

## ■ Review

## Tissue engineering in the treatment of congenital diaphragmatic hernia

### *Konjenital diyafragma herni tedavisinde doku mühendisliği*

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#### ABSTRACT

Congenital diaphragmatic hernia (CDH) is one of the most common major congenital anomalies and is described as the presence of a diaphragmatic defect that leads the herniation of abdominal organs into the chest. Although advanced treatment strategies are introduced over the recent years, they have not really improved the survival rate which stayed at around 70%. Major determinants of poor outcome in CHD are pulmonary hypoplasia and pulmonary hypertension. Various surgical interventions and novel medical therapies are attempted to improve lung function and survival but remains less than desired. Repair of the diaphragmatic defect with prosthetic materials was found to be associated with high rates of complications and recurrences during follow-up. Therefore, regenerative medicine should be considered as an alternative treatment strategy in CDH both by inducing cellular function in the hypoplastic lungs (stem cell therapy) and by developing a functional myogenic patch (tissue engineering). Nearly 30% of infants who have CDH born with severe pulmonary hypoplasia and hypertension which may lead to respiratory failure and prompt mechanical support, since the survival of these newborns relate to the degree of pulmonary hypoplasia, accurate prenatal evaluation of this degree is of paramount importance. The two main diagnostic tools which could be used for this purpose are prenatal ultrasound (US) and magnetic resonance imaging (MRI). Various prenatal treatment strategies have been tried to cure pulmonary hypoplasia and hypertension in CDH. Vitamin A, corticosteroids, antioxidants such as vitamin C, E, N-acetylcystein, phosphodiesterase inhibitors, glucagon-like peptide 1 agonists and tyrosine kinase inhibitors have all been analyzed in animal studies and demonstrated variable results. Since there are very few human studies, further researches should be performed in humans confirming the clinical benefit of these therapies. Due to the advancements in prenatal screening methods, we, now have the ability to detect most of the major genetic disorders in gestation and have chance to provide optimal treatment strategy in the postnatal period. Results of the animal studies regarding the application of regenerative medicine for treatment of children with CDH are encouraging. Hopefully, with the support of further studies focusing especially on safety and ethical issues, the near future will provide us the evidence necessary for their application in our clinical practice.

**Keywords:** congenital, diaphragmatic hernia, tissue engineering,

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## ÖZ

Doğumsal diyafragmatik herni (CDH), en sık karşılaşılan konjenital anomalilerden biri olup abdominal organların göğsüne herniyasyonuna neden olan diyafram kusurunun varlığı olarak tanımlanmaktadır. Son yıllarda ileri tedavi stratejileri getirilmesine rağmen hayatta kalma oran, % 70 civarında kalmış ve geliştirilememiştir. KKH'de kötü sonuçların başlıca belirleyicileri pulmoner hipoplazi ve pulmoner hipertansiyondur. Akciğer fonksiyonlarını ve sağkalımı geliştirmek için çeşitli cerrahi müdahaleler ve yeni tıbbi tedaviler denmektedir, ancak istenilen seviyeden daha az orandadır. Protez materyali ile diyafragma defektinin onarımının, takip sırasında yüksek komplikasyonlar ve rekürrens oranları ile ilişkili olduğu tespit edilmiştir. Bu nedenle, rejeneratif ilaç hem hipoplazi akciğerlerde hücresel fonksiyonu indükleyerek (kök hücre tedavisi) hem de işlevsel bir miyojenik yama (doku mühendisliği) geliştirerek, CDH'de alternatif bir tedavi stratejisi olarak düşünülmektedir. Solunum yetmezliğine sebep olan ciddi pulmoner hipoplazi ve hipertansiyon ile doğan CDH'li yenidoğanların yaklaşık % 30'unun hayatta kalması pulmoner hipoplazi derecesi ile ilişkili olduğundan, bu dereceyi prenatal olarak değerlendirmek büyük önem taşır. Prenatal ultrasonografi (US) ve manyetik rezonans görüntüleme (MRI) iki ana tanı aracıdır. CDH'de pulmoner hipoplazi ve hipertansiyonu iyileştirmek için çeşitli doğum öncesi tedavi stratejileri denendi. Vitamin A, kortikosteroidler, C vitamini, E, N-asetilsistein, fosfodiesteraz inhibitörleri, glukagon benzeri peptid 1 agonistleri ve tirozin kinaz inhibitörleri gibi antioksidanlar hayvan çalışmalarında analiz edildi ve değişken sonuçlar gösterdi. İnsanlar üzerinde çok az çalışma olduğu için, bu terapilerin klinik yararlarını teyit edebilmek için ileri aşama araştırmalar insanlar üzerinde olmalıdır. Doğumdan sonra bebeğe destekleyici tedavisinin yanı sıra solunum yetmezliği durumunda tercihen yüksek frekanslı salınlı ventilasyon ile solunum desteği de uygulanmalıdır. Eksojen kök hücreler, özellikle AFS hücreleri akciğer gelişimini hem çeşitli pulmoner hücre tiplerine entegre ederek, hem de anti-inflamatuvar ve immünomodülatör etkiler yoluyla parakrin modele göre veya doğal progenitör hücreleri aktive ederek geliştirebilir. Ancak, akciğer hasarının altında yatan mekanizmayı ve kök hücrelerin moleküler tepkisini anlamak için, özellikle de insanlarda yapılacak daha ileri araştırmalara ihtiyaç vardır. Doğum öncesi tarama yöntemlerindeki ilerlemeler sayesinde, artık gebelikteki en büyük genetik bozuklukların çoğunu tespit etme ve postnatal dönemde optimal tedavi stratejisi sunma olanağı bulunmaktadır. CDH'li çocukların tedavisinde rejeneratif tıbbın uygulanmasına ilişkin hayvan çalışmalarının sonuçları gelecek vadetmektedir. Yakın gelecekte özellikle güvenlik ve etik konular çerçevesinde yoğunlaşan daha ileri çalışmaların desteğiyle, klinik olarak uygulanması için gerekli kanıtlar bu çalışmalar ile sağlanacaktır.

**Anahtar Kelimeler:** doğumsal, diyafragma hernisi, doku mühendisliği

## Introduction

Although perinatal care and treatment options progress in the last decades, congenital malformations are still causing major morbidity and mortality [1]. The aim of regenerative medicine is to restore damaged organs by repairing and/or replacing involving cells and tissues. The two main approaches in regenerative medicine are; stem cell therapy, which means stimulating regeneration by injecting functional cells into the damaged site, and tissue engineering defined as formation of new tissues by using biocompatible materials [2].

Congenital diaphragmatic hernia (CDH) is one of the most common major congenital anomalies and is described as the presence of a diaphragmatic defect that leads the herniation of abdominal organs into the chest [3]. Although advanced treatment strategies are introduced over the recent years, they have not really improved the survival rate which stayed at around 70% [4, 5]. Major determinants of poor outcome in CHD are pulmonary hypoplasia and pulmonary hypertension. Various surgical interventions and novel medical therapies are attempted to improve lung function and survival but remains less than desired. In addition, repair of the diaphragmatic defect

with prosthetic materials was found to be associated with high rates of complications and recurrences during follow-up [6-8]. Therefore, regenerative medicine should be considered as an alternative treatment strategy in CDH both by inducing cellular function in the hypoplastic lungs [stem cell therapy] and by developing a functional myogenic patch [tissue engineering] [9-12]. We herein review these two popular therapeutic strategies in scope of the latest developments in this topic.

## Pulmonary hypoplasia

Nearly 30% of infants who have CDH born with severe pulmonary hypoplasia and hypertension which may lead to respiratory failure and prompt mechanical support [13]. Since the survival of these newborns relate to the degree of pulmonary hypoplasia, accurate prenatal evaluation of this degree is of paramount importance.

## Diagnosis

The two main diagnostic tools which could be used for this purpose are prenatal ultrasound (US) and magnetic resonance imaging (MRI). Various US parameters have been identified to foresee the extent of pulmonary hypoplasia such as lung-to-head ratio, total fetal lung volume and the position of the liver and

stomach [14, 15]. Commonly used MRI measurements, that have higher sensitivity and specificity for estimating the degree of pulmonary hypoplasia, include total fetal lung volume, predicted lung volumes and liver herniation percentage [16-18].

### Prenatal pharmacologic management

Various prenatal treatment strategies have been tried to cure pulmonary hypoplasia and hypertension in CDH. Vitamin A [19], corticosteroids [20, 21], antioxidants such as vitamin C, E, N-acetylcystein [22], phosphodiesterase inhibitors [23], glucagon-like peptide 1 agonists [24] and tyrosine kinase inhibitors [25] have all been analyzed in animal studies and demonstrated variable results. Since there are very few human studies, further researches should be performed in humans confirming the clinical benefit of these therapies [26, 27].

### Postnatal management

Apart from the supportive therapy of the infant after birth, respiratory assistance preferably with high-frequency oscillatory ventilation, should be implemented in case of respiratory failure [3, 28]. Extra-corporeal membrane oxygenation (ECMO) can be considered as an option when the respiratory failure is severe until proper gas exchange is achieved. However, since there is poor evidence regarding the benefits of ECMO in CDH, it should mainly be used in patients for whom lung hypoplasia would cause inadequate gas exchange or severe circulatory failure [3, 29].

Surgical repair of CDH is not advised before cardio-respiratory functions become stable. The herniated abdominal organs are placed back into the abdomen and the diaphragmatic defect is closed either primarily or with a prosthetic patch via a subcostal or transverse abdominal incision [30]. Minimally invasive approaches via thoracoscopy or laparoscopy are alternative options in infants with less severe symptoms [31, 32].

### Stem cell therapy

Regeneration of the hypoplastic lungs of the infants with CDH by using stem cell therapy has gained popularity in recent years. The lungs continue to develop during postnatal life and have extensive repair and regeneration capability after destruction [33]. Thus, early intervention with cellular based therapies can induce parenchymal development by increasing the number and size of bronchopulmonary segments in patients with hypoplastic lungs and may restore normal function. Lung development is stimulated by exogenous stem cells through two main mechanisms. They may integrate and differentiate, or induce resident stem cells by paracrine actions [34, 35]. Resident stem cell activation seems to be more successful in regenerating the lung tissue compared to using a single exogenous cell source [36]. Recently, De Coppi and Deprest investigated different kind of stem cells including basal cells, multipotent lung stem cells and multipotent cells which expresses tyrosine kinase c-kit,

which all may have the ability to generate different niches in postnatal lung [1]. Among these, the most promising progenitor was found to be multipotent, clonogenic and self-renewing lung stem cells represented by tyrosine kinase c-kit expression as they have the ability to generate bronchiolar, alveolar and pulmonary vessel tissue when injected in mice [35, 37]. Mesenchymal stem cells [MSC] are ideal cells which could be used in pulmonary hypoplasia in CDH due to their immunomodulatory potential [38, 39]. Also, Haaften et al demonstrated in their animal model that MSCs prevent arrested alveolar and vascular growth in part through their paracrine activity [40].

MSCs can be harvested from placenta, fetal bone marrow, cord blood, adipose tissue and amniotic fluid. Amniotic fluid sampling is routinely considered to detect chromosomal and genetic defects in CDH. Thus, using amniotic fluid-based stem (AFS) cells is more ethical than the obtaining samples from other sources. In addition, harvesting stem cells from the above mentioned alternative sources is more challenging and is associated with higher morbidity [39]. Moreover, AFS cells are less immunogenic, feasible for autologous cell-based therapy and available before birth [41]. Their mechanism of action and the potential pathways which activate the hypoplastic lungs have been investigated in many animal models [42, 43]. Pederiva et al showed that lung growth, bronchial motility, and innervation increased with AFS cell exposure in rats [44]. Recently, Di Bernardo et al showed in a nitrofen model that AFS cells induce fetal rodent lung growth by their paracrine action [45].

In summary, exogenous stem cells, especially AFS cells may improve lung development both by integrating and differentiating into various pulmonary cell types and by a paracrine fashion via anti-inflammatory and immunomodulatory effects or by activating the native progenitor cells. However, further studies, especially in humans, are needed to understand the underlying mechanism of lung damage and the molecular response of stem cells.

### Tissue engineering for diaphragmatic repair

#### Rationale of tissue engineering

Although primary closure of the diaphragmatic defects is associated with low recurrence rate, it can be performed in nearly half of the patients. Defect size is considered to be the most significant surgical predictor of morbidity and mortality in CDH and also associated with longer ventilation duration and hospital stay [46, 47]. Repair of large defects could be performed with various surgical techniques including abdominal or thoracic muscle flaps, free fascia lata grafts and different kinds of prosthesis (poly-propylene, poly-tetra-fluoro-ethylene (Teflon), Dacron, and others).

However, most surgeons do not prefer muscle flaps due to the residual defects left in the muscle source, complexity of the procedure and high morbidity [48]. Use of prosthesis for



diaphragmatic repair has been related with higher infection rate, more adhesions, more small bowel obstruction rate and most importantly high recurrence rate, as high as 40-50%, than primary repair [49-51].

The underlying mechanism of hernia recurrence in prosthetic repair is probably the traction and related detachment in the rapidly growing diaphragm of the infant [49]. Therefore, a cell based engineered graft which has the ability of remodeling over time may adapt the rapid growth of the diaphragm and become an ideal alternative for hernia repair.

### Cell Sources

Tissue engineering consists of cells, a supportive 3D scaffold and a bioreactor. While, scaffolds consist of bioactive natural materials which does not have mechanical strength, synthetic materials do not have bioactivity, but are mechanically stronger. These two materials can be engineered and with addition of bioactive properties cellular growth can be achieved [52].

The first diaphragmatic repair by using an engineered construct was reported by Fauza and colleagues in 2001. In that animal study they were able to show that, unlike acellular grafts, engineered cellular diaphragmatic constructs are similar to normal muscle in terms of anatomic and histologic structure [53].

Muscular or tendinous constructs can be used for diaphragmatic repairs. Kunisaki et al compared these two options in their study and showed that tendinous grafts lead to improved structural outcomes when compared to alike muscular grafts [54]. The potential reasons of the preference of tendinous constructs are: (i) the residual diaphragm muscle will continue to grow over time; (ii) normally, most of the diaphragm is tendinous and (iii) function of the muscle construct may decline over time due to lack of innervation.

Fetal cells seem to be the best option that could be used for diaphragmatic engineering. Because; fetal cells (i) multiply more rapidly than postnatal cells, (ii) are more resistant to hypoxia, (iii) survive better through the refrigeration and cryopreservation processes, (iv) have ability to grow in vivo due to their angiogenic properties [55, 56]. There are several kind of fetal cell sources such as placenta, amniotic fluid, Wharton's jelly and umbilical cord blood. Among these amniotic fluid considered to be the safest source. AFS cells can be easily obtained during amniocentesis or birth. Throughout gestation MSCs can be obtained from small amounts of amniotic fluid [57-59]. If the need for patch repair can be foreseen via prenatal US or MRI, cells which are obtained by amniocentesis could be engineered to create an autologous muscle or tendon construct and used to repair the diaphragmatic defect in the postnatal period. Recently, Turner et al provided preclinical efficacy by demonstrating in their animal study that diaphragmatic repair by using autologous tendon engineered with amniotic

mesenchymal stem cells improved outcomes with no local or systemic adverse effects [60].

In summary, according to the results of various animal models so far, diaphragmatic repair with tissue engineered constructs is safe and associated with better outcomes in comparison to acellular bioprosthesis [60-62].

As a conclusion due to the advancements in prenatal screening methods, we, now have the ability to detect most of the major genetic disorders in gestation and have chance to provide optimal treatment strategy in the postnatal period. Results of the animal studies regarding the application of regenerative medicine for treatment of children with CDH are encouraging. Hopefully, with the support of further studies focusing especially on safety and ethical issues, the near future will provide us the evidence necessary for their application in our clinical practice.

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