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Stem cell-based organ replacements—Airway and lung tissue engineering

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ABSTRACT

Tissue engineering requires the use of cells seeded onto scaffolds, often in conjunction with bioactive molecules, to regenerate or replace tissues. Significant advances have been made in recent years within the fields of stem cell biology and biomaterials, leading to some exciting developments in airway tissue engineering, including the first use of stem cell-based tissue-engineered tracheal replacements in humans. In addition, recent advances within the fields of scaffold biology and decellularization offer the potential to transplant patients without the use of immunosuppression.

Introduction: Current options for airway replacement

Considerable progress has been made in recent years within the field of tissue engineering, not least within the field of airway tissue engineering. Tissue engineering, as a branch of regenerative medicine, aims to apply the principles and methods of engineering and the life sciences toward the development of biological substitutes that can restore, maintain, or improve tissue function.[1,2] Tissue engineering, broken down into its constituent components, requires cells and scaffolds on which to seed the cells.[3,4] Recent interest has focused on a third arm of tissue engineering, namely signals, i.e., pharmaceutical agents, endogenous chemicals, or cytokines, which also seem to be critical in the formation of fully functional tissues and organs through tissue engineering.[5] In this review, we shall focus on recent progress within the field of airway tissue engineering, as applied to regeneration and/or replacement of the trachea (windpipe) or larynx (voice box).

Unmet clinical need for tissue-engineered airways

There are a range of clinical disorders affecting the head and neck, including congenital, traumatic, and cancer-related causes, for which there are currently no good conventional therapeutic solutions. Current options for tissue replacement include the use of synthetic materials (alloplastic transplantation), autologous tissues, allotransplantation, or xenotransplantation, but these have significant limitations, as exemplified by previous experience in replacing airways.[6]

Congenital laryngotracheal malformations, with a reported prevalence of 1 in 100,000, form an important subgroup of conditions for which current treatments are suboptimal.[7] No successful therapeutic option has yet been identified for complete agenesis identified at birth. The EXIT procedure ensures adequate oxygenation of the fetus by the maternofetal circulation until a definitive surgical procedure is planned. Current surgical options, if autologous tracheal reconstructive techniques are deemed unsuitable due to a lack of available autologous tissues, include airway replacement, utilizing either the esophagus as a tubular autograft, prosthetic material substitutes, or tracheal homografts.[7]

However, the use of the esophagus requires multiple reconstructive steps to repair the esophagus, and it is associated with significant morbidity and mortality.[8] Although attempted previously, the use of prosthetic materials as airway replacements was associated with migration, dislodgement, extrusion, granulations, infection (and in some cases biofilms), stenosis, and obstruction.[9] Finally, despite the initial optimism associated with pediatric tracheal homografts, cadaveric tracheas are not in widespread use, due principally to shortages in organ donors and concerns regarding prion-related diseases.[10] In addition, pre-transplant

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tracheal preparation (including fixation with chemicals, cryopreservation, irradiation, and lyophilization) is only effective at reducing antigenicity in the short to medium term, is associated with tracheomalacia in some patients, and the growth potential of the tracheal allograft is not known.[10–12] Clearly, alternative strategies are warranted for this important group of pediatric patients.

The key problem in restoring function, as for advanced structural disorders of the larynx, for example, is the lack of availability of anatomically, physiologically, and biomechanically appropriate tissue. Thus, as a result of the inability to provide functioning surgical, or prosthetic, solutions to laryngeal replacement, advanced laryngeal cancer is now treated largely with chemoradiation, despite severe short- and long-term morbidity (which, paradoxically, can include a functionless larynx) and a 3–6% mortality for this modality.[13] Alternative options for functional tissue replacement are clearly warranted.

Alloplastic transplantation (prosthetics)

The use of prosthetic materials has so far failed to provide functional tissues for replacement, as demonstrated by earlier work exploring the use of synthetic materials for tracheal replacement. Thus, many types of materials have been examined for use in airway prostheses since the first report by Daniel in 1948.[9] The apparent simplicity of tracheal replacement encouraged trials of alloplastic transplantation with tubular conduits, initially made of solid materials, such as polytetrafluoroethylene, polypropylene mesh, Dacron polyurethane mesh, and silicone rubber.[9] However, these foreign materials failed to integrate into the surroundings tissues, and there were several disadvantages of this approach including problems of infection, dislodgement, migration, extrusion, and stenosis. Re-epithelialization was predictably impossible on the inner lumen, which led to the formation of granulation tissue and dehiscence at the interface between the prosthesis and the native trachea, usually within a few months. Furthermore, solid tubes can never be removed, because the connective tissue tract formed around them proceeds to obstruct with new connective tissue formation and by contraction in the absence of a stent. For the above reasons, the non-porous tracheal prosthesis is now seldom used clinically.

Attention then turned to porous materials—often meshes of various substances—which theoretically might encourage tissue ingrowth and possibly even epithelialization in time. It was found that a minimal porosity of 40–60 μm was necessary for capillary ingrowth.[9] However, similar complications ensued, and such grafts exhibited delayed re-epithelialization, continued cicatrix formation, bacterial and fungal colonization, obstruction, and stenosis.

Although there have been recent successful reports of tracheal transplantation using a porous bioartificial and non-biodegradable nanocomposite polymer, based on polyhedral oligomeric silsesquioxane nanocages (POSS) covalently bonded to poly(carbonate-urea)urethane (PCU) polymer chains, strictly speaking, this is a tissue engineering approach since the porous polymer was coated with autologous mesenchymal stem cells.[14] Nevertheless, it illustrates the potential of synthetic scaffolds in promoting tissue regeneration of static, tubular structures.

Autologous tissues

Current attempts at airway reconstructive surgery using the patient's own tissues are far from ideal in that they do not fully restore function and are associated with significant donor site morbidity and pain. In addition, there is often a limited availability of tissue for reconstruction. A wide range of tissues have been used for this purpose including pericardial patch repairs, cartilage, and rib grafts.[15] The limiting factor in nearly every procedure remains the complexity and multistage requirement, limiting the practicality of the procedure and often resulting in difficult or negative outcomes, including granulation tissue formation, patch collapse, and restenosis, often necessitating re-intervention.

Within the field of airway reconstruction, Pearson,[16] Delaere et al.,[17] and others have shown using an autologous approach that most of the larynx can be removed (a feature of the supracricoid partial laryngectomy) with preservation of vocal and sphincter functions, provided one side retains movement ("cricoarytenoid-nerve–muscle" unit). However, such attempts fail to fully restore function, are only an option in selected (non-advanced) cases, and bring with them additional concerns in the case of oncological surgery regarding satisfactory tumor clearance (i.e., removal of tumor with a clear margin).

Allotransplantation

To date, vascularized composite allotransplantation has been performed for the trachea,[18] larynx,[19–21] face,[22] limb,[23] tongue,[24,25] and abdominal wall,[26] among others,[27] despite the fact that these procedures were performed for quality rather than quantity of life. In such cases though, the trade-off in side-effects from immunosuppression, consequences of rejection, and reduction in life-span are very serious.[28] One of the proposed advantages of transplantation in these settings, as in the setting of limb transplantation, is the return of normal muscle function, something not possible by static conventional surgical reconstruction, or prosthesis. However, all have required the use of immunosuppressive therapy.

Although these cases have demonstrated that human composite tissue allotransplantation, such as for the larynx, is now technically possible, several drawbacks remain with this approach. For a start, making severed laryngeal nerves function again has proved elusive.[29,30] Furthermore, loss of a larynx due to trauma is rare. Most people who would merit a laryngeal transplant would have a present or a past history of laryngeal carcinoma, and such patients would be subjected to lifelong immunosuppressive therapy in order to prevent rejection, with the increased risk of rapid tumor recurrence. This is not only what happened to the 1969 partial laryngeal transplant patient but also to the world's first tongue transplant recipient who also had advanced squamous cell cancer.[24,25,31] The Leuven Tracheal Transplant Group has recently reported successful tracheal allotransplantation using a heterotopic revascularization approach (using the patient's forearm) followed by withdrawal of immunosuppressive therapy and orthotopic transplantation.[18] However, allogeneic donor cells disappeared shortly after withdrawal of immunosuppression, as determined by fluorescence *in situ* hybridization analysis. In addition, the membranous posterior wall of the allograft underwent avascular necrosis, which may have occurred secondary to immunological rejection. Its "success" must therefore be questioned.

Limitations of allotransplantation include shortages in organ donors, risks of rejection, and the need for lifelong immunosuppressive medications (with the associated risks of infection, malignancy, side-effects of treatment, toxicity, and significant costs associated with the need for lifelong treatment). It has been estimated that lifelong immunosuppression reduces life expectancy, on average, by 10 years.[32] Moreover, these agents do not prevent chronic rejection, the primary cause of late graft loss. For allotransplants such as these, which are rarely life-saving procedures, a completely non-immunogenic allograft with preserved functional and mechanical characteristics is the minimum target for organ replacement. A completely non-immunogenic graft would remove the need for immunosuppression and would increase the potential donor pool compared to conventional organ transplantation.