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Bioengineered transplantable porcine livers with re-endothelialized vasculature

In Kap Ko [a, 1], Li Peng [a, b, 1], Andrea Peloso [a, c, 1], Charesa J. Smith [a], Abritee Dhal [a], Daniel B. Deegan [a], Cindy Zimmerman [a], Cara Clouse [a], Weixin Zhao [a], Thomas D. Shupe [a], Shay Soker [a], James J. Yoo [a, *], Anthony Atala [a]

[a] Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA
[b] Hospital of Nantong University, No.20 Xisi Road, Nantong, 226001 Jiangsu Province, China
[c] IRCCS Policlinico San Matteo, Dept. of General Surgery, University of Pavia, Pavia, Italy

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ABSTRACT

Donor shortage remains a continued challenge in liver transplantation. Recent advances in tissue engineering have provided the possibility of creating functional liver tissues as an alternative to donor organ transplantation. Small bioengineered liver constructs have been developed, however a major challenge in achieving functional bioengineered liver *in vivo* is the establishment of a functional vasculature within the scaffolds. Our overall goal is to bioengineer intact livers, suitable for transplantation, using acellular porcine liver scaffolds. We developed an effective method for reestablishing the vascular network within decellularized liver scaffolds by conjugating anti-endothelial cell antibodies to maximize coverage of the vessel walls with endothelial cells. This procedure resulted in uniform endothelial attachment throughout the liver vasculature extending to the capillary bed of the liver scaffold and greatly reduced platelet adhesion upon blood perfusion *in vitro*. The re-endothelialized livers, when transplanted to recipient pigs, were able to withstand physiological blood flow and maintained for up to 24 h. This study demonstrates, for the first time, that vascularized bioengineered livers, of clinically relevant size, can be transplanted and maintained *in vivo*, and represents the first step towards generating engineered livers for transplantation to patients with end-stage liver failure.

1. Introduction

Liver transplantation represents the only curative treatment for end-stage liver disease. Unfortunately this solution is limited by a critical shortage of donor organs that are suitable for transplant. According to the United Network for Organ Sharing, over 16,000 patients are currently awaiting liver transplant, while less than 7000 donor organs become available annually. This discrepancy between organ supply and demand results in thousands of deaths each year [www.unos.org]. Several treatment strategies are being developed to sustain critically ill patients until a time when a transplantable donor organ is available [1,2]. However, these therapies can only buy a small amount of additional time for patients in liver failure. Recently, the use of non-heart-beating liver donors (NHBD) has been considered, but this would still not close the organ supply/demand gap [3–6]. Clearly, alternative treatments for patients with end stage liver disease need to be investigated.

Over the past decade, the fields of regenerative medicine and tissue engineering have offered new strategies for the generation of engineered organs [7–9]. These new strategies are based upon the use of scaffolds with a preexisting architectural structure that are seeded with an appropriate population of cells [10]. Natural tissue extracellular matrices (ECM) possess a dynamic network of macromolecules with organ-specific anatomical and biochemical properties [11,12]. It would be advantageous to include these properties in any scaffold considered for organ engineering.

Whole organ engineering represents the ultimate solution for completely solving the shortage of transplantable organs [13–20]. For the reasons mentioned above, decellularized whole organ matrices would be the preferred option for a construct scaffold. These scaffolds are easily generated by perfusion of donor organs with mild detergents that remove the cellular components from the organ [21,22]. Importantly, decellularized whole organ matrices

* Corresponding author. Tel.: +1 336 713 7294; fax: +1 336 713 7290.
E-mail address: jyoo@wakehealth.edu (J.J. Yoo).
[1] Co-first authors.

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retain the vascular network and tissue microarchitecture present in the native organ [23,24]. The current study is based on the development of a decellularized liver scaffold that is subsequently repopulated with cells isolated from liver tissue samples. The ultimate goal of these studies is to create a transplantable organ that could replace the functional capabilities of the patient's failing liver [25,26].

The decellularization process for the production of an acellular organ scaffold has been under development for many years, and the results have been quite remarkable [20,21]. However, the recellularization process has proven to be very challenging. One critical obstacle to achieving a transplantable, recellularized organ is reestablishment of a patent vasculature with sufficient endothelial cell coverage to prevent thrombosis. Acellular ECM is potently thrombogenic, and blood clots will form in an insufficiently endothelialized construct, even with the use of standard anticoagulant therapy [27].

The goal of the current study was to re-establish a functional vasculature in bioengineered livers of clinically relevant size. To achieve this goal, we developed a novel re-endothelialization technique based on using anti endothelial cell antibodies to stabilize seeded cells on the vessel walls. We validated the vascular functionality by transplanting the re-endothelialized livers using a heterotopic transplantation model with inflow from the renal artery and the outflow to the renal vein (Fig. 1). The major findings indicated that the re-endothelialized liver scaffolds were able to withstand physiologic blood pressure and maintain blood flow within the bioengineered livers for 24 h. This study shows, for the first time, a strategy for overcoming a major hurdle in the engineering of transplantable liver, the establishment of functional vasculature.

2. Materials and methods

2.1. Decellularization of porcine liver

Native livers were harvested from 5 to 8 kg piglets. The portal vein (PV), common hepatic artery (HA), suprahepatic (SH)- and infrahepatic (IH) inferior vena cava (IVC) were cannulated with smart site connectors (Cole Parmer) attached to 14 G tubing with inflow and outflow adjusted to mimic normal flow through the organ. Detergent solutions (1% Triton X-100 and 0.1% ammonium hydroxide in distilled water) were perfused into the liver tissue using a peristaltic pump (Master fl ex L/S with Master flex L/S easy load pump head, Cole Parmer, Vernon Hills, IL, USA). Decellularization of the liver was performed by perfusing the organ at a flow rate of 0.5 ml/min for 2–3 days, followed by washing with saline for 3–4 days. The decellarized livers were sterilized by gamma irradiation at 1.2 MRad (12,000 Gy) prior to cell seeding.

2.2. Characterization of acellular liver scaffold

To evaluate the efficiency of scaffold decellularization, DNA quantification and histological analysis (H&E) were performed. For DNA quantification, samples were excised from representative lobes of native and decellularized livers. The samples were minced and lyophilized in preparation for analysis (Labconco, Kansas City, MO). DNA was extracted from 5 mg samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) and quantified using Quant-iT PicoGreen (Invitrogen Corp., Carlsbad, CA). Fluorescence from the PicoGreen signal indicates residual DNA and was measured at 525 nm (excitation 490 nm) using a SpectraMax M5 Multi-Mode Microplate Reader (Molecular Devices Inc., Sunnyvale, California, US).

To evaluate the maintenance of vasculatures within the decellularized liver scaffold, angiographic studies were performed using computed tomography (CT) and liver vascular casting combined with electron microscopic analysis. For CT imaging of native and decellularized liver scaffold, a CT contrast agent (MICROFIL, Flowtech, Inc., Carver MA) was infused through the PV at 1 ml/min flow rate, while the artery and veins were clamped. The livers were scanned on a Toshiba Aquilion 32 CT scanner (Toshiba America Medical Systems, Inc., Tustin, CA) and analyzed with CT angiography body and soft tissue deformation processing algorithms. Imaging was conducted using a TeraRecon Aquarius Workstation (TeraRecon Inc., Foster City, CA).

Liver casts were prepared using Batson's compound n° 17 (Polysciences Inc., Warrington, PA) injected through the portal vein with constant flow rate (1 ml/min) using a syringe pump (New Era Pump Systems, Inc., Farmingdale, NY, USA). Following the resin injection, the infused liver was incubated to allow the polymerization within the liver vascular system. After curing, the liver tissue was digested with 20% sodium hydroxide at 37 °C, followed by washing with deionized water. The vasculatures of the liver cast were imaged using scanning electronic microscopy (SEM). The cast segments were mounted on silver plates, sputter coated with gold and imaged at 15 KV.

2.3. Re-endothelialization of acellular liver scaffold

To improve re-endothelialization of vasculatures within the liver scaffold, rat anti-mouse CD31 antibody (BD sciences, Franklin Lakes, NJ) was conjugated to the

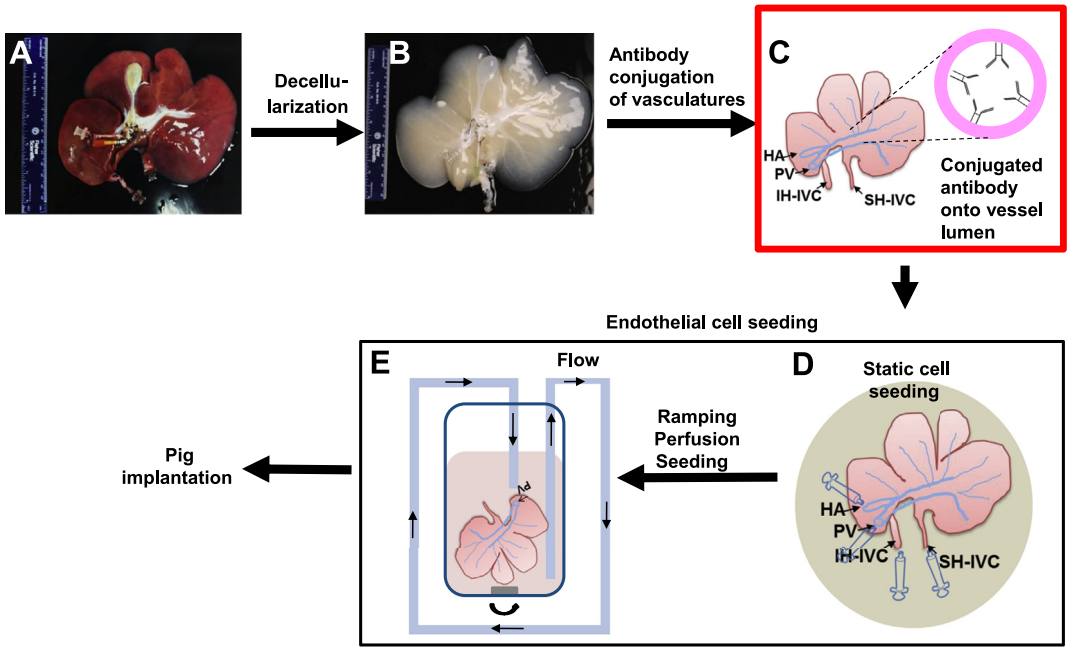

Fig. 1. Schematic diagram of the re-endothelialization processes for decellularized pig liver scaffolds. (A) Native liver harvested from piglets was decellularized using 1% Triton X-100 to obtain a completely decellularized liver scaffold (B). Following antibody conjugation on vasculatures of the liver scaffold (C), the antibody-conjugated scaffold was seeded with endothelial cells (MS1) using a combination of static (D) and perfusion methods (E), and then matured in a bioreactor system. The engineered liver construct was implanted into pigs. PV, HA, SH-IVC, and IH-IVC indicates portal vein, hepatic artery, suprahepatic inferior Vena Cava, and intrahepatic inferior Vena Cava, respectively.