Subcoronary Technique for Orthotopic Implantation of Aortic Scaffold Reinforced with Polytetrafluoroethylene Strips in Sheep: A Pilot Study

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Abstract: Over the time, numerous studies have been reported describing successful pulmonary valve replacement, either with xenografts or allografts, performed in sheep model. In contrast, comparable results have not been achieved yet for aortic valve replacement in orthotopic position, which involves a more difficult surgical technique, high hemodynamic demands for the implanted valve and poor survival of animals undergoing this kind of surgery. To our knowledge, in Romania, these were the first orthotopic allogeneic aortic root implantations using the subcoronary technique, carried out by our team. We established feasible perioperative, anesthetic and surgical protocols, which will be used in future studies to evaluate the in vivo functional performances of decellularized valves compared with autologous adipose derived stem cells seeded valves. Therefore, a unique research core was created, which is currently the only center of this kind in our country, by joining a multidisciplinary team consisting of biomedical engineers, cardiovascular surgeons, anesthesiologists, cardiologists, pathologists, microbiologists and veterinarians, who will continue the research activity in the field of tissue engineering and translate experimental results into clinical activity.

Keywords: decellularized aortic roots, subcoronary technique, sheep, aortic valve implantation

1. Introduction

Over the time, numerous studies have been reported describing successful pulmonary valve replacement, either with xenografts or allografts, performed in sheep model. In contrast, comparable results have not been achieved yet for aortic valve replacement (AVR) in orthotopic position, which involves a more difficult surgical technique, high hemodynamic demands for the implanted valve and poor survival of animals undergoing this kind of surgery [1]. Structural valve deterioration and early leaflet calcification are known as major causes of bioprosthetic valve failure and therefore, increasing the need for reintervention [2]. Furnica et al (2015) suggested that two mechanisms are involved in bioprosthesis valve failure. Besides the chemical mechanism of aortic leaflet calcification, a mechanical one caused by atherosclerotic plaques was also observed [3]. Regarding prosthetic valve types, in human patients undergoing AVR and not only, there are many advantages when comparing stentless valves to

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mechanical or tissue stent provided valves, especially when it comes to a female patient, whose valve annulus is small in size. Of course, there are surgical techniques for valvular annulus enlargement, but these require surgical experience, prolongation of the operative time, as well as a major risk in case a re-intervention for valve replacement is needed. In echocardiographic terms, it translates into a larger orifice and a smaller transvalvular gradient, eliminating the risk of valve stenosis and patient-prosthesis mismatch, therefore, in clinical terms, leading to a better quality of life and an upper functional class of heart failure [4]. Moreover, a stentless valve maintains the dynamic interaction between the elements of the valvular apparatus: the valvular annulus leaflets, Valsalva sinuses and the sino-tubular junction. This synergic motion will lead to a better hemodynamic performance. Lower transvalvular gradients will lead to decreased left ventricular mass and apparently to an improved survival rate [5]. Another advantage is that stentless valves allow the placement of a transcatheter valve-in-valve [6]. Currently, some of the stentless valve models approved for use in clinical practice are: Prima valve® and Toronto SPV® from SJ Medical, Freestyle® from Medtronic (first generation); Super valve® which requires a single suture line (second generation); Sorin Pericarbon® and Equine 3F® (third generation). The surgical technique of implanting a stentless valve may vary depending on the surgical demands and the available valve. Therefore, one can opt for the subcoronary technique in which all sinuses are excised, or the modified subcoronary technique, in which the non-coronary sinus is preserved. "Root inclusion" and "full root technique" (Bentall procedure) involve re-implantation of the coronary arteries [7].

The aim of this study was to describe the surgical technique used in sheep model for orthotopic implantation of decellularized aortic roots, in cardiopulmonary bypass, using the subcoronary technique, as well as the challenges encountered during the surgery and the premises that led to its choice.

2. Materials and methods
2.1. Scaffolds preparation for decellularization

6 aortic roots were isolated from ovine hearts by careful dissection, preserving a portion of approximately 2 cm of the subvalvular muscle. The supravalvular portion was dissected and the ascending aorta was prepared up to the level of the brachiocephalic trunk. (Figure 1-A, B). The coronary arteries were carefully dissected, so that a portion of approximately 1 cm was prepared at each coronary artery. After the coronary artery ligation was performed, the aortic roots were mounted in the decellularization device (Figure 1-C, D).
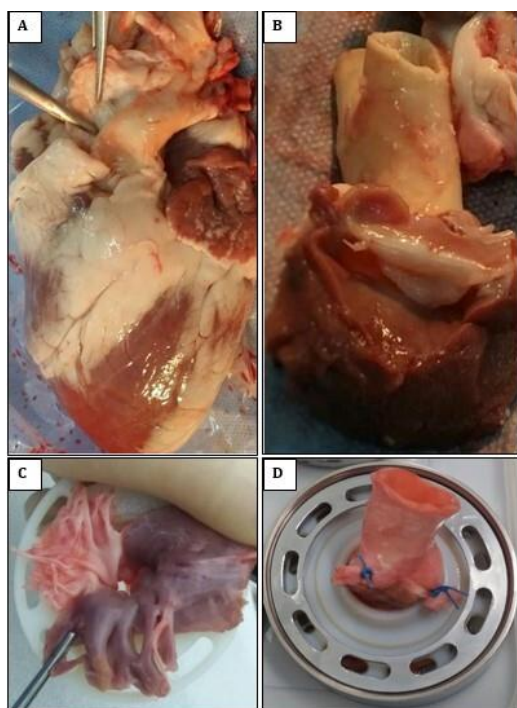

Figure 1. A. Dissection and preparation of the aortic roots; B. Isolated aortic root; C. Fixation of the aortic roots in the decellularization device; D. Coronary artery ligation

The scaffolds were tested for sterility and for the degree of decellularization by: DNA extraction followed by electrophoresis, DAPI and Hematoxylin/Eosin staining. The decellularization protocol and quality control assessment was described in our previous paper [8].

2.2. *In vitro* aortic root replacement

Aortic root replacement was simulated *in vitro* before the first operation in order to choose the most feasible surgical technique. We chose two decellularized aortic roots, considering one of them the native aortic root and the other one the decellularized bioprosthesis, simulating a valve-in-valve implantation. Initially, the root inclusion technique was attempted. The valvular annulus of the bioprothesis was thinned as much as possible and the coronary sinuses were partially excised, leaving the valve annulus containing the valve and the crown corresponding to the sino-tubular junction intact, in order to maintain the geometry of the aortic bulb (Figure 2-A).

The aortic valve of the native aortic root was excised and 3 traction sutures were placed at the top of each aortic commissure to expose the aortic root. 6.0 Polypropylene annular sutures were placed at the level of the aortic leaflets nadir, along the entire circumference of the valvular annulus (Figure 2-B). The sutures were then placed into the annulus of the bioprosthesis (Figure 2-C). When the bioprothesis was lowered into position, we observed that it was too large despite choosing the smallest measure available in the laboratory (14 mm), and the geometry of the aortic bulb changed, the junctional crown became folded. Moreover, the valve did not descend sufficiently, increasing the risk of obstruction of the coronary arteries. Because no smaller valves were available, an incision was made at the level of the non-coronary sinus and the subjacent valvular annulus, as practiced in the aortic annular enlargement procedure (Figure 2-D).
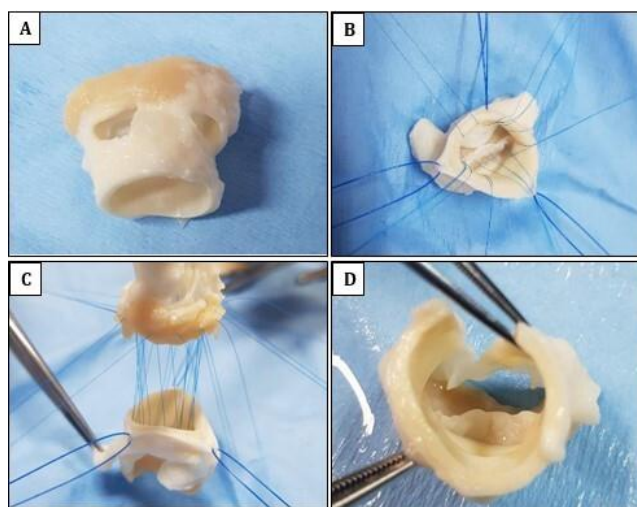

Figure 2. A. Decellularized bioprosthesis with sinuses excised;
B. Isolated sutures placed at the level of the valve annulus;
C. Placing the sutures into the bioprosthesis annulus;
D. Non-coronary sinus and aortic annulus section

Consequently, we adopted the subcoronary technique. The aortic sinuses of the bioprosthesis were excised, leaving three commissural pillars (Figure 3-A). Following the same steps described previously, three traction sutures were placed at the top of each aortic commissure. Annular sutures were placed along the aortic annulus circumference and then passed through the annulus of the bioprosthesis (Figure 3-B). The aortic annulus enlargement allowed us to lower the valve into the right position. Subsequently, the commissural pillars were sutured to the native aortic wall in a "commissure to commissure" manner (Figure 3-C). The final appearance of the prepared aortic root was satisfactory (Figure 3-C and 3-D).
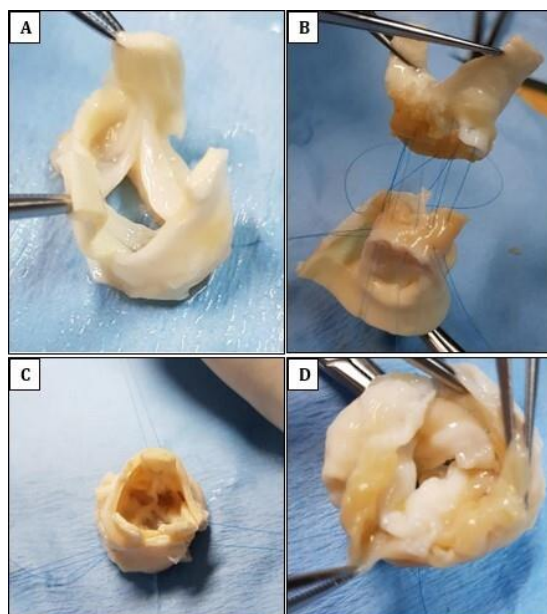
Figure 3. A. Bioprosthesis with three commissural pillars prepared;
B. Sutures placed along the native annulus and passed through the
bioprosthesis annulus; C. Final appearance, endoaortic view;
D. Final appearance - endoventricular view

2.3. Animals and surgical protocol

Two female juvenile sheep (25 and 26 months), weighing 47.0 kg and 43.5 kg respectively, underwent orthotopic aortic valve implantation by the subcoronary technique, using allogeneic aortic roots previously decellularized in our laboratory.

All animal procedures and perioperative care were conducted in accordance to the "Guide for the care and use of laboratory animals" (NIH Publication No. 85-23 revised 1996). This work is a part of a research grant conducted in accordance with the approved protocol no. 131/21.10.2016 by the Ethics Committee of the University of Medicine, Pharmacy, Science and Technology "George Emil Palade" of Tîrgu Mureș.

3. Results and discussions

3.1. Surgical technique and perioperative management

10-14 days before the operation, the animals were kept in separate boxes. During this period, laboratory analysis (complete blood count, biochemistry, blood electrolytes, coagulation tests) were performed. The daily feed ration was reduced to half 48 h before the operation, with complete fasting in the last 24 h and water restriction in the last 4-6 h before anesthesia. After preparation of the animal (trimming, bath with antiseptic detergent), the sheep was light sedated with intramuscularly detomidine (0.04-0.05 mg/kg body weight - bw), EKG electrodes mounted, followed by general anesthesia (oro-tracheal intubation and mechanical ventilation), central venous catheter placed in the jugular vein, femoral artery catheter, peripheral venous catheter placed in the left brachial vein.

The anesthesia induction was performed by intravenous administration of propofol (1.0 mg/kg bw), ketamine (0.04 mg/kg bw) and atropine (0.02 mg/kg bw), general anesthesia being maintained during the surgery with 1%-2.5% sevoflurane. Intraoperative monitoring of hemodynamic and respiratory parameters, temperature and O_2 saturation was performed as follows: heart rate and heart rhythm using 3 lead electrocardiography (ECG); invasive blood pressure with a 20G arterial catheter placed in the right femoral artery; central venous pressure with an 8F 4 lumen catheter placed in the left internal jugular vein; O_2 saturation by pulse-oximetry using a lingual sensor; core body temperature by using an esophageal sensor and diuresis by placing a Foley catheter.

Left thoracotomy was performed in the third intercostal space, followed by pericardiotomy and exposure of the right heart. Systemic heparinization (3mg/kg bw) was performed in order to achieve an activated clotting time (ACT) > 380 s. Cardiopulmonary bypass (CPB) was established. In the first case, arterial cannulation was achieved using one cannula placed in the ascending aorta before the origin of the brachiocephalic trunk, whereas in the second case, an arterial cannula was placed in the brachiocephalic trunk to ensure cerebral perfusion and an additional one was placed distal to the brachiocephalic trunk, in the aortic arch (Figure 4-A). The venous cannula was placed in the right atrial appendage. Under moderate hypothermia, the aorta was cross-clamped and Custodiol cardioplegia solution was administered in the aortic root through an Effler cannula. A transverse aortotomy was performed and the native aortic valve was excised. The decellularized aortic root scaffold was prepared by excising three strips of the aortic wall containing the coronary sinuses, including the two coronary arteries ligated prior to decellularization (Figure 4-B, C). Three polytetrafluoroethylene (PTFE) strips were placed at the level of the decellularized aortic root annulus, in order to reinforce the suture. The scaffold was implanted in the native aortic annulus using 5.0 monofilament polypropylene isolated sutures (Figure 4-D). Double layered aortorrhaphy was performed using 4.0 monofilament Polypropylene running suture. The aortic cross-clamp was removed followed by reperfusion and CPB was gradually weaned off. At this point of surgery, in the first case, the sheep went into cardiac arrest which was unresponsive to resuscitation maneuvers. Protamine was administered to neutralize the effects of heparin. The central cannulas were removed and the cardiotomy sites checked for hemostasis. A pericardial drainage tube was placed, followed by wound closure.
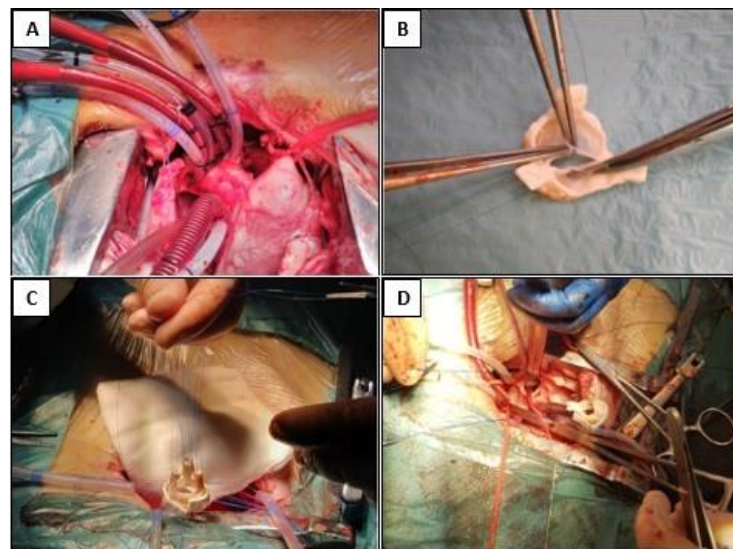

Figure 4. A. Double arterial cannulation; B, C. Decellularized
aortic root scaffold preparation;
D. Scaffold implantation in the native aortic annulus

For the awakening/reversal of anesthesia, the last dose of propofol was administered 60 min before the end of the operation, maintaining the sevoflurane concentration of 1% until the skin was sutured. The animal was manually ventilated until awakening from anesthesia, being extubated when spontaneous breathing and resumption of rumination movements were registered.

In the first case, the total operation time was 300 min with a CPB time of 171 min and a cross-clamp time of 125 min, whereas in the second case, the total operation time was 310 min with a CPB time of 190 min and a cross-clamp time of 95 min.

Among all types of valvular replacements, aortic valve replacements are the most commonly performed, the main cause being their degeneration in about 81% of cases, as opposed to pulmonary valve replacements caused mainly by congenital defects [9].

3.2. Surgery premises

Our goal was to find the best technique for implanting an aortic root, without having to perform a large- scale surgery, such as Bentall procedure or its variants (e.g. Cabrol procedure), in which re-implantation of the coronary arteries should be performed. In the latter case, postoperative outcome may be aggravated by severe complications that are independent of the functionality of the valvular implant, in particular due to obstruction of the coronary arteries characterized by an extremely low caliber and increased friability. One of the proposed models is the one described by Krause (1997) of complete subcoronary technique for implanting a stentless porcine aortic root [10].

In human patients, it is generally accepted that stentless valves show superior hemodynamic characteristics and low transvalvular gradients compared with stented valves [11,12] especially when using the full root replacement technique [13]. However, the full root technique involves re-implantation of the coronary arteries, which can lead to an increased CBP and cross-clamp time and a significant risk of bleeding. Being recognized as a difficult technique, the success of this procedure is highly dependent on the surgeon's experience. Additionally, it has been shown that the surgical technique of implanting the bioprosthesis has an impact on its durability, evaluated by structural valve deterioration. Thus, Mohammadi et al (2014) reported that "full root technique" is associated with a lower re-intervention rate at 10-15 years compared to the subcoronary technique, but does not influence short and long term survival rates [6].

The Hannover research group in heart valves tissue regeneration reported promising results of decellularized valves implantation both in pulmonary position in juvenile sheep and in aortic position in 6-year-old sheep, as an advanced age animal model [14,15]. In the last study they performed full root aortic valve replacement with re-implantation of the coronary arteries in fully grown female sheep with an average weight of 80.3±10 kg. Akhyari et al (2010) performed a surgical technique similar to that performed by our team, of orthotopic aortic implantation in a subcoronary position. The animals (juvenile sheep) were followed up for 5 months and showed excelent functional outcome [1].

Considering all the above-mentioned aspects, as well as the age and weight of the experimental animals, the low caliber and increased friability of the coronary arteries, our team opted for the subcoronary technique.

3.3. Surgery complications (tips and tricks)

It is absolutely necessary to choose a smaller size of the implanted valve in order to be positioned at the level of the aortic annulus and to maintain its geometry. Another important aspect to be considered in the subcoronary implantation technique is to avoid performing the suture line at the level of the commissure between the right and non-coronary leaflet. This ascension of the suture line will decrease the risk of the atrio-ventricular block occurring through the injury of the Hiss bundle, which passes through this region. Song et al (2012) observed that an alternative technique of subcoronary implantation with ascension of the inflow suture line at the level of the right non-coronary commissure to prevent injury of the conduction tissue at this level (membranous septum), significantly reduces the incidence of complete atrio-ventricular block and the need of implanting a pacemaker, compared to the conventional subcoronary technique [16].

In our first case, a technical problem occurred, caused by the fact that the ascending aorta up to the origin of the brachiocephalic trunk was extremely short, the suture site of the commissural pillars requiring a supra-junctional aortotomy, and the presence of the arterial cannula proximal to the origin of the brachiocephalic trunk made the aortorrhaphy difficult. Therefore, a circulatory arrest in moderate hypothermia (23C, 50 min) was established and an ascending aortoplasty using a heterologous pericardial patch was performed. The sheep was gradually warmed up and the CPB resumed. After aortic cross-clamp removal, fine ventricular fibrillation occurred. Resuscitation measures were taken, delivering 4 internal shocks and administering two i.v. epinephrine boluses of 2 mg/mL each respectively two i.v. xiline boluses of 100 mg, but without success. Left coronary artery obstruction was suspected.

Having this particular situation, the arterial cannulation in the second case was performed differently. The first arterial cannula was placed in the brachiocephalic trunk to ensure cerebral perfusion and a second one in the aorta distal to the brachiocephalic trunk, (aortic arch to the descending aorta), thus avoiding the problem encountered in the previous operation. After aortic cross-clamp removal, coarse ventricular fibrillation occurred, but after delivering 2 internal shocks and administering 50 mg of Xiline, the heart rhythm was converted to a sustained sinus rhythm, and the CBP was weaned off with no incidences. The animal was sacrificed after 24 h in order to analyze the harvested aortic root.

3.4. Biomaterials used for suture reinforcement

Synthetic grafts and fibrous biomaterials have been largely used in cardiovascular applications as vascular grafts and prosthetic heart valves. In 1952, Voorhees et al. first reported the use of synthetic polymers as vascular grafts [17]. Polyethylene terephtalate (Dacron) and expanded polytetra-fluoroethylene (PTFE) grafts have been succesfully used for the replacement of large diameter blood vessels; however it was not suitable for small vessels. [18]

Polytetrafluoroethylene (PTFE), also known as Teflon, is a widely used material in cardiovascular surgery for suture reinforcement due to its favorable mechanical properties. [19] It is a synthetic fluoropolymer, similar to polyethylene, obtained by vinyl polymerisation of the tetrafluorethylene monomer. [20] The chemical structure of PTFE was presented in Figure 5 [21].
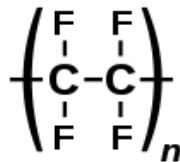

Figure 5. Chemical formula of Polytetrafluoroethylene

Moreover, it has been suggested that PTFE surgical materials have the lowest bacterial load among other suture materials, such as silk, polyglycolic acid (PGA) or nylon (polyamide suture) [22]. The major drawback of PTFE in cardiovascular surgery is adhesion formation in the long term, which can complicate re-interventions [23].

Teflon strips were used in human patients for a modified Bentall technique in order to minimize bleeding at the aortic root, a common issue in aortic surgery [24].

In our experience, given the high friability of the ovine tissue and especially of the aortic wall, we proceeded in a similar manner. For scaffold implantation, three strips of PTFE were placed around the annulus to strengthen the anastomoses, therefore preventing bleeding at suture sites.

Prior to this work, our team published promising results of pulmonary valve replacement in sheep, implanted in extra-anatomic pulmonary position as right ventricle (RV) to pulmonary artery (PA) shunts, using acellular xenogeneic scaffolds and adipose derived stem cells seeded valves, as well as a minimally invasive surgical method for adipose derived stem cells harvesting and isolation, succesfully used to prepare the seeded valves [25,26].

Of course, the surgical experience in subcoronary stentless aortic valve implantation is defining for the long-term outcome. By reaching the learning curve plateau, better results regarding the transvalvular gradient, need for reintervention and survival rate will be achievable [27].

Being the first interventions of this type carried out by our team, we did not intend at this stage to evaluate the short or long term functionality of the implanted decellularized valves, an objective that will be pursued in the future stages of our project.

To our knowledge, these were the first orthotopic decellularized aortic root implantations using the subcoronary technique in Romania, carried out by our team, making thus an important step forward in the field of heart valves regenerative medicine in our country.

4. Conclusions

The pilot study of orthotopic aortic valve replacement in sheep, using the subcoronary technique in cardiopulmonary bypass described in this manuscript, represents a huge leap forward in the field of heart valves tissue engineering in Romania, by creating a research core, which is currently the only center of this kind in our country. A multidisciplinary team was needed, consisting of biomedical engineers, cardiovascular surgeons, anesthesiologists, cardiologists, pathologists, microbiologists and veterinarians, who will continue the research activity in the field of tissue engineering and translate experimental results into clinical activity. We established feasible perioperative, anesthetic and surgical protocols, which will be used in future studies to evaluate the *in vivo* functional performances of decellularized valves compared with autologous adipose derived stem cells seeded valves. Concomitantly, the described technique can help other teams working in the regenerative field of heart valves.

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