IN VIVO TISSUE-ENGINEERED ALLOGENEIC CONNECTIVE TISSUE MEMBRANES AS VASCULAR GRAFTS: OPTIMIZATION OF DECELLULARIZATION AND ANIMAL TRANSPLANTATION EXPERIMENTS.

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Introduction

Aiming for ideal vascular grafts, we have developed the autologous connective tissue grafts constructed in subcutaneous spaces of patients. In 2014, we clinically applied this technology in pediatric pulmonary artery patch augmentation, reporting favorable outcomes.[1] However, the formation of reliable connective tissue membranes may be challenging in high-risk pediatric patients due to limited subcutaneous areas and insufficient regeneration activities. Therefore, we began exploring the option of graft creation in healthy parents for allogeneic transplantation to their children. Furthermore, simplification and shortening of the decellularization process are indispensable to achieve same-day transplantation in the operating room. This study focuses on optimizing decellularization process and conducting preliminary animal transplantation experiments.

Methods

Silicone rod molds were implanted subcutaneously in beagle dogs for four weeks, after which the formed connective tissue tubes were excised. These tissue tubes were decellularized using a 1% sodium lauryl ether sulfate (SLES) solution with horizontal shaking (2h/1h/30min). Following decellularization, DNA quantification and tensile strength measurements in the short-axis direction were performed. The tissues were then trimmed into sheets and transplanted as allogeneic patches into another beagle dog's carotid artery. Post-transplantation assessments were conducted using ultrasound, and the grafts were excised after three months.

Results

Decellularization for more than one hour was required to ensure the complete removal of cellular components from the connective tissue membranes. Tensile strength measurements indicated no significant differences before and after decellularization. During three month-implantation, the grafts did not develop aneurysmal dilation. After extraction, Morphological examination exhibited no thrombus formation of the luminal surface, which was covered with smooth neointima.

Discussion

We successfully simplified and shortened the decellularization process using shaking methods compared to previous perfusion methods.[2] The decellularized connective tissue membranes maintained mechanical properties and excellent regenerative performance comparable to previous autografts, which suggests their potential as substitutes for allogeneic vascular grafts.

Figures

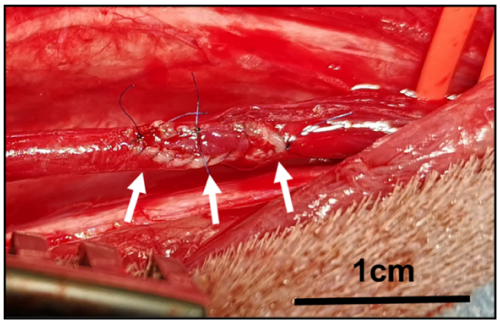

Figure 1: Implantation of patch grafts. Decellularized connective tissues were trimmed to an elliptical sheets of 10 × 8 mm. The resulting sheets were allo-transplanted as vascular patches.

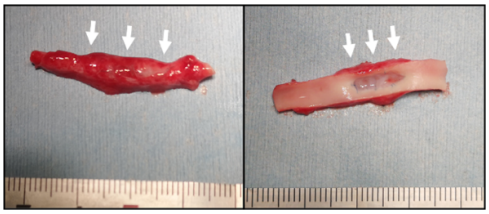

Figure 2: Macroscopic observation of harvested grafts at 3 months after implantation. The inner surfaces of the grafts were almost completely covered with neointima (right).

References

1. Kato N et al, Ann Thorac Surg, 102(4):1387-1390, 2016.
2. Yamanami M et al, Artif Organs, 46(4):633-642, 2022.

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