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Tissue engineering in congenital diaphragmatic hernia

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ABSTRACT

Engineered diaphragmatic repair is emblematic of perinatal regenerative medicine and of the fetal tissue engineering concept. The alternative of a cellularized graft for the repair of a congenital diaphragmatic defect in the neonatal period is both biologically justifiable by the mechanisms behind diaphragmatic hernia recurrence as well as an ideal match for fetal mesenchymal stem cell-based constructs. It has been among the most developed experimental pursuits in neonatal tissue engineering, of which clinical application should be forthcoming.

Diaphragmatic repair is a putative low-hanging fruit of pediatric applications of tissue engineering. In principle, an autologous "living" tissue replacement for a malformed, missing, or, in any way, disrupted diaphragm would constitute a direct, suitable solution against the primary mechanism behind diaphragmatic hernia recurrence, that is, somatic growth. At the same time, the diaphragm is commonly perceived as a relatively simple structure, arguably more amenable to engineered reconstruction even at this early age of regenerative medicine. Finally, engineered diaphragmatic repair epitomizes the therapeutic concept preconized by fetal tissue engineering, in that the construct should ideally be created in parallel to gestation, so as to be promptly available for implantation in the neonatal period. This latter aspect is magnified by the facts that congenital diaphragmatic hernia (CDH) is almost always diagnosed prenatally, and it is among the most common major structural congenital anomalies, with the better controlled studies showing this disease as occurring in 1:2107–1:3163 births.[1–3]

By the same token, diaphragmatic replacement poses a variety of tangible challenges, some of which are rather unique. The following is an outlook of the current status of engineered diaphragmatic reconstruction, along with other pertinent material. Regenerative strategies to enhance or replace pulmonary structures, while evidently also germane to CDH, are beyond the scope of this review.

Background

According to data from the "CDH Registry," the repair of the diaphragm cannot be performed primarily in approximately 51% of the patients with CDH.[4] In these cases, several alternative techniques have been described, for example abdominal or thoracic muscle flaps, free fascia lata grafts, as well as a myriad of prosthesis, such as poly-propylene, poly-tetra-fluoro-ethylene (Teflon), Dacron, acellular human or porcine dermal derivatives, acellular small intestinal submucosa, lyophilized dura-mater, silicone, and others, including combinations of different ones. Notwithstanding a number of advocates, muscle flaps are not favored by most pediatric surgeons (and often families) because of the residual defects left in the abdominal and/or thoracic walls as well as the increased risk for local hemorrhage, particularly if extracorporeal life support is or may be employed. At the majority of referral centers, a prosthesis made of expanded Teflon continues to be favored (Figure 1).

While there has been ample variability in the reported postoperative recurrence rates after CDH repair, the larger series including at least 2 years of median follow-up show that recurrence remains a distinctly prevalent complication, with the vast majority of them occurring in children who could not have had the hernia repaired primarily.[5–7] Repair of CDH with prosthetic patches has also been associated with higher rates of infection, adhesions, small bowel obstruction, and both thoracic and spinal column deformities when compared with primary repair.[5,8–10] The main mechanism behind hernia recurrence is believed to be related to the fast growth rate of the diaphragm in infants, which is thought to lead to traction and eventual detachment of the prosthesis, usually at its posterior-medial aspect.[8] This is in

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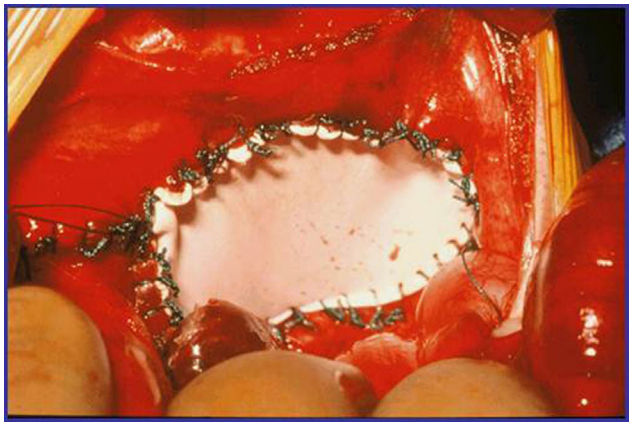

Fig. 1. Repair of a congenital diaphragmatic hernia in a newborn with a prosthetic Teflon patch (white), viewed intra-operatively from the abdomen.

accordance with the lack of impact from the broad changeability in the technical details employed across all the institutions that recognize recurrence as a ubiquitous complication of prosthetic CDH reconstruction as well as with the fact that recurrence is exceedingly rare after prosthetic diaphragmatic repair in adults. In point of fact, the close association between growth and diaphragmatic hernia recurrence forms the basis for the principle behind using a cell-based engineered construct for diaphragmatic repair, in that adequate cell activity is essential to graft remodeling over time, with remodeling being the optimal means to adapt to growth.

Patch disruption is predicted to occur within 18–20 months of age in most patients, especially if little or no muscle was available for prosthetic attachment at the first repair, yet it can happen at any age.[5] The most common clinical manifestations of hernia recurrence are intestinal obstruction and respiratory distress, in that order.[5,11] Asymptomatic recurrences are not uncommon nor are patients with multiple recurrences.[5,9] Diagnosis is usually made through a plain chest radiograph, which should be performed at least once a year post-operatively in every patient who received a prosthesis. However, sometimes a contrast GI radiograph is necessary. Occasionally, depending on the herniated content, ultrasound, CT, or, more rarely, a contrast enema may be of help. Hernia recurrence should be surgically repaired, due to the risk for respiratory distress, incarceration, or strangulation. In select cases of short-term "small" and stable recurrences, re-operation may be postponed, depending on the patient's overall condition.[12]

At the same time that CDH survival rates have improved substantially over the last decade or so, morbidity rates have worsened. This has been a predictable trend, in that, high-risk children who would otherwise have died previously, now survive, often with an assortment of problems in various systems, and need to be followed up by a dedicated, multi-disciplinary team.[4,6,13] In many reported series, including from our institution, there is a direct relationship between the need for prosthetic diaphragmatic repair and the incidence and severity of late complications.[11,14,15]

Diaphragmatic engineering

The first reported attempt at engineering a construct for diaphragmatic replacement involved the use of autologous fetal myoblasts procured from skeletal muscle in a short- to mid-term ovine model of diaphragmatic defects created after birth.[16] That study showed some benefits of an engineered construct when compared with an equivalent acellular bioprosthesis and started a series of other experimental and translational pursuits that have since substantially modified the approach to engineered diaphragmatic repair.

The relevance of experimental developments in diaphragmatic engineering is particularly dependent on the animal model employed. Rodent models bear little to no significance to the clinical scenario in light of the biomechanical, anatomical, and developmental variables at play in CDH repair. Large animal models utilizing growing individuals are paramount to translational impact. Due to their size, growth rate, and the advantages of fetal cells for diaphragmatic engineering (further details below), the ovine model has long been established as an optimal choice.

Engineered tendon vs. muscle

The question as to whether a tendinous or a muscular construct would be better suited for diaphragmatic repair constitutes a germane point in diaphragmatic engineering. Three reasons lend support to the preference for the engineering of a diaphragmatic tendon, rather than a muscle graft, for diaphragmatic repair: (a) the residual rim of native diaphragmatic muscle in CDH appears to develop/grow and function normally in the vast majority of children with this defect; (b) a sizeable portion of the normal diaphragm is comprised of a tendon; and (c) meaningful muscular function driven by substantial nerve ingrowth from the host has yet to be demonstrated in engineered skeletal muscle grafts in large animal models. Over time, a muscle construct tends to become functionally limited due to inadequate innervation, becoming event rated, whereas neural input would not be as critical for the proper function of a tendinous implant. The only study to date comparing these two strategies of diaphragmatic repair has corroborated the notion that the engineering of a tendon should be favored.[17] The experiment, which included analyses for up to a year after implantation soon after birth in a large animal model, showed no advantage of a fetal myoblast-based construct over an equivalent mesenchymal stem cell (MSC)-based one in which the cells were allowed to differentiate to their default fibroblastic phenotype. As importantly, the study also revealed that the donor myoblasts quickly lost their myogenic phenotype *in vivo*, essentially assuming a fibroblastic lineage.

One potential exception to the predilection for a tendinous patch could be the repair of diaphragmatic agenesis. Yet, true diaphragmatic agenesis is rare. It is actually customarily confused with ordinary CDH in which the diaphragmatic defect is particularly large, especially if the residual diaphragm is not properly dissected from the adjacent posterior abdominal wall. Further, diaphragmatic agenesis could conceivably also be repairable with a simpler tendinous graft even if no direct diaphragmatic function would be provided, though this remains to be determined. Still, should large engineered muscle grafts become functionally viable reproducibly, they would likely be preferred for this particular form of reconstruction.

Cell sources

Although different cells with phenotypes compatible with the fabrication of diaphragmatic constructs can certainly be obtained from postnatal sources, fetal cells are justifiably favored for diaphragmatic engineering. On the one hand, the repair of congenital diaphragmatic defects is emblematic of the therapeutic appeal of fetal tissue engineering, which entails the minimally invasive and ethically unobjectionable procurement of fetal cells, which can then be used to engineer a variety of tissue grafts in parallel to the remainder of gestation, so that an infant or a fetus with a prenatally diagnosed birth defect can benefit from having autologous, expanded tissue readily available for surgical