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Generation and transplantation of primary human cholangiocyte organoids on bioengineered scaffolds for repair and replacement of the extrahepatic biliary tree

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Introduction:
Treatment of common bile duct disorders such as biliary atresia is limited to liver transplantation or hepatojejunostomy due to the lack of suitable tissue for surgical reconstruction. Here, we explore the potential of bioengineered biliary tissue consisting of human extrahepatic cholangiocyte organoids (ECOs) and biodegradable scaffolds for transplantation and biliary reconstruction in vivo.

Methods:
Primary human cholangiocytes were isolated by mechanical dissociation from deceased organ donors with ethical approval and informed consent (n=8). Propagation of ECOs was achieved using our established protocol. The Illumina HumanHT-12v4 array was used for transcriptomic analysis. ECOs were seeded on Polyglycolic Acid (PGA) or densified collagen scaffolds. Biliary reconstruction was achieved in immunodeficient NSG mice by partially replacing the gallbladder wall with an ECO populated PGA-scaffold patch (ECO-patch; n=8), or replacing a length of the native common bile duct with ECO populated collagen tubes (ECO-tubes) through end-to-end anastomosis (n=4). Fibroblast-populated (n=5, PGA; n=4, collagen) or acellular scaffolds (n=2, PGA) were used as negative controls. Biliary tree patency was confirmed using magnetic resonance cholangiopancreatography (MRCP) or cholangiography.

Results:
ECOs closely correlate with primary cholangiocytes in terms of transcriptomic profile (*r*: 0.92) and functional properties (ALP, GGT, bile acid transfer). ECO-populated scaffolds form biliary tissue-resembling structures, maintain their functional properties (ALP, GGT) and marker expression (CK7, CK19, HNF1B). All ECO-transplanted animals exhibited prolonged survival (ECO-patch vs. acellular controls, P=0.0027; ECO-tubes vs. fibroblasts, P=0.0082; log-rank test). The transplanted cells integrated in the biliary epithelium, continued expressing biliary markers (CK7, CK19, HNF1B), exhibited ALP activity and a patent lumen. All fibroblast reconstructions failed, the biliary epithelium was replaced by fibrotic tissue and the lumen of the gallbladder or neo-bile duct was occluded.

Discussion:
We demonstrate that ECO-populated biodegradable scaffolds can successfully be transplanted and reconstruct the biliary tree in vivo. To our knowledge, this is the first application of regenerative medicine in cholangiopathies and first report of tissue transplantation and organ reconstruction using human primary cells expanded in vitro.