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Physiologic musculofascial compliance following reinforcement with electrospun polycaprolactone-ureidopyrimidinone mesh in a rat model

Lucie Hympanova[a,b,c], Marina Gabriela Monteiro Carvalho Mori da Cunha[a,b], Rita Rynkevic[a,b,d], Manuel Zündel[e], Monica Ramos Gallego[f], Jakob Vange[f], Geertje Callewaert[a,b,g], Iva Urbankova[a,b,c], Frank Van der Aa[b,h], Edoardo Mazza[e,i], Jan Deprest[a,b,g,*]

[a] Centre for Surgical Technologies, KU Leuven, Leuven, Belgium
[b] Department of Development and Regeneration, KU Leuven, Leuven, Belgium
[c] Institute for the Care of Mother and Child, Charles University, Prague, Czech Republic
[d] INEGI, Faculdade de Engenharia da Universidade do Porto, Porto, Portugal
[e] Institute of Mechanical Systems, ETH Zurich, Zurich, Switzerland
[f] Coloplast A/S, Global R & D, Biomaterials, Holtedam, Humlebæk, Denmark
[g] Pelvic Floor Unit, University Hospitals KU Leuven, Leuven, Belgium
[h] Department of Urology, University Hospitals Leuven, Leuven, Belgium
[i] EMPA, Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland

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ABSTRACT

Purpose: Electrospun meshes may be considered as substitutes to textile polypropylene implants. We compared the host response and biomechanical properties of the rat abdominal wall following reinforcement with either polycaprolactone (PCL) modified with ureidopyrimidinone-motifs (UPy) or polypropylene mesh.

Methods: First we measured the response to cyclic uniaxial load within the physiological range both dry (room temperature) and wet (body temperature). 36 rats underwent primary repair of a full-thickness abdominal wall defect with a polypropylene suture (native tissue repair), or reinforced with either UPy-PCL or ultra-light weight polypropylene mesh (n = 12/group). Sacrifice was at 7 and 42 days. Outcomes were compliance of explants, mesh dimensions, graft related complications and semi-quantitative assessment of inflammatory cell (sub) types, neovascularization and remodeling.

Results: Dry UPy-PCL implants are less stiff than polypropylene, both are more compliant in wet conditions. Polypropylene loses stiffness on cyclic loading. Both implant types were well incorporated without clinically obvious degradation or herniation. Exposure rates were similar (n = 2/12) as well as mesh contraction. There was no reinforcement at low loads, while, at higher tension, polypropylene explants were much stiffer than UPy-PCL. The latter was initially weaker yet by 42 days it had a compliance similar to native abdominal wall. There were eventually more foreign body giant cells around UPy-PCL fibers yet the amount of M1 subtype macrophages was higher than in polypropylene explants. There were less neovascularization and collagen deposition.

Conclusion: Abdominal wall reconstruction with electrospun UPy-PCL mesh does not compromise physiologic tissue biomechanical properties, yet provokes a vivid inflammatory reaction.

1. Introduction

Symptomatic pelvic organ prolapse decreases the quality of life of 11.4% women over 45 years (Slieker-ten Hove et al., 2009). Native tissue repair techniques have been considered suboptimal because of quoted reoperation rates of 30% (Olsen et al., 1997). In order to improve durability of repairs, synthetic meshes were introduced identical to those used in hernia repair. Though anatomical outcomes of mesh-augmented vaginal prolapse repairs are better, its introduction has coincided with the occurrence of more local complications and

Abbreviations: CD, cluster of differentiation; FBGC, foreign body giant cells; H & E, hematoxylin and eosin; i.e., id est; M1, macrophage type 1; M2, macrophage type 2; PCR, polymerase chain reaction; PCL, polycaprolactone; PP, polypropylene; UPy, ureidopyrimidinone; UPy-PCL, ureidopyrimidinone-polycaprolactone

* Corresponding author at: Centre for Surgical Technologies, KU Leuven, Leuven, Belgium.
E-mail addresses: lucie.hympanova@upmd.eu (L. Hympanova), biamori@gmail.com (M.G.M.C. Mori da Cunha), r.rynkevic@gmail.com (R. Rynkevic), zuendel@imes.mavt.ethz.ch (M. Zündel), dkmrg@coloplast.com (M.R. Gallego), dkjv@coloplast.com (J. Vange), gjecallewaert@gmail.com (G. Callewaert), iva.urbankova@upmd.eu (I. Urbankova), frank.vanderaa@kuleuven.be (F. Van der Aa), mazza@imes.mavt.ethz.ch (E. Mazza), Jan.Deprest@uzleuven.be (J. Deprest).

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reinterventions (Maher et al., 2016). Consequently, current techniques in pelvic reconstruction surgery are being re-examined in an effort to find a mechanism to help improve long-term cure rates without increasing the risk of complications (SCENIHR, 2015). Mesh placement is still recommended in specific cases. Nevertheless, the risk of graft related complications and their consequences should be assessed and compared with expected benefits.

Polypropylene (PP) is the most widely used polymer for weaving and knitting of durable implants. At present it is the main recommended polymer for manufacturing vaginal implants (SCENIHR, 2015). Unfortunately, post-implantation complications such as pain, exposure or infection occur in up to 10% of operated women (Abed et al., 2011). These adverse effects are tied to a number of material aspects, such as preimplantation biomechanical mesh properties, its durable nature and subsequent host response, causing a chronic foreign body reaction and inappropriate compliance (Klinge et al., 1999). In an effort to reduce complications, lightweight macroporous mesh, which experimentally induce a more optimal host response, is currently being used (Kelly et al., 2016). This has not prevented complications from occurring. Given the severity of graft related complications, and difficulty to treat them, the search for better materials has been advocated (SCENIHR, 2015; Mangera et al., 2012). An alternative to durable materials is to use non-textile scaffolds with a physiologic matrix structure as used in tissue engineering. Ideally, these would be synthetic, resorbable and should have the potential of being made bioactive. Synthetic materials are easier to obtain in larger quantities, cheaper and do not carry the risk of disease transmission compared to xeno- or allografts. Resorbable implants have the advantage of being eventually completely degraded, hence not maintaining a chronic inflammatory foreign body reaction, and all its related complications. Conversely, following degradation, the material is ideally replaced by neo-tissue that has functional properties as closely as possible to the original tissues without associated local complications (Roman et al., 2016). One strategy is to create implants by a process called electrospinning. This method uses electrostatic forces to draw charge threads of polymer solution to a collector (Morton, 1902). These implants have a large surface, fiber and pore size of the extracellular matrix, which should facilitate cell adherence and their ingrowth (Cipitria et al., 2011). One innovative absorbable, supramolecular material that meets these criteria may be obtained from polycaprolactone modified with ureidopyrimidinone motifs (UPy-PCL). This polymer has received considerable attention as a suitable scaffold due to its elasticity, flexibility, and customizable degradation properties (Felfel et al., 2016). UPy-functionalized polymers have the ability to bind cell-adhesive peptides as one of tissue regeneration approaches (Mollet et al., 2014). We have embarked into manufacturing such prototype implants. The primary objective of this study was to investigate the biomechanical properties of a primary repaired full-thickness abdominal wall defect reinforced with a novel hybrid UPy-PCL mesh types using a well-established rat model. Secondary objectives were to document biocompatibility and the host response to UPy-PCL as compared to polypropylene. We also aimed to compare these post-implantation mesh characteristics to those of the implant prior to implantation.

2. Material and methods

2.1. Implants

Implants were fabricated either from polypropylene (PP, n = 12) or ureidopyrimidinone-polycaprolactone (UPy-PCL, n = 12), the UPy-PCL polymer was obtained from SupraPolix, Eindhoven, The Netherlands. The polypropylene implant used was a commercially available knitted Restorelle® (Coloplast, Humlebaek, Denmark), (Fig. 1B). Investigated UPy-PCL scaffold (Coloplast) was made by electro-spinning (Fig. 1A, see paragraph 2.2. for more details). Constituting fibers of UPy-PCL mesh are organized as a network-like structure with random in-plane orientation and fiber interspaces in the order of a few microns, whereas PP is composed of knitted filaments with a macroporous structure of 2.1 mm diameter and 230 μm interstitial pores (Ulrich et al., 2016). Investigated implants were from the same production lot, sterilized by electron beam irradiation (25 kGy) and packaged with a molecular sieve desiccant in an atmosphere of nitrogen. Main characteristics of polypropylene and UPy-PCL mesh are summarized in Table 1.

2.2. Electrospinning

Our electrospinning method employed a coaxial nozzle with the inner needle (17G) used to pump the polymer and outer (19G) for pure solvent. The purpose is to prevent clogging on the tip. The mesh was spun to an area density of 35–50 g/m^2. After the spinning process, meshes were stored in a vacuum oven overnight at 45 °C/ < 1 mBar. Fiber size distribution was measured with scanning electron microscope. The mesh was cut to size and weighed to determine the exact area density. Fabrication parameters are in details depicted in Table 2.

2.3. Mechanical characterization of implant materials under dry and wet conditions (ex vivo)

Implants with a 4:1 aspect ratio were subjected to uniaxial tensile load in dry and wet conditions. Fig. 1 indicates the loading direction applied in the tests. The implant materials were characterized according to a standardized protocol that we previously used for textile meshes (Supplement 1) (Maurer et al. 2014).

2.4. Animals

Thirty six adult 12 weeks-old female Sprague Dawley rats (250–300 g) were randomly divided in three groups (n = 12/per group) based on power calculation: (1) native tissue primary repair, (2) reinforcement repair with UPy-PCL and (3) polypropylene. Animals were housed per four in cages with unrestricted access to the food, water and chew. They were treated in accordance with current national guidelines on animal welfare. The experimental protocol was approved by the Ethics Commission on Animal Experimentation of the Faculty of Medicine, KU Leuven.

2.5. Surgical procedure and study design

The anesthesia and surgical protocol were reported in detail in previous study (Supplement 2) (Ozog et al., 2009). Postoperatively, animals were allowed to move, eat and drink *ad libitum.* They were clinically examined daily the first week thereafter twice a week and all complications were noted. Harvesting was at 7 and 42 days (n = 6 each group and time point).

2.6. Obduction

Under isoflurane anesthesia, rats were euthanized by intravenous administration of 0.2 mL of a mixture of embutramide 200 mg, mebezonium 50 mg and tetracaine hydrochloride 5 mg (T61; MSD Animal health BVBA; Brussels; Belgium) and underwent obduction. During gross anatomical examination we noted any herniation, fluid collections, infection or implant exposures (loss of epithelial integrity). Further the length and the width within the borders marked by the polypropylene sutures were measured and the contraction was calculated (explant/implant area* 100%). The former surgical area was resected ‘en bloc’ including 1 cm of the neighboring tissue. The presence of adhesions was scored using a scale of 0–3 (Toosie et al., 2000). The explant was cut into 10 mm wide strips, perpendicular to the long axis of the animal. The middle strip (10 × 30 mm) was kept in 0.9%NaCl saline solution at room temperature until biomechanically tested (< 2 h after sacrifice). Another specimen (10 × 10 mm) was fixed in