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Tissue engineering applied to the trachea as a graft

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Abstract

Tissue engineering offers, through new technologies, an ex vivo generation of organs and functional tissues as grafts for transplants, for the improvement and substitution of biological functions, with an absence of immunological response. The treatment of extended tracheal lesions is a substitution of the affected segment; nevertheless, the allogeneic transplant has failed and the use of synthetic materials has not had good results. New tissue engineering technology is being developed to offer a tracheal graft for a posterior implantation. The purpose of this article is to review all the methods and components used by the engineering of tissue for tracheal grafts. (Gac Med Mex. 2016;152:106-8)

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Introduction

Airway obstruction by tracheal stenosis results from lesions caused by prolonged use of mechanical ventilation with orotracheal, nasotracheal or inner cannula tracheostomy tubes in 5-20% of cases[1,2]. Other causes can be malignant or benign tumors, neoformations (granulomas, papillomas, bridles), congenital (tracheomalacia) and infectious (tuberculosis) diseases. Clinically, it manifests by respiratory distress, secretion retention and pneumonias; these lesions can lead to death by asphyxia, which makes its diagnosis and treatment an urgent matter. If the lesion encompasses less than five tracheal rings, treatment involves resection and end-to-end anastomosis, but if extension is larger, tissue has to be replaced[3,4]. Allogeneic transplantation has been unsuccessful due to unavailability of organs in adequate conditions and time, as well as to histocompability and immunosuppressive treatment, which lead to graft loss o stenosis relapse. Synthetic grafting has been tried with multiple materials, but they induce infection, extrusion and stenosis. Autologous tissues have also been tried to be used, but the most common complication is stenosis relapse[5-7] and, hence, tissue-engineering technologies have been implemented to generate replacements of different organs and tissues, which consist in obtaining biological matrices that serve as a scaffold for stem cells of the receptor to grow and obtain organs that are similar to the original in structure, mechanics and functionality[8,9].

The trachea is a cartilaginous conduit that serves for ventilation and secretion dragging. With a broad caliber and thin walls, it connects the larynx with the main bronchi; it is flattened in the posterior and cylindrical in the anterior part, with an 11-cm-length. Cartilaginous rings are incomplete in the posterior part, where they connect to fibrous and muscle tissue, a feature that prevents its collapse with inspiration. Its lumen is lined by a ciliated pseudo-stratified columnar epithelium that enables mucus clearance and regulates fluids and ionic transportation[10-12].

The ideal tracheal graft should be laterally rigid and longitudinally flexible, hermetic, not collapsing with inspiration, biocompatible, non-immunogenic, non-toxic, bacterial colonization-resistant, easy to implant to ensure

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its permanence, and has to have a ciliated epithelium[13]. For that, an adequate matrix has to be available, which must serve as a physical support or scaffold to seed the receptor's cells. The ideal scaffold must allow cell adhesion, growth and differentiation, but it also has to create a tridimensional stable network with a sufficiently porous microenvironment for cell growth. The scaffold can be biological from a donor of the same species (allogeneic matrices), from a donor of different species (xenogeneic matrices), synthetic or combined.

Decellurarized and autologous cells-seeded allogeneic biological matrices, which preserve some of the essential properties of the original tissue, have been successfully used to obtain grafts[14,15]. The matrix is obtained by eliminating the cells and DNA residues from the tracheal segment in order for not to trigger an inflammatory and rejection response; still, cases have been reported where such response develops against these tissues[16,17]. The lack of viability and functionality in tissue construction may be due to structural components destruction such as collagen and elastic fibers, proteoglycans and glycosaminoglycans of the matrix during the decellularization process, which results in resilience and rigidity loss, since this process has been difficult to standardize[18-20]. Proteoglycans are important in tracheal tissue, since they intervene in cell adhesion, migration and proliferation, as well as in extracellular matrix regulation, affecting its integrity, performance and durability; therefore, they play a relevant role in the generation of tissues *in vitro*[21,22]. Preservation time of the matrix is important, since proteolytic activity and degradation start at 4 °C.

Tracheal grafts using animal xenogeneic matrices have not elicited rejection, since, during the decellularization process, immune system-recognized antigens are eliminated and repopulated by receptor cells. Patches of porcine jejune decellularized segments, seeded with muscle cells and human fibroblasts, have been used to replace connective tissue with no rejection[23,24].

Synthetic matrices have also been used in the trachea. In the decade of the 90's, polymeric or hydrogel synthetic materials were used to create cartilage, with poor preclinical results, since they elicited a strong inflammatory response[25]. A structure comprising a silica tubular stent wrapped with epithelial tissue obtained from the internal mucosa of the ear was implanted in rabbits. A polypropylene mesh was used to substitute the tracheal cartilage and the lateral thoracic fascia as vascular supply. After two weeks *in vivo*, rigidity, elasticity, diameter and thickness of the wall were similar to those of native trachea. However, long-term complications include the development of stenosis, glandular growth and secretions accumulation due to the absence of mucociliary function[26]. An alternative approach was proposed using temperature-sensitive polymers as a substrate for epithelial cells, which were used in the lumen of a graft comprised by Dacron-reinforced monofilament polypropylene [27]. Sheep stem cells have also been seeded in a polyglycolic acid scaffold treated with growth factors in order to develop a structure similar to the native one. Receptor stem cells have been seeded in nano-compounds matrices covered with a synthetic polymer[28,29].

In comparison with synthetic scaffolds, natural biodegradable matrices have strong advantages, since they preserve the extracellular matrix natural composition, they don't release toxic products and play an active role in cell behavior regulation, which affects its proliferation, migration and differentiation[30,31].

Currently, the effort lies in the creation of artificial or hybrid materials mimicking the extracellular matrix using a biological matrix fortified by synthetic materials[32]. The combination of synthetic and biological materials has already been successfully assayed in dogs. A hybrid graft was used in a pediatric patient to treat a congenital tracheal stenosis by associating a polpoly-ε-caprolactone biopolymer seeded with autologous stem cells and applying transforming growth factor β on the cartilage to induce chondrocyte proliferation. A 2-year clinical follow-up was carried out with no complications observed[33]. An important factor in graft generation is the bioreactor as a device to contain the matrix, the cells and the molecules required to enable its interaction in tissue regeneration under ideal conditions. The culture environment has to be dynamic, controllable and reproducible, and it is essential for even cell distribution in the scaffold, as well as for nutrient supply and detritus elimination. It also provides with hemodynamic tension and shear forces to promote tissue growth, such as angiogenesis, ciliary function, metabolic activity and cell differentiation.

Tracheal grafts have been able to be constructed in 24 h, where the matrix surface is already covered by cells. Construction success depends on the type of used cell, culture conditions and scaffold properties. The range of cell sources for tissue engineering keeps on growing. The most widely used stem cells in these procedures are fat tissue-, peripheral blood- and bone marrow-derived; the latter possess high differentiation capacity. Chondrocytes have been obtained from auricular and costal cartilage, nasal septum, bone or joints to develop cartilaginous tissue. Epithelial cells can be obtained from nasal or tracheal mucosa; these allow for the epithelial regeneration required as a physical barrier that also regulates the respiratory tract metabolic functions. During the first

attempts to obtain a tracheal bioprosthesis in pigs, only chondrocytes were used, which conferred mechanical resistance to the graft *in vivo*, but got infected due to the lack of epithelial cells serving as a barrier against microorganisms. With epithelial cells addition in the internal part of the graft, health tissue was generated, with long-term survival[34,35]. A new *in vitro* culture system has been developed, known as co-culture, which seeds nasal chondrocytes and respiratory epithelial cells onto opposite sides of the collagen membrane[36].

Recently, amniotic fluid and umbilical cord-obtained stem cells have been experimentally used for the treatment of congenital stenotic conditions[37]. Walles et al. decellularized a matrix without altering the structure and the basal membrane, and seeded it with autologous endothelial progenitor cells to allow adequate revascularization, as well as with bone marrow cells, costal chondrocytes and respiratory epithelial cells. The authors demonstrated that obtaining all tracheal cell elements functioning, such as chondrocyte proliferation, the cartilaginous matrix, muscular tissue and presence of ciliated epithelial cells is possible[38].

Discussion

Tissue engineering is a rapidly growing interdisciplinary field with considerable potential to significantly contribute to regenerative medicine. The development of new strategies and technologies intended to create tissues and complete organs ex vivo is exciting. The future of tissue engineering lies in the search of new alternatives in the generation of matrices without stopping from being commercially viable, considering that a complex interaction of factors is required, such as the variety of spatial and temporal signals. Thus, the biology of the tissue and organ to be designed has to be better known, especially the trachea.

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