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Original research

# Aortic valve conduit implantation in the descending thoracic aorta in a sheep model: The outcomes of pre-seeded scaffold

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## HIGHLIGHTS

- Bone marrow-derived MSC has much potential in cardiovascular surgery.
- Pre-seeded AVCs may provide a new era of biological grafts.
- Pre-seeded valves are well organized and functional with the least calcification.

## ARTICLE INFO

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## ABSTRACT

*Background:* We evaluated the outcomes of implanting pre-seeded decellularized aortic valve conduit (AVC) with bone marrow-derived mesenchymal stem cells (MSCs) in a sheep model.
*Methods:* Eight sheep AVCs were obtained under sterile conditions and decellularized by using detergent-based methods. Decellularized AVCs were seeded with autologous bone marrow-derived MSCs in a dynamic bioreactor system. Pre-seeded AVCs were implanted in the descending thoracic aorta in a sheep model. In all sheep, a decellularized pericardial patch was also anastomosed to the proximal part in order to reduce the incidence of rupture. Pathological evaluations, echocardiography, multislice computed tomography (CT), and CT angiography were performed for the evaluation of implanted AVCs.
*Results:* The longest survival period was 19 months in pre-seeded animals with complete recellularization at the long-term follow-up. Immunohistochemical staining for desmin, smooth muscle actin, and cytokeratin was significantly positive in the pre-seeded samples and reached near normal ranges. CT angiography revealed no intimal tearing after 18 months of follow-up.
*Conclusion:* Pre-seeded AVCs with bone marrow-derived MSCs may have satisfactory results in post-operative cell seeding capabilities with promising functional potentiality. This modality may be beneficial and may provide a new era of biological grafts in cardiovascular surgery.


## 1. Introduction

Congenital heart disease affects approximately 20,000 infants annually in the United States [1]. Aortic valve (AV) replacement is essential in patients with degenerative calcified AVs or irreparable damages. Lifestyle, comorbidity, and the potential for reoperation are among the most important factors that can affect the consequences of heart valve implantation [2]. Calcification, degeneration, and limited durability are among the most noticeable

*Abbreviation:* AVC, Aortic valve conduit; MSC, Bone marrow-derived mesenchymal stem cell; ECM, Extracellular matrix; SDS, Sodium dodecyl sulfate; PBS, Phosphate-buffered saline; SMA, Smooth muscle actin; SEM, Scanning electron microscopy.

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complications after the implantation of nonviable bioprosthetic valves [3–5]. Therefore, it is important to discover a biocompatible substitute for synthetic valves to reduce the postoperative complications.

Tissue-engineering approaches for aortic valve conduit (AVC) replacement are likely to realize clinical success in the near future. Decellularized tissues have the advantage of evoking a platform for host cells to migrate to the empty scaffold and remodel the tissue. Certainly, migrated autologous cells should be capable of sustaining the durability of the engineered tissue. Nevertheless, cellular debris and residues after the decellularization may cause calcification and provoke cellular immune responses, resulting in an unorganized migration of appropriate cells and graft failure [6].

A biologically suitable and applicable AV scaffold with preserved and nonantigenic extracellular matrix (ECM) and totally removed cells may overcome these obstacles and preclude the probable reoperation, particularly in younger patients. No method of AV decellularization is quite effective for eliminating the occurrence of calcification. An experimental animal study has demonstrated the efficacy of heart valve decellularization in preventing allogenicity and even xenogenicity [7,8]. Recently, the potential of in vivo recellularization of decellularized heart valves has been verified [9].

The objective of the current study was to estimate the morphological, histopathological, and functional features of pre-seeded AVC scaffolds with bone marrow-derived mesenchymal stem cells (MSCs) in the descending thoracic aorta in a sheep model.

## 2. Materials and methods

### 2.1. AVC procurement

The committee on animal welfare in the University of Medical Sciences approved the study. All animals were treated in compliance with NIH guidelines for the care and use of laboratory animals. Fresh ovine pericardium and AVCs (5 cm in length) were precisely dissected under sterile conditions from eight male sheep (aged 165 ± 51 days) with a mean body weight of 42 ± 6 kg. Samples were treated with 1% Triton X-100 (v/v) for 24 h. Then, they were placed in 1% sodium dodecyl sulfate (SDS) for the next 24 h. Decellularized AVCs were rinsed with phosphate-buffered saline (PBS) in order to wash the detergents away for further cell seeding process.

For decellularization of the pericardial tissues, each sample was separately treated with 0.5% SDS for 12 h. Subsequently, all the samples were rinsed with PBS. All solutions were autoclaved prior to administration to have an effective ECM for further cell seeding process.

### 2.2. Biomechanical properties of pericardium

Longitudinal tensile properties of both natural and decellularized pericardial samples were evaluated by using a universal test machine (Zwick/Roell, Model: Hct 400/25, Radeberg, Germany) with a constant elongation rate of 4 mm/min. The results were recorded at the maximal force of longitudinal fibers. The specimens were clenched in sample holders, and the test was performed at room temperature.

### 2.3. Bone marrow aspiration, isolation, and expansion of ovine MSCs

After local anesthesia and procedural sedation, ovine bone marrow-derived MSCs were obtained from the iliac crest of eight sheep for the cell seeding process and further autologous implantation. An approximate amount of 15–40 mL of bone marrow was aspirated from the anterosuperior iliac spine. Bone marrow-derived MSCs were isolated at a density of $1 \times 10^5/cm^2$, as previously described [10,11]. In brief, the aspirated specimen was centrifuged at 1200 rpm for 10 min. Bone marrow cells were resuspended in 1 mL of culture media. Culture plates were maintained in an incubator with 5% $CO_2$and 95% air at 37 °C, for 3 subsequent days. The medium was refreshed at 3–4 days intervals. Expanded MSCs were applied for in vitro cell seeding, when the cells reached 80%confluence.

### 2.4. Seeding and 3D cultivation on AVCs (bioreactor design)

Four AVCs were rinsed for 60 min in PBS and Hank's balanced salt solution (HBSS) before the cell seeding process. The bioreactor equipment was sterilized and kept in a conventional cell incubator in an atmosphere of 5% $CO_2$ and 95% air at 37 °C. The AVCs were loaded with $2 \times 10^6$ cells following the cell seeding process. The pulsatile circulation was started at a rate of0.1 L/min. The initial flow rate was increased by 0.15 L/min until a maximal flow of 2.0 L/min (the cycle rate mimicked the physiological conditions: 60 beats/min) was reached. The mean system pressure was maintained at 100 mmHg during dynamic cultivation (48 h).

### 2.5. Surgical technique

Eight sheep with a mean body weight of 48 ± 4 kg were prepared for the operation. The sheep received the in vitro recellularized AVCs with bone marrow-derived MSCs. The sheep were anesthetized by administration of atropine sulfate (1 mg/kg), propofol (5 mg/kg), diazepam (0.27 mg/kg), and isoflurane (2.5%). Next, the sheep were heparinized and intubated via oral endotracheal tubes.

Left thoracotomy was performed between the fourth and fifth ribs, with the sheep in the right lateral decubitus position. After dissecting the fascia and pleura, the heart was exposed. Before clamping the aorta, heparin (100 mg/kg) was administered, and a shunt was placed between the aortic arch and descending aorta to reduce the risk of ischemia in the organs and extremities. The pre-seeded AVCs were implanted between the recipient descending thoracic aortas by using Prolene 5-0 sutures (China, Mainland) with a pericardial patch located at the proximal side of the graft. The field was observed carefully for intraoperative bleeding, and then the chest was closed with placement of a chest tube.

### 2.6. Echocardiography

The graft structure and hemodynamic parameters were assessed with a transthoracic echocardiographic technique. Transthoracic echocardiography with the depth optimized (SonoSite MicroMaxx, China, Mainland) and equipped with a 7.5 MHz phased-arrayed transducer was performed 48 h and 18 months postoperatively to evaluate hemodynamic performance of the implanted pre-seeded AVCs. Images included the conduit and leaflets from a long- and short-axis view.

### 2.7. Multislice computed tomography and CT angiography

Multislice computed tomography (CT) scanner (SIEMENS) (New York, USA) was used for evaluation of the implanted AVCs. A standardized protocol was used for acquiring data (250–300 mA, 120 kV, a collimation of 8 × 0.5 Mm, and a rotation time of 400 ms). A multisegment algorithm was applied for image reconstruction. For CT angiography, three vials of iodixanol (China, Mainland) were injected via an intravenous tube, and the images were taken in the arterial phase.