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Title: Intravital microscopy confirmed microvascular and ECM preservation in the decellularized rat kidney directly after transplantation

Running title: Microvascular and ECM preservation after transplantation

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Abstract

The aim of the present study was to determine whether decellularized rat kidney microvascular and extracellular matrix (ECM) integrity could be preserved under *in vivo* conditions directly after transplantation. Whole kidneys were harvested from the Sprague Dawley rat and were decellularized by perfusion with 0.5% sodium dodecyl sulfate (SDS) for 24 hours, followed by phosphate-buffered saline (PBS) for an additional 24 hours. Decellularized kidneys were then transplanted into recipients and vascular high-molecular-weight (150-kDa) FITC dextrans were infused via the jugular vein. Blood was then allowed to flow through the decellularized transplant. Intravital multiphoton microscopy confirmed the suitable confinement of the dextrans within vascular tracks and preservation of the decellularized architecture that was monitored in the short-term post transplantation.

New and Noteworthy: The study confirmed *in vivo* microvascular and ECM preservation in the short-term post transplantation.

Keywords: intravital microscopy; decellularized rat kidney; microvascular; ECM; transplantation

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INTRODUCTION

Transplantation is the ideal solution for end stage renal disease (ESRD) [1]. The exponential increase in ESRD has accentuated the demand for alternative solutions like the bioartificial kidney.

Decellularization is a promising organ bioengineering strategy that supports the development of the bioartificial kidney[2]. Angiographic[3] and histologic[4] techniques have examined whole kidney scaffold structure and function in various settings. Such investigations have provided evidence that the decellularization process successfully removes native cellular components and preserves the macrovascular and extracellular matrix (ECM) architectures. Nevertheless, there is still a limited understanding of the dynamic events that occur post-transplantation.

Intravital multiphoton microscopy offers a unique opportunity to explore these events, as it provides subcellular resolution to characterize live morphological and functional features within the kidney[5-7]. As a result, the aim of this study was to provide real-time confirmation of microvascular and ECM preservation in the decellularized rat kidney directly after transplantation.

MATERIALS AND METHODS

Rats

Experiments were performed on 200-400 g male Sprague Dawley rats (Envigo, Indianapolis, IN) in accordance with the Institutional Animal Care and Use Committee at the School of Medicine, Wake Forest University, Animal Research Oversight Committee at Khalifa University of Science and Technology and ARRIVE guidelines.

Fluorescent Probes

Jugular venous infusates were prepared by combining 50 µl of 150-kDa fluorescein isothiocyanate (FITC) dextrans (TdB Consultancy, Uppsala, Sweden) and 30-50 µl of Hoechst 33342 (Invitrogen, Carlsbad, CA) in 0.5-1 ml of saline.

Rat Kidney Deellularization and Sterilization

Whole kidneys were extracted with intact renal arteries, veins, and ureters. The arteries were cannulated using PE-50 polyethylene catheter tubing (Clay Adams- BectonDickson, Parsippany, NJ) and a 14-gage cannula and then secured with a 4/0 silk suture. Kidneys were flushed with 0.5-1 ml of heparinized PBS and then attached to a peristaltic pump (Cole-Palmer, Vernon Hills, IL, USA). After that, they were suspended in PBS and perfused via the renal artery at a rate of 0.4 ml/min with 0.5% sodium dodecyl sulfate (SDS, Sigma-Aldrich Co. LLC)[8] for 24 hours, followed by phosphate-buffered saline (PBS) for an additional 24 hours. The scaffolds were then sterilized with 10.0 kGy gamma irradiation[9].

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Decellularized Rat Kidney Transplantation and Intravital Multiphoton Microscopy

Rats were anesthetized with intraperitoneal injections of 50 mg/kg of pentobarbital (Hospira, Inc., Lake Forest, IL). Body temperature was controlled, and the internal jugular vein was cannulated. Incisions were created to expose the renal artery and vein in the recipient, and a unilateral nephrectomy was conducted. The renal artery and vein of the decellularized kidney were anastomosed end-to-end to the remains of the left renal artery and vein of the recipient after nephrectomy, and the decellularized kidney was left exposed for imaging as outlined in the literature[10]. The infusates were introduced via the jugular vein and fluorescent micrographs were collected using an Olympus FV1000MP multiphoton/intravital microscope from decellularized and normal kidneys.

RESULTS AND DISCUSSION

The results collected from the perfusion-based decellularization process (Figure 1) were consistent with previous findings[8]. Orthotopic transplantation of the decellularized rat kidney provided a unique opportunity to investigate morphological and functional features within decellularized whole organs *in vivo*. Intravital multiphoton micrographs obtained from the decellularized rat revealed signs of reduced tissue autofluorescence in scaffolds (Figure 2A), as compared to the high level of autofluorescence present in the normal kidney (Figure 2I). This reduction in autofluorescence can be attributed to the vast removal of cellular components and the residual collagen structures within the ECM[11], but it also obscured the ability to differentiate between the various renal tubular compartments.

The introduction of Hoechst 33342 directly confirmed the removal of cellular components from the decellularized scaffold, as its signature fluorescent pattern was absent along tubular structures and within their lumen (Figures 1A through 1H). However, robust Hoechst 33342 fluorescent signaling was observed within proximal (PT) and distal (DT) tubules in the normal rat kidney (Figure 2I) and provided a direct comparison of renal features before and after decellularization.

The fluorescent patterns outlined by the the150-kDa FITC dextrans also provided a way to track microvascular and ECM integrity *in vivo* directly after transplantation. Continuous imaging, which began at the start of perfusion and ended roughly 5 minutes later (Supplemental Video 1), revealed the appropriate confinement of the high-molecular-weight FITC dextrans within remnant vascular (V) tracks of peritubular capillaries within the scaffold. Similar results were observed in the control kidney.

However, the level of FITC fluorescence within the microvasculature of the decellularized kidney was lower than that of the normal kidney. The dextrans appeared to be the inhomogeneous distributed within the vessel walls of the scaffold. These findings suggest the possibility that less

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high-molecular-weight FITC dextrans could have entered the scaffold, or at least within the area that was imaged. This issue could be linked to the potential for clotting since no measures were taken to inhibit the onset of thrombosis, which is known to occur shortly after transplantation[12].

CONCLUSION

In conclusion, this study provided real-time confirmation of microvascular and ECM preservation in the decellularized rat kidney directly after transplantation. These results contribute to the current understanding of the dynamic events that occur post-transplantation, improve our knowledge on decellularized scaffold durability *in vivo*, and will ultimately support the future development of a functional bioartificial kidney. Future studies will focus on obtaining a better understanding of the deformation characteristics that accompany thrombi formation.

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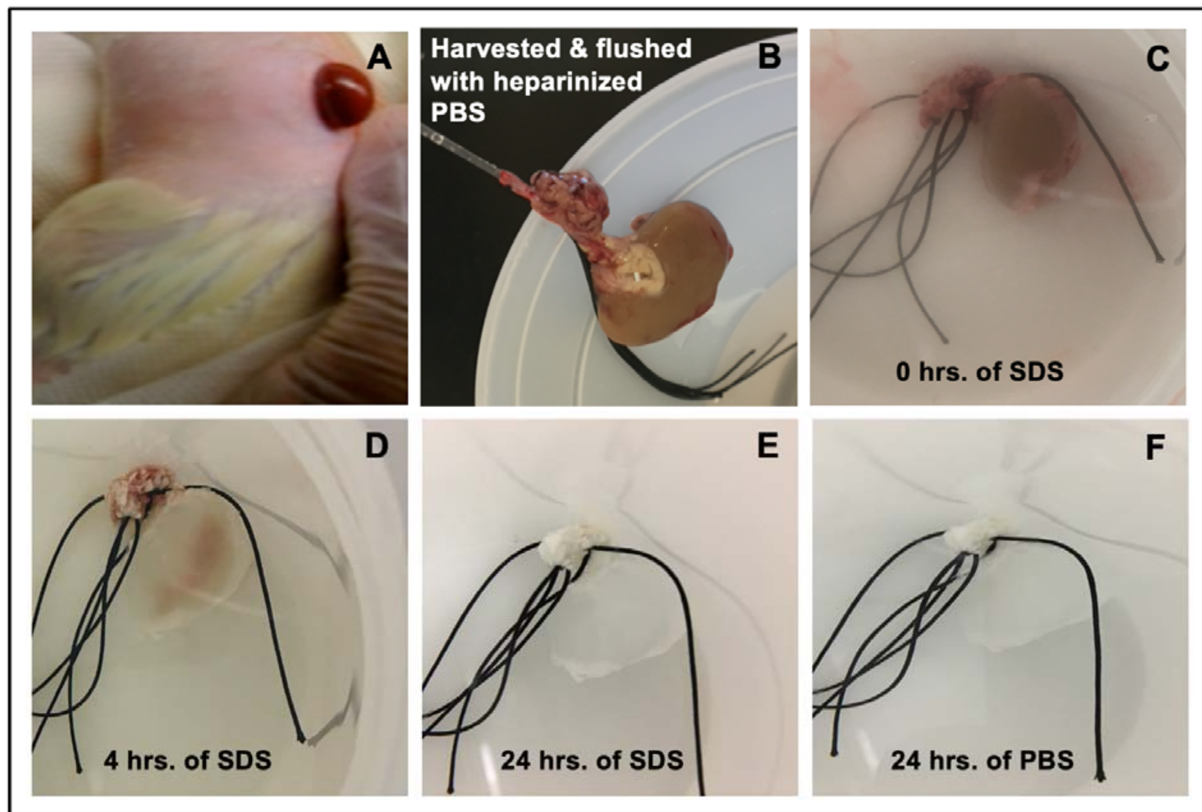

Figure 1. The whole rat kidney before and after perfusion-based decellularization. (A) Exteriorized left kidney of a rat prior to its extraction, (B) cannulated kidney after being flushed with heparinized PBS, (C) kidney suspended in PBS at the start of perfusion with SDS, (D) kidney after 4 hours of perfusion with SDS, (E) kidney after 24 hours of perfusion with SDS, and (F) kidney after its subsequent perfusion with PBS.

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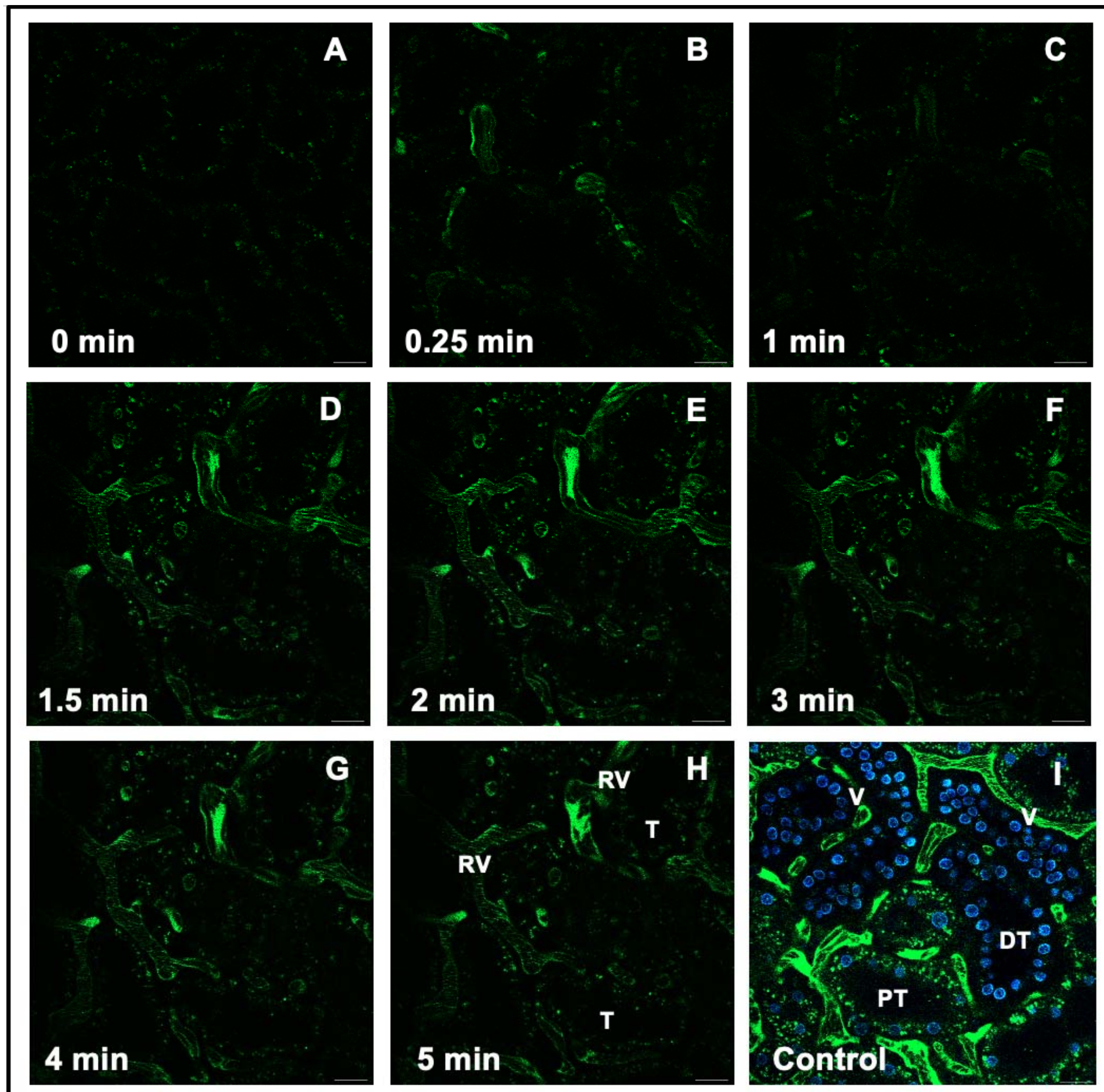

Figure 2. Structural and functional assessment of the microvasculature and ECM of the decellularized rat kidney. Images (A) through (H) highlight the absence of Hoechst 33342 fluorescent signal within tubular (T) structures of the decellularized rat kidney, and image (H) shows presence of the nuclear stain within proximal (PT) and distal (DT) tubules in the normal (control) rat kidney. All images show the confinement of the high-molecular-weight FITC dextrans within remnant vasculature (RV) of the decellularized kidney peritubular capillaries within the first 5 minutes of transplantation, and in the normal kidney peritubular capillary vasculature (V). Scale bar represented 20 μm.

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AUTHOR CONTRIBUTIONS

PRC conceived and designed research and was responsible for conducting all experiments and preparing the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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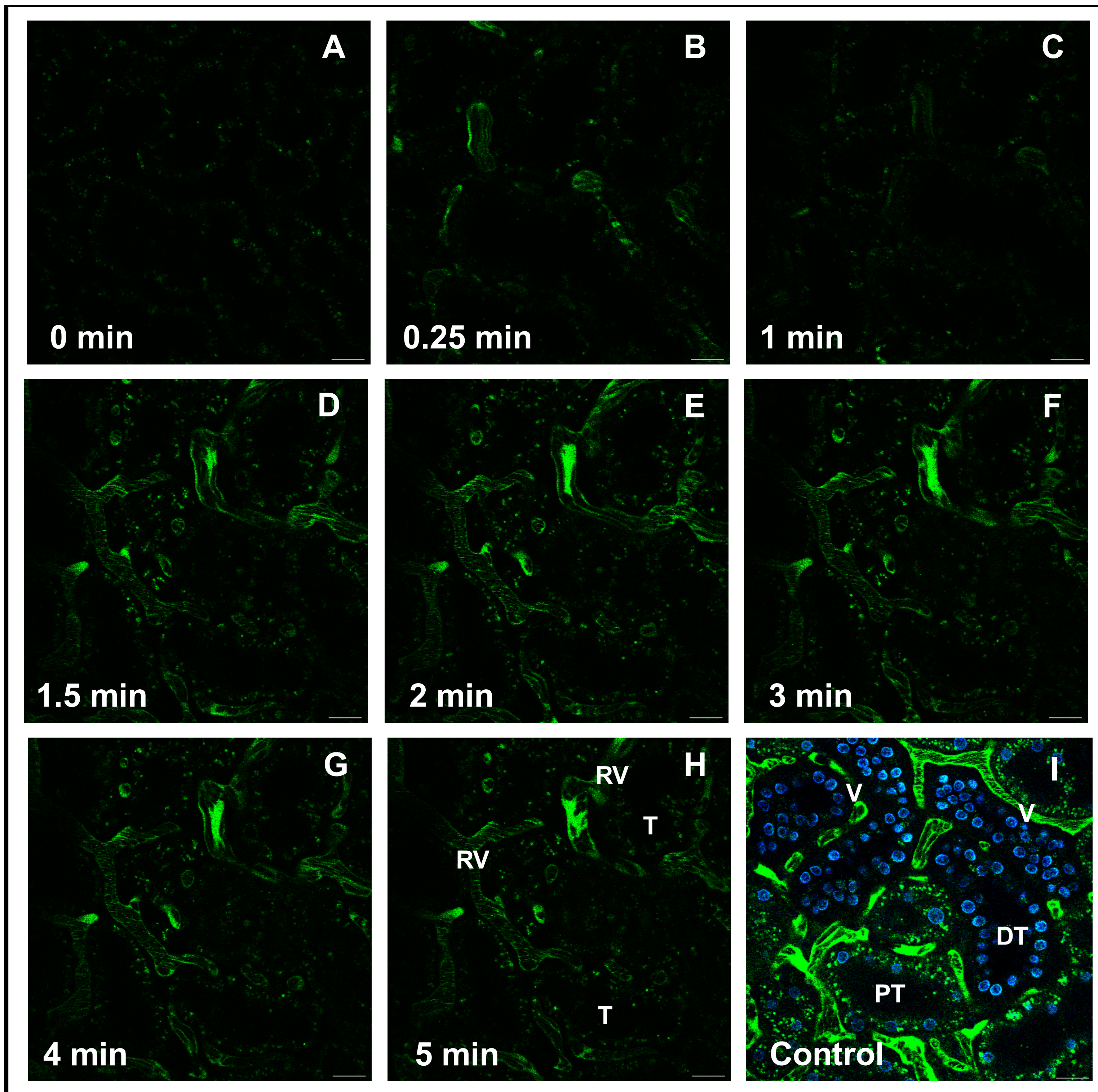

Figure 2

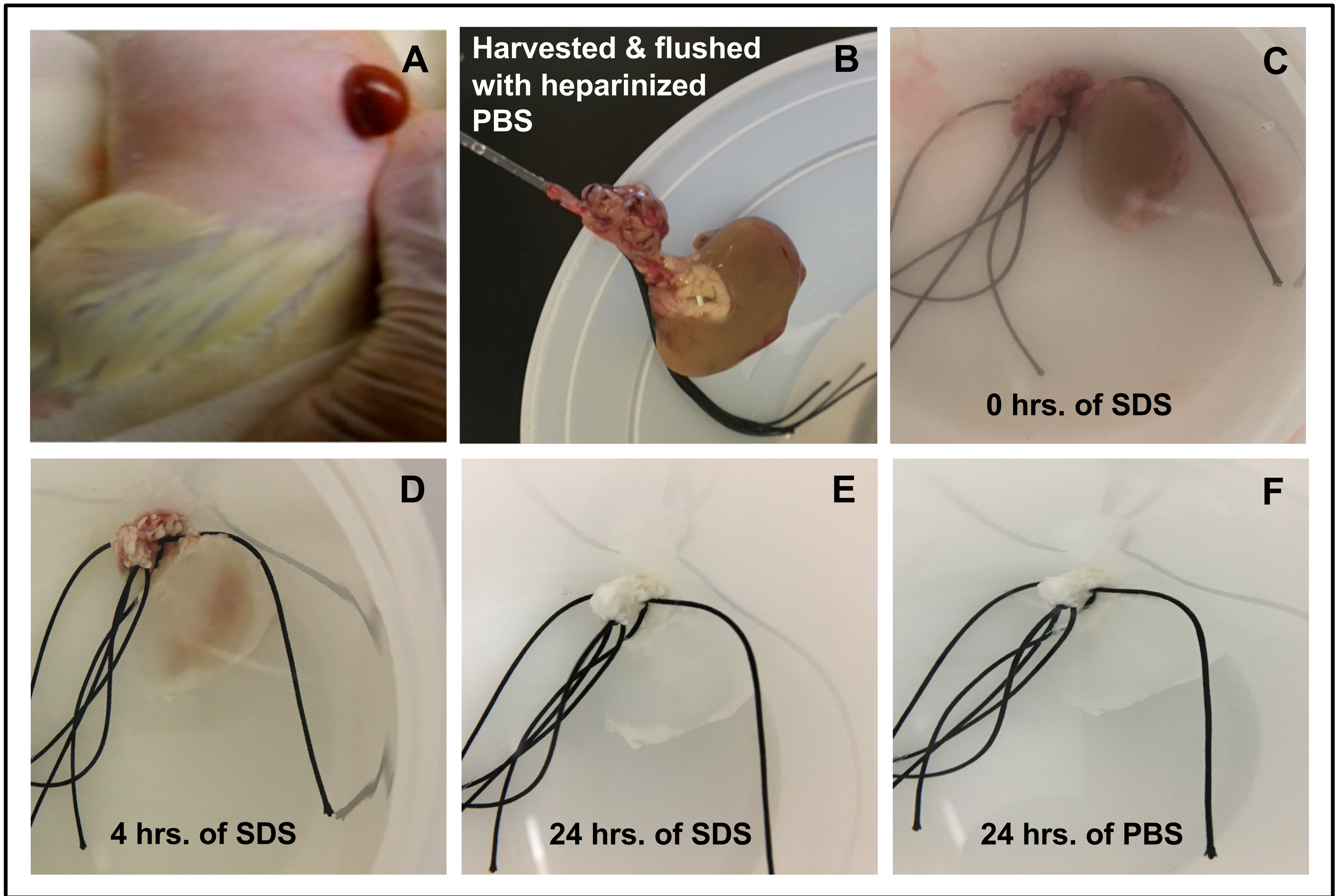

Figure 1