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Tissue response to collagen containing polypropylene meshes in an ovine vaginal repair model

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ABSTRACT

Pelvic Organ Prolapse (POP) is the herniation of pelvic organs into the vagina. Despite broad acceptance of mesh use in POP surgical repair, the complication rate is unacceptable. We hypothesized that collagen-containing polypropylene (PP) mesh types could modulate mesh-tissue integration and reduce long-term inflammation, thereby reducing mesh-associated complications. This study compared the long-term tissue response to an unmodified PP mesh and two collagen containing meshes in an ovine model which has similar pelvic anatomy and vaginal size to human. Three commercially available macroporous PP meshes, uncoated PP mesh (Avaulta Solo) (PP), the same textile PP mesh layered with a sheet of cross-linked porcine acellular matrix (Avaulta Plus) (PP-ACM) and a different yet also macroporous PP (Sofradim) mesh coated with solubilized atelocollagen (Ugytex) (PP-sCOL) were implanted in the ovine vagina and tissue explanted after 60 and 180 days. The macrophage phenotype and response to implanted meshes, and vascularity were quantified by immunostaining and morphometry. We quantified changes in extracellular matrix composition biochemically and collagen organisation and percentage area around the interface of the mesh implants by Sirius Red birefringence and morphometry. PP-ACM induced a more sustained inflammatory response, indicated by similar $CD45^+$ leukocytes but reduced $CD163^+$ M2 macrophages at 60 days ($P < 0.05$). PP-sCOL increased Von Willebrand Factor (vWF)-immunoreactive vessel profiles after 60 days. At the micromolecular level, collagen birefringence quantification revealed significantly fewer mature collagen fibrils (red, thick fibrils) at the mesh-tissue interface than control tissue for all mesh types ($P < 0.001$) but still significantly greater than the proportion of immature (green thin fibrils) at 60 days ($P < 0.05$). The proportion of mature collagen fibrils increased with time around the mesh filaments, particularly those containing collagen. The total collagen percent area at the mesh interface was greatest around the PP-ACM mesh at 60 days ($P < 0.05$). By 180 days the total mature and immature collagen fibres at the interface of the mesh filaments resembled that of native tissue. In particular, these results suggest that both meshes containing collagen evoke different types of tissue responses at different times during the healing response yet both ultimately lead to physiological tissue formation approaching that of normal tissue.

Statement of Significance

Pelvic organ prolapse (POP) is the descent of the pelvic organs to the vagina. POP affects more than 25% of all women and the lifetime risk of undergoing POP surgery is 19%. Although synthetic polypropylene (PP) meshes have improved the outcome of the surgical treatment for POP, there was an unacceptable rate of adverse events including mesh exposure and contracture. It is hypothesized that coating the PP meshes with collagen would provide a protective effect by preventing severe mesh adhesions to the wound, resulting in a better controlled initial inflammatory response, and diminished risk of exposure. In this study we assessed the effect of two collagen-containing PP meshes on the long-term vaginal tissue response using new techniques to quantify these tissue responses.

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1. Introduction

Pelvic organ prolapse (POP) is defined as the downward descent or herniation of pelvic organs into the vagina [1]. Common symptoms of POP are pressure or bulge in the pelvic area, urinary incontinence and sexual dysfunction [2]. POP affects more than 25% of all women and it is estimated that the lifetime risk of undergoing surgery for POP is 19% [3,4]. Reconstructive surgery is the main treatment for POP and augmentation using synthetic polypropylene (PP) biomaterial meshes has improved the outcome of the surgical treatment for POP [5], although this is considered controversial. Lightweight, macro porous monofilament PP meshes were the most common non-degradable synthetic material used in transvaginal POP surgery as these showed lower foreign body tissue responses and reduced material stiffness [6]. While these mesh implants resulted in greater cure rates than native tissue surgery, there was an unacceptable rate of adverse events [7–9] including mesh exposure and contracture. The Food and Drug Administration (FDA), and later in Europe the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) issued warnings on the transvaginal placement of synthetic implants [10], resulting in companies withdrawing some mesh products from the market. New guidelines from the FDA and the International Urogynecological Association (IUGA) recommend testing new meshes in preclinical models to improve outcomes for any new mesh products being developed before translation to the clinic [11].

In addition, although mesh implantation induces a pro-inflammatory response, which is followed by fibrosis with a concomitant increase in the strength of surgical repair, this fibrotic response can cause pain and discomfort. In order to minimize fibrosis and pain, many investigations have examined the modification of mesh designs including, pore size (large versus small pore size), weight (lightweight or heavyweight) and collagen coating [12]. One approach to improve the biocompatibility of mesh was to coat the mesh with extracellular matrix (ECM) proteins. ECM coated meshes are degradable and can allow tissue remodelling and formation of appropriate soft tissue rather than fibrosis by inducing angiogenesis and promoting the accumulation of progenitor cells at the site of implantation [13]. It has been reported that coating the PP mesh with acellular porcine collagen resulted in less erosion and inflammation. It was hypothesized that the collagen would provide a protective effect by preventing severe mesh adhesions to the wound thereby decreasing direct mesh-tissue contact with the polymer, resulting in a better controlled initial inflammatory response, and diminished risk of exposure [14,15].

The host response to surgically implanted biomaterials is a critical determinant of its success or failure [16]. The early tissue response to a synthetic mesh is acute inflammation, characterised by an influx of neutrophils followed by pro-inflammatory (referred to as M1) macrophages. After the acute response following departure of the neutrophils, chronic inflammation develops that can vary in time and extent, particularly the rate at which M1 macrophages differentiate into an anti-inflammatory M2 macrophage phenotype, which will ultimately influence the wound healing response and the quality of new tissue formation [16].

An appropriate animal model is necessary to test new meshes to fulfil the IUGA requirement for preclinical studies. Although rodents are widely used because of their low cost and ease to work with, the small size of their vagina has prompted the recent shift to larger animal models [14,17]. The ovine model is attractive because the pelvis has similar anatomy and size as the human pelvis and has similar pelvic support structures as in women [18,19]. Ewes also have prolonged labours with relatively large foetuses and may also spontaneously develop postpartum POP [20,21]. The limitation of the ovine model is their quadrupedal posture, which reduces intra-abdominal pressure experienced by the pelvis. However, it has been suggested that their ruminant physiology and tendency to ruminate facing uphill increases the pressure on the pelvic structures including the vaginal wall [19]. Moreover, their large size and hence vaginal capacity allows the examination of mesh with dimensions more representative of those implanted into women. In addition, several meshes can be implanted into the same animal [14].

The objective of this study was to evaluate whether the format of collagen coating on the PP mesh affects the chronic inflammatory host response and healing process in the long term to the implanted mesh. Sixty days was chosen to examine the tissue response to the effect of differential degradation of the collagen coating between the two modified PP meshes that used two different collagen formats and 180 days was chosen as a longer term steady state end point to examine if any differences were still apparent. We assessed the effect of two collagen-containing PP meshes on the long-term vaginal tissue response, evaluating the chronic inflammatory macrophage response, vascular response, the total collagen and glycosaminoglycans (GAG) content, and the organisation and density of collagen at the mesh-tissue interface in explanted vaginal tissues. We used standard techniques to measure these parameters and a new technique to quantify the collagen response at the mesh tissue interface by quantifying red (mature) and green (immature) collagen fibril deposition by assessing birefringence in 100 and 200 μm increments in an ovine model.

2. Methods

2.1. Mesh

Three commercially available so called Amid-I macroporous PP meshes were used where the PP component was the same in all; 1. Avaulta Solo (PP) (Bard Medical, Convington, GA, USA, 58 g/m^2), 2. Avaulta Plus (PP-ACM) (composed of the same fabric as PP with a hydrophilic slowly degrading cross-linked porcine acellular matrix (ACM) sheet comprising collagen, 0.5 mm thick and 1.8 mm pores, 100 g/m^2) and 3. PP-sCOL (PP-sCOL) (Sofradim International, Trevoux, France, 38 g/m^2), comprising PP monofilaments coated with reconstituted enzyme-purified solubilized atelocollagen, polyethylene glycol and glycerol (Supplementary Fig. 1)

2.2. Animal implantation

Animal surgery and mesh implantation was undertaken at the KU Leuven in accordance with protocols approved by the local Animal Ethics Committee as previously reported [17]. Thirty-six multiparous Texel ewes underwent surgical implantation of mesh (n = 12/mesh type). Briefly, following general anaesthesia, antibiotic prophylaxis, and hydro-dissection of the rectovaginal space, a single vaginal incision was made and a 35 × 35 mm flat mesh was inserted and fixed to underlying tissue of the posterior wall with multiple interrupted 4/0 polypropylene sutures (Prolene; Ethicon, Zaventem, Belgium). We chose 35 × 35 mm mesh size to avoid graft related complications associated with implanting larger mesh sizes, including exposure and contraction [9]. The vaginal incision was closed with continuous 2/0 polyglactin 910 (Vicryl; Ethicon). Tissues were explanted after 60 and 180 days and processed as previously described to assess the effect of collagen after partial/complete resorption of collagen on the collagen-containing meshes and the chronic rather than acute inflammatory response [17]. Control tissues were collected from non-operated regions adjacent to the implants and at the same distance from the introitus as there are regional differences cranio-caudally and between