

Human Tracheal Allograft Banking: A Singapore Experience and Review on Recent Progress

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Abstract

While experimental studies on tracheal transplantation are numerous, these have failed to translate into wider clinical application due to various factors. This review discusses on the current techniques of processing, decontamination and storage of human tracheal allografts for clinical transplantation. We also present our lack of success in our attempt in tracheal allograft banking. With few allografts available for research, it is difficult to validate the effectiveness of various decontamination and preservation protocols. Some tissue banks adopt the use of controversial chemicals and irradiation for decontamination. While these methods may completely eliminate the risk of transmission of infectious diseases and micro-organisms, their effect on the integrity of the allograft and possible long-term side-effects to the patients remains unknown.

Keywords: Tracheal stenosis; Allograft; Revascularisation of allografts; Tissue banking

Introduction

Tracheal stenosis is a rare and often life-threatening disease. While conventional therapeutics options, such as primary resection of affected segment with end-to-end anastomosis, balloon dilatation, stenting and slide tracheoplasty exist for short and medium segment stenoses [1], these management approaches are unsuitable for patients with long segment or recurrent tracheal stenosis [2]. In these patients, tracheal replacement with a tracheal substitute after an extensive resection becomes necessary.

The trachea may appear to be a simple connection between the larynx and bronchi. However, in reality, it has a complex structure with functions in ventilation and clearance of respiratory secretions [3]. Hence, a tracheal substitute must fulfill the requirements of (1) providing structural support in the form of a laterally rigid but longitudinally flexible tube to maintain airway patency [3-6], (2) being non-toxic, non-antigenic and non-carcinogenic [5,6], (3) having an airtight conduit which integrates into surrounding tissues and does not dislodge or wear-off over time [5], (4) being able to withstand late stenosis and buckling [5,6], (5) preventing the accumulation of secretions and resistance to bacterial colonization [5,6].

As a result of these stringent criteria, the human tracheal allograft remains the most common substitute in use currently as it is also biocompatible and contains cartilaginous rings to maintain a patent airway [7,8]. Besides, tracheal replacement with human tracheal allograft can be performed repeatedly if necessary [2]. This has given rise to the emergence of tracheal allograft banks in Miami (United States of America), London (England) and Bonn (Germany) to meet the increasing demand [2,9].

In this review, the current techniques of processing, decontamination and storage of tracheal allografts for clinical transplantation are briefly discussed. We also present our lack of success in our attempt in tracheal allograft banking at the National Cardiovascular Homograft Bank (NCHB).

Donor Screening

The tracheal allografts are recovered from brain dead donors or non-beating heart donors. Recovery takes place within a specific

timeframe, usually within 24 hours of death or within 48 hours of the total ischaemic time, according to the American Association of Tissue Banks guidelines [10]. Contraindications include donors who are Human Immunodeficiency Virus (HIV) positive, Hepatitis B or C positive, syphilis positive, or have malignancies, viral diseases of unknown etiology, and thoracic diseases/trauma involving the trachea [2,9,11]. In addition, our tissue bank also excludes donors who have active tuberculosis or dengue infection. These two criteria are essential in our context due to the prevalence of the diseases in Southeast Asia and the adverse effects they may have on our recipients [12].

Despite the various methods of tracheal allograft decontamination from chemical or antibiotic decontamination to irradiation, the stringent evaluation of a potential donor remains the most crucial safety net to prevent the transmission of infectious diseases to the recipients. Bujia et al. reported that HIV remains in tissues that have been treated with thimerosal, formaldehyde or cialit although it was uncertain if the virus was in the active or dormant form [13]. Similarly, Costain and Crawford also questioned whether the standard dosage of 25 kGy of gamma-irradiation could destroy HIV, and recommended that a dosage of up to 50 kGy was required to inactivate HIV [14].

Preservation Methods

Tracheal allografts usually contained a high bioburden as a result of its constant exposure to the atmosphere. Therefore, the use of fresh allografts is uncommon because of its higher risk of microbial transmission, increased allogenicity and the necessity to subject recipients to immunosuppressive therapy. Due to these concerns,

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allografts are usually treated prior to implantation to eliminate any micro-organism and reduce their antigenicity.

Chemical preservation

Tracheal allograft reconstruction using chemically preserved human allograft was first reported in 1980 [15]. Since then, this has been the most common way to preserve the tracheal allografts due to a lack of suitable alternative method to process them [2].

One chemical method describes the immersion of allografts in isotonic saline solution with gentamicin or vancomycin at 1°C to 10°C. They are then soaked in 4% to 5% formalin solution at 1°C to 10°C for 14 days. Next, they are transferred to 4 g thimerosal dissolved in 1 L Dulbecco phosphate-buffered saline. After 42 to 56 days, the final step involves storing the allografts in acetone to dissolve adipose tissue for a minimum of 10 days to a year before implantation [1,2,9,16].

The chemically preserved allograft serves as a bioprosthesis, which do not provoke an intense inflammatory reaction seen in artificial prostheses. They also avoid the requirement of immunosuppressive therapy, as the chemical preparation has been shown to destroy the allograft's immunogenic antigens [2]. Bujia et al. reported that while unpreserved human allografts expressed the human leukocyte antigen class II antigens, these were completely destroyed after 7 days in formaldehyde and 42 days in cialit and thimerosal [17]. In addition, histological examinations had demonstrated that all cells in these allografts were non-viable and all major histocompatibility complex markers, which cause antigenicity, were lost [9]. Jacobs et al. also reported that the patients who survived tracheal allograft reconstruction did not experience any rejection or require immunosuppression. There was also no clinical or radiographical indication of allograft calcification, which is an endpoint to irreversible mechanical and immunological damage to the tissue, in their surviving patients [2,9]. However, as result of chemical preservation, cell regeneration and growth in the complex structure of the trachea might not occur. This means paediatric patients who receive these chemically treated allografts are at risk of needing a re-operation [2,5]. This problem related to allograft growth has been mitigated by the use of adult-oversize allografts in paediatric patients [2,9].

Despite the relatively good outcomes (Table 1), there is a high risk of infection, as reported by Propst et al. About 61.5% of allograft procedures were complicated by infection despite antibiotic therapy after transplantation of the allograft [11]. Moreover, the duration of follow-up in the studies reporting the use of chemically preserved allografts have been relatively short, which prevents the proper evaluation of the long-term durability of chemically preserved allografts in tracheal reconstruction.

Cryopreservation

While cryopreserved tracheal allografts have been used in several experimental models of long segment tracheal replacement, clinical application of this procedure is limited as contradictory results have been reported [18].

The possible advantages of cryopreserved allografts include (1) reduced allogenicity caused by degeneration of epithelium, which in turn contributes to the loss of human leukocyte antigen class II antigen expression during freeze-thaw [7,8], (2) no requirement of immunosuppression for recipients [19], (3) easy management of the allograft during grafting as it retains the original elasticity, (4) increased protection by the internal lumen laid with mucosa from external environment such as micro-organisms and effects of air-drying, (5) easy availability from tissue banks, and (6) capacity for long-term storage [20]. While a longer period of cryopreservation may result in lower viability of the allograft, this may be offset by the decreased antigenicity and thus the extent of rejection in the recipient [8]. However, cryopreserved allografts could shrink longitudinally after months of implantation [19].

Moreover, the types of cryopreservation protocols were also discovered to influence the integrity of human trachea. Mabrut et al. studied the characteristics of the human trachea treated in 20% glycerol with Hydroxyethyl Starch (HES), 15% Dimethyl Sulfoxide (DMSO) with HES, 15% DMSO with 4% albumin, and HES only. They found out that while there was no apparent morphological change by macroscopic inspection, light microscopic examination and histological examination revealed better preservation of mechanical characteristics of the tracheal cartilage for allografts preserved using glycerol with HES, or DMSO with albumin. They also presented their observation on the presence of occasional microfissures in arterial grafts stored in -196°C, in contrast to those stored in -140°C, which had none [7].

Decontamination Methods

Antibiotics

While some tissue banks choose to rinse tracheal allografts in antibiotics before subjecting them to chemical treatment, our bank has been evaluating the efficacy of other non-chemical decontamination regimens. Our preference in using only antibiotics is due to our concern that certain chemicals used, such as thimerosal, may yield toxic residues that may result in side-effects in recipients. The current antimicrobial agent thimerosal is controversial owing to the toxicity of its ethyl mercury breakdown product [21]. Therefore, we solely rely on a variation of our current antibiotic regimen for allograft decontamination. We had previously validated the same regimen for the decontamination of cardiovascular homografts.

Initially, our tracheal allografts were decontaminated using 50 IU/mL penicillin G and 50 ug/mL streptomycin. They were incubated at 37°C for 6 to 12 hours before controlled rate freezing. The allografts were then cryopreserved in 10% DMSO and stored in vapour phase liquid nitrogen at -196°C. However, positive microbiological cultures suggested the ineffectiveness of this antibiotic regimen. We observed an allograft contaminated with *Candida guilliermondii* and *Pichia ohmeri* before and after incubation. Similarly, another allograft was contaminated with *Cladosporium* in the post-decontamination specimen.

Author and reference no.	Year of publication	Duration of follow-up	% of survival rate after tracheal allograft transplantation	% of surviving patients without airway problem
Elliot et al. [16]	1996	9-18 months	80% (4/5)	75% (3/4)
Jacobs et al. [2]	1996	5-10 years	83% (20/24)	80% (16/20)
Jacobs et al. [9]	1999	0.57-2.44 years	83% (5/6)	100% (5/5)
Jacobs et al. [9]	1999	5-14 years	84% (26/31)	84% (22/26)
Propst et al. (11)	2011	3.32-7.55 years	90% (9/10)	-

Table 1: Post-operative outcome of transplantation of chemically preserved tracheal allografts worldwide.

In an attempt to resolve this persisting problem, a new regimen consisting of an antifungal drug, Caspofungin, together with stronger, broader spectrum antibiotics was implemented. Tissue decontamination was performed using 100 ug/ml amikacin, 50 ug/ml vancomycin and 0.1 mg/ml Caspofungin at 4°C for 48 to 52 hours. Caspofungin was selected because it is a novel class of antifungal agent, which displays similar efficacy to amphotericin B in the treatment of invasive candidiasis and aspergillosis. Besides, it has an excellent safety profile [22]. In contrast to amphotericin B, which usage had been discontinued in some tissue banks due to its deleterious effects on human tissue viability and cell damage [23], an advantage of Caspofungin is that it does not appear to result in such adverse effects. This was observed from histological examination performed by our tissue bank, which revealed no appreciable difference in the quality of tissue between pre-incubated tracheal tissues and tissues incubated in Caspofungin at 4°C for 24 hours.

The microbiological outcome of allografts decontaminated with the new regimen was intriguing. This was because findings suggested that a strong antibiotic regimen failed to kill a particular species of sensitive bacteria. While *Klebsiella* and *Acinetobacter baumannii* were isolated in the post-recovery tissue specimens, *Klebsiella* continued to be isolated from the tissue specimens post-decontamination. When the antibiotic sensitivity test was performed, it was revealed that the *Klebsiella* isolated was purportedly sensitive to many antibiotics, including amikacin. Yet, the incubation process with amikacin was unable to eliminate the bacteria. Although we have successfully obtained a negative microbiological culture post-decontamination in another allograft treated with this procedure, a shortage of allografts makes it difficult to ascertain if this antibiotic regimen is truly effective (Table 2).

Irradiation

Tracheal allograft contains cartilaginous rings, mucosal lining and fibrous tissue between the rings and *trachealis* muscle posteriorly, which would most likely activate a strong immunologic response in the recipient. To counteract this problem, the method of denaturing the tracheal cartilage to reduce its antigenicity and destroy all micro-organisms was proposed [20].

The novel use of irradiated cryopreserved tracheal allografts was first reported by Kunachak et al. in 2000. The allografts were initially kept at 57°C for 20 minutes before being placed in a -70°C chamber for two or more days. They were then gamma-irradiated for 5 hours at a dose of 25 kGy and stored at -70°C until implantation. The patients were not administered immunosuppressants [20,24].

The main advantage of irradiation as a decontamination procedure as compared to a chemical method is a shorter processing time of less than a week [20]. Gamma-irradiation has been used in terminal sterilisation of bone allografts for clinical applications. A radiation dose of 25 kGy is believed to destroy all micro-organisms and reduce the

antigenicity of the allograft. Although it is not known if the combination of radiation dose and cryopreservation would have any consequence on cell viability, a preliminary histological examination prior to grafting revealed evidence of viable chondrocytes [20,24]. However, it is also important to evaluate if gamma-irradiation will adversely affect tracheal allografts and the dosage at which it will damage the tissue. This is because several authors have previously reported the impact of irradiation on the biological and biomechanical integrity of bone allografts by causing hydrolysis of polypeptide chains in the collagen molecules resulting in degradation [14,25]. In addition, for allografts which contain moisture, irradiation can also cause radiolysis of water molecules, which release free radicals that can induce cross-linking reactions in collagen molecules [25]. Experimental studies have also shown that tracheal allografts have poor long-term durability after high dose irradiation at 1 kGy [26]. Unfortunately, there is no international consensus on the optimum radiation dose due to the different confounding factors and individual decisions by tissue banks. This has resulted in the application of doses ranging from 15 kGy to 35 kGy [25].

The post-operative results of the small number of recipients implanted with allografts irradiated at 25 kGy appear promising. Overall, 75% of the patients with severe subglottic tracheal stenosis who underwent segmental tracheal allograft reconstruction were successfully decannulated after 18 to 20 months of follow-up [20]. Nevertheless, in another study, Kunachak et al. discovered that although the transplanted site appeared to have good mucosal healing, the mucosa of donor trachea did not survive at the transplanted site. On the contrary, the apparent normal post-operative mucosal lining represents migration of the recipient mucosa [24].

Revascularisation of Allografts

A critical aspect of successful tracheal transplantation is effective vascularisation of the allograft. Experimental studies revealed the fatal result of ischaemic necrosis of epithelium, submucosa and cartilage caused by transplantation of devascularised allografts, regardless of preservation methods [5]. Often, due to the lack of revascularisation, the blood supply to the transplanted allograft becomes too slow to prevent necrosis or stenosis [6].

Hence, to facilitate blood delivery, different revascularisation approaches are employed. The techniques include direct revascularisation by using arteries or veins, or more commonly, indirect revascularisation through the wrapping of allograft with the recipients' viable tissues to stimulate neoangiogenesis [19,27,28]. However, direct revascularisation is challenging and has only been attempted in experimental models. This is because the human trachea lacks a large arterial or venous vessel that allows for direct vascular anastomosis to the blood vessels of the recipient, as it is supplied by a complex network of small blood vessels instead [19,29].

A common surgical approach to accelerate the revascularisation

Trachea no.	Antibiotic regimen	Post-recovery tissue or solution culture	Post-antibiotic incubation tissue or solution culture
1	Penicillin and streptomycin	Candida guilliermondii, Pichia ohmeri	Candida guilliermondii, Pichia ohmeri
2	Penicillin and streptomycin	Negative	Cladosporium sp.
3	Amikacin, vancomycin and caspofungin	Klebsiella sp., Acinetobacter baumannii	Klebsiella sp.
4	Amikacin, vancomycin and caspofungin	Coagulase-negative Staphylococcus, Propionibacterium acnes	Negative

Table 2: Type of micro-organisms isolated from tracheal allografts recovered by National Cardiovascular Homograft Bank (NCHB).

process is by wrapping fresh or preserved allografts with viable tissue from the recipient. In the first allotransplantation using revascularised tracheal allograft performed by Rose et al. in 1979, the donated trachea was implanted heterotopically into the sternocleidomastoid muscle of the recipient and transferred into the orthotopic position after 3 weeks [30]. This was followed by Levashov et al. and Klepetko et al, who used omentum to wrap donor trachea for revascularisation before implanting it orthotopically [19,31]. Delaere et al. chose to cover the allograft in vascularised fascia in the recipient's forearm [29], and He et al. reported covering a chemically preserved allograft with pedunculated greater omentum and greater pectoral muscles instead [32]. Strap muscle or *rectus abdominis* flaps have also been used to achieve the same objective [1].

Revascularisation significantly contributes to satisfactory graft outcome. The revascularised trachea explanted by Klepetko et al. revealed that it was mechanically stable and macroscopically intact. Its cartilage was covered by respiratory epithelium and it had excellent vascularisation of the tracheal wall [19].

However, the disadvantages of this technique are (1) it is a lengthy process as the allograft needs to be implanted in a heterotopic position for a period of time, (2) initial immunosuppressive therapy for the patient is required (3) There is a shortage of donors and allografts and (4) probable longitudinal shrinking of allograft [3]. Immunosuppression for recipients is necessary during heterotopic transplantation of the tracheal allograft to prevent immunologic rejection, although the first recipient was not administered immunosuppressant [30]. Another advantage of a revascularised allograft is its ability to support the growth of the recipient's cells, as demonstrated in chimerism as the recipient's buccal mucosa cells grew to replace the necrotic posterior tracheal wall of the allograft [29].

Conclusion

While experimental studies on tracheal transplantation are numerous, these have failed to translate into wider clinical application due to various factors.

Firstly, long-segment tracheal stenosis is an extremely rare condition affecting mainly paediatric patients with congenital airway abnormalities or adults with thoracic malignancies or chronic airway infections such as tuberculosis. Secondly, tracheal reconstruction has always been a surgical challenge since the beginning of the past century [3]. The prevention of necrosis at anastomosed sites due to retarded revascularisation is a serious issue affecting patient outcome. Therefore to accelerate revascularisation, various techniques are employed, which includes the tissue-wrapping techniques. Finally, there is the constant problem of donor shortage, which limits the number of tracheal allografts that tissue banks can recover.

There are numerous challenges in elucidating the most effective antibiotic regimen to disinfect cryopreserved trachea suitable for transplantation. In our tissue bank's context, with few allografts available for research, it becomes extremely difficult to validate the effectiveness of various decontamination and preservation protocols. Some tissue banks adopt the use of controversial chemicals, and even irradiation for decontamination or sterilisation. While these methods may completely eliminate the risk of transmission of infectious diseases and micro-organisms, their effect on the integrity of the allograft and possible long-term side-effects to the patients remains unknown. Although the short-term outcome of a majority of the patients receiving such allografts appears promising, the long-term outcome is uncertain, and requires further study.

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