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Systemic Decellularization for Multi-organ Scaffolds in Rats

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ABSTRACT

Introduction. Bioscaffolds derived from animal organs are promising materials for xenotransplantation and regenerative medicine. For effective generation of biological scaffolds from diverse organs, there have been many technical challenges. In this study, we introduced a novel approach to create multiorgan bioscaffolds through systemic decellularization.

Methods and materials. To obtain acellular bioscaffolds, the healthy adult rats were systemically perfused with ionic detergent through the carotid artery. Additional liver perfusion was set up to prevent potential obstruction from the influx of the decellularized debris via the portal vein. The perfusion system was controlled to maintain a constant physiological cardiac output of approximately 50 mL/min and was designed to minimize air entrapment. After decellularization, every organ designated for bioscaffold was harvested for evaluation of vascular structure and histology.

Results. The perfusion times were different for each organ. In our histological analysis, the decellularized bioscaffolds harvested from most organs including major solid organs (ie, heart, liver, and kidney) as well as the others (such as stomach, intestines, spleen, etc) represented no evidence of residual cellular materials. Furthermore, the well-preserved collagen materials and intact vascular structures were also confirmed.

Conclusion. The results from this study suggested that this systemic decellularization has the advantages to obtain a variety of bioscaffolds from single donor, and we can even decellularize organs with complex influx vascular structures. This method may also be used to study organ bioengineering for patients who need simultaneous combined organ transplantation.

THE NUMBER OF PATIENTS who need organ transplantation have increased, but donation has not kept pace. Therefore, alternative sources of transplantable grafts have been investigated.[1,2] Among these, tissue engineering has come into the spotlight due to expectations to overcome possible xenotransplantation complications.[1]

One of the tissue engineering methods, the decellularized bioscaffold, possesses many advantages: eliminating all potentially immunogenic cells, preserving not only the extracellular matrix (ECM) containing several bioactive molecules but also the vascular structure essential for oxygen and nutrient supply to the organ, supporting the three-

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dimensional structure of the intact organ.[1,3] Initially, decellularization technique had been limited to thin tissues such as intestines,[4] heart valve,[5] vocal folds,[6,7] and vessels.[8,9] In recent years, it has been reported that whole solid organs could be decelluarized to generate bioscaffolds that preserve the intact organ geometry.[10–13] Over the years, although these acellular organ bioscaffolds have provided a tool for tissue bioengineering and organ regeneration,[2,12,14,15] a number of hurdles remain to be resolved for preclinical and clinical application.

So far, not many studies have been done on organs with complex inflow and outflow vascular structures such as spleen, stomach, prostate, and urinary bladder due to difficulty in decelluarization using perfusion methods. Furthermore, a suitable tool to study the combined organ transplantation is required for patients who need simultaneous multiorgan transplantation such as pancreas-kidney and heart-lung. According to the United Network for Organ Sharing, over 100,000 patients in United States are awaiting a transplantable graft; about 2000 individuals who have multiple health problems needed simultaneous combined organ transplantation such as kidney-pancreas and thoracic-heart-lung transplantation. For these reasons, we demonstrated an efficient and novel approach to obtain the various scaffolds from one donor by systemic decellularization in the present study.

MATERIALS AND METHODS

We used adult male Sprague-Dawley rats weighing 250 to 350 g. Before this surgical application, rats were fasted at least for 12 hours. Anesthetized rats were systemically anticoagulated with heparin (200 U, Chungwae-pharma corporation). All animal work was approved by the Institutional Animal Care and Use Committee (Kangwon National University, Chuncheon).

Briefly, catheters were inserted in the carotid artery and portal vein for systemic decelluarization. We made several different outflows for flushing, on canial vena cava, suprahepatic and infrarenal caudal vena cava, pulmonary artery and vein, and portal vein, after catheterization. Subsequently, the caudal parts of the infrarenal caudal vena cava and aorta were temporarily clamped using microbulldog clamp for blocking detergent inflow into some organs located caudal parts such as large intestines, urinary bladder, and prostate, which need a relatively short time to make decellularized organ. The cranial mesenteric artery was also temporarily clamped to prevent influx of detergent into stomach, spleen, intestines, and liver due to the same reason. For cardiac decelluarization, pulmonary veins from lung were dissected to prevent transfer of air bubbles from the lungs to the heart. The liver was perfused through the portal vein, then we cut it just caudal to the site of catheter insertion to flush out detergent from the other abdominal organs including the intestines.

The perfusion system was controlled to maintain a constant physiological cardiac output of approximately 50 mL/min and was designed to minimize air entrapment.[2] Catheters were inserted in the carotid artery and portal vein, then detergent was perfused individually. After systemic flushing with phosphate to-buffered saline (PBS), 1% sodium dodecyl sulfate (SDS) in PBS was used for decellurization through carotid artery at a rate of 50 mL/min. Four hours later, hepatic perfusion via the portal vein was started at a speed of approximately 17 mL/min. During the process, cellular debris flowed out from the incision site of the vessels. The final step included multiple rinsing with PBS to dispose all of the potentially cytotoxic chemicals.[2]

To verify the intact vascular structure, x-ray fluoroscopic examination was also performed. The contrast medium was injected to large vessels of major organs (aorta in heart, portal vein in liver, and renal artery in kidney, respectively). Each organ designated for bioscaffold analysis was fixed in 10% formalin, and all organ sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome staining for microscopic examinations (Fig 2). During the perfusion process, we evaluated the decellularization rate of major organs every 4 hours by gross findings.

RESULTS

In the current study, we applied a novel approach for systemic decellularization to produce a multiorgan scaffold.

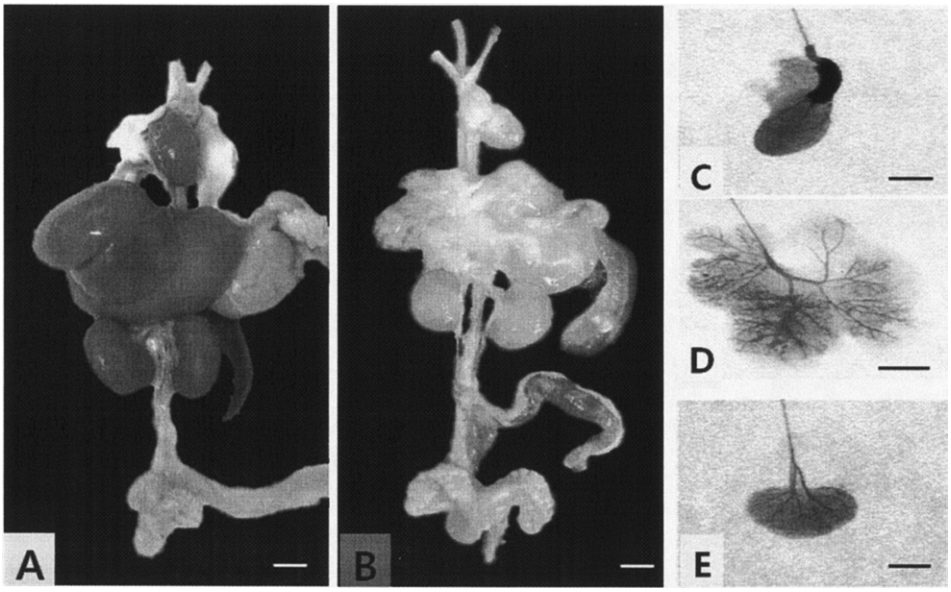

Fig 1. Gross findings of native adult rat organs **(A)** and decellularized organs after systemic sodium dodecyl sulfate perfusion **(B)**. Most organs were nearly transparent after decellularization. X-ray fluoroscopic image of the vascular architectures of the decellularized organs **(C–E)**. The intact coronary arteries and aortic valves in decellularized heart **(C)**, portal vein in decellularized liver **(D)** and renal arteries and small branch in decellularized kidney **(E)** were confirmed, respectively. Scale bar: 10 mm.