Tracheal reconstruction with a composite graft: fascial flap-wrapped allogenic aorta with external cartilage-ring support

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Abstract

OBJECTIVES: Animal and clinical studies have demonstrated the feasibility of tracheal replacement by silicone-stented allogenic aortas. In clinical trials, however, this graft did not show mature cartilage regeneration into the grafts as was observed in animal models. To solve this issue, we investigated tracheal replacement with a composite graft based on a fascial flap-wrapped allogenic aorta with external cartilage-ring support in a rabbit model.

METHODS: Seven male ‘Géant des Flandres’ and ‘New Zealand’ rabbits served as donors of aortas and cartilage rings, respectively. Nineteen female ‘New Zealand’ rabbits were used as recipients. First, in nine animals, neoangiogenesis of the composite graft following a wrap using a pedicled lateral thoracic fascial flap and implantation under the skin of the chest wall was investigated. Animal sacrifice was scheduled at regular intervals up to 38 days. Second, 10 animals underwent tracheal replacement with the composite graft after a 7-to-9 day revascularization period, and were followed-up to death. Macroscopic and microscopic examinations were used to study the morphology, stiffness and viability of the construct.

RESULTS: There was one operative death after tracheal replacement. The first group of animals was found to have a satisfactory tubular morphology and stiffness of their construct associated with preserved histological structure of cartilages and moderate to severe aortic ischaemic lesions. In the group of rabbits having undergone tracheal replacement, the anatomical results were characterized by a discrepancy between the severity of ischaemic lesions involving both allogenic aorta and cartilage rings and the satisfactory biomechanical characteristics of the graft in 7 of 10 animals, probably due to cartilage calcification deposits associated with inflammatory scar tissue ensuring the stiffness of the construct.

CONCLUSIONS: Our investigations demonstrate the feasibility of the replacement of circumferential tracheal defects using our composite graft. Future experiments using therapeutic bronchoscopy tools are required to draw conclusions regarding the effectiveness of this tracheal substitute in the long-term.

Keywords: Animal model • Trachea • Transplantation • Tracheal replacement

INTRODUCTION

Despite significant progress in the field of tracheal allotransplantation, the ideal tracheal substitute remains to be found [1, 2]. In this setting, focusing on alternatives to tracheal allografts requiring immunosuppressive therapy, experimental studies in sheep and pig models showed that replacement of the trachea with either fresh or cryopreserved aortic allografts (AA) produced a conduit consisting of elements of mature tracheal tissue without the need for immunosuppression [3–5]. However, Tsukada et al., attempting to replace trachea with fresh AAs failed to reproduce these results. In their series of 10 sheep, no actual tracheal regeneration occurred and only a conduit of fibrotic tissue was observed in the four animals that survived >3 months [6]. In clinical trials of tracheal replacement with silicone-stented AA for extensive tumours, despite long-term satisfactory oncological results, the four surviving patients (out of six) did not show evidence of graft cartilage regeneration and none of them currently has a graft stiff enough to allow definite stent withdrawal [7–9]. Our hypothesis was that this graft insufficiency could be due to ischaemic phase prior neoangiogenesis. Therefore, we recently investigated, in rabbits, the efficacy of the fascial wrap as a provider of blood vessels for AA revascularization, and the construction of a tube-shaped graft [10]. Despite the fascial flap wrapped...
vascular pedicle: juxtaposition of recipient's fascial vascular carrier as an outer layer wrapped around allogenic cartilage rings and aorta. Figure 1: (A) Diagram of the composite construct with a unique transferable fascial vascular carrier as an outer layer wrapped around allogenic cartilage rings and aorta. (B) Operative view showing the composite construct with an inserted silicone tube, before graft wrap using the quadrangular-shaped fascial flap.

around the AA as an outer layer, this composite graft was not stiff enough to allow a potential tracheal replacement without the need for a complementary stenting. To address this issue, we hypothesized that the addition of allogenic tracheal rings around the AA as external support could achieve a more reliable tracheal substitute (Fig. 1A), and we report the investigation of this novel construct in a rabbit model.

MATERIALS AND METHODS

The experiment was conducted in two phases. We first investigated the efficacy of the rabbit lateral thoracic fascial flap wrap for neoangiogenesis of the AA graft-based tracheal substitute and consecutive overlapping of allogenic cartilage rings, and tracheal replacement with this composite revascularized construct.

The experimental protocol was approved by the regional ethical board on experimental use of animals (Comité d’Ethique en Experimentation Animale Nord-Pas-de-Calais). Experiments were performed in compliance with standard guidelines of the French Ministry of Agriculture and Fisheries (Ministère de l’Agriculture et de la Pêche) that regulates animal research in France.

Animals

Twenty-six syngenetic adult New Zealand (NZ) white rabbits, weighing 2725-4730 g and seven syngenetic adult male ‘Geant des Flandres’ (GF) rabbits, weighing 6110-6640 g were used (C.E. G.A.V La Passerie, 61350 Saint-Mars-D’Egrenne, France). All animals were housed in our institution at the University Hospital Department of Experimental Research.

Cryopreserved allogenic aortic grafts and tracheas

The seven male GF rabbits served as AA donors. They were anaesthetized with an intramuscular injection of ketamine [50 mg/kg, and xylazine (2.5 mg/kg)], and then euthanized using an intracardiac injection of embutramide, mebezonium and tetracaine (T61; Intervet, Beaucouzé, France). In these animals, the entire thoracic aorta (average length: 82.5 ± 11.6 mm) was harvested through a sternotomy.

After euthanasia, as described above, seven male NZ white rabbits served as donors of the entire trachea that was harvested through a cervical and transmediastinal approach. These grafts shared a mean of 32 ± 2.68 cartilage rings. The tracheal mucosa was peeled off the underlying cartilage, to obtain an epithelium-denuded trachea for harvesting cartilage rings.

All the grafts were transferred to the European Homograft Bank (EHB) in ice-cold isotonic sterile saline within 24 h of harvesting, for the cryopreservation process as previously described [10]. Likewise, the thawing process prior to use has also been previously described [10].

Anaesthesia of recipient animals

Anaesthesia of recipient female NZ rabbits was induced by an intramuscular injection of ketamine [50 mg/kg, and xylazine (2.5 mg/kg)], and was maintained using inhalation of isoflurane and oxygen administered through a face mask during spontaneous ventilation throughout procedures. Isoflurane discontinuation allowed animal awakening after approximately 25–30 min.

Postoperative analgesia was provided with two intramuscular injections of nalbuphine (1 mg/kg) on Day 1, and an additional transdermal patch of fentanyl (12.5 μg/h) in the recipients who underwent tracheal replacement.

Operative technique

Graft preparation. During the first phase, each GF rabbit’s thoracic aorta was divided into three segments (length: mean 28.6 mm; range 26–36) and prepared by the insertion of a 16f outer diameter silicone tube to maintain a patent graft lumen and by ligation of the extremities using 2/0 absorbable POLYSORB (Covidien France, La Clef St Pierre, France) sutures. Five to seven cartilage rings were harvested from each NZ rabbit’s epithelium-denuded trachea. They were laid over and around the aortic segments (Fig. 1B) and sutured into place with 6/0 absorbable PDS II (Ethicon France, Issy Les Moulineaux, France) interrupted sutures to avoid displacement.

During the second phase, similar composite grafts were prepared identically save that 5-mm outer diameter 25-mm long silicone stents (Tracheobronxane, Dumen BB, Novatech SA, La Ciotat, France) were inserted into the aortic segments to maintain lumen patency.

Graft fascial wrap. During the first phase, the graft fascial wrap was performed as an isolated procedure in order to study the revascularization process in a heterotopic position. Nine female NZ rabbits were used as recipients (Table 1). With the animals in the supine position, a vertical skin thoracic incision along the left mammary line was performed. The thoracic skin was dissected laterally from the underlying lateral thoracic fascia. A grossly quadrangular-shaped fascial flap was elevated from the underlying chest wall muscles and pedicled on the lateral thoracic vessels, as previously described (Fig. 1B) [10, 11]. The edge of the fascial flap was rolled around the composite graft
Cervical incisions were closed with 2/0 absorbable Optime R (Péters Surgical, Bobigny, France) interrupted sutures. During resection and reconstruction, cross-fascial wrapping of the composite aortic and tracheal allografts in control rabbits. A graft sample was cryopreserved, and transverse and longitudinal sections were cut at the level of the graft and flap pedicle (and adjacent trachea after the second phase) following 2 days of formalin fixation. Specimens were embedded in paraffin, cut into 3-μm slides and stained with haematoxylin–eosin–saffron (HES) for microscopic examination. The findings were compared with the normal morphology of both cryopreserved aortic and tracheal allografts in control rabbits.

**Follow-up**

The rabbits were housed in individual cages and allowed standard rabbit diet ad lib. Antibiotic prophylaxis (erythromycin 10/kg) was administered postoperatively over 3–5 days in both operation stages, and no immunosuppressive drug was given. After the first phase, sacrifice of the nine animals was scheduled at regular intervals, and after tracheal replacement, the 10 animals were followed-up to death. Therapeutic bronchoscopy tools were not available for this group of animals.

**Macroscopic evaluation**

Table 1: Clinical and pathological findings in nine recipient rabbits (composite-graft fascial wrap in a heterotopic position)

<table>
<thead>
<tr>
<th>Rabbit (weight, g)</th>
<th>Graft length (mm)</th>
<th>Number of cartilage rings</th>
<th>Complication</th>
<th>Sacrifice day</th>
<th>Cartilage viability</th>
<th>Aortic graft necrosis</th>
<th>Neoangiogenesis</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (4440)</td>
<td>27</td>
<td>6</td>
<td>–</td>
<td>3</td>
<td>+++</td>
<td>30%</td>
<td>+</td>
<td>(L, E)</td>
</tr>
<tr>
<td>2 (4430)</td>
<td>29</td>
<td>6</td>
<td>–</td>
<td>4</td>
<td>+++</td>
<td>50%</td>
<td>+</td>
<td>(L, E)</td>
</tr>
<tr>
<td>3 (3685)</td>
<td>29</td>
<td>6</td>
<td>–</td>
<td>5</td>
<td>+++</td>
<td>30%</td>
<td>+</td>
<td>(E)</td>
</tr>
<tr>
<td>4 (4730)</td>
<td>29</td>
<td>6</td>
<td>–</td>
<td>6</td>
<td>+++</td>
<td>90% (C)</td>
<td>–</td>
<td>+ (E, N)</td>
</tr>
<tr>
<td>5 (3290)</td>
<td>26</td>
<td>5</td>
<td>Limited skin necrosis</td>
<td>10</td>
<td>+++</td>
<td>90% (C)</td>
<td>+</td>
<td>++ (E, L)</td>
</tr>
<tr>
<td>6 (4315)</td>
<td>36</td>
<td>7</td>
<td>Wound dehiscence</td>
<td>13</td>
<td>+++</td>
<td>90%</td>
<td>+</td>
<td>++ (E, L)</td>
</tr>
<tr>
<td>7 (3630)</td>
<td>26</td>
<td>6</td>
<td>Wound dehiscence</td>
<td>20</td>
<td>+++</td>
<td>90% (C)</td>
<td>+</td>
<td>+ (N)</td>
</tr>
<tr>
<td>8 (3460)</td>
<td>29</td>
<td>6</td>
<td>Wound dehiscence</td>
<td>24</td>
<td>+++</td>
<td>30%</td>
<td>+</td>
<td>+ (E, L)</td>
</tr>
<tr>
<td>9 (4515)</td>
<td>26.5</td>
<td>6</td>
<td>–</td>
<td>38</td>
<td>+++</td>
<td>100% (C)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

C: calcifications; L: lymphocytes; E: eosinophils; N: neutrophils.

**Histological examination**

After the graft fascial wrap 19 animals survived the experiments. Six animals, however, experienced wound dehiscence (32%): three after the first phase (fascial wrap), and three after the first stage (revascularization) of the second phase, respectively. During the first phase, a limited skin necrosis was also observed (Tables 1 and 2). At the beginning of tracheal replacement procedures, one animal experienced cardiac arrest, probably as a consequence of hypoxemia and died at the end of the procedure. Rabbit 11 died of tracheal dehiscence of the upper anastomosis with cervical sepsis on Day 8. The other animals died of acute respiratory distress between Day 9 and Day 47, due to mucus plug obstruction extending to the major airways (rabbits 13, 17) or located in the stent (rabbits 14–16, 18). Last, rabbits 12 and 19 died as a result of an obstruction at the edge of the stent due to fibrin deposit or granulation tissue, respectively.

**RESULTS**

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rabbits were found to have a tubular morphology of their construct with the whitish internal face of the aortic graft surrounded by a thick wall including well-recognizable cartilage rings (Fig. 3A) such as that obtained by cartilage tissue engineering [12]. The grafts showed a satisfactory rigidity and elasticity in comparison with native tracheas from rabbits.

After tracheal replacement, the macroscopic study of upper airway specimens showed a similar morphology and stiffness of the construct in 7 of 10 animals (rabbits 10–14, 17, 19). The adjacent tracheal mucosa was usually congestive (Fig. 3B). However, the three rabbits (15, 16, 18) that experienced wound dehiscence after the first stage were found to have graft malacia with no clearly identifiable cartilage rings.

Pathological findings

Microscopic examination in the nine rabbits of the first phase showed that all the cartilage rings were viable up to Day 38. In four animals (rabbits 1–3, 8), the aorta was partially viable (Fig. 4) with areas involved in partial necrosis, limited to 30–50% of the aortic graft circumference. In the other five animals (rabbits 4–7,
The need for tracheal replacement is of the utmost importance in both benign and malignant pathologies. Tracheal transplantation is a particularly challenging surgical problem due to the lack of an identifiable vascular pedicle, which makes allografts unsuitable for direct revascularization. This issue has been previously addressed by using indirect revascularization techniques in the heterotopic position with the recipient’s epiploon [1], or forearm fascial flap allowing successful (albeit patchy) tracheal allotransplantation by transferring the wrapped graft to the orthotopic position with an intact vascular pedicle that was sutured to the neck vessels [2]. Given the need for immunosuppressive therapy, these transplantation techniques, however, cannot be considered for cancer patients. Furthermore, the latter method was found to have an additional limitation, since it does not allow the replacement of circumferential tracheal defects.

Tissue-engineered trachea seems to be a promising alternative [13]. Numerous experimental studies in animal models have been conducted mainly in the field of in situ tissue engineering with semi-synthetic grafts, but to our knowledge, the clinical application has been limited to a unique case of replacement of the right half of three rings of the trachea [14, 15]. Using a stem-cell seeded human airway scaffold is another solution supported by experimental studies and clinical applications. Unfortunately, clinical and pathological data are lacking and outcomes of patients are unknown [16–18].

Focusing on the value of AAs as tracheal substitutes in animal models, which were found to have regeneration of a mature tracheal tissue into their graft, we previously performed tracheal replacement with a silicone-stented AA in patients with extensive tracheal tumours [3–8]. Of the six patients we treated, four are free of disease (follow-up period: mean 65.5 months; range 59–72), but no graft developed sufficient stiffness to allow definitive stent removal. We hypothesized that critical ischaemia prior to neoangiogenesis of the AA might lead to this graft insufficiency, and that its indirect revascularization in a heterotopic position before tracheal replacement could overcome this problem. Indeed, a study we conducted in the NZ rabbit model demonstrated the efficiency of graft fascial wrap for revascularization of the AA and the construction of a viable tube-shaped graft with a transferable vascular pedicle, potentially available for tracheal replacement [10]. Some potential weaknesses, however, might be predictable in this model. First, the inadequacy between the tracheal and pathological in a signiﬁcant timeframe in rabbits than in our previous model. First, the inadequacy between the tracheal and fascial wrap for revascularization of the AA and the construction of a viable tube-shaped graft with a transferable vascular pedicle, potentially available for tracheal replacement [10]. Some potential weaknesses, however, might be predictable in this model. First, the inadequacy between the tracheal and fascial wrap for revascularization of the AA and the construction of a viable tube-shaped graft with a transferable vascular pedicle, potentially available for tracheal replacement [10]. Some potential weaknesses, however, might be predictable in this model. First, the inadequacy between the tracheal and
aortic diameters in the NZ rabbit (the latter being smaller in size of about 1/3), therefore leading us to conduct the present tracheal replacement study using larger rabbit species (GF weighing \( \approx 6400 \) g) as aorta donors. Second, the thinness of the aortic wall measuring only 100–150 µm, which makes the potential regeneration of a mature tracheal tissue with biomechanical properties allowing a tracheal replacement without the need for permanent stenting improbable. Thus, we hypothesized that the addition of a cartilage-ring external support might improve the stiffness of the construct.

After the graft wrap in a heterotopic position, the microscopic examination of specimens showed a satisfactory viability of cartilage rings up to Day 38, in line with both older and more recent landmark studies demonstrating that intact cartilage allografts are resistant to rejection and that tracheal cartilage remains viable when surrounded by well-vascularized recipient tissue [2, 19]. Despite signs of neoangiogenesis at the adventitial side of the aortic grafts appearing from the third day, in seven of nine animals, the AAs were found to be involved in partial or complete necrosis. A possible cause may be that the cartilage rings act as a barrier to sufficient vessel in growth, as has been previously described during experimental revascularization of tracheal autografts [20].

In the group of rabbits that underwent tracheal replacement, the anatomical results were characterized by a discrepancy between the severity of ischaemic lesions involving both AA and cartilage rings and the satisfactory biomechanical properties of the graft in the majority of animals probably due to cartilage calcification deposits associated with inflammatory scar tissue ensuring the stiffness of the construct. The calcification deposits might be the consequence of ischaemia of cartilage rings resulting in cartilage cell apoptosis or necrosis, and consecutive dystrophic calcification. Finally, despite graft ischaemia, late fistula or cervical sepsis was not shown in the animals, demonstrating the efficiency of the fascial flap wrap into preventing these potential complications.

The present study having been conducted in two phases allowed us to compare the fate of the graft itself in both heterotopic and orthotopic positions, and to discuss the mechanism of graft ischaemic lesions. During both phases, none of the animals showed evidence of acute graft rejection such as lymphocyte inflammatory reaction and vascular thrombosis of either neocapillaries or pedicled fascial vessels. After tracheal replacement, given the flap vessels patency observed upon subsequent histological examination in all the animals, a possible plication of the flap pedicle after elevation and rotation must be discarded as a mechanism of graft ischaemia. We hypothesize that the environment of the graft might play a major role. In a heterotopic position and inside a sterile environment, the observed inflammatory reaction, mainly composed of polymorphonuclear

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**Table 3:** Tracheal transplantation and replacement in rabbit models

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Procedure</th>
<th>Animals (number)</th>
<th>Peroperative mortality</th>
<th>Three-week mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbognani et al. [21]</td>
<td>TR with cryopreserved allogenic aorta</td>
<td>10</td>
<td>1</td>
<td>18*</td>
</tr>
<tr>
<td>Dodge-Khatami et al. [22]</td>
<td>TR (silicone-metallic prosthesis)</td>
<td>7</td>
<td>–</td>
<td>3*</td>
</tr>
<tr>
<td>Tanaka et al. [23]</td>
<td>Tracheal transplantation (cryopreserved allografts)</td>
<td>7</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>Weidenbecher et al. [24]</td>
<td>TR (tissue-engineered composite graft)</td>
<td>6</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Present study</td>
<td>TR (composite graft: AA and cartilage rings)</td>
<td>10</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*Euthanasia scheduled from Day 7 to Day 21.

*Euthanasia for respiratory distress.
leucocytes, was moderate, and associated with moderate to complete aorta necrosis, and complete conservation of the cartilage viability. In an orthotopic position, the graft was exposed to oro-pharyngo-tracheal microbiological contamination. The observed inflammatory reaction was acute, leading to more severe ischaemic lesions involving both graft components (Fig. 5A and B). This detrimental role of a potentially contaminating environment is supported by the fact that the most necrotic graft lesions were observed in the animals that experienced wound complication after the flap wrap procedure.

Survival after tracheal replacement ranged from 8 to 47 days, with a 3-week mortality rate of 40%, in line with data from either tracheal transplantation or replacement in rabbit models showing a 3-week mortality rate of 43–100% (Table 3) [11, 21–24]. Given the short survival with no technical possibility of bronchoscopic stent removal, we were not able to investigate whether the respiratory epithelium regenerated or if scar tissue formed within the lumen of the neo conduit such as previously described by Carbognani et al. [21] after tracheal replacement with cryopreserved AA, and Weidenbecher et al. [24] after tracheal replacement using a tissue-engineered cartilage graft, respectively [21, 24]. Likewise, the survival was too short to investigate whether the graft maintained its stiffness in the long-term through the calcification deposits.

Future issues of particular interest will be: (i) conducting the experiments using appropriate therapeutic bronchoscopy tools to increase the survival and observation time of animals, and (ii) investigating the efficacy of growth factors to improve graft neoangiogenesis, such as the platelet-derived growth factor which has been shown in the pig model to improve healing and neovascularization [25].

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Conflict of interest: none declared.

REFERENCES

[21] Carbognani P, Spaggiari L, Solli P, Corradi A, Cantoni AM, Barocelli E et al. Bronchoscopic stent removal, we were not able to investigate whether the respiratory epithelium regenerated or if scar tissue formed within the lumen of the neo conduit such as previously described by Carbognani et al. [21] after tracheal replacement with cryopreserved AA, and Weidenbecher et al. [24] after tracheal replacement using a tissue-engineered cartilage graft, respectively [21, 24]. Likewise, the survival was too short to investigate whether the graft maintained its stiffness in the long-term through the calcification deposits.

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