Airway Tissue Engineering for Congenital Laryngotracheal Disease

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Abstract

Regenerative medicine offers hope of a sustainable solution for severe airway disease by the creation of functional, immunocompatible organ replacements. When considering fetuses and newborns, there is a specific spectrum of airway pathologies that could benefit from cell therapy and tissue engineering applications. While hypoplastic lungs associated with congenital diaphragmatic hernia (CDH) could benefit from cellular based treatments aimed at ameliorating lung function, patients with upper airway obstruction could take advantage from a *de novo* tissue engineering approach. Moreover, the international acceptance of the EXIT procedure as a means of securing the precarious neonatal airway, together with the advent of fetal surgery as a method of heading off postnatal co-morbidities, offers the revolutionary possibility of extending the clinical indication for tissue-engineered airway transplantation to infants affected by diverse severe congenital laryngotracheal malformations. This chapter outlines the necessary basic components for regenerative medicine solutions in this potential clinical niche.

Clinical Background

Tracheal failure is devastating in any patient group, but is particularly emotive within the neonatal population. Structural congenital anomalies (**Table 1**) are the most common cause of airway obstruction and insufficiency in this period [1], with malformations forming a spectrum of severity affecting any portion of the upper respiratory tract from face to bronchi [2]. Although complete congenital laryngotracheobronchial obstruction is rare - the European Organization for Rare Diseases reports the prevalence of tracheal agenesis to be around 1 in 100,000 births - these anomalies are universally lethal without intervention.

Presentation and diagnosis of extreme laryngotracheal birth defects usually occurs following routine prenatal ultrasound scanning with confirmation by *in utero* MRI [3], but can present later with immediate respiratory distress at birth. Without immediate surgical intervention, the lack of a patent proximal airway is unsurvivable unless a bypassing pathway exists for intubation of the bronchi via associated broncho-oesophageal fistulae. Fortunately, rates of prenatal diagnosis are improving allowing planned delivery via the EXIT procedure, where a precarious neonatal airway may be salvaged or established *de novo* via anaesthetic techniques or tracheostomy prior to cutting the umbilical cord [4]. An increasing number of children are being born alive with previously 'unsurvivable' airway defects, with the clinical team's intention to treat by subsequent airway reconstructive procedures (**Figure 1**).

Anatomical malformations of the fetal trachea may occur in association with a constellation of other mediastinal abnormalities; cardiac, vascular and oesophageal malformations are particularly common. Definitive surgical correction of the trachea requires careful timing and coordination between otolaryngology, cardiothoracics, obstetrics, pediatric surgery and neonatal intensive care for maximal chances of survival [5]. In some cases, babies with proximal airway anomalies are also found to have an underlying chromosomal abnormality [6-8]. The argument for pre- and perinatal treatment of these children is even more nebulous given the potential presence of other underlying unsurvivable co-morbidities, and for this reason chromosomal and genetic testing via amniocentesis provides vital information for counseling parents on treatment options.

Despite perinatal intervention, death is sadly inevitable for many of these babies for two main reasons. The first of these is the *in utero* development of secondary severe congenital lung disease. Without a patent connection between mouth and lungs to provide a route of escape, the constant production of lung fluid overdistends the lungs throughout gestation, leading to congestive heart failure in the fetus (hydrops) [9]. In cases of extensive tracheo-oesophageal connections or CDH, the opposite problem where babies are born with hypoplastic lungs may also occur because the fetus has been unable to generate intra-pulmonary negative pressure *in utero* [10]. Fetal surgical techniques have gained acceptance in a variety of conditions including CDH [11, 12], and fetal tracheostomy is gaining momentum as a viable treatment option to relieve tracheal obstruction in some fetal surgical centers [13].

The second, as-yet insurmountable, obstacle to survival occurs if insufficient salvageable trachea exists to reconstruct a functional airway. Anterior augmentation surgery, for example using tracheal donor allografts [14], has been largely superseded by the highly successful slide tracheoplasty technique [15, 16], but resections are still limited to 30% of the total tracheal length [17]. Stents may be of help in cases of malacia and recurrent stenosis, but carry a large inherent morbidity [18]. Nevertheless, existing treatments fail or are insufficient in a small proportion of babies who therefore require whole-scale tracheal replacement. Tracheal replacement by conventional organ transplantation has generally not been possible in the neonatal setting, due to the paucity of appropriate-sized donor organs and the generally poor condition of donor tracheae following prolonged end-of-life intubation and ventilation [14]. The decision to subject a child to the accompanying lifelong immunosuppression with its multiple risks and comorbidities would also not be taken lightly.

Concept of Tissue Engineering

Tissue engineering unites the fields of cell biology, materials science and engineering towards a common goal of creating substitutes to repair, replace or regenerate tissues and organs [19]. The basic principle is to create a biocompatible scaffold in which growth of the patient's cells can be encouraged, either by seeding prior to implantation or by subsequent recruitment into the scaffold in its *in vivo* position. The prerequisite for the graft to be free from immunogenicity is another key objective of tissue engineering as a personalized

therapy, and distinguishes the field from conventional transplantation where immunosuppression is required to prevent rejection.

Tissue-engineered trachea could be of particular value in the context of a prenatal tracheal diagnosis because an organ replacement could be built to individual fetal dimensions during gestation, ready for use in the perinatal or immediate postnatal period (**Figure 2**).

The trachea was initially considered, perhaps naively, to be a convenient 'starter organ' on which to concentrate tissue engineering efforts, due to its relatively simple anatomy as a hollow air-conduction organ with no moving parts [20]. However, early attempts in animals to replace the trachea with simple silicon, Dacron or metal [21, 22] tubes proved disastrous, as the trachea occupies a frontline immunological position with the body's external environment. The pseudostratified ciliated epithelium of the proximal airways is highly specialized to trap and remove inhaled pathogens and debris, and lack of a functional mucociliary escalator post-transplantation is therefore highly problematic. Establishment of a blood supply is essential for epithelialization of any graft, but with a finely segmental native blood supply in place of a vascular pedicle amenable to anastomosis, bioengineered tracheal grafts are relatively slow to establish connections capable of supporting cell growth and integration with host tissues. Notwithstanding these issues, tracheal tissue engineering raises fewer potential ethical objections as an experimental therapy than in other clinical niches, given the total lack of alternative treatment options, and the greater intrinsic regenerative potential of pediatric patients compared to adults increases the likelihood of success. As such, regenerative approaches in the trachea have outstripped other organs in terms of high profile clinical compassionate cases both in adults [23], and in children [24, 25].

Organ Scaffolds

Scaffold design, to create a framework on which cells can engraft and differentiate, is a core component of tissue engineering. Scaffold constructs provide structural and functional cues for the regenerative process and should allow for sustained integration when transplanted. An ideal scaffold for airway transplantation would be:

- inert, non-carcinogenic and non-immunogenic;

- biocompatible and capable of promoting cell engraftment, proliferation and differentiation;
- mimic the macroscopic and microscopic architecture of airway region being replaced;
- mechanically stable and resistant to collapse following transplantation;
- able to rapidly recapitulate a vascular system;
- readily available.

More specifically for a neonate or child, the scaffold needs to accommodate significant growth and adapt to developmental requirements. A neonatal trachea is typically 5 cm in length and 5 mm in diameter and grows 5mm a year to full adult length of approx. 15 cm and 20 mm diameter at 16 years of age [26]. The ultimate aim of tissue engineering is to create an organ that grows with the child, thereby avoiding multiple surgeries to transplant upsized replacements. Integration of the scaffold into the surrounding host tissue by remodeling and regeneration (rather than by stenotic scarring) is a final key aim, and is particularly pertinent in pediatric airway surgery where serial dilation of stenosis forms a large part of the surgical workload.

The optimal scaffold material is still a subject of debate and may vary depending on the specific clinical indication [27]. A variety of scaffold materials have been tested for airway transplantation which have been traditionally classified into two types, namely those derived from native biological material [28] and those that are wholly synthetic [29]. Biological scaffolds are created from native tissues and organs, through a decellularisation process whereby resident cells are disrupted and removed using mechanical forces, nucleases, and/or detergents in combination or in repeated cycles. The resulting product is a bare extracellular matrix framework derived from the original tissue or organ that is devoid of the immunogenic cell epitopes normally found in allografts. For the airway, these decellularised scaffolds offer a significant advantage over other materials in that they are closely biomimetic, retaining both macro- and microscopic architecture. Increasing evidence suggests that functional cues are also retained in the form of small molecular proteins within both tracheal and lung matrix promoting differentiation of engrafted cells [24, 30, 31].

Whilst the benefits of decellularised tissues and organs are undisputed, these scaffolds are dependent on donors; as in adult allotransplantation, the supply of neonatal and pediatric-sized tissues and organs are also severely limited [32]. Despite those limitations, decellularised tissue has lead to clinical translation of tissue engineered airways with worse outcome associated to the use of synthetic materials, particularly in pediatrics [33]. Alternative donor sources, such as scaffolds derived from xenografts, may be promising substitutes [34]; however, this strategy comes with its own hurdles, such as the difficulties of ensuring that the xenograft is a suitable anatomical substitute, the potential for zoonotic infections, and the possibility for long-term scaffold rejection due to xenospecific epitopes [35].

Synthetic scaffolds may overcome these problems in the future, but current technology limits scaffold design being matched for macroscopic features only. Furthermore synthetic scaffolds for neonates and children are again limited by the need to accommodate growth leading to the necessity of multiple operations. Early work has begun to address this with the utilization of biodegradable material to create scaffolds that aim to be completely replaced by recipient tissue. Materials developed so far have been shown to be biocompatible and were able to support extracellular matrix formation in engineered cartilage in an animal model [36, 37]. Future scaffold development may ultimately combine the best of both decellularised and synthetic strategies to create composite or hybrid constructs.

Other considerations specific for airway tissue engineering applications are the scaffold requirements for the anatomical region of interest. The trachea, as a tubular hollow organ construct, is often considered a simple structure to recreate; however the remaining parts of the airway are infinitely more challenging. The larynx has the added complexity of requiring fine motor control for vocal cord function; for a fully functional organ replacement, engineering for vocal cord 'moving parts', either by surgical reinnervation, or by using soft robotic 'pacing' implants will be required for voice, swallow and airway protection. Nevertheless, initial attempts to engineer laryngeal muscle and cartilage have shown promising results. Halum *et al* implanted synthetic PCL constructs into surgically created hemi-larynx defects in rats, and saw that implants seeded with muscle progenitor cells

differentiated to express motor end plates demonstrated local neuronal growth and bursts of motor unit potentials with a similar firing timing and intensity to the contralateral native adductor muscle complex, whilst implants seeded with undifferentiated muscle progenitor cells or myotubules did not [38]. Jacobs *et al* performed laryngotracheal reconstructions in rabbits using tissue-engineered cartilage consisting of 1% hyaluronic acid constructs seeded with autologous auricular chondrocytes, crosslinked by UV photopolymerisation following seeding. Thirteen out of 15 animals receiving such grafts survived to 12 weeks, and the tissue-engineered cartilage functioned well with similar epithelialisation, chondrocyte survival, mechanical strength and histology to control cartilage [39]. The full-scale phase I/II RegenVOX clinical trial to implant autologous stem-cell-seeded decellularised hemilarynges are currently in progress in the UK to assess this therapy's efficacy and safety in adult patients with severe acquired laryngotracheal stenosis [27].

Recellularisation of Bioengineered Airway

The functionality of tissues *in vivo* relies heavily upon the presence of a range of differentiated cell types. In the airways, chondrocytes populate cartilage to provide structural support whilst an epithelial layer lines the luminal surface, allowing clearance via the mucociliary escalator [40]. Ideally, tissue-engineered airway transplantation strategies should incorporate methods to restore these functions as quickly as possible following surgery to minimize the risk of airway collapse or infection [31, 41].

In the pediatric setting, the use of autologous cells to restore tissue function is of particular importance in order to avoid the need for lifelong immunosuppression. However, in fetal or neonatal cases, access to autologous tissue to extract these cells poses a challenge. Practically, the amniotic fluid represents a source of prenatal cells with great promise. Our laboratory and others have shown that stem cells can be isolated from human amniotic fluid [42] and that these cells can be reprogrammed to pluripotency, i.e. gain the potential to differentiate into all cell lineages [43, 44]. Cells could be isolated prenatally, reprogrammed and driven towards the cell lineages of interest for airway bioengineering for application in neonatal procedures. The time taken to generate iPSCs and subsequent tissue-specific derivatives is considered a major hurdle to translation of these techniques [45] but this may be less critical in neonatal procedures where the diagnosis is usually made early in gestation,

typically at around 21 weeks, several months before a therapeutic approach is usually needed.

IPSC technology continues to improve at a rapid rate but much work remains to improve the safety profile of cultured cells for transplantation [46]. A second strategy to generate autologous epithelial cells for airway bioengineering from amniotic fluid is to culture cells directly. Fetal epithelia, including the respiratory tract, are in contact with amniotic fluid, which contains a variety of poorly characterized epithelial cell types [47-49]. Further, advances in surgical techniques mean that it is now also possible to obtain tracheal aspirates [50], which may contain a higher abundance of respiratory progenitors and would be particularly valuable in patients with congenital high airways obstruction syndrome (CHAOS) whose obstruction may impede shedding of respiratory cells into the bulk amniotic fluid.

Any strategy reliant upon amniotic fluid as a cell source depends on early prenatal diagnosis but in cases presenting postnatally, autologous tissue is more readily accessible. iPSCs derived from human dermal fibroblasts differentiate to form both proximal and distal airway epithelial cell types under culture conditions which mimic developmental processes [51-55]. Given that iPSCs can also generate chondrocytes [56, 57], these may represent an opportunity to generate from one original cell, multiple autologous cell types for airway transplantation.

Endogenous airway stem cells represent an alternative postnatal cell source. Basal epithelial cells are tissue-specific stem cells that regenerate differentiated goblet cells, which produce airway mucus, and ciliated cells, which create motile force to move mucus across the epithelial surface [58, 59]. Basal epithelial cell cultures can be generated from endobronchial biopsies and recent work in our laboratory suggests that under optimized culture conditions these can multiply sufficiently in culture for bioengineering applications (Butler et al., in press). Currently, bone marrow-derived mesenchymal stromal cells (MSCs) represent the predominant source of autologous cartilage for postnatal tissue engineering as a result of the limited *in vitro* expansion potential of chondrocytes [60]. The use of MSCs in tissue-engineered grafts may also provide beneficial immunomodulatory effects, anti-apoptotic effects and/or stimulate ingrowth of the host cells surrounding the graft [61].

Conclusions/Perspectives

Despite considerable logistical and technical obstacles, airway tissue engineering is rapidly emerging as a serious potential therapy in adults. The potential for application of this technology to the fetal and neonatal population is particularly encouraging, as the ability to surgically correct serious airway malformations at an early stage may be able to considerably improve survival and surgical outcomes in the area of congenital airway disease.

Table 1: Examples of congenital anomalies leading to severe pediatric airway insufficiency.

Anomaly	**Examples of Conditions**
Congenital High Airway Obstruction Syndrome (CHAOS)	- Laryngeal or tracheal agenesis - Laryngeal webs or cysts - Congenital neck masses e.g. cystic hygromas, teratomas
Abnormal aerodigestive tract connections	- Type IV laryngeal clefts - Tracheo-oesophageal fistulae
Extrinsic mediastinal compression	- Vascular ring or sling
Stenosis of the trachea and/or bronchi	- Complete tracheal rings
Poor tracheal quality	- Tracheomalacia (in isolation or downstream of airway stenosis)

Figure 1: Severe congenital airway defects A) Laryngeal agenesis viewed at bronchoscopy. B) Type IV laryngeal cleft viewed at open repair surgery.

Figure 2: Concept schematic for a fetal/neonatal airway tissue engineering strategy

References

[1] Lioy J, Sobol SE. Disorders of the Neonatal Airway. 2015.
[2] Walker P, Cassey J, O'Callaghan S. Management of antenatally detected fetal airway obstruction. International journal of pediatric otorhinolaryngology. 2005;69:805-9.
[3] Sichel J-Y, Ezra Y, Gomori JM, Eliashar R. Prenatal Magnetic Resonance Imaging of a Cervical Lymphangioma for Assessment of the Upper Airway. Annals of Otology, Rhinology & Laryngology. 2002;111:464-5.
[4] Liechty KW. Ex-utero intrapartum therapy. Seminars in Fetal and Neonatal Medicine: Elsevier; 2010. p. 34-9.
[5] Saadai P, Jelin EB, Nijagal A, Schecter SC, Hirose S, MacKenzie TC, et al. Long-term outcomes after fetal therapy for congenital high airway obstructive syndrome. Journal of pediatric surgery. 2012;47:1095-100.
[6] Fokstuen S, Bottani A, Medeiros PF, Antonarakis SE, Stoll C, Schinzel A. Laryngeal atresia type III (glottic web) with 22q11. 2 microdeletion: report of three patients. American journal of medical genetics. 1997;70:130-3.
[7] Kanamori Y, Kitano Y, Hashizume K, Sugiyama M, Tomonaga T, Takayasu H, et al. A case of laryngeal atresia (congenital high airway obstruction syndrome) with chromosome 5p deletion syndrome rescued by ex utero intrapartum treatment. Journal of Pediatric surgery. 2004;39:E25-E8.
[8] Mesens T, Witters I, Van Robaeys J, Peeters H, Fryns J-P. Congenital High Airway Obstruction Syndrome (CHAOS) as part of Fraser syndrome: ultrasound and autopsy findings. Genetic counseling (Geneva, Switzerland). 2012;24:367-71.
[9] Hedrick MH, Ferro MM, Filly RA, Flake AW, Harrison MR, Adzick NS. Congenital high airway obstruction syndrome (CHAOS): a potential for perinatal intervention. Journal of pediatric surgery. 1994;29:271-4.
[10] Leboulanger N, Garabédian E-N. Laryngo-tracheo-oesophageal clefts. Orphanet J Rare Dis. 2011;6:81.
[11] Deprest J, Gratacos E, Nicolaides K. Fetoscopic tracheal occlusion (FETO) for severe congenital diaphragmatic hernia: evolution of a technique and preliminary results. Ultrasound in obstetrics & gynecology. 2004;24:121-6.
[12] Deprest JA, Flake AW, Gratacos E, Ville Y, Hecher K, Nicolaides K, et al. The making of fetal surgery. Prenatal diagnosis. 2010;30:653-67.
[13] Paek BW, Callen PW, Kitterman J, Feldstein VA, Farrell J, Harrison MR, et al. Successful fetal intervention for congenital high airway obstruction syndrome. Fetal diagnosis and therapy. 2002;17:272-6.
[14] Jacobs JP, Quintessenza JA, Andrews T, Burke RP, Spektor Z, Delius RE, et al. Tracheal allograft reconstruction: the total North American and worldwide pediatric experiences. The Annals of thoracic surgery. 1999;68:1043-51.
[15] Elliott M, Hartley BEJ, Wallis C, Roebuck D. Slide tracheoplasty. Current Opinion in Otolaryngology & Head and Neck Surgery. 2008;16:75-82.
[16] Butler CR, Speggiorin S, Rijnberg FM, Roebuck DJ, Muthialu N, Hewitt RJ, et al. Outcomes of slide tracheoplasty in 101 children: a 17-year single-center experience. J Thorac Cardiovasc Surg. 2014;147:1783-9.
[17] Grillo HC. Tracheal replacement: a critical review. The Annals of thoracic surgery. 2002;73:1995-2004.
[18] Jacobs JP, Quintessenza JA, Botero LM, van Gelder HM, Giroud JM, Elliott MJ, et al. The role of airway stents in the management of pediatric tracheal, carinal, and bronchial disease. European journal of cardio-thoracic surgery. 2000;18:505-12.

[19] Atala A, Kasper FK, Mikos AG. Engineering complex tissues. Sci Transl Med. 2012;4:160rv12.
[20] Delaere PR, Van Raemdonck D. The trachea: the first tissue-engineered organ? J Thorac Cardiovasc Surg. 2014;147:1128-32.
[21] Roomans GM. Tissue engineering and the use of stem/progenitor cells for airway epithelium repair. Eur Cell Mater. 2010;19:20571996.
[22] Wykoff TW. A preliminary report on segmental tracheal prosthetic replacement in dogs. The Laryngoscope. 1973;83:1072-7.
[23] Crowley C, Birchall M, Seifalian AM. Trachea transplantation: from laboratory to patient. J Tissue Eng Regen Med. 2015;9:357-67.
[24] Elliott MJ, De Coppi P, Speggiorin S, Roebuck D, Butler CR, Samuel E, et al. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. Lancet. 2012;380:994-1000.
[25] Hamilton NJ, Kanani M, Roebuck DJ, Hewitt RJ, Cetto R, Culme-Seymour EJ, et al. Tissue-Engineered Tracheal Replacement in a Child: A 4-Year Follow-Up Study. Am J Transplant. 2015;15:2750-7.
[26] Griscom N, Wohl M. Dimensions of the growing trachea related to age and gender. American Journal of Roentgenology. 1986;146:233-7.
[27] Fishman JM, Lowdell M, Birchall MA. Stem cell-based organ replacements—Airway and lung tissue engineering. Seminars in pediatric surgery: Elsevier; 2014. p. 119-26.
[28] Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng. 2011;13:27-53.
[29] Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol. 2005;23:47-55.
[30] Ott LM, Weatherly RA, Detamore MS. Overview of tracheal tissue engineering: clinical need drives the laboratory approach. Annals of biomedical engineering. 2011;39:2091-113.
[31] Weiss DJ, Elliott M, Jang Q, Poole B, Birchall M. Tracheal bioengineering: the next steps. Proceeds of an International Society of Cell Therapy Pulmonary Cellular Therapy Signature Series Workshop, Paris, France, April 22, 2014. Cytotherapy. 2014;16:1601-13.
[32] Wright JC, Barlow AD. The current status of neonatal organ donation in the UK. Archives of Disease in Childhood-Fetal and Neonatal Edition. 2015;100:F6-F7.
[33] Fishman JM, De Coppi P, Elliott MJ, Atala A, Birchall MA, Macchiarini P. Airway tissue engineering. Expert opinion on biological therapy. 2011;11:1623-35.
[34] Zani A, Pierro A, Elvassore N, De Coppi P. Tissue engineering: an option for esophageal replacement? Seminars in pediatric surgery: Elsevier; 2009. p. 57-62.
[35] Manji RA, Lee W, Cooper DK. Xenograft bioprosthetic heart valves: Past, present and future. International Journal of Surgery. 2015.
[36] Komura M, Komura H, Kanamori Y, Tanaka Y, Ohatani Y, Ishimaru T, et al. Study of mechanical properties of engineered cartilage in an in vivo culture for design of a biodegradable scaffold. The International journal of artificial organs. 2010;33:775-81.
[37] Teoh G, Crowley C, Birchall M, Seifalian A. Development of resorbable nanocomposite tracheal and bronchial scaffolds for paediatric applications. British Journal of Surgery. 2015;102:e140-e50.

[38] Halum SL, Bijangi-Vishehsaraei K, Zhang H, Sowinski J, Bottino MC. Stem Cell–Derived Tissue–Engineered Constructs for Hemilaryngeal Reconstruction. Annals of Otology, Rhinology & Laryngology. 2014;123:124-34.
[39] Jacobs IN, Redden RA, Goldberg R, Hast M, Salowe R, Mauck RL, et al. Pediatric laryngotracheal reconstruction with tissue‐engineered cartilage in a rabbit model. The Laryngoscope. 2015.
[40] Rackley CR, Stripp BR. Building and maintaining the epithelium of the lung. J Clin Invest. 2012;122:2724-30.
[41] Hamilton N, Bullock AJ, Macneil S, Janes SM, Birchall M. Tissue engineering airway mucosa: a systematic review. Laryngoscope. 2014;124:961-8.
[42] De Coppi P, Bartsch G, Jr., Siddiqui MM, Xu T, Santos CC, Perin L, et al. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol. 2007;25:100-6.
[43] Li C, Zhou J, Shi G, Ma Y, Yang Y, Gu J, et al. Pluripotency can be rapidly and efficiently induced in human amniotic fluid-derived cells. Hum Mol Genet. 2009;18:4340-9.
[44] Liu T, Zou G, Gao Y, Zhao X, Wang H, Huang Q, et al. High efficiency of reprogramming CD34(+) cells derived from human amniotic fluid into induced pluripotent stem cells with Oct4. Stem Cells Dev. 2012;21:2322-32.
[45] Neofytou E, O'Brien CG, Couture LA, Wu JC. Hurdles to clinical translation of human induced pluripotent stem cells. J Clin Invest. 2015;125:2551-7.
[46] Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, et al. Somatic coding mutations in human induced pluripotent stem cells. Nature. 2011;471:63-7.
[47] Virtanen I, von Koskull H, Lehto VP, Vartio T, Aula P. Cultured human amniotic fluid cells characterized with antibodies against intermediate filaments in indirect immunofluorescence microscopy. J Clin Invest. 1981;68:1348-55.
[48] Ochs BA, Franke WW, Moll R, Grund C, Cremer M, Cremer T. Epithelial character and morphologic diversity of cell cultures from human amniotic fluids examined by immunofluorescence microscopy and gel electrophoresis of cytoskeletal proteins. Differentiation. 1983;24:153-73.
[49] Regauer S, Franke WW, Virtanen I. Intermediate filament cytoskeleton of amnion epithelium and cultured amnion epithelial cells: expression of epidermal cytokeratins in cells of a simple epithelium. J Cell Biol. 1985;100:997-1009.
[50] Pereira-Terra P, Deprest JA, Kholdebarin R, Khoshgoo N, DeKoninck P, Boerema-De Munck AA, et al. Unique tracheal fluid microRNA signature predicts response to FETO in patients with congenital diaphragmatic hernia. Ann Surg [Epub ahead of print]. 2015.
[51] Mou H, Zhao R, Sherwood R, Ahfeldt T, Lapey A, Wain J, et al. Generation of multipotent lung and airway progenitors from mouse ESCs and patient-specific cystic fibrosis iPSCs. Cell Stem Cell. 2012;10:385-97.
[52] Wong AP, Chin S, Xia S, Garner J, Bear CE, Rossant J. Efficient generation of functional CFTR-expressing airway epithelial cells from human pluripotent stem cells. Nat Protoc. 2015;10:363-81.
[53] Wong AP, Bear CE, Chin S, Pasceri P, Thompson TO, Huan LJ, et al. Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. Nat Biotechnol. 2012;30:876-82.
[54] Huang SX, Islam MN, O'Neill J, Hu Z, Yang YG, Chen YW, et al. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells. Nat Biotechnol. 2014;32:84-91.

[55] Firth AL, Dargitz CT, Qualls SJ, Menon T, Wright R, Singer O, et al. Generation of multiciliated cells in functional airway epithelia from human induced pluripotent stem cells. Proc Natl Acad Sci U S A. 2014;111:E1723-30.
[56] Inui A, Iwakura T, Reddi AH. Human stem cells and articular cartilage regeneration. Cells. 2012;1:994-1009.
[57] Lee J, Taylor SE, Smeriglio P, Lai J, Maloney WJ, Yang F, et al. Early induction of a prechondrogenic population allows efficient generation of stable chondrocytes from human induced pluripotent stem cells. FASEB J. 2015;29:3399-410.
[58] Hogan BL, Barkauskas CE, Chapman HA, Epstein JA, Jain R, Hsia CC, et al. Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. Cell Stem Cell. 2014;15:123-38.
[59] Teixeira VH, Nadarajan P, Graham TA, Pipinikas CP, Brown JM, Falzon M, et al. Stochastic homeostasis in human airway epithelium is achieved by neutral competition of basal cell progenitors. Elife. 2013;2:e00966.
[60] He X, Fu W, Zheng J. Cell sources for trachea tissue engineering: past, present and future. Regen Med. 2012;7:851-63.
[61] Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med. 2013;45:e54.