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Title

Antithrombotic revascularization strategy of bioengineered liver using a biomimetic polymer

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Running title

Revascularization of liver scaffolds with a biomimetic polymer

Abbreviations

3D, three-dimensional

BD, bile duct

DAB, 3, 3'-diaminobenzidine

ECM, extracellular matrix

ELISA, enzyme-linked immunosorbent assay

ESLD, end-stage liver disease

FITC, fluorescein isothiocyanate

HE, hematoxylin and eosin

HUVEC, human umbilical vein endothelial cell

IHVC, infra hepatic vena cava

LESC, liver sinusoidal endothelial cell

MPC, 2-metacryloyloxyethyl phosphorylcholine

NHS, N-(methacryloyloxy)tetra(ethylene oxide)oxycarbonyl succinimide

PENHS, poly (MPC-co-NHS)

PH, primary hepatocyte

PV, portal vein

RA, renal artery

REDV, Arg-Glu-Asp-Val

TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling

Abstract

A bioengineered liver has the potential to save patients with end-stage liver disease, and a three-dimensional decellularized scaffold is a promising approach for practical use. The main challenge in bioengineered liver transplantation is thrombogenicity during blood perfusion. We aimed to apply a novel antithrombotic polymer to revascularize liver scaffolds and evaluate the thrombogenicity and biosafety of the polymer-treated scaffolds. A biomimetic polymer, 2-metacryloyloxyethyl phosphorylcholine (MPC) was prepared for modification of the extracellular matrix (ECM) in liver scaffolds. The polymer was injected into the rat liver scaffolds' portal vein (PV) and could extensively react to the vessel walls. In an ex-vivo blood perfusion experiment, we demonstrated significantly less platelet deposition in the polymer-treated scaffolds than non-treated or re-endothelialized scaffolds with human umbilical vein endothelial cells (HUVECs). In the heterotopic transplantation model, liver volume was better maintained in the polymer-treated groups and platelet deposition was suppressed in these groups. Additionally, the polymer-treated liver scaffolds maintained the metabolic function of the recellularized rat primary hepatocytes during perfusion culture. The MPC polymer treatment efficiently suppressed thrombus formation during blood perfusion in liver scaffolds and maintained the function of recellularized hepatocytes. Revascularizing liver scaffolds using this polymer is a promising approach for bioengineered liver transplantation.

Impact statement

Thrombogenicity is one of the most critical challenges in bioengineered liver transplantation using decellularization techniques. We developed a new strategy for

antithrombotic revascularization of bioengineered livers with a biomimetic MPC polymer to the ECM. The polymer treatment was demonstrated to be safe for recellularized hepatocytes. This novel approach should be a key for clinical application of bioengineered liver transplantation.

Introduction

End-stage liver disease (ESLD) causes approximately 2 million annual deaths worldwide.¹ Liver transplantation is the only curative and well-established treatment strategy for patients with ESLD. However, donor shortages remain a chronic problem, which is difficult to resolve. This problem was addressed through the development of cellular transplantation and bioartificial liver.²

Decellularized liver scaffold-based bioengineering was recently developed as a promising approach to help these patients.³⁻⁵ The decellularized liver scaffold consists of an extracellular matrix (ECM), which preserves the microvasculature and biological properties and can serve as a three-dimensional (3D) framework for recellularization, that is, seeding new liver cells such as hepatocytes, cholangiocytes, and endothelial cells.^{6, 7}

Several studies have attempted transplantation of a bioengineered liver.^{3, 8-13} However, one of the main challenges faced during this procedure is thrombogenicity of the liver tissue during blood perfusion.¹⁴ To overcome this issue, re-endothelialization and various coating agents such as heparin-gelatin, Arg-Glu-Asp-Val (REDV) peptide, and anti-CD31 antibodies or aptamers were explored.^{8-11, 15} However, the difficulty of complete re-endothelialization and the clinical applicability of those agents are still significant concerns.

The synthetic biomimetic 2-metacryloyloxyethyl phosphorylcholine (MPC) polymers contain a representative phospholipid polar group.¹⁶ They have high biocompatible properties such as resistance to protein adsorption, low thrombogenicity, and suppression of immunoresponses. Due to this, they have been utilized in the development of various medical devices and research in tissue engineering.¹⁷

Therefore, we propose a new working hypothesis in which the ECM surface in contact

with blood is coated with the MPC polymer to control the thrombogenic reaction.

This study aimed to apply the new functional MPC polymer to a decellularized liver scaffold and to evaluate its biocompatibility, especially its antithrombogenicity during blood perfusion, and safety for recellularized hepatocytes.

Materials and Methods

Animals

Lewis rats (200–300 g; SLC, Hamamatsu, Japan) were used to prepare the 3D liver scaffold for primary hepatocyte isolation and ex vivo blood perfusion. Male Lewis rats (400–450 g; SLC) were used for blood collection and heterotopic transplantation of 3D liver scaffolds. The rats were maintained on a standard laboratory diet and water ad libitum and were housed in a temperature- and humidity-controlled environment under a constant 12-hour light / dark cycle in the animal facility at Kyoto University. All animal experiments were approved by the Kyoto University Animal Experimentation Committee and were performed in accordance with the Kyoto University Animal Protection Guidelines.

Liver harvest and decellularization

Liver harvesting and the preparation of decellularized liver scaffolds were performed according to our previous report.¹⁸. Briefly, the harvested livers previously cannulated with a 24-gauge cannula in the bile duct (BD) and a 20-gauge cannula in the portal vein (PV) was washed with PBS. The liver was frozen at –80°C and thawed overnight at 4°C. Subsequently, the liver was perfused with 0.25 w/v% Trypsin-1 mmol/L EDTA-4Na solution with phenol red (Wako, Osaka, Japan) at 37°C for 1 h, followed by 0.1% polyoxyethylene octyl phenyl ether (Wako)/ 0.05% EDTA (Sigma, St. Louis, USA) solution at a flow rate of 0.5 mL/min for 48 hours. The decellularized liver was sterilized by perfusion with 0.1% peracetic acid (Sigma) for 2 h and washed with sterilized PBS.

Treatment with MPC polymer on decellularized liver scaffold

The MPC polymer was synthesized by a conventional radical copolymerization of MPC and N-(methacryloyloxy) tetra(ethylene oxide) oxycarbonyl succinimide (NHS) (PENHS).¹⁶ The obtained polymer was soluble in aqueous medium and the composition of the MPC and NHS units in the polymer was 80 mol% and 20 mol%, respectively. The NHS units can react with amino groups of proteins in the ECM. A MPC polymer bearing a PENHS unit and a small amount of fluorescein group in the side chain was also synthesized and used for fluorescence evaluation. The MPC polymer was dissolved in PBS at 0.50 wt% concentration and the solution was perfused via the PV of the decellularized liver. The liver scaffold was incubated overnight at 4°C, then washed with PBS via PV to remove the unattached polymer.

Recellularization of HUVECs and perfusion culture

HUVEC recellularization was performed as previously reported.¹⁵ In total, 1.5×10^7 HUVECs suspended in 3mL of EGMTM-2 BullekitTM (Lonza Sales Ltd., Basel, Switzerland) was injected into the PV of liver scaffolds at a flow rate of 1mL/min. Perfusion cultures were performed as previously reported.¹⁸ Briefly, the cannula inserted into the PV was connected to the circulation culture system using a peristaltic pump (Perista pump, ATTO, Tokyo, Japan) in a customized chamber after 3 h of static culture for cell attachment. EGMTM-2 BullekitTM was continuously perfused to the recellularized liver at 0.7–1.0 mL/min at 37°C. After 2 days of perfusion culture, ex-vivo blood perfusion was performed.

Ex-vivo blood perfusion of the decellularized liver scaffold

Fresh rat blood was collected in 10 mL syringes via the IVC, supplemented with 5 IU/mL heparin and perfused via the PV of the decellularized liver scaffold in the non-

treated, HUVECs-recellularized, and polymer-treated groups. The cannula inserted into the PV of scaffold was connected to a circulating device using the same device as mentioned above and continuously perfused with blood at 1 mL/min for 6 h. Three independent experiments were performed in each group.

Recellularization of primary hepatocytes and perfusion culture

Rat primary hepatocytes (PH) were isolated using a 2-step collagenase perfusion technique as previously described.¹⁹ A total of 3.0×10^7 PHs were suspended in 30 mL of HCM™ (Lonza Sales Ltd., Basel, Switzerland) and injected via the BD of the polymer-treated or non-treated liver scaffolds at 1 mL/min. Perfusion cultures were performed as mentioned above. HCM™ was continuously perfused to the recellularized liver at 0.7–1.0 mL/min at 37°C. The medium was replaced every 48 h, and perfusion culture was performed for 7 days. Three independent experiments were performed in each group.

Heterotopic transplantation of the decellularized liver graft

DONOR OPERATION

The liver harvesting method was modified for donor operations. Lewis rats (female; 200–250 g; SLC) were anesthetized with vaporized isoflurane and placed in the supine position. Following access to the abdominal cavity through a midline incision, a 70% partial hepatectomy (median and left lateral lobectomy) was performed. After heparinization, PBS was perfused via the abdominal aorta to remove blood from the residual liver (right lateral and caudate lobe). The PV was dissected and cannulated with a 24-gauge catheter. The infra hepatic vena cava (IHVC) was dissected at the bifurcation site of the left renal vein after ligation and dissection of the right renal vein.

The BD and hepatic arteries were ligated and dissected. After removing the liver, the supra-hepatic vena cava was ligated.

GRAFT PREPARATION

The 30% partial liver (right lateral and caudate lobes) was used as a graft for transplantation. Decellularization and MPC polymer treatment were performed using the same method as described above. Ringer's Lactate containing 5 IU/mL heparin was perfused via the PV immediately before transplantation.

RECIPIENT OPERATION

Lewis rats (male; 400–450 g; SLC) were anesthetized with vaporized isoflurane and placed in a supine position. Following access to the abdominal cavity through a transverse incision, right nephrectomy was performed. The IHVC (graft) and IHVC (recipient) were anastomosed by end-to-side anastomosis using 8-0 Prolene[®], and the PV (graft) and right renal artery (RA) (recipient) were anastomosed using an internal stent of the 24-gauge catheter. To compensate for the temporal dead volume in the graft, 4 mL of fresh rat blood containing 5 IU/mL heparin was transfused immediately before blood perfusion into the graft. After 30 min of blood perfusion, the grafts were removed by washing with PBS. Transplantation was performed in the polymer- and non-treated groups. Three independent experiments were performed in each group.

Histological analysis

Blood-perfused decellularized liver grafts and cultured recellularized liver grafts were fixed in 4% paraformaldehyde for 24 h at 4°C and subsequently embedded in paraffin.

Paraffin-embedded sections (4 μm) were dewaxed, rehydrated, and subjected to hematoxylin and eosin (HE) staining or immunohistochemical staining. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining was performed in three sections, each prepared from three independent experiments using an *In situ* Apoptosis Detection Kit (Takara Bio, Shiga, Japan) according to the manufacturer's protocol. The samples were incubated with 3, 3'-diaminobenzidine (DAB) substrate (Dako, Glostrup, Denmark) and counter-stained with methyl green. For immunofluorescence analysis, antigen retrieval was performed by autoclaving for 15 minutes at 121°C, and the sections were incubated in PBS containing 10% donkey serum. All the antibodies were diluted in PBS containing 5% donkey serum. The sections were incubated with primary antibody overnight at 4°C, followed by incubation with secondary antibody for 1 h at 25°C and mounted using ProLong™ Gold Antifade Mountant with DAPI (Invitrogen, Waltham, USA). The stained sections were visualized under an Olympus BX 50F4 microscope (Olympus Optical, Tokyo, Japan). The deposition of platelets was compared between the polymer- and non-treated groups by measuring fluorescence intensities of integrin αIIb (five random periportal and central vein areas in three sections each prepared from three independent experiments per group) using ImageJ software version 1.53k, as evaluated in previous reports.²⁰

Liver function analysis

Albumin content in the medium was measured using a rat albumin ELISA quantification kit (Bethyl Laboratories, Montgomery, TX). Urea content was analyzed using the QuantiChrom Urea Assay Kit (BioAssay Systems, Hayward, CA, USA)

according to the manufacturer's protocol.

Statistical analysis

Data are expressed as the mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as $p < 0.05$. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling-positive cells and fluorescence intensity of integrin α IIb were compared using the unpaired *t*-test. The metabolic activity of the recellularized liver grafts was compared using the Wilcoxon rank-sum test.

Results

Decellularization of a rat's whole liver

A translucent acellular liver scaffold was generated 48 h after decellularization (Fig. 1A). Histological analysis of the decellularized liver scaffolds confirmed the absence of nuclei or cytoplasmic components in the decellularized liver scaffold (Fig. 1B). Crystal violet staining of the PV showed that the vascular system was intact after decellularization (Fig. 1C). The injected dye spread into the parenchymal space and finally drained into the central venous system.

MPC polymer treatment on decellularized liver scaffold

The structural formulas of the MPC polymer used in this study are shown in Fig. 2A. We injected the polymer via the PV and let the scaffold stand overnight at 4°C. The treated polymers were visualized by immunofluorescence using an anti-FITC antibody.

The polymer was extensively attached to the PV and central venous lumen (Fig. 2B).

Evaluating antithrombotic ability of the MPC polymer after *ex vivo* blood perfusion

Ex vivo blood perfusion of the decellularized liver scaffold in the non-treated, HUVECs-recellularized, and MPC polymer-treated groups was performed for 6 h using a circulation device (Fig. 3A). The perfused blood spread through the PV system to the entire liver and finally drained into the IVC. Although some clots were observed in the gross appearance of the decellularized liver, they were insufficient to stop blood flow, even after 6 h. Furthermore, PBS injected into the PV after the experiment was drained into the IVC without any obvious leakage from the surface of the decellularized liver, suggesting the presence of patent routes of blood circulation. In the gross appearance, the liver scaffold treated with MPC polymer tended to have fewer blood clots (Fig. 3B). In HE staining and immunofluorescence images, extensive thrombus deposition was observed in the scaffold of non-treated group. In the HUVECs-recellularized group, HUVECs were attached to the portal lumen of liver scaffolds but did not provide full coverage. Although thrombi appeared to be less extensive than the non-treated group, they were observed in both areas with and without HUVEC coverage. Conversely, low thrombus deposition was observed in the MPC polymer-treated scaffold (Fig. 3C, D). Furthermore, we quantified platelet deposition by measuring the fluorescence intensity of integrin αIIb to evaluate the antithrombotic ability of the MPC polymer in the decellularized scaffold. The polymer-treated groups showed significantly reduced platelet deposition compared to the non-treated and HUVECs-recellularized groups ($p < 0.001$; Fig. 3E).

Evaluating the safety of MPC polymer for hepatocytes

After the MPC polymer treatment of the decellularized scaffold, we recellularized rat primary hepatocytes via the BD and proceeded with perfusion culture for 6 days (Fig. 4A). During recellularization via BD, no obvious differences were observed in the way hepatocytes spread into the parenchymal space of the decellularized scaffold (Fig. 4B). The culture had an appropriate distribution of hepatocytes in the polymer- and non-treated groups on day 2 of perfusion according to HE staining (Fig. 4C). We confirmed that the polymer was attached to the lumen of the vasculature in the polymer-treated group (Fig. 4D). These findings indicated that the polymer treatment did not interfere with the distribution of the recellularized hepatocytes. We compared the metabolic activity of the hepatocytes between the polymer- and non-treated groups to assess the safety of the polymer for the hepatocytes. The ELISA results showed no significant differences in albumin and urea concentrations in the perfusion medium collected on days 2, 4, and 6 between the two groups ($p = 0.700$; Fig. 4E). Furthermore, the number of TUNEL-positive cells among hepatocytes on day 2 of the perfusion culture was not significantly different between the groups ($p = 0.808$; Fig. 4F). These results suggested that the polymer had no hepatotoxicity.

Application of MPC polymer to the heterotopic transplantation model

Heterotopic graft transplantation was performed to evaluate in vivo antithrombotic potential of the polymer after MPC polymer treatment of the decellularized liver scaffold (Fig. 5A). The graft was filled with blood via anastomosis between the RA (donor) and PV (recipient) without leakage from the anastomosis and graft surface (Video S1; Fig. 5B). In the non-treated groups, the graft began to gradually shrink after approximately 10 min of blood perfusion, and clots appeared after 30 min. In the

polymer-treated groups, graft shrinkage and blood clots were lower in terms of gross appearance (Fig. 6A). Thick thrombus deposition on the vasculature was observed in the polymer non-treated groups, whereas little thrombus was observed in the polymer-treated groups according to HE staining (Fig. 6B). The fluorescence intensity of integrin α IIb was significantly less in the polymer treated groups ($p < 0.001$; Fig. 6C, D), suggesting antithrombogenicity of the polymer for the decellularized liver scaffold in the *in vivo* transplantation model.

Discussion

This is the first report of a novel approach to revascularization of a decellularized liver scaffold using a functional MPC polymer. We applied this polymer to decellularized tissue and confirmed its excellent compatibility with decellularized tissue. Platelet deposition was significantly suppressed in the polymer-treated scaffolds compared with that in the polymer-untreated scaffolds. Furthermore, metabolic functions of recellularized hepatocytes were maintained in the polymer-treated scaffolds, suggesting that the polymer was not toxic to the recellularized cells.

Bioengineered livers based on decellularization techniques can potentially regenerate transplantable liver grafts.^{3-5, 21-23} One of the techniques that must be developed to achieve the practical use of a bioengineered liver is revascularization. This is considered necessary because collagenous fibers from the vascular basement membrane activate the clotting cascade during blood perfusion, which results in graft failure after transplantation.²⁴⁻²⁶ We previously reported that liver sinusoidal endothelial cells (LSEC) were efficient cell sources for re-endothelialization to reduce thrombus formation in decellularized scaffolds and maintain the function of co-recellularized hepatocytes.¹⁵ However, the problems of a complicated procedure of cell preparation for recellularization remained followed by incomplete re-endothelialization of the liver scaffold.

In view of these issues, we considered a novel technology besides re-endothelialization to reduce the thrombus. The MPC polymers have already been used in various medical devices such as artificial hearts, intravascular stents, and artificial joints.¹⁷ Furthermore, their biocompatibility in humans is established.²⁷⁻³⁰ These polymers effectively prevent unfavorable biological responses and reactions such as platelet deposition in contact with plasma or whole blood.^{17, 31, 32} These properties are

owed to the extreme hydrophilicity and electrically neutral nature of the polymers, as well as the ability of phosphorylcholine to induce bulk-like behavior in the surrounding environment.^{17, 31} We focused on the excellent antithrombogenicity and biosafety of MPC polymers and developed a reactive polymer compatible with decellularized tissues. The antithrombogenicity of the MPC polymer was superior to that of re-endothelialization with HUVECs in the *ex-vivo* study, attributed to the superior vascular coating ability of the MPC polymer. There is no example for the treatment of natural tissue and scaffold using the reactive MPC polymer to improve their biocompatibility. Another advantage was the simplicity of the treatment procedures.

This study demonstrated the antithrombogenicity of a novel MPC polymer for decellularized liver scaffolds using *ex vivo* and *in vivo* models. In the heterotopic transplantation model, polymer-untreated scaffolds collapsed and formed blood clots after 30 min of blood perfusion at the same heparin concentration (5 IU/mL) as in the *ex vivo* models. This result is consistent with a previous report showing that non-heparinized decellularized porcine liver scaffolds lose blood flow after 20 min of blood perfusion in a heterotopic transplantation model.³³ In contrast, platelet deposition was significantly suppressed in the polymer-treated vessel walls of the liver scaffolds in the transplant model. Thus, our antithrombogenic approach using MPC polymers is feasible. Furthermore, considering its affinity for ECM in decellularized tissue, this polymer treatment is suggested to be a promising approach for achieving antithrombotic effects in decellularized liver and variable decellularized tissues.

Several studies have attempted to overcome the thrombogenicity of decellularized liver scaffolds. Heparin was primarily examined for antithrombotic purposes in decellularized tissues. Hussein et al. reported that a heparin-gelatin mixture improved the ability of endothelial cells to migrate and adhere to vessels, which could cover the

vasculature lumen and maintain their function.⁸ However, the anticoagulation ability of heparin-coated scaffolds is temporary, and the replenishment of heparin is impossible *in vivo*.³⁴ Therefore, the re-endothelialization technique was investigated to provide bioengineered livers with long-term antithrombotic properties. Several biomaterials were developed to improve the efficiency of re-endothelialization. Meng et al. reported that gelatin hydrogel perfusion significantly improved the re-endothelialization of decellularized rat liver scaffolds, which enabled active blood flow for 8 days after transplantation.³⁴ Devalliere et al. reported an approach to improve the revascularization of liver scaffolds by utilizing the REDV-ELP peptide, which could facilitate cell-specific binding of endothelial cells.¹¹ Ko et al. reported that conjugating anti-endothelial cell antibodies (anti-CD31 antibody) enhanced the attachment of vascular endothelial cells in a porcine model.²⁰ They heterotopically transplanted revascularized liver scaffolds and confirmed active blood flow for 24 h. There were concerns regarding its mass-producing ability and immunogenicity although the anti-CD31 antibody was efficient for enhanced attachment of endothelial cells. Kim et al. utilized anti-CD31 aptamers as a coating agent of liver scaffolds that have lower immunogenicity and stronger binding affinity for endothelial cells.¹⁰ They achieved higher endothelial coverage in the aptamer-coated group than in the aptamer-uncoated group. They also demonstrated low levels of integrin α IIb in the vessel walls of the aptamer-coated scaffolds in a rat blood perfusion model after 3 h. Thus, several biomaterials have been developed to improve endothelial cell coverage. In our study, the MPC polymer itself allowed thrombus inhibition in the liver scaffolds without endothelial coverage, and the antithrombogenicity was superior to HUVEC re-endothelialization. This polymer treatment is a simpler method than that used in previous reports. Furthermore, the established safety of MPC polymers in humans is

one of the advantages of these biomaterials for future clinical applications.

A limitation of this study was that the long-term hemocompatibility of the MPC polymer for the bioengineered liver was not examined. To overcome this limitation, this parameter should be evaluated in a large animal transplantation model to advance our approach for clinical applications.

In conclusion, our study demonstrated a novel approach for the revascularization of a decellularized liver scaffold using a MPC polymer. We demonstrated that the polymer could extensively coat vessel walls and efficiently suppress thrombus formation. Furthermore, the polymer was non-toxic to hepatocytes. Revascularization of liver scaffolds using this polymer should be a promising approach for bioengineered liver transplantation.

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Disclosure

The authors declare no conflicts of interest.

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Data sharing statement

The data that support the findings of this study are available from the corresponding author, K.F., upon reasonable request.

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Figure legends

Fig. 1. Characterizing the decellularized liver scaffold. (A) Gross appearance of rat whole liver before and after decellularization. (B) Hematoxylin and eosin staining and Masson's trichrome staining images showing no remaining nuclei or cytoplasm in the scaffold. (C) Intrahepatic portal tree and bile duct dyed with crystal violet. Scale bars = 100 μm .

Fig. 2. MPC polymer treatment on the decellularized liver scaffold. (A) The structural formula of the MPC polymer. PENHS units were added to allow adhesion to ECM. (B) Immunofluorescence staining of MPC polymer attached to the vasculature lumen (arrows). Scale bars = 50 μm .

Fig. 3. Evaluation of antithrombogenicity of MPC polymer during ex-vivo blood perfusion. (A) Decellularized liver scaffold connected to the blood circulation device by a cannula inserted into the PV. (B) Gross appearance of the decellularized liver scaffold of the non-treated, HUVECs-recellularized, and polymer-treated groups after ex-vivo blood perfusion. (C) Hematoxylin and eosin staining images of vasculature of the specimen after ex-vivo blood perfusion. (D) Platelet deposition evaluated with integrin αIIb staining (arrows). Arrowheads indicate the vasculature surface covered with HUVECs or the MPC polymer. (E) Quantifying platelet deposition based on the average fluorescence intensities of integrin αIIb . Scale bars = 10 mm (B) and 50 μm (C, D). All error bars represent the standard error of the mean. *: $p < 0.001$; a.u., arbitrary unit.

Fig. 4. Evaluating the safety of MPC polymer for primary hepatocytes. (A) Protocol for polymer treatment, recellularization of rat primary hepatocytes, and perfusion culture. (B) Gross appearance of recellularized liver before perfusion culture with or without the polymer treatment. Hematoxylin and eosin staining (C) and immunofluorescence (D) of recellularized liver after 48 hours of perfusion culture with or without the polymer treatment. (E) Cumulative albumin secretion and urea synthesis during perfusion culture. (F) TUNEL-positive cells (arrows), and their counts on day 2 of perfusion culture. All scale bars = 100 μ m; all error bars represent the standard error of the mean.

Fig. 5. Heterotopic transplantation of decellularized liver scaffold. (A) Scheme of the heterotopic transplantation model. (B) Gross appearance of the decellularized liver graft before and after in-vivo blood perfusion.

Fig. 6. Evaluating antithrombogenicity of MPC polymer for decellularized liver scaffold during in-vivo blood perfusion. (A) Gross appearance of decellularized liver grafts with or without the polymer treatment after *in vivo* blood perfusion. (B) Hematoxylin and eosin staining images of vasculature of the specimen with or without the polymer treatment after *in vivo* blood perfusion. (C) Platelet deposition evaluated with integrin α IIb staining (arrows). Arrowheads indicate the vasculature surface treated with the polymer. (D) Quantifying platelet deposition based on the average fluorescence intensities of integrin α IIb. Scale bars = 10 mm (A) and 50 μ m (B, C). All error bars represent the standard error of the mean. *: $p < 0.001$; a.u., arbitrary unit.

Figure 1

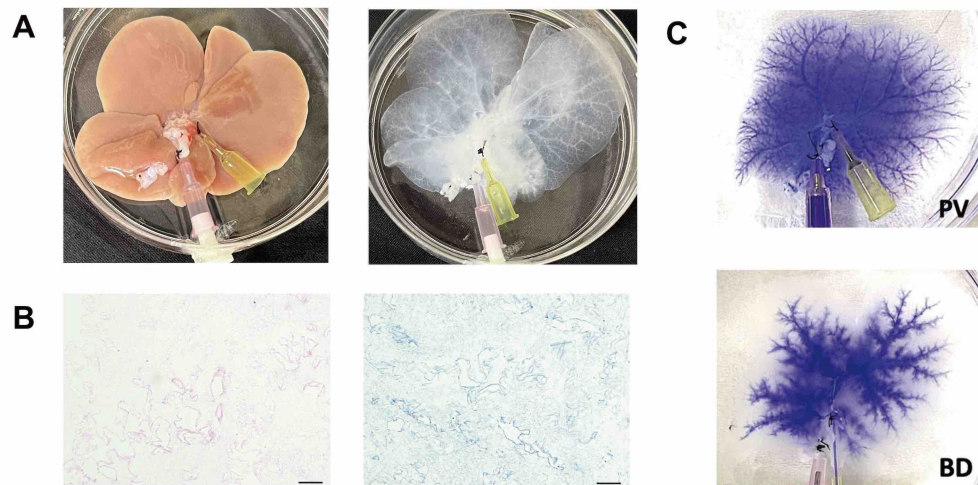
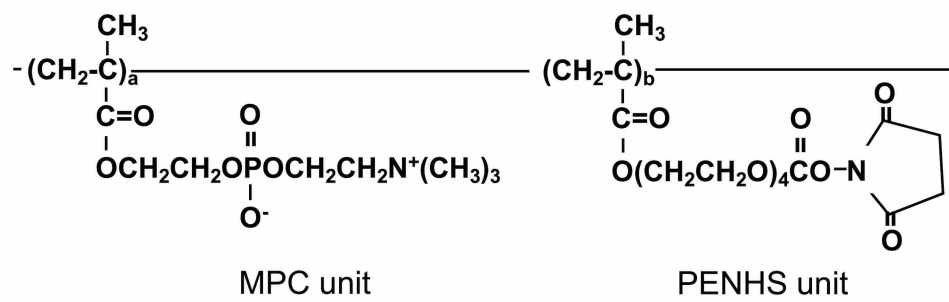


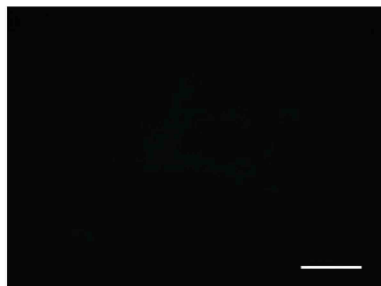
Figure 2

A



B

Polymer (-)



Polymer (+)

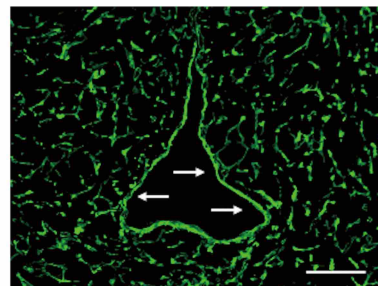


Figure 3

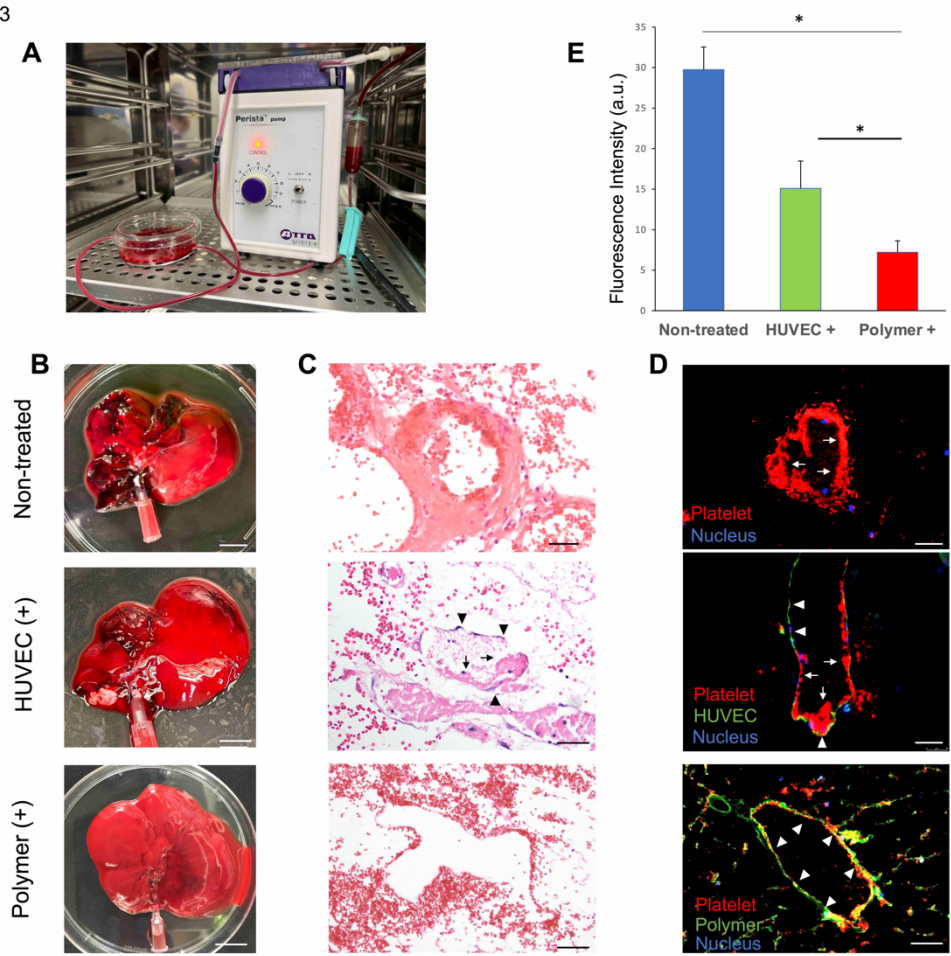


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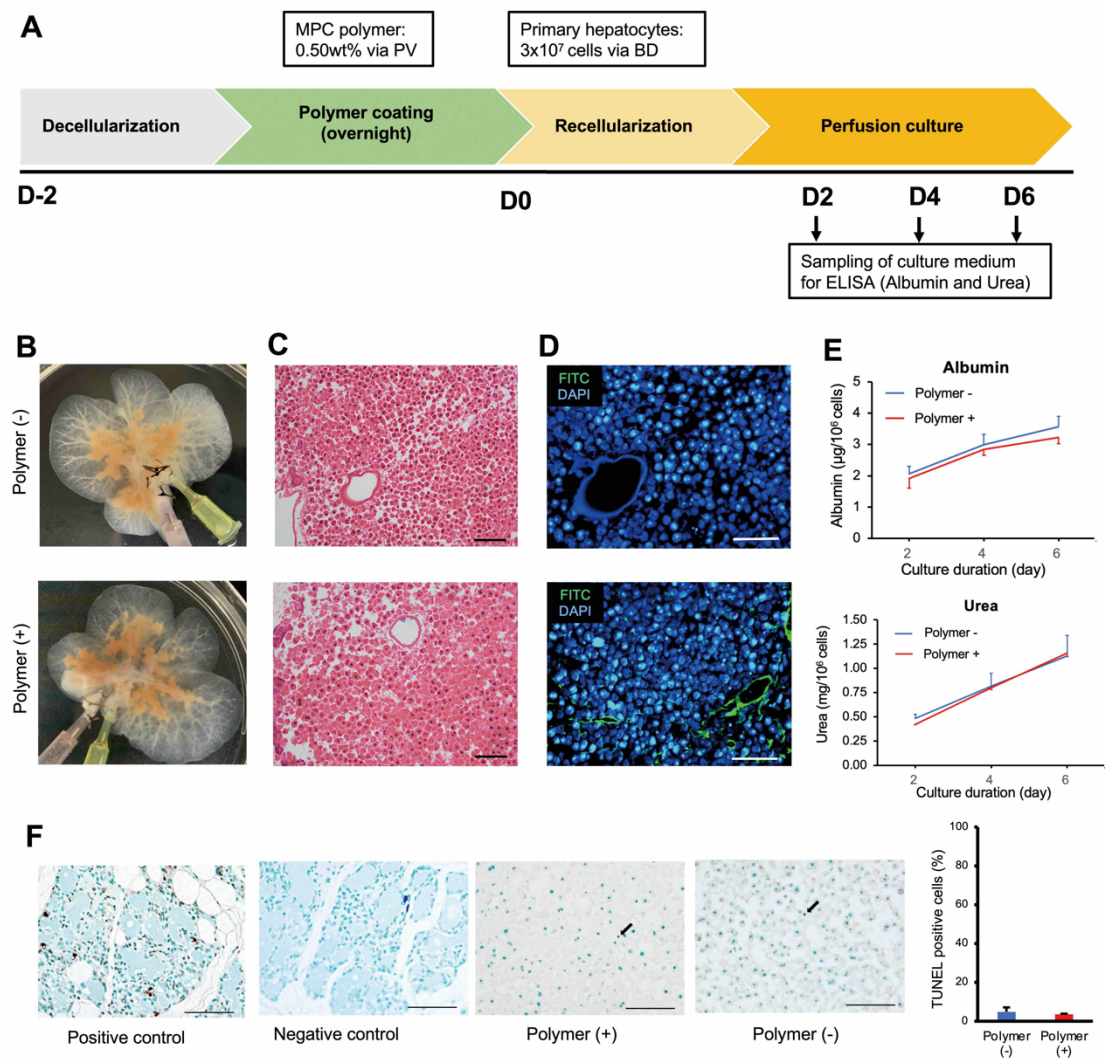


Figure 5

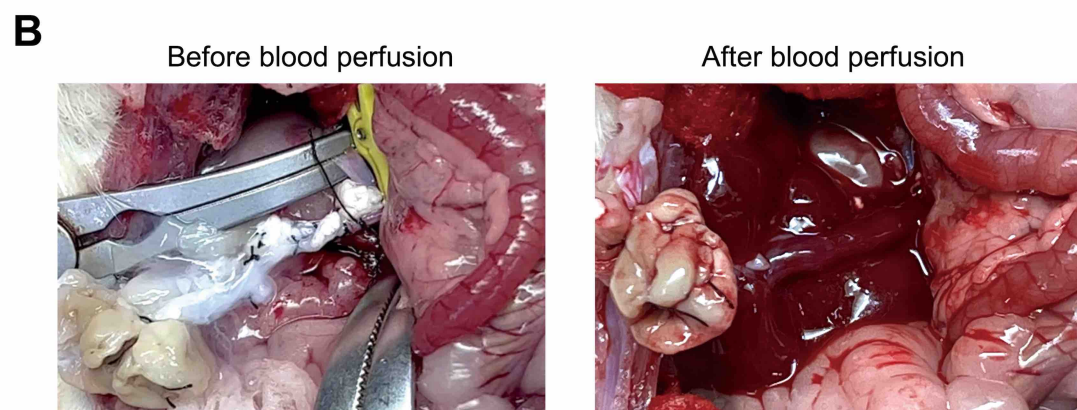
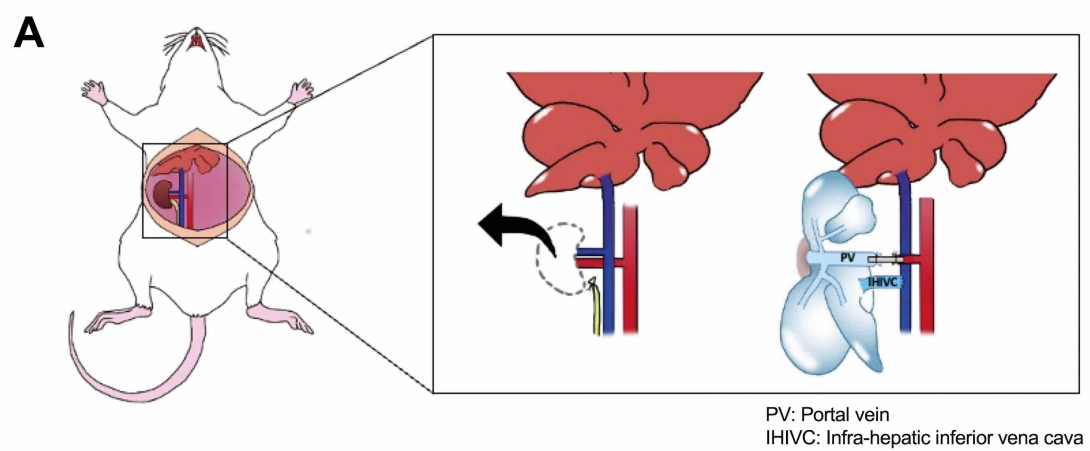


Figure 6

