

Extrahepatic bile duct regeneration through decellularized organ-specific scaffolds. A pilot study

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Introduction

Cholecystectomy is one of the most common intraabdominal surgical procedures. Around 750,000 procedures are carried out in patients per year only in the United States. Nevertheless, between 0.3% to 0.6% of surgically intervened patients have developed life-threatening iatrogenic-induced bile duct injuries [1,2,3]. These injuries require surgical reconstruction, which is usually attempted through Roux-en-Y hepaticojejunostomy [4,5]. However, this major procedure can lead to complications such as stenosis, recurrent cholangitis and secondary biliary cirrhosis [6] these conditions can be produced by the absence of a barrier that isolates the intestinal content from the bile duct as the Oddi`s sphincter would do it under normal conditions [7]. Therefore, it is necessary to produce a better treatment that could preserve the anatomy and physiology of this anatomical region. One tissue-engineered approach which could replace the bile duct with a biological scaffold is the decellularized tissues[8]. Decellularization is an attractive technique to obtain acellular scaffolds for organ and tissue regeneration, due to the possibility to retain the architecture and biofactors present in the extracellular matrix (ECM) of the native tissue[9], which are necessary for cell survivor, proliferation and differentiation. In this work, our aim was to obtain a **Tissue-Engineered Scaffold with Preserved Matrix Architecture and Cytocompatibility .** We describe a method for porcine bile duct decellularization, going from tissue harvesting to evaluation of the microarchitecture, and demonstrating the biological properties of decellularized extracellular matrix bile ducts (dECM-BD)scaffolds.

Methods

Decellularization Protocol

For these experiments, a total of 10 young Landrace male pigs (weight 25–30 kg) were employed . All animals were euthanized by anesthesia overdose (sodium pentobarbital).All animals were euthanized by anesthesia overdose (sodium pentobarbital).

The selection of decellularization reagents was based on modifying preexistent protocols presented by Giraldo et al. and Cheng et al. [8,10], The process started with perfusion of SDS 1% solution in Milli-Q water, this solution was recirculated for 96 hours, and it was changed every 24 hours. Then a solution of PBS (1X) was perfused through the bile ducts for 24 hours to rinse the tissue from any remains of SDS detergent. After this, the samples were perfused by DNase-I solution (10mg/mL) for 24 hours. Final perfusion with Milli-Q water was carried out for 24 hours as a final rinse from any cell debris (Fig. 1C-D).

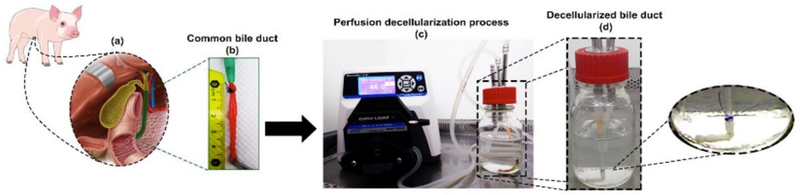


Figure 1. Extraction of bile ducts and decellularization Protocol with peristaltic pump. a-d) [11]

Resullts

Decellularization Assessment

Macroscopically, a gradual change of color was observed during the perfusion decellularization process. Nuclei labeling with DAPI showed an effective decrease in the number of positively stained particles after the completion of decellularization process (Figure 2a,b). The presence of fluorescence after decellularization in DAPI images (Figure 2b) is partially explained by the autofluorescence from collagen in the samples.Quantification of the perfusion decellularization process is shown by DAPI images quantification and DNA quantification (Figure 1c,d). H&E stains confirmed the removal of cells (**e,f**).

As additional assessment of the decellularization process, immunostaining of HLA-A and CK7 positive cells confirmed a decrease of both proteins in the dECM-BD when compared to native tissue (Figure 2g–j). Furthermore, dECM-BD retained the lumen structures of blood vessels in the vascular plexus, marked by arrows (Figure 2g,h). Quantification of CK7 mean values were 17.55 ± 3.31% and 0.18 ± 0.08% for native tissue and dECM-BD, respectively. For HLA-A, mean values were 26.04 ± 4.28% and 1.60 ± 0.36% for native and dECM-BD samples, respectively (Figure 2k,l, respectively).

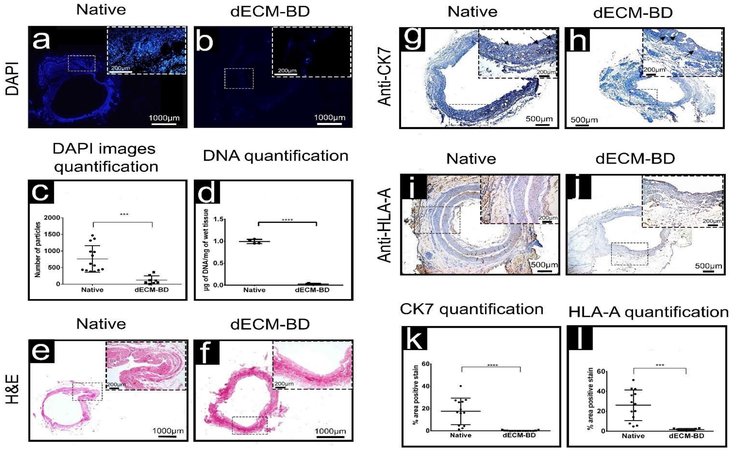


Figure 2 Decellularization Assessment. Nuclei labeling with DAPI and DNA quantification (a-d) H&E stains (e-f) and immunostaining of HLA-A and CK7 (g-i) [11]

Cell Infiltration into dECM-BD

Infiltration of cells into the dECM-BD after 1 week in culture under static conditions was confirmed by Masson's trichrome staining. Presence of cells was observed in the sub-mucosa of dECM-BD, shown with arrows in Figure 3. [11]

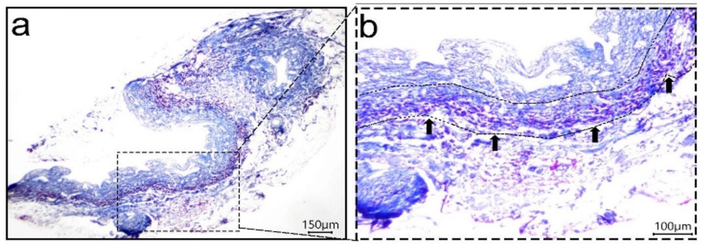


Figure 3 Cell Infiltration into (Luminal side is in the upper side of the images). dECM-BD 7 days post seeding a) Magnification shows cells infiltration b) [11]

Conclusion

The decellularization process of bile ducts described in the current work is a fast and effective method that results in the removal of cellular components but that preserves the non-cellular components of the tissue: the ECM. The most important features of the current protocol are that ECM architecture alterations are minimal, and the perivascular lumen is preserved. The potential of regenerating tissues using xenogeneic sources may overcome the current shortage in organ transplantation medicine.

Future studies will assess the cell adhesion of endothelial cells to the vascular plexus of these decellularized matrices and their survival under dynamic conditions. Long-term viability studies may indicate if the dECM-BD scaffold allows not only cell infiltration as demonstrated here but also their proliferation and subsequent survival. These assessments may further characterize the in vitro recellularization of the biliary ducts before in vivo implantation. Bile duct regeneration with organ-specific natural scaffolds that retain a biological and biochemical memory are a novel alternative to common bile duct reconstruction and represent a milestone in general and Hepato- Pancreato-Biliary surgery.

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