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# Temporal changes in the biomechanical properties of endometrial mesenchymal stem cell seeded scaffolds in a rat model

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ABSTRACT

Use of synthetic clinical meshes in pelvic organ prolapse (POP) repair can lead to poor mechanical compliance in vivo, as a result of a foreign body reaction leading to excessive scar tissue formation. Seeding mesh with mesenchymal stem cells (MSCs) prior to implantation may reduce the foreign body reaction and lead to improved biomechanical properties of the mesh–tissue complex. This study investigates the influence of seeding human endometrial mesenchymal stem cells (eMSCs) on novel gelatin-coated polyamide scaffolds, to identify differences in scaffold/tissue biomechanical properties and new tissue growth following up to 90 days' implantation, in a subcutaneous rat model of wound repair. Scaffolds were subcutaneously implanted, either with or without eMSCs, in immunocompromised rats and following 7, 30, 60 and 90 days were removed and assessed for their biomechanical properties using uniaxial tensile testing. Following 7, 30 and 90 days' implantation scaffolds were assessed for tissue ingrowth and organization using histological staining and scanning electron microscopy. The eMSCs were associated with altered collagen growth and organization around the mesh filaments of the scaffold, affecting the physiologically relevant tensile properties of the scaffold–tissue complex, in the toe region of the load–elongation curve. Scaffolds seeded with eMSCs were significantly less stiff on initial stretching than scaffolds implanted without eMSCs. Collagen growth and organization were enhanced in the long-term in eMSC-seeded scaffolds, with improved fascicle formation and crimp configuration. Results suggest that neo-tissue formation and remodelling may be enhanced through seeding scaffolds with eMSCs.


## 1. Introduction

Synthetic knitted meshes have been used to repair abdominal hernias since 1958 [1]. These meshes provide structural reinforcement to the weakened tissue and provoke a foreign body reaction, which leads to the formation of scar tissue, and additional reinforcement. However, an excessive fibrotic response of unorganized tissue around the implanted mesh can cause pain, leading to the need for surgical revision and occasionally removal of the mesh. More recently, lightweight meshes with large pores have been used. These meshes have a reduced total surface area, and so stimulate lesser foreign body reaction, with reduced scar tissue formation [2]. The large pore size also leads to a decrease in bridging fibrosis: fibrous tissue formation between the fibres of the mesh [3]. As a consequence, the meshes are more flexible and elastic in vivo. However, despite these improvements synthetic meshes continue to have complications such as recurrence of herniation, wound infection and adhesion formation between mesh and tissue [4].

Similar knitted meshes are used to provide support for weakened vaginal fascia and musculature, as seen in pelvic organ prolapse (POP). Use of synthetic mesh has significantly reduced recurrence rates of anterior vaginal wall POP, compared with native tissue surgical repair operations [5]. Problems associated with the use of POP mesh include mesh exposure and pain, which are not rare, and erosion into nearby viscera [6,7]. These complications are more common for vaginally placed mesh, compared to abdominal hernia mesh, because of the larger area covered by the mesh in the vagina, the thinner vaginal wall, the lack of sterility of the vagina, and the mobility of this tissue compared with the

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abdominal wall. Ideally, these meshes should primarily support the damaged tissue, but also promote new tissue ingrowth and neovascularization, with limited fibrous capsule and scar tissue formation. It is desirable that this new connective tissue comprises collagen in sufficient quantities and organization to ensure the optimum biomechanical properties of strength and elasticity [8]. It has been reported that poor mechanical compliance between implanted mesh and tissue may lead to stress shielding [9] and potentially contribute to complications such as erosion into surrounding tissue [8,10] and dyspareunia [11].

One way of reducing the foreign body reaction and excessive fibrosis might be to seed meshes with cells prior to implantation, particularly utilizing cells with anti-inflammatory and immunomodulatory properties, e.g. mesenchymal stem/stromal cells. Additional benefits of cell seeding meshes prior to implantation include increased total collagen content [12,13], enhanced innervation [13], improved vascularization [14,15], reduced inflammatory reaction [14] and decreased mechanical stiffness [14], all likely due to the paracrine actions of mesenchymal stem cells. For reconstruction of the vagina due to trauma or a congenital defect using a tissue engineering approach, epithelial cells [16,17] and smooth muscle cells [13,16] have been explored. Similarly, repairing damaged vaginal tissue due to POP, vaginal fibroblasts [18], muscle-derived stem cells [19], and mesenchymal stem cells (MSCs) [14] have been used. MSCs have a particular advantage over differentiated cells, largely due to their ability to control inflammation and encourage repair caused by injury, through complex paracrine immunomodulatory pathways involving both the innate and adaptive immune responses [20]. We have identified, characterized [21,22] and expanded [23] a unique source of human mesenchymal stem cells from endometrium (eMSCs) that are highly clonogenic and proliferative, self-renew and differentiate into multiple mesodermal lineages. These characteristics augment successful neo-tissue regeneration through paracrine actions [14,24].

We have previously fabricated a range of novel meshes with improved mechanical properties, compared to some commonly used clinical polypropylene meshes [25] and assessed them using a rat abdominal hernia model [26]. We found that our gelatin-coated polyamide (PA + G) scaffold evoked a milder early inflammatory response, with better tissue integration and new collagen deposition, and greater sustained neovascularization, compared to Polyform™ clinical mesh (Boston Scientific, USA) [26]. In a further study, when seeded with eMSCs and subcutaneously implanted into immunocompromised rats for up to 90 days, these novel constructs promoted significantly earlier neovascularization and elicited a lesser foreign body reaction with fewer macrophages and leukocytes surrounding the mesh filaments in the long term, compared to unseeded scaffolds. Cell-seeded scaffolds were also less stiff than those without cells, after 7 and 90 days' implantation [14].

The purpose of this study was to undertake more detailed analyses of how seeding human eMSCs on PA + G scaffolds impacts on key biomechanical properties following implantation in immunocompromised rats for up to 90 days. Measured biomechanical properties include toe and linear region stiffness, inflection strain and maximum load. These biomechanical properties were related to the extent and organization of new tissue ingrowth, noting differences in collagen deposition and organization between cell-seeded and non-cell-seeded scaffolds.

## 2. Materials and methods

### 2.1. PA+G scaffold fabrication

Polyamide meshes (Fig. 1A) were warp knitted to a mass per unit area, thickness and a large pore size of 85 ± 3 g $m^{-2}$, 0.41 ± 0.01 mm and 1.31 ± 0.11 mm, respectively [25]. The knitted polyamide mesh had a calculated porosity of 82%. Meshes were gelatin coated by dip-coating in 12% porcine gelatin in water and cross-linked with 0.025% (w/v) glutaraldehyde in phosphate-buffered saline (PBS), as previously described [14]. The applied gelatin spanned the pores of the knitted scaffold (Fig. 1B), creating a platform for cell attachment and growth. Gelatin-coated polyamide scaffolds were of mass per unit area and thickness 145 ± 32 g $m^{-2}$ and 0.45 ± 0.03 mm, respectively [14]. These scaffolds were cut into 10 mm × 25 mm pieces, with the longitudinal axis cut in the knit machine direction and gamma sterilized at 25 kGy.

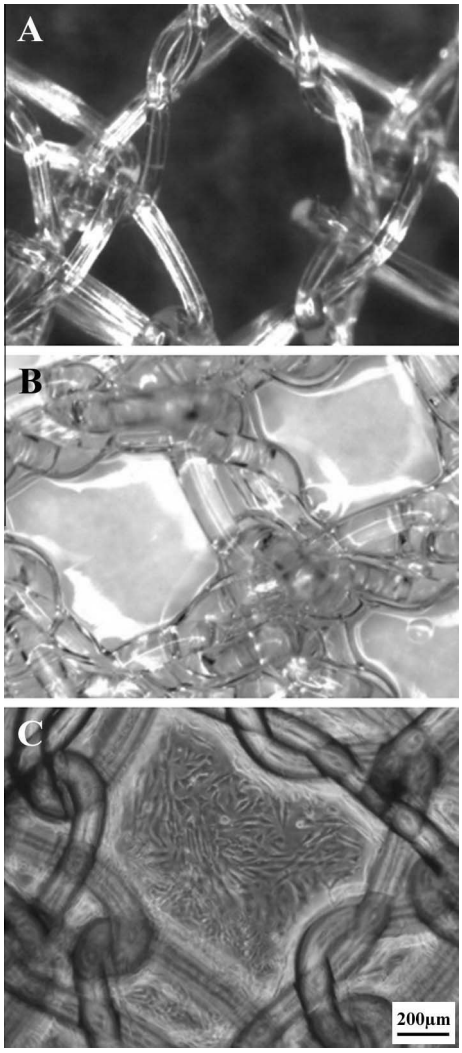


**Fig. 1.** Optical micrographs of (A) PA mesh, (B) PA + G scaffold and (C) PA + G scaffold seeded with eMSCs. Scale bar is 200 µm.

### 2.2. Cell seeding of scaffolds

This project was approved by the Monash Health Human Research Ethics Committee B (10103B). Human endometrial tissue was obtained from patients undergoing hysterectomy who had previously given written informed consent and used as a source of eMSCs. Cells were isolated as previously described [14,27]. Briefly, eMSCs from two patients were pooled and cultured in DMEM/F12 medium (Life Technologies, Mulgrave, Australia) containing 10% fetal calf serum, 1% antibiotic–antimycotic (Life Technologies; 100 U $ml^{-1}$ penicillin, 100 µg $ml^{-1}$ streptomycin, 0.25 µg $ml^{-1}$ fungizone®) and 2 mM glutamine (culture medium)

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