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Full thickness abdominal wall defect in growing rats as a model for congenital diaphragmatic hernia prosthetic repair☆,☆☆,★

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ABSTRACT

Background: Large congenital diaphragmatic hernia may require prosthetic correction. Acellular collagen matrices were introduced to avoid complications owing to the use of synthetic patches. We tested 3 different ACM for reconstruction of an abdominal wall defect in an animal model that mimics the fast growth during infancy.

Methods: Pelvisoft® (CR Bard, Covington, GA) and 2 investigational ACM were used for primary reconstruction of a full thickness abdominal wall defect. 3 months-old rats ($n = 26$) were allowed to survive for 90 days after implantation. Anatomical, tensiometric and histological analyses were performed. Based on good outcomes, we did the same with 1 month-old rats ($n = 54$). Unoperated rats were used for obtaining reference tensiometric values of selected native tissues.

Results: Major wound complications were exclusively observed in 1 month-old rats. All explants in both groups thinned significantly ($p < 0.03$) and had an elastic modulus increasing over time, far above that from native tissues at 90 days of life. Both investigational ACM induced a more vigorous foreign body reaction than Pelvisoft®.

Conclusions: The shift from 3 to 1 month-old rats was associated with wound complications. Pelvisoft® showed a better biocompatibility than the 2 investigational ACM. Passive biomechanical properties of all explants were still not comparable to that of native tissues.

Congenital diaphragmatic hernia (CDH) is a birth defect characterized by the presence of a full thickness muscular defect in the diaphragm. CDH occurs sporadically; the incidence is 1/2500 to 1/5000 of newborns [1]. Despite advances in neonatal care, mortality rates reach 20%–40% [2]. Following neonatal stabilization survivors require a surgical repair that may either be primary closure or graft augmented reconstruction of the muscular defect. Early studies reported long-term recurrence rates after primary closure of up to 22.4% [3]. Patch repair may reduce but not exclude that risk [4–6]. Current patch repair rates are around 50% [7]. The need for a patch use is dependent on the size of the defect, hence predictive of outcome [2]. There are some early reports on a high patch rates in babies with severe CDH that underwent antenatal intervention, though the latter remains an investigational procedure [8,9].

For diaphragmatic reconstruction, both synthetic materials like Gore-tex© (Gore-Tex, W.L. Gore & Associates, Inc., USA). polytetrafluoroethylebe, PTFE) as well as xenogenic acellular collagen matrices (ACM) have been used [10]. A number of complications may relate to the use of patches, such as small bowel obstruction or scoliosis [3,5,11]. Patch-repairs may also fail and this for a variety of reasons. For instance, endogenous collagenases may degrade ACM [12]. Also the lack of physiological adaptation, i.e. the fast growth of the young host, may cause dehiscence [11,13]. Because the currently used materials are still not ideal, research into novel materials as well as applying tissue engineering technology is ongoing. Herein we aimed to evaluate a candidate collagen matrix, which eventually could also be used for cell seeding prior to the operation, by comparing its performance to a clinically used implant [14,15]. Experimental

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★ Evaluation of acellular collagen matrix for muscular defect surgical correction requires biocompatibility analysis and tensiometric comparison with native tissues.

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Table 1
Summary of the different rat groups and implanted materials.

Implant/time point of primary operation	Operated at 90 days of life	Operated at 30 days of life
Pelvisoft	8	18
ACM, strong cross linking	9	18
ACM, mild cross linking	9	18

evaluation prior to clinical use is key in the life cycle of novel implant materials. Skipping this step can lead to dramatic unanticipated effects as recently witnessed in other fields [16,17].

1. Materials and methods

1.1. Overall study design

We used an earlier described rat model for abdominal wall reconstruction, which we have used extensively in the past [18,19], however using nonadult animals. We first did a primary mesh augmented repair of two full thickness defects in 26 male rats at the age of three months, which is just prior to adulthood (average weight: 425 g), and allowed them to survive for 90 days. As no relevant complications were observed, we felt comfortable to lower the bar age-wise for the primary intervention. We then operated 54 male rats at the age of 1 month (average weight: 150 g) (Table 1). At that age it was felt that only one similar sized defect could be induced given the limited size of the experimental animal (Fig. 1). The expected weight gain over the next 90 days would be 350% (Harlan Laboratories, Dublin, VA). Additionally unoperated rats at 30, 60 and 90 days of life were used for obtaining reference tensiometric values of selected native tissues (diaphragm, abdominal wall and latissimus dorsi) ($n = 12$).

1.2. Investigated implants

Pelvisoft® (CR Bard, Covington, GA) is a fenestrated porcine dermal ACM cross-linked with hexamethylenedi-isocyanate (HMDI) that is commercially available and widely used in urogynaecology [20]. This product has 4–5 mm long, narrow slits which are evenly dispersed, run 2 mm apart in parallel lines along the longitudinal axis of the implant, improving ingrowth of host tissue [21]. Under lateral tension, these slits open into diamond shaped pores. Two other purpose designed prototype ACM, manufactured by Matricel GmbH, were made of highly purified porcine type I collagen containing low amounts of other natural fiber forming proteins like elastin and collagen type III. The in vitro and in vivo stability of these materials was tailored using a nontoxic cross-linking method based on the carbodiimide 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC) in the presence of N-hydroxysuccinimide (NHS). The manufacturer was asked to produce an ACM with biomechanical properties as close as possible to that of intact native tissues. Also we asked to make the matrix resistant to degradation for over 90 days. This was achieved by using two different degrees of cross-linking, i.e. prototype 1 was strongly and prototype 2 was mildly cross-linked.

All implants were from the same production lot and purchased via the hospital pharmacy, with the exception of investigational explants. All materials were hydrated for 10 min in sterile saline prior to implantation.

1.3. Surgical procedure

Rats were anesthetized with 2.5% isofluorane mask inhalation (oxygen at 0.5 L/min). We first disinfected the skin with polyvidone iodine 7.5% (Braunol; B. Braun Medical, Machelen, Belgium) and covered the animal with sterile drapes. A vertical midline incision was made and skin flaps raised. Using a grid, a longitudinal full-thickness defect (3.5×2.0 cm) was created in the anterior abdominal wall, consisting of fascia, muscle and peritoneum. The implants were fixed tension-free to the abdominal wall with continuous polypropylene 4/0 (Prolene, Ethicon, Dilbeek, Belgium) at an interrun distance of 5 mm, after initial fixation at the corners. The subcutis and skin were closed with continuous resorbable 3/0 polyglactin sutures (Vicryl, Ethicon, Zaventem, Belgium). The wound was disinfected with polyvidone iodine and covered with aluminum spray [27]. Rats were allowed to recover on a heating pad, and returned to their cages with free access to chew and water. They were clinically followed initially daily, and then later weekly.

1.4. Necropsy

Animals were sacrificed at prefixed time points (i.e. 30, 60 and 90 days post implantation), using intercostal intracardial injection of 1 mL of a solution containing of embutramide 200 mg, mebezonium

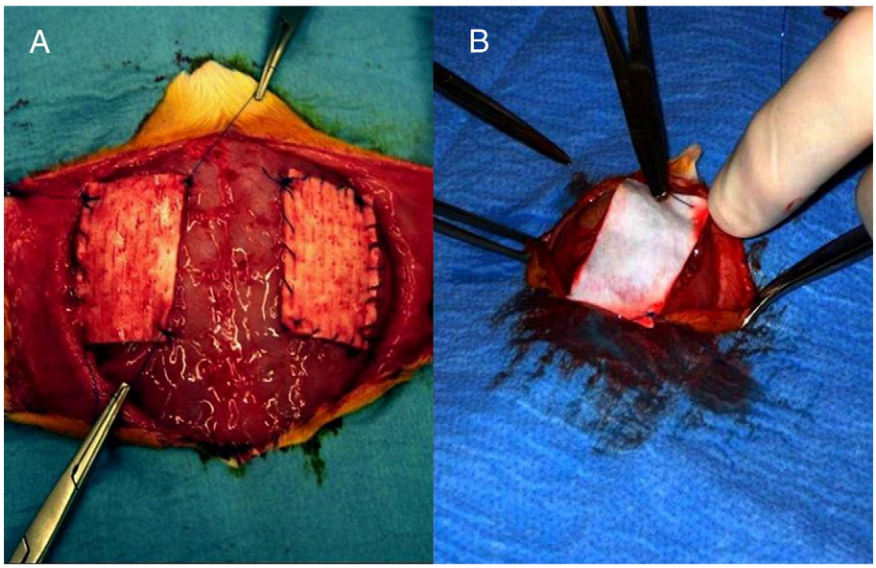

Fig. 1. Rat model of full thickness abdominal wall defect. (A) Double ACM implantation in a 3 months old rat. (B) Single ACM implantation in a 1 month old rat.