Stem Cell-Based Therapies and Tissue Engineering of Trachea as Promising Therapeutic Methods in Mustard Gas Exposed Patients

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ABSTRACT

Tissue engineering and cell-based therapies are promising therapeutic approaches in structural and functional defects of the trachea. Researchers have focused on these approaches to overcome the complications related to such diseases. Patients exposed to mustard gas suffer from massive damage to the respiratory system. Current treatment plans are only palliative and include anti-inflammatory drugs, broncholytics, long-acting β_2-agonists, and inhaled corticosteroids. As mustard gas exposure leads to chronic airway inflammation, it seems that tracheobronchomalacia, because of chronic inflammation and weakness of the supporting cartilage, is an important factor in the development of chronic and refractory respiratory symptoms. The previous studies show that regenerative medicine approaches have promising potential to improve the life quality of patients suffering from tracheal defects. It seems that the engineered tracheal graft may improve the respiratory function and decrease symptoms in patients who suffer from asthma-like attacks due to mustard gas exposure. There are several successful case reports on the transplantation of stem cell-based bioartificial grafts in structural airway diseases. Therefore, we hope that the reconstruction of tracheobronchial structure can lead to a decrease in respiratory difficulties in mustard gas-exposed patients who suffer from tracheomalacia. In the present review, we summarize the main aspects of tracheal tissue engineering and cell-based therapies and the possibilities of the application of these approaches in mustard gas-exposed patients.

KEYWORDS: Stem cell; Cell-based therapies; Tissue engineering; Trachea; Mustard gas

INTRODUCTION

Tissue engineering and cell-based therapies are promising treatments for degenerative diseases of the trachea. These methods can overcome the problems in the shortage of organ for transplantation and tissues incompatibility between donor and recipient. Researchers are trying to find appropriate protocols to build viable and functional engineered trachea to eliminate the morbidity and mortality of patients.

Tracheal injury caused by mustard gas exposure leads to progressive and life-threatening conditions in patients. Current therapeutic methods are based on anti-inflammatory drugs and broncholytics. However, these methods are not efficient to repair or regenerate this tissue. Therefore, many efforts have so far been made to generate tracheal substitute.

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In order to fabricate an engineered trachea, it is necessary to consider its native structure. Trachea is a cartilaginous and membranous tube, which connects the larynx to the right and left main bronchi. U-shaped hyaline cartilage rings of the trachea support its fibroelastic wall. The free ends of the U-shaped cartilages are attached together by trachealis muscles [1]. Mustard gas exposure leads to tracheal inflammation and necrosis. This chemical agent causes detachment of tracheal epithelial cells [2]. On the other hand, some patients develop tracheal collapse [3].

The selection of appropriate cell sources is one of the most important items in tracheal cell-based therapies. The application of autologous cells can definitely remove the need for immunosuppression. However, the selection of the best cells among different sources has not been clearly identified [4]. Researchers reported their findings on different sources of cells, including pluripotent stem cells [5-7], mesenchymal stem cells (MSCs) [8-10], and terminally differentiated cells [11], for tracheal regeneration. On the other hand, different tracheal reconstruction methods such as decellularized trachea [12], injectable matrix [13], electrospun nanofibers [14], and bio 3D printing system [15] have been investigated to optimize the engineered trachea. Although many efforts have been devoted to fabricate a perfect engineered trachea, there is no optimal and reproducible method.

Due to the limitation of current therapeutic methods in tracheal defects, it is important to focus on modern methods. In the present review article, we summarize advances in regenerative medicine of the trachea and discuss the challenges in this area.

INSIGHT INTO TISSUE ENGINEERING AND CELL-BASED THERAPIES OF THE TRACHEA

Nowadays, investigators are focusing on stem cells to support the regeneration of the airway tissues. Although the mechanism of this supportive effect is not entirely clear, it appears that stem cells can differentiate and substitute the damaged cells. Moreover, MSCs show anti-inflammatory properties and facilitate tissue regeneration [16]. Some studies show that mature cells, such as epithelial cells, chondrocytes, and smooth muscle cells can be used for tracheal regeneration [16, 17]. However, the isolation process of these cells leads to low yield and the cells are unstable in vitro. Therefore, it is important to focus on the native environment of these cells to manage the conditions in vitro.

On the other hand, for producing a functional trachea, the establishment of a suitable platform is essential. The first attempts to reconstruct the trachea have been started in late 19th century. To achieve this purpose, researchers have investigated decellularized platforms [18-20], and synthetic scaffolds [14, 21]. Decellularized trachea has been suggested as a natural platform to produce efficient bio-engineered trachea. In this situation, the extracellular matrix (ECM) 3D architecture is maintained. Moreover, during the decellularization protocols, the tissue antigenicity decreases, leading to minimal allograft rejection [22]. MacChiarini, *et al*, reported the first human transplantation of a bioengineered airway tissue in a 58-year-old man [23]. They used a decellularized porcine proximal jejunum segment and seeded the muscle cells and fibroblasts on this structure. The patient follow-up showed that clinical examination was normal and the patient's life quality returned to a normal condition.

The difficulty and non-reproducibility of decellularization technique, the shortage of organs for decellularization, and limitation of the animal's tissue application demonstrate the necessity of alternative scaffolds. Researchers have used natural and synthetic biomaterials to produce scaffolds in the trachea bioengineering. In order to achieve this purpose, attention to the native structure of the trachea and native environment of different cells in the trachea is very important. Therefore, the reconstructed trachea should reveal the mechanical stability and efficient ciliated epithelium to provide appropriate anatomical

and physiological functions and decrease complications [22].

A number of synthetic and natural biomaterials have been used in this field. Synthetic biomaterials, such as polypropylene [24], polytetrafluoroethylene [25], polyester-urethane [26], and poly (ε-caprolactone) [27] can provide the mechanical stability of the trachea. On the other hand, natural biomaterials, such as collagen [28, 29], chitosan, and gelatin [30], can provide bioactive properties to interact with cells. A composition of natural and synthetic biomaterials seems to be more efficient due to the combination of their unique properties. Recently, Wu, *et al*, tested a bilayer tubular scaffold based on electrospun poly(L-lactide-co-caprolactone)/collagen fibers seeded with autologous tracheal epithelial cells and chondrocytes in a rat trachea injury model. They showed that this construct can improve the tracheal tissue regeneration [21].

One of the very interesting methods to produce bio-engineered trachea is 3D bioprinting. In this technology, 3D structures are fabricated by the positioning of bioactive molecules and living cells, mimicking the native structure. In 2014, researchers reported an artificial tracheal construct, using this technology for the repair of the partial tracheal defects. They produced the artificial tissue by coating of polycaprolactone 3D-printed scaffold with seeded MSCs in fibrin and transplanted them in rabbit animal model. This structure showed a stable shape and function and did not trigger any graft rejection after eight weeks [31]. Recently, Park, *et al*, proposed a novel bio-engineered trachea mechanically similar to native trachea [32]. They confirmed that it is possible to design a bio-engineered trachea with appropriate behavior.

Generally, tissue engineering of the trachea is a laborious process, because trachea is a complex organ with different tissue types including cartilage, muscle, and vascular and nervous tissues. Strength and stiffness are essential factors for optimal function of the trachea. Therefore, it seems that using scaffold is important to achieve this goal [33]. However, researchers reported scaffold-free trachea tissue engineering by 3D bio-printing technology [34, 35]. Matsumoto, *et al*, recruited different combinations of human cartilage, fibroblasts, umbilical vein endothelial cells, and MSCs for constructing the scaffold-free trachea. After printing of multicellular aggregates of these cells, they obtained small diameter tracheal tubes. The implantation of tubes consisted of human cartilage cells, umbilical vein endothelial cells, and MSCs demonstrated more ECM than others [34]. They concluded that this technology could produce a more similar structure to the native trachea. Recently, Taniguchi, *et al*, showed that scaffold-free trachea can be created by bio-printed spheroids containing MSCs and chondrocytes. This artificial trachea was functional several weeks after transplantation in an animal model [36].

Blood supply is another crucial issue in the success of tissue engineering. Technical difficulty of tracheal graft revascularization has led to poor results. Researchers tried to develop tracheal transplantation using vascularized units. To date, the only acceptable way of tracheal blood supply is the application of vascularized soft tissue flap and then the establishment of anastomoses between this flap and the trachea adventitia [22, 37]. However, cartilaginous tissue is a barrier for the ingrowth of blood vessels toward the mucosal lining. Therefore, the intercartilaginous ligaments incision is required to improve revascularization process [37]. There are several methods in tissue engineering to establish blood supply. The application of endothelial cells into the bioengineered trachea can probably expedite neovascularization. In 2004, investigators studied a decellularized porcine jejunum segment containing autologous endothelial cells as a tissue-engineered trachea. After three weeks, the vascular network of the graft demonstrated uniform re-endothelialization with autologous endothelial cells [38]. However, further analysis is required to confirm the functionality of this vascular network. Another approach to improve vascularization is the incorporation of angiogenic factors such as vascular endothelial growth factor

[39], platelet-driven growth factor [31], and basic fibroblast growth factor [40], in bio-engineered structure. Nanoparticles [41] and exosomes [40] can be recruited for angiogenic factors delivery. Recent reports show that they can transfer bioactive molecules in a protected manner and nanoparticles can act smartly. To date, there is no study on using nanoparticles to induce vascularization in engineered trachea. Nanoparticles can act as a safe carrier for essential factors of this process. Incorporation of these factors with endothelial cells seems to improve the processes involved in blood supply of the trachea. Considering the advances made in tracheal engineering, it is now possible to manage the patients who suffer from mustard gas complications.

TRACHEAL PROBLEMS IN MUSTARD GAS-EXPOSED PATIENTS

Mustard gas [bis(2-chloroethyl) sulfide] is an alkylating agent and was first used during World War I [41, 42]. There are several documented cases of using mustard gas as a chemical weapon against both civilians and troops worldwide [43, 44]. The chemicals used in weapons are usually vaporized and affect the exposed organs such as the skin, respiratory tract, and eyes [45]. In contact with nucleic acids, the alkylating agents add an alkyl group to the nitrogen ring and extracyclic oxygen of the molecules, leading to loss of cellular structural integrity. This usually causes blistering on the affected epithelium [46, 47]. Survivors are often faced with chronic skin lesions, ocular sores, and respiratory problems [45]. Inhalation of alkylating agents leads to massive damage to the tracheobronchial tract and lungs. Therefore, victims often die within the first hours of suffocation. However, some survivors endure chronic deterioration of the pulmonary function [48]. Chronic obstructive pulmonary disease, scars and strictures of the central airways, tracheobronchomalacia, interstitial lung disease, emphysema, pulmonary fibrosis, bronchiectasis, and susceptibility to opportunistic infections are commonly reported [3, 49, 50]. Treatment plans are only palliative and include anti-inflammatory drugs, broncholytics, long-acting β_2-agonists, inhaled corticosteroids and proton pump inhibitors [51, 52]. However, there is evidence of a relative inefficacy of β_2-agonists in many patients. Kabir, *et al*, believe that desensitization of β-adrenergic receptors is the main reason [53]. Another important subject may be the concurrence of gastro-esophageal reflux and respiratory symptoms [54]. Paroxysmal dyspnea and asthma-like symptoms are of the most complicating problems with which chest physicians are faced regarding patients with chronic complications of mustard gas respiratory exposure. It seems that tracheobronchomalacia, as a result of chronic inflammation and weakness of the supporting cartilage, is an important factor. Therefore, application of a tracheal graft may be an effective way to alleviate refractory symptoms attributed to obstructive bronchopneumonopathy.

As respiratory symptoms in patients with exposure to mustard gas are similar to those of obstructive pulmonary disease, some authors have suggested using β_2-agonists for treatment of asthma-like attacks [51]. Anecdotal animal experiments have shown that long-term treatment with β-agonists is associated with a decrease in β_2-adrenergic receptor mRNA levels [55, 56]. Other studies have indicated that chronic use of β_2-adrenergic receptor agonists in the treatment of obstructive lung disease is associated with reduction of response. The possible mechanism proposed is tachyphylaxis [57, 58]. Recently, Kabir, *et al*, indicated that 2-choloroethyl ethyl sulfide exposure was associated with desensitization of β_2-adrenergic receptors as well [53]. This can be the reason for the fact that regular use of β-agonists is not related to clinical improvement. Moreover, previous research studies have proved that gastroesophageal reflux (GER) is more common in people who suffer from respiratory problems [59, 60]. In addition, GER is a risk factor for exacerbation of chronic obstructive pulmonary disease (COPD) [61]. Although the definite pathophysiology is not clear, it seems that microaspiration is a probable mechanism, which leads to airway irritation and increased resistance due to vagally mediated bronchoconstriction [62]. In anoth-

er study, Ghanei, *et al*, proved the increased incidence of GER in mustard gas-exposed patients [63]. Aliannejad, *et al*, hypothesized that GER might be an important factor for the exacerbation of respiratory symptoms in these patients. However, they believe that it is still unclear that an inverse correlation could also be involved [54]. In fact, GER could be the result of raised intra-abdominal pressure due to chronic cough. Besides these facts, all researchers agree that mustard gas exposure leads to chronic airway inflammation. It seems that diffuse airway inflammation is associated with tracheobronchomalacia [64]. Some reports suggested the role of acquired form of tracheobronchomalacia in development of chronic respiratory symptoms. Palombini, *et al*, indicated that in about 14% of patients with chronic cough the symptoms were directly associated with tracheobronchomalacia [65]. Ghanei, *et al*, reported a high prevalence of air trapping in high-resolution chest computed tomography (CT), at least 15 years after being exposed to sulfur mustard [66]. Moreover, they hypothesized that tracheobronchomalacia is an important consequence of exposure to sulfur mustard which is associated to air trapping and development of chronic obstructive pulmonary disease [3].

Tracheomalachia is defined as structural abnormality in the hyaline cartilages of the trachea. It can be either malformation or deficiency of the supporting cartilages, which is associated with the decrease of cartilage to muscle ratio. This abnormality causes narrowing and shortening of the trachea during exhalation. Consequently, the collapsed trachea creates expiratory flow obstruction and even cessation of secretion clearance, which can lead to some discomfort such as expiratory stridor and hoarseness [67]. Therefore, the problem of refractory respiratory symptoms in mustard gas-exposed patients can be explained by this pathological mechanism.

THE UTILITY OF REGENERATIVE MEDICINE IN MUSTARD GAS-EXPOSED PATIENTS

In the last few decades, researchers have focused on the regenerative medicine to find new methods in the treatment of chronic and degenerative diseases [68-70]. Tracheal replacement has been investigated for the treatment of patients who suffer from tracheal defects. In 2005, researchers reported the formation of a trachea-shaped structure from in vivo tissue-engineered human hyaline. They used human nasal septum cartilage to harvest chondrocytes. The chondrocytes were mixed with hydrogel Pluronic F127 and painted over high-density polyethylene in shape of a trachea. The composite was implanted in athymic mice for eight weeks. The macroscopic evaluation showed the coverage of the construct with a cartilage-like structure. The up-regulation of type II collagen in the engineered trachea indicates that this structure is similar to native trachea [71]. In another study, the effectiveness of a luminar remodeling type of artificial trachea was evaluated. The cervical tracheas of beagle dogs were replaced with prosthesis made of a polypropylene mesh tube and a polypropylene spiral and atelocollagen layer. The collagen layer was soaked with peripheral blood, bone marrow aspirate, or bone marrow MSCs. Better results, including faster epithelialization, lack of stenosis, and fewer problems were reported in those who received either bone marrow aspirate or bone marrow MSCs. They suggested that these cell sources can improve the tracheal mucosa generation on this platform [72].

In 2017, decellularized dermis was suggested as a platform for airway mucosa engineering in tracheal transplantation. In this study, human bronchial epithelial cells and human respiratory fibroblasts were cultured onto a decellularized dermis. After three weeks, the engineered structures were implanted onto a decellularized trachea in New Zealand white rabbits. A muscle wrap had been also applied for prevascularization. The findings showed the formation of pseudo-stratified ciliated layer 24 hours after implantation [73]. Moreover, there are some successful case reports in the field of human airway transplantation with

stem cell-based bioartificial grafts for structural airway diseases. The first human case of regenerative technique of tracheal tissue was done on a 78-year-old woman. In this study, the defective area of the trachea was replaced with a Marlex mesh tube covered by collagen sponge. The epithelialization was observed after two months and continued to cover all areas for two years. They did not report any complications during two years [74]. In 2008, Macchiarini, *et al*, reported a 30-year-old woman who suffered from severe hypoxia and right to left ventilatory shunt. This was due to destruction of the upper respiratory tract by tuberculosis that had led to expiratory collapse, despite primary placement of Dumon stent. After removal of the stent, transplantation of tissue-engineered airway was planned. A trachea segment was received from a deceased person. All loose connective tissues of the donor's trachea were removed, decellularization protocol was done for 25 cycles and its matrix was colonized by epithelial cells, which were obtained by the culture of the recipient's nasal epithelium. Then, chondrogenic MSCs were added to the matrix. This engineered tissue was successfully implanted. Moreover, follow up was done for the next four months with full recovery [75]. In 2012, Elliott, *et al*, reported a 12-year-old boy with long-segment congenital tracheal stenosis. He underwent autologous patch tracheoplasty, but due to intolerance to the patch, it was replaced with a balloon-expandable intraluminal stents in the 6th year of his life. Then again, it was removed after three years due to aortic erosion. Consequently, implantation of the tracheal homograft was planned. However, one year later, airway hemorrhage caused by aorto-tracheal fistula proposed the idea of replacing the trachea by allogenic stem cell-based tissue-engineered tracheal graft. A tracheal segment was received from a deceased body and decellularized as the scaffold. Bone marrow-derived MSCs were obtained from the patient and together with patches of autologous epithelium were added to the scaffold. Additionally, the tissue-engineered trachea was saturated by human recombinant erythropoietin and transforming growth factor β in order to stimulate angiogenesis and chondrogenesis, respectively. A bronchoscopy was performed after one week and it showed neovascularization. Eighteen months later, a chest CT showed tracheal growth. The patient was followed up for two years with full recovery [76].

In contrast, in 2011, The Lancet published a research article on a 36-year-old man who had undergone tracheaectomy due to mucoepidermoid tracheal cancer; tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite was performed successfully [77]. However, about five years later, in 2016, the journal editors expressed their uncertainty about the validity of this paper [78].

Recently, researchers reported adipose-derived MSCs transplantation in a patient after long-term exposure to sulfur mustard. In this clinical trial, 400×10^6 cells were injected during two months and precise evaluations were performed. The systemic infusion of these cells did not lead to toxicities and serious problems. On the other hand, patient's physical activity and 6MWT, BSDA, SGRQ, and CAT scores improved. These findings show the potency of adipose-derived MSCs in sulfur mustard-exposed patients. However, further clinical trials are required to confirm these results [79].

It seems that airway transplantation with stem cell-based bio-artificial graft is possible for patients suffering from respiratory problems due to exposure to mustard gas. We believe this technique may improve the respiratory function and decrease symptoms in patients who suffer from asthma-like attacks due to mustard gas exposure. Furthermore, by modifying the function of the trachea, complications such as recurrent respiratory tract infections could be alleviated. Moreover, appropriate use of bronchodilator drugs after trachea transplantation can decrease resistance to β-agonists, which is a considerable problem in this setting. Another advantage is cough control and consequently preventing the increase of intra-abdominal pressure and GER. In previous case reports, replacement of respiratory airways was shown to be safe and well-tolerated. Therefore, we can hope that reconstruction of tracheobronchial structure could theoretically lead to de-

crease of respiratory difficulties in patients who suffer from tracheomalacia.

The above-mentioned studies show that tissue engineering approaches have a promising potential and they can help patients suffer from tracheal defects due to mustard gas exposure. Although tissue engineering can improve the life quality of these patients, their lung tissues are also affected by mustard gas. Therefore, it is essential to think about the management of the whole respiratory system in these patients.

There are many advances in tracheal tissue engineering, but to achieve a perfect bio-engineered trachea, it is essential to focus on the native structure of the trachea and overcome the obstacles regarding graft vascularization and tissue functionality.

In conclusion, mustard gas exposure leads to the respiratory system tissue damage. In this study, we focused on the advances made in and limitations of tissue engineering approaches in trachea defects and the possibility of this approach in mustard gas-exposed patients suffering from tracheomalacia. To achieve a perfect management and treatment for these patients, precise and multilateral studies, that cover lung tissue in addition to airway tract, need to be considered.

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REFERENCES

1. Snell RS. *Clinical anatomy by regions.* Lippincott Williams & Wilkins, **2011**.
2. Lorenzen JM, Kaucsar T, Schauerte C, *et al*. MicroRNA-24 antagonism prevents renal ischemia reperfusion injury. *J Am Soc Nephrol* 2014;**25**:2717-29.
3. Ghanei M, Moqadam FA, Mohammad MM, Aslani J. Tracheobronchomalacia and air trapping after mustard gas exposure. *Am J Respir crit care med* 2006;**173**:304-9.
4. Kojima K, Vacanti CA. Tissue engineering in the trachea. *Anat Rec (Hoboken)* 2014;**297**:44-50.
5. Imaizumi M, Nomoto Y, Sugino T, *et al*. Potential of induced pluripotent stem cells for the regeneration of the tracheal wall. *Ann Otol Rhinol Laryngol* 2010;**119**:697-703.
6. Imaizumi M, Nomoto Y, Sato Y, *et al*. Evaluation of the use of induced pluripotent stem cells (iPSCs) for the regeneration of tracheal cartilage. *Cell transplant* 2013;**22**:341-53.
7. Coraux C, Nawrocki-Raby B, Hinnrasky J, *et al*. Embryonic stem cells generate airway epithelial tissue. *Am J Respir Cell Mol Biol* 2005;**32**:87-92.
8. Yan B, Zhang Z, Wang X, *et al*. PLGA–PTMC–Cultured Bone Mesenchymal Stem Cell Scaffold Enhances Cartilage Regeneration in Tissue-Engineered Tracheal Transplantation. *Artif Organs* 2017;**41**:461-9.
9. Wang J, Sun B, Tian L, *et al*. Evaluation of the potential of rhTGF-β3 encapsulated P (LLA-CL)/collagen nanofibers for tracheal cartilage regeneration using mesenchymal stems cells derived from Wharton's jelly of human umbilical cord. *Mater Sci Eng C Mater Biol Appl* 2017;**70**(Pt 1):637-45.
10. Moravej A, Karimi M-H, Geramizadeh B, *et al*. Mesenchymal stem cells upregulate the expression of PD-L1 but not VDR in dendritic cells. *Immunol Invest* 2017;**46**:80-96.
11. Kojima K, Bonassar LJ, Roy AK, *et al*. A composite tissue-engineered trachea using sheep nasal chondrocyte and epithelial cells. *FASEB J* 2003;**17**:823-8.
12. Knobeloch T, Abadi SEM, Bruns J, *et al*. Injectable polyethylene glycol hydrogel for islet encapsulation: an in vitro and in vivo characterization. *Biomed Phys Eng Express* 2017;3. pii: 035022.
13. Pham-Hua D, Padgett LE, Xue B, *et al*. Islet encapsulation with polyphenol coatings decreases proinflammatory chemokine synthesis and T cell trafficking. *Biomaterials* 2017;**128**:19-32.
14. Mahoney C, Conklin D, Waterman J, *et al*. Electrospun nanofibers of poly (ε-caprolactone)/depolymerized chitosan for respiratory tissue engineering applications. *J Biomater Sci Polym Ed* 2016;**27**:611-25.
15. Supavekin S, Zhang W, Kucherlapati R, *et al*. Differential gene expression following early renal ischemia/reperfusion. *Kidney Int* 2003;**63**:1714-24.
16. Jungebluth P, Macchiarini P. Airway transplantation. *Thoracic surgery clinics* 2014;**24**:97-106.
17. Walles T, Giere B, Hofmann M, *et al*. Experimental generation of a tissue-engineered functional and vascularized trachea. *J thorac cardiovas surg* 2004;**128**:900-6.

18. Hung SH, Su CH, Lin SE. Preliminary experiences in trachea scaffold tissue engineering with segmental organ decellularization. *Laryngoscope* 2016;**126**:2520-7.
19. Sun F, Jiang Y, Xu Y, *et al*. Genipin cross-linked decellularized tracheal tubular matrix for tracheal tissue engineering applications. *Sci Rep* 2016;**6**:24429. doi: 10.1038/srep24429.
20. Den Hondt M, Vanaudenaerde B, Vranckx J. Tracheal tissue-engineering: Epithelial grafting of gently-decellularized rabbit trachea. *Plast Reconstr Surg Glob Open* 2017;**5**(4 Suppl):22-3.
21. Wu T, Zheng H, Chen J, *et al*. Application of a bilayer tubular scaffold based on electrospun poly (L-lactide-co-caprolactone)/collagen fibers and yarns for tracheal tissue engineering. *J Mater Chem B* 2017;**5**:139-50.
22. Law JX, Liau LL, Aminuddin BS, Ruszymah BHI. Tissue-engineered trachea: A review. *Int J Pediatr Otorhinolaryngol* 2016;**91**:55-63. doi: 10.1016/j.ijporl.2016.10.012.
23. MacChiarini P, Walles T, Biancosino C, Mertsching H. First human transplantation of a bioengineered airway tissue. *J Thorac Cardiovasc Surg* 2004;**128**:638-41.
24. Suh SW, Kim J, Baek CH, *et al*. Replacement of a tracheal defect with autogenous mucosa lined tracheal prosthesis made from polypropylene mesh. *ASAIO J* 2001;**47**:496-500.
25. Cull DL, Lally KP, Mair EA, *et al*. Tracheal reconstruction with polytetrafluoroethylene graft in dogs. *Ann Thorac Surg* 1990;**50**:899-901.
26. Yang L, Korom S, Welti M, *et al*. Tissue engineered cartilage generated from human trachea using DegraPol® scaffold. *Eur J Cardiothorac Surg* 2003;**24**:201-7.
27. Lin C-H Su J-M, Hsu S-H. Evaluation of type II collagen scaffolds reinforced by poly (ε-caprolactone) as tissue-engineered trachea. *Tissue Eng Part C Methods* 2008;**14**:69-77. doi: 10.1089/tec.2007.0336.
28. Kobayashi K, Suzuki T, Nomoto Y, *et al*. A tissue-engineered trachea derived from a framed collagen scaffold, gingival fibroblasts and adipose-derived stem cells. *Biomaterials* 2010;**31**:4855-63.
29. Okumura N, Nakamura T, Shimizu Y, *et al*. Experimental study of a new tracheal prosthesis made from collagen-grafted mesh. ASAIO J 1991;37:M317-M8.
30. Risbud M, Endres M, Ringe J, *et al*. Biocompatible hydrogel supports the growth of respiratory epithelial cells: possibilities in tracheal tissue engineering. *ASAIO Trans* 1991;**37**:M317-9.
31. Chang JW, Park SA, Park JK, *et al*. Tissue-engineered tracheal reconstruction using three-dimensionally printed artificial tracheal graft: preliminary report. *Artif Organs* 2014;**38**:E95-E105. doi: 10.1111/aor.12310.
32. Park JH, Hong JM, Ju YM, *et al*. A novel tissue-engineered trachea with a mechanical behavior similar to native trachea. *Biomaterials* 2015;**62**:106-15.
33. Dikina AD, Strobel HA, Lai BP, *et al*. Engineered cartilaginous tubes for tracheal tissue replacement via self-assembly and fusion of human mesenchymal stem cell constructs. *Biomaterials* 2015;**52**:452-62.
34. Matsumoto K, Taura Y, Yamasaki N, *et al*. Scaffold-Free Trachea Tissue Engineering Using Bioprinting. *Am J Respir Crit Care Med* 2015;**191**:A5343.
35. Machino R, Taniguchi D, Takeoka Y, *et al*. Development Of Scaffold-Free Trachea Tissue Engineering Using Bio 3D Printing System. *Am J Respir Crit Care Med* 2016;**193**:A7421.
36. Taniguchi D, Matsumoto K, Tsuchiya T, *et al*. Corrigendum to: 'Scaffold-free trachea regeneration by tissue engineering with bio-3D printing'. *Interact Cardiovasc Thorac Surg* 2018;**26**:713. doi: 10.1093/icvts/ivy025.
37. Delaere P, Van Raemdonck D. Tracheal replacement. *J Thorac Dis* 2016;**8**(Suppl 2):S186-96. doi: 10.3978/j.issn.2072-1439.2016.01.85.
38. Chen F-M, Zhang M, Wu Z-F. Toward delivery of multiple growth factors in tissue engineering. *Biomaterials* 2010;**31**:6279-308.
39. Wagner Q, Offner D, Idoux-Gillet Y, *et al*. Advanced nanostructured medical device combining mesenchymal cells and VEGF nanoparticles for enhanced engineered tissue vascularization. *Nanomedicine* 2016;**11**:2419-30.
40. Yongjun L, Shen Y, Ashraf M, *et al*. Electrical Stimulation Enhances Stem Cells-Mediated Angiogenesis via Exosomes. *Circulation* 2016;**134**:A17819-A.
41. Somani S, Babu S. Toxicodynamics of sulfur mustard. *Int J Clin Pharmacol Ther Toxicol* 1989;**27**:419-35.
42. Beebe GW. Lung Cancer in World War I Veterans: Possible Relation to Mustard-Gas Injury and 1918 Influenza Epidemic 2. *J Natl Cancer Inst* 1960;**25**:1231-52.
43. Ghanei M, Harandi AA. Long term consequences from exposure to sulfur mustard: a review. Inhal Toxicol 2007;**19**:451-6.
44. Ghabili K, Agutter PS, Ghanei M, *et al*. Mustard gas toxicity: the acute and chronic pathological effects. *J Appl Toxicol* 2010;**30**:627-43. doi: 10.1002/jat.1581.
45. Khateri S, Ghanei M, Keshavarz S, *et al*. Incidence of lung, eye, and skin lesions as late complications in 34,000 Iranians with wartime exposure to mustard agent. *J Occup Environ Med* 2003;**45**:1136-43.
46. Shulman L. The biology of alkylating-agent cellular injury. *Hematol Oncol Clin North Am* 1993;**7**:325-35.
47. Watson A, Griffin G. Toxicity of vesicant agents scheduled for destruction by the Chemical Stockpile Disposal Program. *Environ Health Perspect* 1992;**98**:259-80.

48. Emad A, Rezaian GR. Immunoglobulins and cellular constituents of the BAL fluid of patients with sulfur mustard gas-induced pulmonary fibrosis. *Chest* 1999;**115**:1346-51.

49. Freitag L, Firusian N, Stamatis G, Greschuchna D. The role of bronchoscopy in pulmonary complications due to mustard gas inhalation. *Chest* 1991;**100**:1436-41.

50. Hefazi M, Attaran D, Mahmoudi M, Balali-Mood M. Late respiratory complications of mustard gas poisoning in Iranian veterans. *Inhal Toxicol* 2005;**17**:587-92.

51. Ghanei M, Shohrati M, Harandi AA, *et al*. Inhaled corticosteroids and long-acting β2-agonists in treatment of patients with chronic bronchiolitis following exposure to sulfur mustard. *Inhal Toxicol* 2007;**19**:889-94.

52. Helden van HP, Kuijpers WC, Diemel RV. Asthma-like symptoms following intratracheal exposure of Guinea pigs to sulfur mustard aerosol: therapeutic efficacy of exogenous lung surfactant curosurf and salbutamol. *Inhal Toxicology* 2004;**16**:537-48.

53. Kabir SM, Mukherjee S, Rajaratnam V, *et al*. Desensitization of β-adrenergic receptors in lung injury induced by 2-chloroethyl ethyl sulfide, a mustard analog. *J Biochem Mol Toxicol* 2009;**23**:59-70. doi: 10.1002/jbt.20265.

54. Aliannejad R, Hashemi-Bajgani S-M, Karbasi A, *et al*. GERD related micro-aspiration in chronic mustard-induced pulmonary disorder. *J Res Med Sci* 2012;**17**:777-81.

55. Mak J, Nishikawa M, Shirasaki H, *et al*. Protective effects of a glucocorticoid on downregulation of pulmonary beta 2-adrenergic receptors in vivo. *J Clin Invest* 1995;**96**:99-106.

56. Hadcock JR, Malbon CC. Regulation of beta-adrenergic receptors by" permissive" hormones: glucocorticoids increase steady-state levels of receptor mRNA. *Proc Natl Acad Sci U S A*. 1988;**85**:8415-9.

57. Hanania NA, Sharafkhaneh A, Barber R, Dickey BF. β-agonist intrinsic efficacy: measurement and clinical significance. *Am J Respir Crit Care Med* 2002;**165**:1353-8.

58. Taylor DR, Drazen JM, Herbison GP, *et al*. Asthma exacerbations during long term β agonist use: influence of β2 adrenoceptor polymorphism. *Thorax* 2000;**55**:762-7.

59. Mokhlesi B, Morris AL, Huang C-F, *et al*. Increased prevalence of gastroesophageal reflux symptoms in patients with COPD. *Chest* 2001;**119**:1043-8.

60. Gao F, Hobson AR, Shang ZM, *et al*. The prevalence of gastro-esophageal reflux disease and esophageal dysmotility in Chinese patients with idiopathic pulmonary fibrosis. *BMC gastroenterol* 2015;**15**:26.

61. Takada K, Matsumoto S, Hiramatsu T, *et al*. [Relationship between chronic obstructive pulmonary disease and gastroesophageal reflux disease defined by the Frequency Scale for the Symptoms of gastroesophageal reflux disease.] *Nihon Kokyuki Gakkai Zasshi* 2010;**48**:644-8. [in Japanese]

62. Terada K, Muro S, Ohara T, *et al*. Abnormal swallowing reflex and COPD exacerbations. *Chest* 2010;**137**:326-32.

63. Ghanei M, Khedmat H, Mardi F, Hosseini A. Distal esophagitis in patients with mustard-gas induced chronic cough. *Dis Esophagus* 2006;**19**:285-8.

64. Ikeda S, Inoue Y, Fujino S, *et al*. [A case of diffuse panbronchiolitis associated with tracheomalacia.] *Nihon Ronen Igakkai Zasshi* 1993;**30**:974-7. [in Japanese]

65. Palombini B, Villanova C, Araujo E, *et al*. A pathogenic triad in chronic cough: asthma, postnasal drip syndrome, and gastroesophageal reflux disease. *Chest* 1999;**116**:279-84.

66. Ghanei M, Mokhtari M, Mohammad MM, Aslani J. Bronchiolitis obliterans following exposure to sulfur mustard: chest high resolution computed tomography. *Eur J Radiol* 2004;**52**:164-9.

67. Datey A, Chaskar A, Sarma L. Tracheobronchomalacia post-pneumonectomy: A late complication. *Indian J Palliat Care* 2013;**19**:107-9. doi: 10.4103/0973-1075.116713.

68. Kaviani M, Azarpira N. Insight into microenvironment remodeling in pancreatic endocrine tissue engineering: Biological and biomaterial approaches. *Tissue Eng Regen Med* 2016;**13**:475-84.

69. Azarpira MR, Vazani K, Ayatollahi M, *et al*. Comparison of healing intra-articular fracture of distal femur using a Kirschner wire and autologous fibrin glue in an animal model. *J Pediatr Orthop B* 2017;**26**:454-7.

70. Wiggenhauser PS, Schantz JT, Rotter N. Cartilage engineering in reconstructive surgery: auricular, nasal and tracheal engineering from a surgical perspective. *Regen Med* 2017;**12**:303-14. doi: 10.2217/rme-2016-0160.

71. Ruszymah BHI, Chua K, Latif MA, *et al*. Formation of in vivo tissue engineered human hyaline cartilage in the shape of a trachea with internal support. *Int J Pediatr Otorhinolaryngol* 2005;**69**:1489-95.

72. Nakamura T, Sato T, Araki M, *et al*. In situ tissue engineering for tracheal reconstruction using a luminar remodeling type of artificial trachea. *J Thorac Cardiovasc Surg* 2009;**138**:811-9. doi: 10.1016/j.jtcvs.2008.07.072.

73. Hamilton NJ, Lee DDH, Gowers KH, *et al*. Use of a decellularised dermis scaffold and human bronchial epithelial cells to tissue engineer airway mucosa suitable for tracheal transplantation. *Lancet* 2017;**389**:S43. doi:https://doi.org/10.1016/S0140-6736(17)30439-7.

74. Omori K, Nakamura T, Kanemaru S, *et a*l. Regenerative medicine of the trachea: the first human case. *Ann Otol Rhinol Laryngol* 2005;**114**:429-33.

75. Macchiarini P, Jungebluth P, Go T, *et al*. Clinical

transplantation of a tissue-engineered airway. *Lancet* 2008;**372**:2023-30.

76. Elliott MJ, De Coppi P, Speggiorin S, *et al*. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. *Lancet* 2012;**380**:994-1000.
77. Jungebluth P, Alici E, Baiguera S, *et al*. Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. *Lancet* 2011;**378**:1997-2004.
78. Lancet E. Expression of concern--Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. *Lancet* 2016;**387**:1359. doi: 10.1016/S0140-6736(16)30091-5.
79. Nejad-Moghaddam A, Ajdari S, Tahmasbpour E, *et al*. Adipose-Derived Mesenchymal Stem Cells for Treatment of Airway Injuries in A Patient after Long-Term Exposure to Sulfur Mustard. *Cell Journal (Yakhteh)* 2017;**19**:117.