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Review

Tissue-engineered trachea: History, problems and the future[☆]Qiang Tan^{a,*}, Rudolf Steiner^b, Simon P. Hoerstrup^c, Walter Weder^a^a Clinic of Thoracic Surgery, University Hospital Zurich, Zurich, Switzerland^b Division of Oncology, University Hospital Zurich, Zurich, Switzerland^c Division of Surgical Research and Clinic for Cardiovascular Surgery,
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Summary

This review tries to summarize the efforts over the past 20 years to construct a tissue-engineered trachea. After illustrating the main technical bottlenecks faced nowadays, we discuss what might be the solutions to these bottlenecks. You may find out why the focus in this research field shifts dramatically from the construction of a tubular cartilage tissue to reepithelialization and revascularization of the prosthesis. In the end we propose a novel concept of ‘in vivo bioreactor’, defined as the design of a perfusion system inside the scaffold, and explain its potential application in the construction of a tissue-engineered trachea.

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1. Introduction

Tissue engineering has emerged as a thriving new field in medical science. It is an interdisciplinary field in which the principles of engineering and life science meet each other to generate biological substitutes for creation, preservation or restoration of lost organ functions [1]. Unlike organ transplantation, tissue engineering offers off-the-shelf tissue and organ substitutes by seeding a patient’s own cells on a biodegradable scaffold, representing a promising future of medical science.

The experimental and clinical tracheal repair dates back to as early as the late 19th century, yet till today no clinically convincing tracheal replacement method has been established [2–6]. Belsey [7] summarized the requirements for tracheal replacement to consist of, first, a laterally rigid but longitudinally flexible tube; second, a surface covered with ciliated respiratory epithelium. At the very beginning, many scientists were led to believe in the illusionary simplicity in tissue-engineered trachea reconstruction and took it as no more than developing a tubular cartilage tissue. Later, animal examinations demonstrated the importance of an intact epithelial line since it prevents the in-growth of granulomatous tissue, which leads to fatal airway obstruction. The cilia also

help to expel the mucosal fluids and the adhering micro-particles. Therefore, the focus in the tissue-engineered trachea researches today has been shifted towards the substitute reepithelialization [8–12]. In this review we will discuss what has been done, what is going on, where the obstacles are, and what might be the practical solutions in the reconstruction of a tissue-engineered trachea.

2. What has been achieved: formation of a tubular cartilage tissue

Although the techniques of tubular cartilage tissue construction are available, there are still some problems involved in each step.

2.1. Sources of chondrocytes

Reconstruction of cartilage tissues has become one of the most popular research topics today in tissue engineering, mainly due to the fact that chondrocytes rely on the permeation of tissue fluid for nutrition supply, and thus exempting cartilage tissues from revascularization. Thanks to their low antigenicity, allogeneic chondrocytes can be used to repair cartilage tissue defects [13,14]. In many countries, scientists are exploring the possibility of establishing cell banks to guarantee a stable supply of chondrocytes [15,16]. Regarding autologous sources, besides mature chondrocytes isolated from nose or rib hyaline cartilage tissues, adult stem cells (ASCs), with their high rate of

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proliferation and ease of isolation, have proven to be a promising alternative. While the ideal donor site for the isolation of ASCs remains controversial, bone marrow aspirates and adipose-derived stem cells so far stand out as appropriate sources [17]. The antigen markers can be used for ASCs isolation including CD166, CD105, CD55, CD54, CD44 and CD13, among which CD105, the transforming growth factor- β (TGF- β) receptor, is identified as chondrogenic potential related cell marker [18]. Meanwhile recent researches demonstrated that CD14⁺ mononuclear cells obtained from peripheral blood can also differentiate into a mesenchymal progenitor phenotype [19]. Many research teams around the world have successfully induced ASCs into chondrocytes in vitro. Theoretically, ASCs can be obtained without limitation but the induction rate is still on the low side these days [20–22].

2.2. Scaffolds

Tissue engineers favor porous biodegradable scaffolds for they can facilitate the penetration of seeded cells, nutrients, and the clearance of biological waste products. But a high porous rate will inevitably sacrifice the initial stiffness of the scaffold, which is of utmost importance in the case of the tissue-engineered trachea. Researchers have tried many materials, both synthetic and natural, including polyglycolic acid (PGA), polylactide-co-glycolide acid (PLGA), DegraPol, Pluronic F-127, acellular cartilage tissue matrices, as well as some combination of them all [23–32]. Unfortunately, none of these materials has worked wonder. For example, the biodegraded molecules from PGA often leads to a low pH environment, which is detrimental for the survival of seeded cells; the porous rate of acellular tracheal matrices is hard to be controlled; and nearly all these scaffolds show inadequate mechanical strength for a circumferential trachea replacement and usually need an external support device such as a silicon tube.

2.3. Bioreactors

Bioreactors play a key role in almost every step in the reconstruction of tissue-engineered tissues: cell proliferation on a large scale, cell seeding process, 3D cell-scaffold constructs culture [33]. There are various designs ranging from the simplest stirred vessels to much more complex double chamber bioreactors for composite tissue formation. Regarding their application in cell culture, microcarriers are frequently used to increase the culture surface with the advantage of less enzymatic subcultivation steps, which turn out to be the main cause of cell dedifferentiation [34]. Recent studies have demonstrated that perfusion of a cell suspension directly through the pores of 3D scaffolds resulted in higher efficiencies and more uniform cell distributions inside the scaffold [35]. In spite of all these improvements, in most cases the cartilage tissues formed in these bioreactors are thinner and softer than native ones. This is mainly due to the insufficient infiltration of nutrition and oxygen into the center part of newly-formed tissues, as well as the obstruction of biological waste elimination [36,37]. In addition, contamination remains another critical issue especially when a long-term in vitro culture is required.

The essential purpose of bioreactor designs, effectively simulating the in vivo regeneration condition in vitro for the off-the-shelf tissues formation, has proven to be extremely difficult to achieve. One possible makeshift is to use nude mice as living bioreactors [38,39]. The results are inspiring, however this approach has a serious limitation in terms of the tissue size: since a tracheal resection less than 6 cm in length can be handled easily by direct anastomosis, tissue-engineered cartilage tissues of small size are therefore clinically irrelevant in tracheal replacement.

3. Problems faced: reepithelialization and revascularization

Now that a tubular bioengineered cartilage tissue is available, is it really a perfect tracheal prosthesis? Earlier animal experiments underwent circumferential replacement of trachea with tubular cartilage tissues only to show frustrating results: most recipients died within 1 week from airway obstruction caused by either the in-growth of granulation tissues or the sputum retention [40]. These results were not surprising at all, since the entire history of trachea replacement has highlighted the importance of a complete reepithelialization process in building a functional tracheal substitute. The experience gained from tissue-engineered skin demonstrated that epithelial cells have better survival chances on a well vascularized wound surface, a clear proof that revascularization is the essence of reepithelialization [41–44].

Oncologists have also done lots of researches on the angiogenesis and vasculogenesis mechanisms, however, with an ironically opposite purpose: they go all out to prevent the in-growth of capillary networks into tumor tissues. On the other hand, tissue engineers work hard to facilitate and accelerate these processes based on the same biological mechanisms [45–48]. Two approaches are frequently used to accelerate the revascularization process these days. In one approach, various growth factors (GFs), such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (BFGF), and platelet-derived growth factor (PDGF) are administrated into the ischemia area to induce the in-growth of vessels from surrounding normal tissues [49–53]. In the other approach, endothelial cells or their progenitors are applied either systemically or topically in the hope that these cells may contribute to the reconstruction of the capillary network inside the ischemia tissues or organs [54–58]. Many studies showed that both hypotheses work well but some disadvantages still exist.

Regarding the first approach, there are two main challenges, namely, how to maintain the working concentration of these GFs after implantation, and how to terminate their function in time to avoid side effects caused by the GFs over expression? Biochemical engineers suggest integrating GFs into biodegradable scaffolds in the hope that these embedded GFs will be slowly released during the scaffold biodegradation process after implantation. This technique, often known as 'in situ tissue engineering', however, is not only technically demanding but has size limitation in neotissue formation without pre-seeded cells inside the scaffold [59–63]. Meanwhile, many promising experiments suggest

the possibility of GFs gene delivery. Unfortunately in these experiments, both viral method (transduction) and nonviral method (transfection) showed technical limitations: the former often goes with likelihood of mutagenesis, carcinogenesis and immune response to viral infection or viral proteins while the latter still suffers from low efficiency of gene delivery into the target cell population [64–68]. The tragic death of a patient due to massive cytokine release gives rise to heated ethical debate in trial gene therapy recently.

Regarding the endothelial cell delivery approach, the dilemma is whether it is really worth to make time-consuming efforts to add the endothelial cells that hardly culture and usually die soon after implantations due to insufficient nutrient supply inside the scaffold.

In addition to revascularization, we still face many other problems in reepithelialization, such as the harvest and proliferation of human respiratory epithelial cells, which are mainly terminal cells unsuitable to be further passaged [69]. Even if we could identify the epithelial stem cell, there are still no widely accepted seeding methods, which can facilitate these cells to rapidly spread and completely cover the inner surface of tubular scaffolds. In early experiments, epithelial cells were simply injected into the lumen of a tubular scaffold placed subcutaneously on the back of a nude mouse. The results, with a maximal surface cover rate of 80%, were disappointing and unacceptable for clinic applications. Recent studies suggested directly suturing cell sheets onto the inner surface of the tissue-engineered trachea substitutes [70], but how to maintain the survival of these epithelial cells during the initial ischemia period remains a challenge.

All these being said, what next?

4. Future perspectives

Let's get down to fundamentals and take a look at normal histological structure of trachea. Between the epithelial layer and the hyaline cartilage tissues we can find a submucosal layer of capillary networks. Normal cartilage tissue preventing the in-growth of capillary vessels is separated from other connective tissues (i.e., submucosa) by a fibrous membrane [71,72]. Unlike parenchymal organs, the trachea is supplied with a network of small vessels inaccessible to direct revascularization through vessel anastomosis. Even immediate autologous orthotopic trachea replacement is bound to fail, despite that such a fresh autograft substitute is far more physiological than even the most optimal tissue-engineered trachea. The capillary net can only penetrate into the implant across the two anastomoses for no more than 2 cm (4 cm bilaterally) and this revascularization process easily takes months [2]. In an implant longer than 3–4 cm, the epithelial cell in the middle will die and thus failing to maintain an intact basement membrane. As a result, granulation tissue hyperplasia into the lumen causes tracheal stenosis.

Such being the case we advanced a novel concept of 'in vivo bioreactor' defined as the design of a perfusion system inside the scaffold for tissue-engineered trachea reconstruction. [Fig. 1] In our opinion, a tissue-engineered trachea scaffold, ideally, should follow the physiological structure mentioned above and leave a porous middle layer facilitating

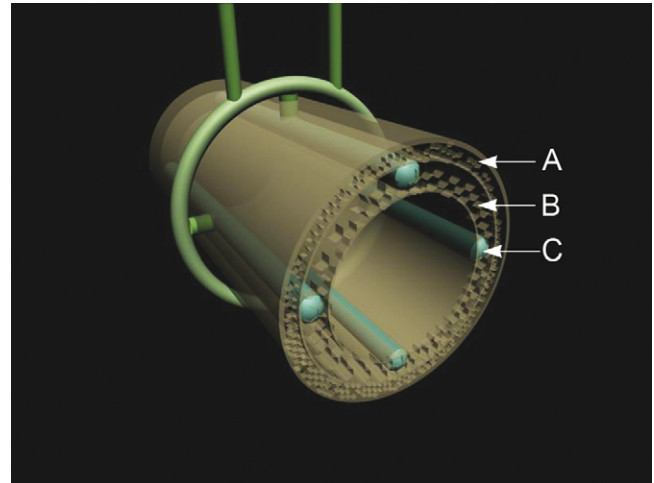


Fig. 1. Sketch of novel tissue-engineered tracheal scaffold with a perfusion system inside. This 'in vivo bioreactor' contains two layers with different density. The inner low-density layer (B) is embedded with porous catheters (C) and allows for in-growth of capillary net, while the outer high-density layer (A) provides the scaffold with desirable mechanical strength.

blood vessels' in-growth between the cartilage and epithelial layers. It may also be of advantage if we would add 3 or 4 porous feeding tubes inside this middle layer with lower density. These feeding tubes will be connected to an extracorporeal pump system after implantation in order to form a perfusion system. Through this perfusion system we continuously administrate medium into the scaffold. The medium infiltrates throughout the scaffold and thus maintains the survival of both epithelial cells seeded on the inner surface and the chondrocytes seeded on the outer layer, which has a relatively high density to prevent the tissue-engineered trachea from a collapse.

Contrary to traditional bioreactors where the medium immerses cell-scaffold composites, in our design the medium actually flows inside the scaffold to mimic the way blood stream flows inside the normal tissue. Since we are not yet able to imitate in vivo regeneration environment successfully in vitro, this in vivo bioreactor seeded with cells needs to be implanted as soon as possible to leverage on the recipient's regenerative capabilities. Thus, the recipient serves as her own bioreactor for the maturation of tissue-engineered organ and that is how we are inspired to name our design 'in vivo bioreactor'. To avoid the coagulation-related problems, the perfusion system will be connected to two extracorporeal pumps instead of being directly anastomosed to the recipient's circulation system. One pump will deliver medium while the other will drain the waste. Like a heart–lung machine widely used in cardiac operations, this in vivo bioreactor is supposed to work as an 'artificial heart' to the tissue-engineered trachea until normal revascularization is established. Obviously GFs and epithelial cells can be added into the perfusate to facilitate revascularization process; artificial oxygen carriers, in addition, such as perfluorocarbon (PFC) emulsion, can be added to increase the oxygen incidence inside the medium. The expression level of the GFs can be readily adjusted by changing their medium concentrations. By combining the in vivo and the in vitro parts of tissue engineering researches, which were traditionally

separated, this concept of 'in vivo bioreactor' would hopefully be applied as a more physiological and more clinically practical way in organ regeneration.

Regarding the epithelial cell source for reepithelialization, our hypothesis is as follows: the key issue for a clinically successful reepithelialization of the tissue-engineered trachea may not be the original type of the epithelial cells but rather the environmental signals and an intact basement membrane layer. Allogenic trachea transplantation, in addition to preclinical and clinical research work using autologous skin or aorta, clearly showed that no matter what kind of epithelial cell type was initially chosen they would be replaced by normal tracheal epithelial as long as there was a sufficient blood supply and an intact basement membrane underneath [73–76]. Therefore for the epithelial cell source, we suggest using autologous skin keratinocytes instead of the ciliated tracheal epithelial cells. Fortunately, in the field of tissue engineering, tissue-engineered skin is by far the most successful branch.

To summarize, the tissue-engineered tracheal substitute is still far away from wide clinical application. The source of epithelial cells, and the retarded revascularization and reepithelialization process of the tissue-engineered tracheal substitute are currently the main obstacles. With the idea of 'in vivo bioreactor' we organically combine the traditionally separated in vivo and in vitro parts of tissue engineering research. In our opinion, the inner structure of the scaffolds might be the key to solve some of these pertinent problems. Being a more physiological way in tissue reconstruction, this novel approach may also find itself in clinic applications in many other areas of tissue engineering research, such as tissue-engineered bone.

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