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Review Article

Tissue-engineered trachea: A review

Jia Xian Law [a], Ling Ling Liau [b], Bin Saim Aminuddin [a, c], Bt Hj Idrus Ruszymah [a, b, *]

[a] Tissue Engineering Centre, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000, Cheras, Kuala Lumpur, Malaysia
[b] Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000, Cheras, Kuala Lumpur, Malaysia
[c] Ear, Nose & Throat Consultant Clinic, Ampang Puteri Specialist Hospital, 68000, Ampang, Selangor, Malaysia

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ABSTRACT

Tracheal replacement is performed after resection of a portion of the trachea that was impossible to reconnect via direct anastomosis. A tissue-engineered trachea is one of the available options that offer many advantages compared to other types of graft. Fabrication of a functional tissue-engineered trachea for grafting is very challenging, as it is a complex organ with important components, including cartilage, epithelium and vasculature. A number of studies have been reported on the preparation of a graftable trachea. A laterally rigid but longitudinally flexible hollow cylindrical scaffold which supports cartilage and epithelial tissue formation is the key element. The scaffold can be prepared via decellularization of an allograft or fabricated using biodegradable or non-biodegradable biomaterials. Commonly, the scaffold is seeded with chondrocytes and epithelial cells at the outer and luminal surfaces, respectively, to hasten tissue formation and improve functionality. To date, several clinical trials of tracheal replacement with tissue-engineered trachea have been performed. This article reviews the formation of cartilage tissue, epithelium and neovascularization of tissue-engineered trachea, together with the obstacles, possible solutions and future. Furthermore, the role of the bioreactor for *in vitro* tracheal graft formation and recently reported clinical applications of tracheal graft were also discussed. Generally, although encouraging results have been achieved, however, some obstacles remain to be resolved before the tissue-engineered trachea can be widely used in clinical settings.

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* Corresponding author. Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000, Cheras, Kuala Lumpur, Malaysia.
E-mail addresses: ruszyidrus@gmail.com, ruszy@medic.ukm.my (B.H.I. Ruszymah).

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1. Introduction

The loss and damage of a tissue/organ resulting from an injury or disease can cause serious health problems, as the transplantation of tissue/organs in these patients is extremely limited due to the lack of donors [1]. This situation seriously deteriorates the patient's quality of life and increases the medical and social costs. Even if a patient is fortunate enough to receive an allograft, lifelong immunosuppression is essential. Alternative treatments such as mechanical devices or artificial prostheses do not restore tissue/organ function. In addition, artificial implants suffer from the shortcomings of having a limited lifespan and may promote allergic reactions due to material abrasion [2].

Dissatisfaction with conventional therapies has led to a shift in interest to a relatively new discipline called tissue engineering. Tissue engineering is defined as an interdisciplinary field that applies the principles of bioengineering, materials science and life sciences toward the assembly of biological substitutes that restore, maintain or improve tissue/organ function [3]. The success in creating functional engineered tissues lies in the integration of cells, biomaterials and signaling systems, also known as the tissue engineering triad [4]. Another important aspect that is essential for successful tissue formation *in vitro* is the bioreactor, which should provide perfusion and physical stimuli to improve cell viability and tissue function.

Stem cells are a key component of tissue regeneration due to their ability to proliferate and self-renew. Stem cells can be recruited to the injured area via two mechanisms: incorporation into an engineered tissue or attraction to the wound site with the help of biomaterials and/or soluble factors (including growth factors, chemokines and cytokines). The development of a scaffold requires the selection of the right biomaterials and fabrication methods, as tissue formation is greatly affected by biocompatibility, bioactivity (e.g. cell attachment, proliferation and differentiation), mechanical properties, architecture (e.g. sheets, fleece and fibers) and the 3-D environment (e.g. porosity, pore size and pore interconnectivity) of the scaffold. The scaffold must possess the appropriate degradation rate that matches the tissue formation rate, with non-toxic degradation products. Bioreactors can provide mechanical stimuli to cells that mimic *in vivo* conditions. These mechanical cues are important in regulating cell function and tissue remodeling, to produce an engineered tissue that closely resembles the native tissue. Furthermore, bioreactors help with nutrient perfusion, which is crucial in supporting cell survival in a 3-D construct.

The trachea or windpipe acts as a conduit for ventilation and to clear tracheal and bronchial secretions. Severe injury or damage to the trachea can result in a significant decrease in quality of life due to problems with breathing, speaking and swallowing. Direct anastomosis is impossible when a tracheal segment longer than 6 cm needs to be resected due to the high mechanical tension at the anastomosis site, which can lead to severe and fatal postoperative complications [5,6]. Conventionally, there is no satisfactory solution to this disorder. Although an allogeneic trachea can be used as a replacement, this is accompanied by the shortcoming of lifelong immunosuppressant therapy, which greatly increases the risk of infection. Tracheal xenografts also suffer from the same disadvantage. Currently, tissue engineering has emerged as a potential alternative to tackle this problem. Reconstruction of the trachea requires a layer of ciliated epithelium supported by a laterally rigid but longitudinally flexible tube [7]. The “new” tissue should be able to self-repair, remodel, revascularize and regenerate, without the risk of rejection [8].

A number of studies on tissue-engineered tracheae have been published. Investigators have come out with plenty of new findings that may make inroads toward the clinical application of tissue-engineered tracheae. Nevertheless, there are still some obstacles that need to be overcome before this becomes reality.

2. Key components in the tissue-engineered trachea

2.1. Formation of tubular cartilage tissue

Cell-scaffold interactions have a great influence on cell behavior [4]. A good scaffold should possess properties that support cell adhesion, migration, proliferation and differentiation. The scaffold should also be able to promote tissue regeneration and remodeling, without eliciting an inflammatory or immunogenic response which may compromise healing [9,10]. The scaffold is fabricated into a 3-D porous structure to allow seeded cells to penetrate, attach and proliferate, as well as to aid in nutrient delivery and clearance of metabolic waste products, and to promote the ingrowth of new blood vessels. However, the scaffolds cannot be too porous as this will decrease the surface area available for cell attachment.

For tissue-engineered tracheae, the mechanical strength of the scaffold is very important in preventing the collapse of the airway, which would cause serious postoperative complications. The mechanical strength of a scaffold largely depends on its composition and architecture [11,12]. A number of materials have been tested as scaffolding for tissue-engineered tracheae. Synthetic biomaterials normally give excellent mechanical strength, but they lack the bioactivities offered by natural biomaterials. Nowadays, a combination of natural and synthetic materials has become the most popular trend. This combination joins the advantages of natural and synthetic materials to yield a scaffold with excellent bioactivities and mechanical properties.

Polypropylene and polytetrafluoroethylene are two examples of non-biodegradable materials used to fabricate the scaffold [13–15]. The biodegradable materials that have been investigated for tracheal repair include poly(ε-caprolactone), poly-lactic-glycolic acid, polyglycolic acid, a poly(L-lactide-co-caprolactone), fibrin/hyaluronan composite, a polyglycolic acid/alginate composite and polyester-urethane (DegraPol®) [16–22]. For the biodegradable scaffold, its main function is to give temporary support to the airway to prevent it from collapsing while the chondrocytes or chondrogenic cells seeded on the scaffold build the ECM to form cartilage tissue. The biodegradable scaffold for a tissue-engineered trachea must possess a degradation rate that is proportional to the cartilage formation rate in order to maintain the construct's mechanical strength. A rapidly degrading scaffold leads to a smaller scaffold size and porosity, making it difficult to fit the construct into the implantation site and impeding cell infiltration into the construct. *In vivo*, a rapidly degrading scaffold loses mechanical strength in a short period of time, resulting in the collapse of the transplanted graft and blockage of the airway.

The chondrocyte is the most common cell choice for the formation of cartilage tissue. Chondrocytes are normally isolated from non-weight bearing sites such as the nasal septum, external ear and rib [23,24]. Kojima et al. used both nasal and trachea-derived chondrocytes for the fabrication of tissue-engineered tracheae and found that they had similar mechanical properties to natural tissue [25]. This showed that the use of chondrocytes harvested from easily isolated tissues did not compromise the quality of the tissue-engineered trachea. Wu et al. used a specially designed culture method to produce sheet-like chondrocyte macroaggregates that were wrapped around a silicon tube and implanted subcutaneously in a rabbit for 8 weeks to stimulate tissue maturation [26]. It was found that the implanted tissue retained the tube-like structure upon removal of silicon tube, although the mechanical strength and glycosaminoglycan content were significantly lower compared to a native trachea. Nonetheless, the results