenterol. Hepatol.*, vol. 28, no. S1, pp. 26–32, 2013.

[51] N. Kono and Y. Nakanuma, "Ultrastructural and immunohistochemical studies of the intrahepatic peribiliary capillary plexus in normal livers and extrahepatic biliary obstruction in human beings," *Hepatology*, vol. 15, no. 3, pp. 411–418, 1992.

[52] P. Deltenre and D.-C. Valla, "Ischemic cholangiopathy," in *Seminars in liver disease*, 2008, vol. 28, no. 3, pp. 235–246.

[53] L. Evensen, D. R. Micklem, A. Blois, S. V. Berge, N. Aarsæther, A. Littlewood-Evans, J. Wood, and J. B. Lorens, "Mural cell associated VEGF is required for organotypic vessel formation," *PLoS One*, vol. 4, no. 6, p. e5798, 2009.

[54] X. Chen, A. S. Aledia, S. A. Popson, L. Him, C. C. W. Hughes, and S. C. George, "Rapid anastomosis of endothelial progenitor cell--derived vessels with host vasculature is promoted by a high density of cotransplanted fibroblasts," *Tissue Eng. Part A*, vol. 16, no. 2, pp. 585–594, 2009.

[55] G. M. Mitchell and W. A. Morrison, "In Vitro and In Vivo Approaches for Pre-vascularization of 3-Dimensional Engineered Tissues," in *Vascularization for Tissue Engineering and Regenerative Medicine*, Springer, 2017, pp. 1–27.

[56] P. Au, J. Tam, D. Fukumura, and R. K. Jain, "Bone marrow--derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature," *Blood*, vol. 111, no. 9, pp. 4551–4558, 2008.

[57] K. M. Chrobak, D. R. Potter, and J. Tien, "Formation of perfused, functional

microvascular tubes in vitro," *Microvasc. Res.*, vol. 71, no. 3, pp. 185–196, 2006.

[58] M. Cabodi, N. W. Choi, J. P. Gleghorn, C. S. D. Lee, L. J. Bonassar, and A. D. Stroock, "A microfluidic biomaterial," *J. Am. Chem. Soc.*, vol. 127, no. 40, pp. 13788–13789, 2005.

[59] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, and others, "Rapid casting of patterned vascular networks for perfusable engineered 3D tissues," *Nat. Mater.*, vol. 11, no. 9, p. 768, 2012.

[60] A. W. Justin, R. A. Brooks, and A. E. Markaki, "Multi-casting approach for vascular networks in cellularized hydrogels," *J. R. Soc. Interface*, vol. 13, no. 125, p. 20160768, 2016.

[61] K. Arcaute, B. K. Mann, and R. B. Wicker, "Stereolithography of three-dimensional bioactive poly (ethylene glycol) constructs with encapsulated cells," *Ann. Biomed. Eng.*, vol. 34, no. 9, pp. 1429–1441, 2006.

[62] O. Rivas, M. Romero, J. Can, and T. Poyatos, "´ A ESPAN ˜ OLA Original article Bile Duct Reconstruction Using 3-Dimensional Collagen," vol. 1, pp. 590–594, 2013.

[63] P. Nau, J. Liu, E. C. Ellison, J. W. Hazey, M. Henn, P. Muscarella, V. K. Narula, and W. S. Melvin, "Novel reconstruction of the extrahepatic biliary tree with a biosynthetic absorbable graft," *HPB*, vol. 13, no. 8, pp. 573–578, 2011.

[64] Y.-L. Liang, Y.-C. Yu, K. Liu, W.-J. Wang, J.-B. Ying, Y.-F. Wang, and X.-J. Cai, "Repair of bile duct defect with degradable stent and autologous tissue in a porcine model," *World J. Gastroenterol. WJG*, vol. 18, no. 37, p. 5205, 2012.

[65] M. Rosen, J. Ponsky, R. Petras, A. Fanning, F. Brody, and F. Duperier, "Small intestinal submucosa as a bioscaffold for biliary tract regeneration," *Surgery*, vol. 132, no. 3, pp. 480–486, 2002.

[66] A. Cushieri, P. R. Baker, R. J. Anderson, and M. P. Holley, "Total and subtotal replacement of the common bile duct: effect of transhepatic silicone tube stenting.," *Gut*, vol. 24, no. 8, pp. 756–760, 1983.

[67] C. H. Scudamore, C. D. Becker, J. S. Fache, R. Bianco, C. R. Shackleton, H. J. Burhenne, D. A. Owen, M. T. Schechter, and D. Seccombe, “Human amnion as a bioprosthesis for bile duct reconstruction in the pig,” *Am. J. Surg.*, vol. 155, no. 5, pp. 635–640, 1988.

[68] Q. Li, L. Tao, B. Chen, H. Ren, X. Hou, S. Zhou, J. Zhou, X. Sun, J. Dai, and Y. Ding, “Extrahepatic bile duct regeneration in pigs using collagen scaffolds loaded with human collagen-binding bFGF,” *Biomaterials*, vol. 33, no. 17, pp. 4298–4308, 2012.

[69] M. Christensen, H. B. Laursen, M. Rokkjær, P. F. Jensen, Y. Yasuda, and F. V. Mortensen, “Reconstruction of the common bile duct by a vascular prosthetic graft: an experimental study in pigs,” *J. Hepatobiliary. Pancreat. Sci.*, vol. 12, no. 3, pp. 231–234, 2005.

[70] G. Mazza, K. Rombouts, A. Rennie Hall, L. Urbani, T. Vinh Luong, W. Al-Akkad, L. Longato, D. Brown, P. Maghsoudlou, A. P. Dhillon, B. Fuller, B. Davidson, K. Moore, D. Dhar, P. De Coppi, M. Malago, and M. Pinzani, “Decellularized human liver as a natural 3D-scaffold for liver bioengineering and transplantation,” *Sci. Rep.*, vol. 5, no. 1, p. 13079, Oct. 2015.

[71] G. Mazza, W. Al-Akkad, A. Telese, L. Longato, L. Urbani, B. Robinson, A. Hall, K. Kong, L. Frenguelli, G. Marrone, and others, “Rapid production of human liver scaffolds for functional tissue engineering by high shear stress oscillation-decellularization,” *Sci. Rep.*, vol. 7, 2017.

[72] G. Wittrin, M. Clemens, M. Arndt, and D. Rühland, “Replacement of the common bile duct by an autologous vein (author’s transl),” *Res. Exp. Med. (Berl).*, vol. 173, no. 1, pp. 95–103, 1978.

Figure legends

Figure 1

Schematic representation of the micro-anatomy of the bile duct. A monolayer of cholangiocytes is supported by a layer of connective tissue and a smooth muscle layer best identified in the distal end of the common bile duct, while oxygen and nutrients are provided through the peri-biliary plexus vessels.

Cells	Scaffold	Synthetic/ Biological	Animal	Advantages	Limitations	Post-operative follow up	Authors	Year
Acellular	Collagen membrane	Biological	Pig	Bioabsorbable, allows for cellular ingrowth	Limited to patch	0.5 - 4 months	Tao, L. *et al.* [33]	2015
Acellular	Collagen membrane	Biological	Pig	Bioabsorbable, allows for cellular ingrowth	Limited to patch	1 - 3 months	Li, Q. *et al.* [68]	2012
Acellular	Collagen sponge and polypropylene mesh	Hybrid	Dog	Successful re-epithelialization of the graft, allows for cellular ingrowth	Non-resorbable, biliary strictures	1 - 12 months	Nakashima, S. *et al.* [34]	2007
Acellular	Collagen tube coated with agarose hydrogel	Biological	Guinea Pig	Successful re-epithelialization of the graft, bioabsorbable, allows for cellular ingrowth	Reduced expression of biliary markers in neo-duct, non-resorbable	0.5 - 6 months	Alonso, A.J.P. *et al.* [32]	2013
Acellular	Proprietary mesh of polyglycolic acid and trimethylene carbonate	Synthetic	Dog	Bioabsorbable, allows for cellular ingrowth	Foreign body reaction, obstruction, cholangitis	6 - 12 months	Nau, P. *et al.* [63]	2011
Acellular	Expanded polytetrafluoroethyelene or woven Dacron	Synthetic	Dog	Allows for cellular ingrowth	Obstruction and migration, non-resorbable	6 weeks	Mendelowitz, D.S. and Beal, J.M. [28]	1982
Whole tissue	Degradable stent of poly[sebacic acid-co-(1,3-propanediol)-co-(1,2-propanediol)] with autologous tissue around stent	Hybrid	Pig	Bioabsorbable	Fibrosis at the site of anastomosis and mildly abnormal liver function (GGT)	1 - 4 months	Liang, Y. *et al.* [64]	2012
Whole tissue	Autologous vein graft	Biological	Dog	Autologous graft	Bile leak and strictures	2 - 12 months	Wittrin, G. *et al.* [72]	1978
Whole tissue	Collagen-based small intestinal submuscosa	Biological	Dog	Autologous graft	Strictures, bile leak, limited to patch	0.5 - 5 months	Rosen, M. *et al.* [65]	2002
Whole tissue	Human amnion with polyglyolic acid mesh	Hybrid	Pig	Autologous graft	Bile leak and strictures	1 - 4 months	Scudamore, C.H. *et al.* [67]	1988
Whole tissue	Autologous vein graft and silicon stent	Hybrid	Pig	Autologous graft	Strictures - stenting required	2 - 12 months	Cushieri, A. *et al.* [66]	1983
Autologous bone marrow cells (BMCs)	Polycaprolactone and polylactic acid copolymer, reinforced with polyglycolic acid fibers	Synthetic	Pig	Successful re-epithelialization of the graft, allows for cellular ingrowth, bioabsorbable	Lengthy process, contribution of BMCs unclear	6 months	Miyazawa, M. *et al.* [27]	2004
Primary human cholangiocytes	Densified collagen gel	Biological	Mouse	Successful epithelialization of the graft with human cells, high functionality, allows for cellular ingrowth, bioabsorbable	Small animal model	1 month	Sampaziotis, F. *et al.* [18]	2017

Table 1. Comparison of approaches to bioengineering the bile duct.