Acta Biomaterialia 10 (2014) 5012–5020

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Induction of endometrial mesenchymal stem cells into tissue-forming cells suitable for fascial repair

Kai Su [a], Sharon L. Edwards [a], Ker S. Tan [b], Jacinta F. White [a], Shital Kandel [a], John A.M. Ramshaw [a], Caroline E. Gargett [b,1], Jerome A. Werkmeister [a,*,1]

[a] CSIRO Manufacturing Flagship, Bayview Avenue, Clayton, Victoria 3169, Australia
[b] MIMR-PHI Institute, 27–31 Wright Street, Clayton, Victoria 3168, Australia

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 15 August 2014
Accepted 27 August 2014
Available online 4 September 2014

Keywords:
Pelvic organ prolapse
Tissue engineering
Endometrial mesenchymal stem cells
Polyamide
Gelatin

ABSTRACT

Pelvic organ prolapse is a major hidden burden affecting almost one in four women. It is treated by reconstructive surgery, often augmented with synthetic mesh. To overcome the growing concerns of using current synthetic meshes coupled with the high risk of reoperation, a tissue engineering strategy has been developed, adopting a novel source of mesenchymal stem cells. These cells are derived from the highly regenerative endometrial lining of the uterus (eMSCs) and will be delivered in vivo using a new gelatin-coated polyamide scaffold. In this study, gelatin properties were optimized by altering the gelatin concentration and extent of crosslinking to produce the desired gelation and degradation rate in culture. Following cell seeding of uncoated polyamide (PA) and gelatin-coated meshes (PA + G), the growth rate of eMSCs on the PA + G scaffolds was more than that on the PA alone, without compromising cell shape. eMSCs cultured on the PA + G scaffold retained their phenotype, as demonstrated by W5C5/SUSD2 (eMSC-specific marker) immunocytochemistry. Additionally, eMSCs were induced to differentiate into smooth muscle cells (SMC), as shown by immunofluorescence for smooth muscle protein 22 and smooth muscle myosin heavy chain. eMSCs also differentiated into fibroblast-like cells when treated with connective tissue growth factor with enhanced detection of Tenascin-C and collagen type I as well as new tissue formation, as seen by Masson's trichrome. In summary, it was demonstrated that the PA + G scaffold is an appropriate platform for eMSC delivery, proliferation and differentiation into SMC and fibroblasts, with good biocompatibility and the capacity to regenerate neo-tissue.

1. Introduction

Pelvic organ prolapse (POP), a common condition affecting a large proportion of women, is defined as the descent of one or more of the anterior or posterior vaginal wall, the vaginal apex or the uterus [1]. It is estimated that nearly 50% of all parous women between the ages of 50 and 79 have POP [2]. The lifetime risk for a woman to undergo surgical reconstruction of prolapsed pelvic organs for urinary or bowel incontinence correction is 11–25%, with 30% of patients in need of reoperation [3,4]. In the US, ~300,000 procedures for correction of POP are performed each year [5]. Typical POP repair involves augmentation of the damaged fascia with a knitted mesh. Direct fascial abutment is often unsuccessful, owing to the compromised state of the damaged tissue, reducing its ability to heal directly across the two sides. Therefore, a mesh is used to provide a scaffold to support the weakened tissue and allow cells to migrate, stabilize and remodel the compromised fascia, without excessive physical tissue tension [6].

Meshes available to surgeons include biological and synthetic types. It is important that clinicians choose a mesh type based on the profile of long-term surgical outcomes, which may vary as a function of material composition and structure [7]. Long-term success of an implanted surgical mesh relies on seamless integration with the host tissue, forming a physical support through which appropriate cells remodel the compromised tissue. Of particular concern during this integrative phase is the extent to which the intrinsic physical and chemical properties of the material influence inflammation and the host tissue response [8]. Implanted meshes are interrogated by leukocytes of various classes, and it is imperative that these cells are pacified into a constructive wound-healing phase rather than a destructive chronic inflammatory mode, which can accelerate material degradation and cause unwanted damage to healing tissues [9]. In contrast to an earlier publication in 2008 [10], the FDA reported in 2011 that it had received 4000 reports of complications related to the insertion of transvaginal polypropylene (PP) mesh in the previous six years [11]. In this

* Corresponding author.
E-mail address: jerome.werkmeister@csiro.au (J.A. Werkmeister).
[1] Equal senior authors.

http://dx.doi.org/10.1016/j.actbio.2014.08.031
1742-7061/

updated statement, it warned patients and doctors that complications from transvaginal mesh used for surgical treatment of POP are not rare. The newly formed tissue resulting from the use of conventional surgical PP mesh often develops into scar tissue instead of normal healthy tissue, which may be responsible for the serious complications (e.g. mesh contraction, erosion and pain) reported in up to 29% of cases [12].

Tissue engineering offers a promising regenerative medicine approach, involving the use of the appropriate biomedical materials in combination with cellular therapy. Recent tissue engineering approaches for POP and hernia surgery have included the use of mouse muscle-derived stem cells cultured on porcine small intestinal submucosa collagen [13], and synthetic Vicryl® (polyglactin 910) hernia meshes seeded with bone marrow mesenchymal stem cells [14]. The present authors recently identified human endometrial mesenchymal stem cells (eMSCs) [15,16], as an easily obtainable and readily available source of mesenchymal stem cells (MSCs). The procurement method of eMSCs is minimally invasive by endometrial biopsy via the cervix without the need for anaesthetic, resulting in minimum pain and morbidity [17]. The present authors have developed a simple one-step method to isolate the eMSCs prospectively using the magnetic beads and W5C5 antibody, with specificity to the SUSD2 marker, and serum-free scale-up culture conditions for clinical application [16,18]. More recently, given the complications associated with current clinical PP meshes, more compliant meshes such as polyamide (PA) have been developed [19], which may be suitable for fascial tissue repair. Polyamide 6 (PA6) polymers are stronger than PP, and are biocompatible, permitting cell attachment and proliferation [19]. PA meshes were knitted using a warp knitting machine. This pattern was of diamond tulle construction and was chosen because it most closely resembled that of the clinical PP meshes. One of these, a gelatin-coated polyamide scaffold (PA + G), improved tissue integration in a rat abdominal hernia model, compared with an uncoated clinical PP clinical mesh [20]. The present authors previously reported the favourable growth of eMSCs cultured on gelatin matrix and on gelatin/fibronectin matrices, both on two-dimensional (2-D) TC plastic as well as on microcarrier beads [18]. Additionally, eMSC-seeded PA + G scaffolds promoted early neovascularization in vivo, reduced inflammation in the long term and promoted a regenerative wound-healing response, with new tissue integration and improved biomechanical properties [21].

The aim of this study was to optimize PA + G scaffold properties and determine their in vitro biocompatibility with eMSCs. Further, the study investigated the capacity of eMSCs seeded on these scaffolds to differentiate into smooth muscle cells (SMC) and fibroblasts (FB), cells essential for generating neo-tissues, which could confer the necessary tissue elasticity and strength for effective repair of the damaged fascial tissue in POP.

2. Materials and methods

2.1. Materials

PA 6, a nylon most commonly used for PA extrusion, was used in this study. It is synthesized from a single monomer by ring opening polymerization of the six carbon caprolactam. PA meshes were warp knitted from 100 μm monofilament, giving a mass/area of 85 g m^{-2} and a large pore diameter of ~1.3 mm. These meshes were coated with porcine gelatin (Type A, 300 bloom; Sigma, USA) as previously described [19].

2.2. eMSC isolation and culture

Informed written consent was obtained from each patient, and ethics approval was obtained from the Monash Health Human Research and Ethics Committee B. Human endometrial tissue was collected from nine patients undergoing hysterectomy, and isolated cells were obtained as published previously, using enzyme digestion, Ficoll-Paque centrifugation to remove red blood cells and magnetic beads labelled with the single marker antibody W5C5 to select eMSCs [16]. The cells were cultured in serum-supplemented medium [SS-Medium, containing DMEM/F12 base medium, 10% foetal bovine serum (FBS), 1% antibiotic–antimycotic and 2 mM L-glutamine (Life Technologies)] with 10 ng ml^{-1} basic fibroblast growth factor (bFGF) up to passage 3 (P3) to obtain sufficient cells for seeding onto scaffolds. Serum-free conditions were then established for the preparation of eMSC-seeded constructs. Cells subjected to serum-starved conditions were weaned off serum by gradual transfer from 10% FBS to 5% FBS to 1% FBS-supplemented DMEM/F-12 media for 2–3 days each, and then serum-free medium [SF-medium, composed of DMEM/1% antibiotic–antimycotic, 1% L-glutamine, 0.5% bovine serum albumin AlbuMAX I, 10 nM linoleic acid, 50 μM 2-β-mercapethanol, 1% insulin-transferrin-selenium-sodium pyruvate (ITS-A, Invitrogen) and 100 μg ml^{-1} heparin, 100 μM l^{-1}-ascorbic acid] with 10 ng ml^{-1} bFGF and 10 ng ml^{-1} epidermal growth factor (EGF) for at least 3 days before experimental use.

2.3. Preparation of eMSC-seeded constructs

Briefly, PA meshes were cut into 10 × 3 cm pieces for easy handling. Meshes were dipped in 60 °C gelatin solution for 6 s and drained briefly. Meshes were laid gently and horizontally onto ice-cold glutaraldehyde solution (diluted from a stock of 50% w/v, ProSciTech, Australia) for 15 min to crosslink the gelatin. Following stepwise soaking in glycine (2% w/v in H_2O, Merck), hydrogen peroxide (2% v/v in H_2O, Merck) and glycerol (4% v/v in H_2O, Merck) with immersion in H_2O between, meshes were dried overnight. PA + G scaffolds were cut into samples of dimensions 1.5 × 1.5 cm and secured in holders (CellCrown™ 24, Scaffdex) compatible with 24-well plates (Iwaki®, Crown Scientific), as shown in Fig. 1. The cell culture plates composed of PA + G meshes were gamma irradiation sterilized at 25 kGy. Prior to cell seeding, scaffolds were rehydrated in PBS with 2% antibiotic–antimycotic (Life Technologies), rinsed with culture medium and immersed in medium with fibronectin (10 μg ml^{-1}; BD Biosciences) overnight at 4 °C. To permit cell attachment eMSCs (50,000 cells cm^{-2}) were seeded onto scaffolds in 100 μl of medium per scaffold and cultured overnight. The eMSC-seeded meshes were then incubated, with media changed every second day. Constructs were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.4. Optimization of gelatin coating on PA mesh

It has been shown that gelatin is an ideal substrate for effective adhesion and growth of eMSCs on tissue culture plates [18]. Gelatin coatings were further optimized on PA meshes with respect to an appropriate concentration and extent of crosslinking [22,23] necessary to allow eMSC adhesion, growth and differentiation on these scaffolds. To optimize the gelatin coating on the PA + G scaffold in PBS at 37 °C, a range of gelatin (5%, 8% and 10% w/v in H_2O) and glutaraldehyde concentrations (0.025%, 0.05% and 2% w/v in PBS) were investigated. Resultant gelatin coatings were examined under an optical microscope (Kyowa Optical) for level of gelation and coverage. To compare the gelatin degradation rate of prepared scaffolds stabilized with various glutaraldehyde crosslinking concentrations, gelatin was quantified for hydroxyproline content after acid hydrolysis (6 N HCl at 115 °C for 4 h) and reacted with ρ-dimethylaminobenzaldehyde and chloramine-T with the absorption at 560 nm measured as described previously [24]. The percentage of gelatin remaining in each mesh at five time points