SURGICAL TRACHEAL REPLACEMENT AND THE ROLE OF TISSUE ENGINEERING

TRAKEA REPLASMANI VE DOKU MÜHENDİSLİĞİNİN ROLÜ

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Abstract

To date researches on the ideal substitute for tracheal replacement have not given satisfactory results due to tracheal peculiar mechanical, anatomical and biological features. Several techniques have been tried and reported using prostheses (artificial and biological), autogenous tissues modelled as trachea or with the use of allografts. Inconsistent long-term outcomes of these studies pushed the researches on tissue engineered trachea. Biological scaffold using cadaver donor trachea seeded with recipient autologous cells in bioreactor have been tried in anecdotal case in a clinical setting with promising outcomes. On the other hand, complete artificial scaffolds were tried only on animal models and their clinical use raised ethical issues. Developments in the understanding of the relationship between scaffold, extracellular matrix and tissues together with advances in minimally invasive surgical technology are necessary for the creation of a functional tracheal substitute.

Keywords: Tissue engineering, tracheal replacement, surgery

Özet

Trakea replasmanının alternatifine ilişkin güncel araştırmalar, trakeanın kendine özgü mekanik, anatomik ve biyolojik özellikleri nedeniyle tatmin edici sonuçlar vermemiştir. Protezler (yapay ya da biyolojik), trakea olarak modellenen otojen dokular veya allogreftlerin kullanımı ile çeşitli teknikler denenmiş ve raporlanmıştır. Bu çalışmaların tutarsız uzun vadeli sonuçları, araştırmacıları doku mühendisliğine yöneltmiştir. Biyoreaktörde alıcı otolog hücreler ile ekilen kadavra donör trakea kullanılan biyolojik iskele, umut verici sonuçlarla klinik ortamda anekdotsal vakada denenmiştir. Diğer taraftan, tamamen yapay iskeleler sadece hayvan modellerinde denenmiş ve klinik kullanımları etik sorunları ortaya çıkarmıştır. Fonksiyonel trakea alternatifleri yaratmak için iskele, ekstraselüler matriks, dokular ve minimal invaziv cerrahi teknolojisindeki gelişmelerin birlikte anlaşılması gerekmektedir.

Anahtar kelimeler: Doku mühendisliği, trakea replasmanı, cerrahi

INTRODUCTION

It is widely accepted that limits of tracheal resection both in case of malignant or benign disease account for 6 cm in adults and 30% of the total extension in children (1); moreover, in case of previous radiation treatment the maximum extent of resection can reach 4 cm due to scar tissue and secondary retraction. In case of a disease that involves a longer tract of the trachea, an end-to-end anastomosis is not generally technically feasible and safe; in these cases, substitution of the whole tract with a new one could solve the problem. Nevertheless, as precisely pointed out by Belsey (2) trachea has well defined peculiar features that must be replicated by any potential substitute: it must be laterally rigid, but longitudinally flexible and it should preferably have a surface of ciliated respiratory epithelium; the conduit should also be airtight and should become integrated with the adjacent tissues. In addition, some authors (3,4) pointed out that material used for tracheal replacement must be biocompatible, nonimmunogenic, noncarcinogenic and nontoxic. Finally, trachea has a segmental vascularization that make the replacement even more challenging (5).

In the light of these premises, several attempt to find the "holy grail" of tracheal substitution have been made with disappointing or anyway non-satisfactory outcomes. Recent

in vitro and in vivo experiments seem to highlight the role of tissue engineering as the answer of this recurring and challenging question.

TECHNIQUES AND MATERIALS FOR TRACHEAL REPLACEMENT

Prostheses

Two main types of artificial prostheses are available: solid and porous. They can both be constituted by several different materials that can be even meshed together according to their characteristics (5). The main drawbacks of prostheses are dislodgement, obstruction due to formation of granulation tissue and subsequent scar tissue and infection. Moreover, solid prostheses can also cause native tracheal wall erosion and a consequent possible fistula with neighbour organs or vessels (5).

Porous prostheses have been conceived in order to allow epithelium migration (3) and development of new tracheal tissue. Unfortunately, only short segments showed to be covered by viable epithelium, while long segments remained uncovered (6) and prone to bacterial infections. As a result, prosthesis might be helpful to maintain patency of airways for some time, even without a real tissue healing beneath, but favourable outcomes are not predictable (5).

A third type of prostheses are bio-prostheses. The most common bio-prostheses that have been used in experimental clinical setting is the aorta graft; although mechanical features of these grafts seems to be acceptable for airways replacement, they often showed a linear contraction of the graft with substantial deformation of the conduit (5). Nevertheless, a French group recently reviewed outcomes of 20 patients operated on over a eight-year period (7) with aortic graft as replacement of large airways defect, reporting more promising results; moreover, authors find the presence of cartilage and respiratory epithelium development inside the aortic graft after stent removal.

Autogenous Tissues

Since the fifties, experiments on animal models and attempts in a clinical setting on humans have been proposed with inconsistent results (5,8). Techniques are based on free flaps with or without the support of foreign material, vascularized tissue flaps or tube construction with autogenous elements. The first two case reports using free flaps of fascia lata with stainless steel wire after resection of a lateral segment of trachea were published by Belsey in 1950 (2); one of the patients survived for more than six years and afterwards underwent a second similar procedures; the patient was eventually sent back home alive and breathing spontaneously.

Vascularized flaps of muscles or skin with the addition of cartilage grafts or foreign materials were used either for reparation of airways' "window" or circumferential resections; microvascular anastomosis could be necessary in case distant flap positioning (5). Compared to free flaps, vascularized flaps have the advantage of being less prone to necrotize. Similarly, cutaneous tubes have been realized with the support of rigid material (cartilage or foreign material) and then implanted in the recipient (9). Recently, Fabre and his colleagues (10) described the use of a autologus free flap of forearm shaped with the use of rib cartilage. Authors described 12 cases over an eight-year period with promising results in terms of long terms outcome, but they acknowledge the lack of respiratory ciliar epitelium as the main drawback, complicating mucus clearance from the bronchi. Authors conclude that this technique should be reserved to patients with a good preoperative pulmonary function. Finally, some authors described the use of oesophagus or small bowel (11,12), but this may dramatically increase intra- and postoperative morbidity to the procedure.

Tracheal Transplantation

Trachea transplantation have been excited generations of thoracic surgeons, but so far, no satisfactory long terms outcomes have been reached (5,8). The first issue to be solved is concerning the long-lasting immunosuppressive treatment that theoretically exclude patients with tracheal malignancies. Moreover, vascularization of the graft has showed to have a paramount importance in the possible outcomes (5,8). As a matter of fact, fresh or preserved devascularized autograft and allograft have shown a constant fail in animal models; only short segments were able to resist without necrosis, but turning into scar tissue (13,14). In humans, few studies with cadaver tracheal graft have been tried. Two different studies from Jacobs (15) and Propst (16) reported a relatively large experience in transplantation of the cartilaginous part of the trachea from cadaveric donor in children. Results show good survival rate and acceptable quality of life, even though all patients received subsequent stenting procedures and decannulation rate were 60%.

As it has already been addressed before, vascularization of the trachea relies on small vessel and it is mostly segmental, so that revascularization of a graft is challenging. Indirect vascularization by mean of omentopexy for free tracheal graft has been proposed with promising results, even if, in case of long segments, the central part often suffered from ischemia (17,18). In humans, case reports show unsatisfactory results (19-21). More recently, Delaere and colleagues (22) report a similar protocol with implantation of the allograft in the recipient forearm with a gradual withdrawal

of immunosuppressive therapy after implantation. Cryopreserved rather than fresh allograft seems to reduce or even inhibit allogenicity, allowing a significant reduction of acute rejection and an early revascularization.

Lastly, direct revascularization has been tried on animal models by several authors showing good results. In a clinical setting, Strome and colleagues reported the use a of a fresh larynx graft en bloc with five tracheal rings, thyroid and parathyroids which received arterial, venous and neural anastomosis. After 40 months the patient was reported to be in good clinical conditions and he regained deglutition and vocal cord motion (23).

TISSUE ENGINEERED TRACHEA

In the current scenario with inconsistent and anecdotal good long-term results, bioengineering seemed to be a possible source of solution. Different approaches are possible in order to construct a bioengineered trachea. The trachea can be made by a bio-prosthesis, which might be a cadaver donor trachea, or it can be totally artificial with the use of bioengineered scaffolds (5).

Scaffold and Extracellular Matrix

The most challenging issues regard the mechanical and biological features of the scaffold that on one hand should reflect all the characteristics of a native trachea and on the other hand should allow regrowth and regeneration of the native epithelium. Moreover, it should be non- or only minimally immunogenic, biocompatible and biodegradable with a programmed and controllable rate with no toxic catabolites (24,25).

The paramount importance of the scaffold mainly relies in the Extra-Cellular Matrix (ECM), that give a 3D framework for cell growth and have crucial influence in the cellular differentiation and tissue formation, modulating cell migration and proliferation and differentiation of repairing cell (26,27). In this perspective, all scaffolds should be highly biocompatible permitting the ingrowth of host cells.

Complete artificial scaffold using nanocomposite and build up with the aid of 3D printers have been proposed. They are based on various biodegradable synthetic materials, including polyglycolic acid (PGA), polylactic acid/polyglycolic acid (PLA/PGA), poly (lactic-co-glycolic acid) (PLGA), polyester urethane, polycaprolactone (PCL). The advantages of whole artificial scaffold would be mainly two: they do not require cadaveric organs, which might not always be available, and it might be precisely tailored on patients features. The majority of the studies report in vitro experiments or on animal models (28-30) with promising results, even though, to date, reconstruction of functional multi-layered trachea seems to be unfortunately still not possible. Moreover, clinical use has been limited by promising, but inconsistent experimental results and consequent ethical and scientific issues (31).

Compared with complete artificial scaffold, decellularized bio-prostheses maintain native natural ECM simplifying tissue regeneration (32). For this reason, allografts or xenografts bio-prostheses have currently being studied extensively. As far as trachea bio-prostheses are concerned, a complete decellularization is anyway difficult, in particular for the cartilaginous parts which might be the cause of graft rejection (33). Decellularization has been seen as the first critical step of the procedure as it should decellularize the entire organ without compromising the ECM. In fact, although it was firstly reported that no significant changes in trachea mechanical features were seen after decellularization process (34,35), it was eventually reported that up to 30% of bio-prosthetic scaffold tended to collapse unpredictably in in vivo models (36,37). Interestingly, biological vascularized matrices have been realized to support complex biological implants; nevertheless, to date, they are most commonly used in the field of reconstructive surgery, and their possible role in tracheal reconstruction is limited (24).

Tissue Components

Besides ECM and scaffold, complex organs such as trachea require a mix of different tissues that should be organized altogether in order to execute their own function. Cartilage, respiratory epithelium, smooth muscles fibres and vascular elements should be managed in order to give their own place to each of them in the frame of a tissue engineered trachea (24). Unfortunately, to date it is still not possible to manipulate cells' behaviour in order to create complex organs such as trachea (8). For example, chondrocytes tend to dedifferentiate in a 3D model and their bioartificial experimental cartilage do not last for long time (24). Cartilage is the backbone of the trachea and its bioengineered construction is a key point for future developments (38).

As pointed out before, respiratory epithelium is an important element in a replaced trachea, as it might help to prevent infections and to clear lung secretion. Epithelial implants have been successfully achieved by Macchiarini and collagues (39) but only for shot tracheal segments.

Experimental and Clinical Experiences

Bioengineered trachea has been tested in animal models, while their clinical use is to date anecdotal (28-30). In 2008 Macchiarini (40) transplanted the first bioengineered

trachea segment as left main bronchus. His technique was based on a cadaver allograft, that underwent several cycles of decellularization; after that, the bio-prostheses was inserted in a specific bioreactor containing both a fluid culture of recipient nasal mucosa cells and air in order to seed the new organ. The bioengineered organ was then transplanted, showing a new vascularization after few days. After five years (41) the patient was in acceptable clinical conditions, even if the proximal anastomosis between native trachea and bioengineered transplant developed a new stenosis with necessity of repeated stenting procedures. The same authors pursued further clinical experience on a completely artificial bio-engineered trachea using a similar technique and bio-reactors, but his research were criticized on ethical and schientific issues (31).

Concurrently, two paediatric patients affected by congenital airways defects received a tracheal transplantation (37,42,43). Authors used cadaveric donor trachea as scaffold and they isolated mononuclear cells from patients' bone marrow that was then seeded in the graft. Moreover, the tracheal rings of the graft were injected with tissue transforming growth factor beta and trachea was then soaked in human recombinant erythropoietin and granulocyte colony stimulating factor. Although patients underwent several stenting procedures after transplant, authors reported the presence of ciliated epithelium on the graft after four years.

FUTURE PERSPECTIVES

Research on bio-materials is developing tirelessly. The way to the solution for a suitable substitute of tracheal tissue might be still long, but the direction seems to be right. Concurrently, minimally invasive surgery and more precise surgical instruments allow more accurate and detailed surgical interventions. The shared effort of biotechnical research and surgery might bring to clinical results soon, with patient-tailored solutions.

CONCLUSION

To date, tracheal replacement is still a chimera for thoracic surgeons due to peculiar anatomical, physiological and biological features. Nevertheless, the development of tissue engineering has helped to clarify limitations and strength of available techniques and it opened new perspective, joining biological and artificial materials all together. New developments will probably disclose new ethical issues that will need to be faced. On the other hand, until a functional solution will be found, these procedures should be reserved to highly selected patients.

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