Bioengineering of the Uterus

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Abstract
Impairment of uterine structure and function causes infertility, pregnancy loss, and perinatal complications in humans. Some types of uterine impairments such as Asherman’s syndrome, also known as uterine synechiae, can be treated medically and surgically in a standard clinical setting, but absolute defects of uterine function or structure cannot be cured by conventional approaches. To overcome such hurdles, partial or whole regeneration and reconstruction of the uterus have recently emerged as new therapeutic strategies. Transplantation of the whole uterus into patients with uterine agenesis results in the successful birth of children. However, it remains an experimental treatment with numerous difficulties such as the need for continuous and long-term use of immunosuppressive drugs until a live birth is achieved. Thus, the generation of the uterus by tissue engineering technologies has become an alternative but indispensable therapeutic strategy to treat patients without a functional or well-structured uterus. For the past 20 years, the bioengineering of the uterus has been studied intensively in animal models, providing the basis for clinical applications. A variety of templates and scaffolds made from natural biomaterials, synthetic materials, or decellularized matrices have been characterized to efficiently generate the uterus in a manner similar to the bioengineering of other organs and tissues. The goal of this review is to provide a comprehensive overview and perspectives of uterine bioengineering focusing on the type, preparation, and characteristics of the currently available scaffolds.

Keywords Tissue engineering · Uterus · Endometrium · Stem cells · Scaffold

Introduction

In 2014, transplantation of a uterus resulted in the successful birth of a child in a patient with uterine agenesis [1]. That study represents the ultimate treatment for congenital and acquired uterine defects [2]. However, uterine transplantation has many obstacles, such as the shortage of donors, possible organ rejection, and the long-term use of immunosuppressive drugs [3].

Bioengineering of a whole or partial uterus may overcome these limitations [3, 4]. In uterine tissue engineering, a uterus-like biomaterial is grafted into patients with uterine factor-associated reproductive and perinatal disorders, including infertility and recurrent pregnancy loss (Fig. 1). The material consists of either an acellular tissue-supporting material—termed a scaffold—alone or a scaffold repopulated with the patient’s own cells or those from an immunocompatible donor. The scaffold is necessary to support the repopulating cells structurally and functionally before or after grafting, although transplantation of cells or tissues such as organoids without support by the scaffold may have a potential for at least partial regeneration of the tissue. Because the acellular scaffold basically consists of extracellular matrices (ECM) alone, it exhibits no or very little immunogenicity even when it is derived from a mismatched unrelated donor. If an acellular scaffold were repopulated with the patient’s own cells, there would be no need for immunosuppressive drugs.

Based on the basic principles of organ tissue engineering, the following issues must be resolved at each step of uterine bioengineering. (1) What type of scaffold or template will be used? (2) How will the scaffold be prepared? (3) Will the scaffold be repopulated prior to grafting? (4) How will (re)cellularization be achieved? (5) What type of cells will be used for (re)-cellularization? (6) How will the uterus-like materials be grafted? [3, 4]. The gold standard of each step has yet to be established. In this review, we provide an overview and perspectives of bioengineering of the uterus, focusing on the type, preparation and characteristics of currently available scaffolds.
General Aspects and Current Status of Organ Tissue Engineering

Basic Strategy

The aim of organ tissue engineering (OTE) is to generate biological tissues and organs to treat a variety of medical conditions involving structural and functional impairment. The typical process of OTE consists of preparation of a cell/tissue-supporting material termed a scaffold, implantation of cells into the scaffold, repopulation, and remodeling of the scaffold by the cells, and thereafter grafting of the organ/tissue-like scaffold into a patient [5–7]. Alternatively, the scaffold can be grafted directly into the patient without repopulation and remodeling of the cells. The acellular scaffold supports the cells that migrate to it from the recipient’s body, allows the migrating cells to proliferate and differentiate, and eventually gives rise to the regenerated tissue and organ [5–7]. An example of the strategy used in a general OTE is shown in Fig. 1, focusing on the repair of a uterus.

Cell Source

The typical strategy for OTE includes three processes: determining a proper cell source, processing the cells and choosing an appropriate supportive scaffold. As for the proper cell source, stem/progenitor cells are ideal because they have potential for generation of the desired types of tissues and organs through self-renewal and multilineage differentiation [8]. Adult stem cells (ASCs), embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are the most likely candidates for the cell source for OTE [8].

Adult Stem Cells

Among the adult stem cells (ASCs), mesenchymal stem cells (MSCs) show promise for a wide range of OTE and regenerative medicine applications [9]. MSCs can be isolated from numerous tissues, including bone marrow and adipose tissues. They can be cultured prior to clinical use [9]. Depending on the specific application, suspensions of MSCs collected from...
MSC-enriched tissue of the patient or an immune-compatible donor may then be introduced intravenously or by local injection to achieve the desired therapeutic effects, such as treating autoimmune diseases or stimulating local tissue repair and vascularization [9]. Indeed, MSCs achieve tissue repair without engraftment and differentiation but instead through paracrine signaling and communication through cell-cell contacts responsible for angiogenesis and immunomodulation [9].

MSCs may also be utilized for tissue engineering by first promoting their differentiation toward a desired cell type (e.g., osteoblasts, chondrocytes, and adipocytes) prior to surgical implantation, often along with scaffold material. Initial animal studies, however, revealed that MSC-derived chondrocytes do not show regenerative abilities, resulting in a failure of engraftment [10]. Thus, practical application of MSCs to OTE appears limited.

Besides MSCs, tissue-specific stem cells are also candidates for OTE. Tissue-specific stem cells produce differentiated cells that function as a part of their specific tissues and organs and also govern the maintenance of their tissue of origin. Thus, given the specified differentiation and regeneration potential of tissue-specific stem cells, it is reasonable to utilize them for OTE. However, there are several limitations in that (1) tissue-specific stem or progenitor cells have not been clearly identified in all types of tissues and organs, (2) they are often inaccessible and difficult to isolate and handle even if identified, and (3) they are difficult to expand in vitro and in vivo.

### Induced Pluripotent Stem Cells

To overcome the limitations of ESCs, particularly the risk of immune rejection and ethical problems associated with the use of human embryos, induced pluripotent stem cells (iPSCs) have emerged as a promising alternative cell source for regenerative medicine, including OTE. iPSCs can be generated from adult somatic cells and acquire ESC-like pluripotency upon reprogramming through the forced expression of factors for maintenance of the defining ESC properties [13]. The reprogramming efficiency to generate iPSCs is, however, still not high [14]. Also, the differentiation efficiency of iPSCs into particular types of cells is not high, at least in part, because of heterogeneity in iPSCs and a lack of established protocols for induction of differentiation [15]. Furthermore, there is a possible risk of generating tumors in iPSC-based therapies [15]. Nevertheless, there is no requirement for human embryos and no or very little risks of immune rejections when using autologous or HLA-matched iPSCs, which has dramatically facilitated preclinical and clinical trials together with basic studies using iPSCs. Indeed, more than 70 clinical trials have been conducted or are ongoing [12]. The first clinical trial involved transplantation of a sheet of retinal pigment epithelial cells differentiated from autologous iPSCs in a patient with neovascular age-related macular degeneration [16]. Many observational or interventional studies involving ESCs and/or iPSCs have been registered in public databases. However, only a small part has focused on the actual transplantation of cells [12].

### Scaffolds

To efficiently achieve regeneration and reconstruction of organs and tissues, a supporting biomaterial(s) termed a scaffold is needed to endow a 3D structure that enables cell engraftment, tissue growth and differentiation. Ideally, the scaffolds should satisfy the following requirements: no adverse immunogenicity, good biocompatibility, no toxicity, timely biodegradability and appropriate biomechanical properties. Current scaffolds can be divided into 3 categories: natural materials, synthetic materials and natural acellular extracellular matrices after complete removal of the cells (decellularized matrices).

### Natural and Synthetic Materials

Natural biomaterials consist of pre-existing macromolecules that are present in ECM. They include collagen, gelatin, hyaluronic acid hydrogels, fibrin, glycosaminoglycans, alginate, Matrigel, silk, hydroxyapatite, and others [17]. These materials exhibit specific advantages, including mechanical and adhesive properties similar to natural ECM. In addition to good biocompatibility, they show less immune responsiveness and possess little capacity for initiating signals. These

### Embryonic Stem Cells

Embryonic stem cells (ESCs) are derived from the inner cell mass of blastocyst-stage embryos. They retain the ability to proliferate indefinitely in culture and retain their pluripotency, i.e., the capacity to differentiate into many cell types. Thus, the use of ESCs has long been considered an important therapeutic strategy for regenerative medicine, including OTE [11]. The establishment and availability of both mouse and human ESCs have facilitated this therapeutic strategy [11]. Indeed, approximately 30 clinical trials and numerous basic OTE studies using ESCs have been conducted or are ongoing [12]. ESC-based OTE, however, has limitations because of potential tumorigenic risks, the possibility of immune rejection, and ethical problems associated with the use of human embryos [6]. Furthermore, despite the pluripotency of ESCs, the efficiency of induction of differentiation into a desired cell type is less than 100% [6]. As a result of the inefficiency, tumors might arise from a small fraction of residual undifferentiated cells even after differentiation induction. These limitations have delayed clinical translation of ESC research [6].
materials have some shortcomings, including batch variability, a short degradation period, difficulty in purification, and quality control.

To overcome the obstacles associated with natural biomaterials, synthetic scaffolds have been developed and now can be divided into 4 types: polymers, ceramics, metals and graphene [17]. Although there are differences in properties among these materials, the general advantages of the synthetic scaffolds include easy modification, designable properties and good mechanical strength. Conversely, they are characterized by poor cell adhesion properties, poor biological signals and poor bio-compatibility. No or poor bioresorbability of synthetic materials can be either beneficial or detrimental for OTE.

Among the synthetic materials, polymers are the most prevalent type including polyactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), polyethylene glycol (PEG), polyhydroxyl ethyl methacrylate (PHEMA), and polyvinyl alcohol (PVA) [17]. Lactic acid polymers were invented in the eighteenth century and are now widely used in a variety of fields. PLA and PLGA are superior to the other synthetic polymers in terms of biocompatibility, biodegradability, bioreabsorbability, low immunogenicity and low toxicity. Thus, PLA and PLGA are favorably applied as 3D scaffolds in various medical fields, including dentistry and plastic surgery. In addition to the simple use of one synthetic material, combinatory use of synthetic materials together with or without bioactive substances improves the scaffolds’ properties resulting in successful OTE through facilitation of cell fabrication, proliferation and differentiation [5]. For instance, PCL was mixed with PLA to improve the thermal resistance and mechanical properties of engineered tissues [18].

As an alternate to a scaffold-based OTE, cell sheet tissue engineering is a scaffold-free strategy for creating transplantable two-dimensional (2D) and three-dimensional (3D) tissues and organs [19]. Cell sheet technology consists mainly of a “thermo-responsive culture dish” that is coated with poly(N-isopropylacrylamide) (PiPAAm). This material changes from a hydrophilic state to a hydrophobic state when the temperature is dropped from 37°C to 32°C. This culture dish enables reversible cell adhesion and detachment by thermoresponsive hydrophobicity of the surface. This material permits non-destructive harvest of cultured cells as an intact monolayer cell sheet, including the deposited ECM. Layering of these cell sheets enables the fabrication of a 3D tissue. Cell sheet-based tissues and their transplantation are used in many settings, such as the heart, cornea, esophagus, periodontal procedures, the middle chamber of the ear, knee cartilage and lung [19].

Decellularized Matrices

Decellularization is defined as a multi-step process of removing the viable cellular components from a human or animal organ or tissue to create a scaffold with retained macrostructure and microstructure of the ECM components, including collagen, elastin, microfibrils, proteoglycans, glycosaminoglycans (GAGs) and various growth factors [20].

Decellularizing procedures involve a blend of chemical, physical, and enzymatic treatments and vary depending on the origin and property of the tissue being processed [21]. Chemical treatments include acids and bases, hypotonic and hypertonic solutions, detergents such as Triton X-100, Triton X-200, sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC), sulfobetaine-10 and -16, and solvents such as alcohols, acetone, ethylenediaminetetraacetic acid and tributyl phosphate. Physical methods include freeze-thaw cycles, direct application of force and pressure, and electroporation. Enzyme treatments include nuclease, trypsin, and Dispase. The reagents and methods used for decellularization may damage the microstructure and composition of the resultant scaffold and therefore may affect the biological and mechanical properties of the final product [21]. Thus, the choice of the reagents together with the methods is critically important.

In addition to the use of ECM derived from decellularized tissue, 3D ECM scaffolds prepared by whole organ decellularization have been explored in regenerative medicine and tissue engineering strategies [21]. ECM-based clinical products are prepared from various allogeneic or xenogeneic tissue sources, including dermis, urinary bladder, small intestine, mesothelium, pericardium, and heart valves, and from several different species, some of which are commercially available [21].

Uterine Tissue Engineering

This review focuses on studies that aim to develop uterine tissue engineering with and without the use of exogenous cells. Various types of engineered 3D uterine tissue culturing systems have been developed and employed to study the mechanisms underlying endometrial differentiation and embryo implantation [3]. We also address the development of tissue culturing system.

Cell source for Bioengineering of the Uterus

When employing exogenous cells for the OTE of the uterus, ASCs including MSCs, ESCs, and iPSCs are the most likely candidates for repopulating the structure.

Uterus-Specific Stem Cells

Various types of uterine stem/progenitor cells have been isolated and identified [22, 23]. The main components of the uterus are the endometrium and myometrium. Thus, those tissues have been used as sources of stem/progenitor cells [24, 25].
Several types of transplantable, i.e., prospectively isolatable endometrial stem/progenitor cells have been identified, including CD140b+/CD146+ or SUSD2+ endometrial mesenchymal stem cells (eMSCs), N-cadherin+ endometrial epithelial progenitor cells and side population (SP) cells, a heterogeneous population predominantly comprised of endothelial cells [23]. In particular, SP cells have several stem/progenitor cell properties. Unfortunately, they are present at low frequencies in the original tissue and organ and therefore, it is extremely difficult to obtain a sufficient number of SP cells for OTE [26, 27]. Furthermore, endometrial SP cells require appropriate an environment and supporting cells, i.e., a niche, to maximally support stem/progenitor cell activities, including cell differentiation [28]. Indeed, in vivo endometrial tissue reconstitution activity is low when SP cells alone are transplanted into immunodeficient mice [27]. However, the activity increases when they are cotransplanted with whole endometrial cells [28].

Like endometrial stem/progenitor cells, several types of myometrial stem/progenitor cells have been identified: SP cells [29], CD34+/CD49f+ cells [30], CD44+/Stro-1+ cells [31] and CD140b+/CD146+ or SUSD2+ cells [32]. Although the percentage of these stem cells varies, only 3% of whole myometrial cells are myometrial SP cells or CD34+/CD49f+ cells [29, 30]. Furthermore, the myometrial tissue reconstitution ability of these stem cells alone is low [29, 30].

Thus, although endometrial and myometrial stem/progenitor cells are attractive and promising candidate cell sources for bioengineering of the uterus, there remain several problems, including the difficulty of in vitro and in vivo expansion, that make it difficult to use them for clinical applications.

**ESCs and iPSCs for Bioengineering of the Uterus**

As previously mentioned, ESCs and iPSCs can proliferate indefinitely, maintaining their stemness. Therefore, the use of these cells could theoretically overcome the difficulties described above. If a proper method of differentiation of ESCs and iPSCs into each component of the uterus were developed, the use of ESCs and iPSCs would be valuable for the bioengineering of the uterus. Human ESCs have the potential for generating endometrial cells both in vitro and vivo [33, 34]. Furthermore, Miyazaki et al. successfully directed the differentiation of human iPSCs through intermediate mesoderm, coelomic epithelium, and Müllerian duct to endometrial stromal fibroblasts under molecularly defined embryoid body culture conditions using specific hormonal treatments [35].

**Scaffolds for the Bioengineering of the Uterus**

Similar to the bioengineering of other organs, natural, synthetic or decellularized ECM materials have been used in both basic and clinical studies of the bioengineering of the uterus (in Tables 1 and 2).

**Synthetic Materials, Natural Materials, or Cell Sheet-Based Strategy**

Since the early 2000’s, synthetic materials natural and cell sheets have been explored for bioengineering of the uterus (Table 1) [34, 36–80]. Most studies have used collagen-based or collagen-containing natural materials [34, 37–49, 58, 64]. Target species have included humans [36, 42, 44, 45, 49–61, 63, 64, 69, 70], rats [34, 37–41, 43, 46, 48, 65–68, 71, 73, 75, 77–80] and others. The target tissue of most studies is the endometrium [38, 42, 44, 45, 47, 49, 55, 56, 62, 64–72, 74, 75, 78–80]. Stem cells, including MSCs and ESCs, have been used for in vitro culture, repopulation of scaffolds and/or in vivo transplantation [34, 41, 43, 45, 47, 59, 60, 72]. Two clinical trials have been conducted to explore the regeneration of endometrium and pregnancy using tissue engineering technologies [44, 45]. Zao et al. used a collagen scaffold to treat human patients with severe Asherman’s syndrome [44]. They aspirated the patients’ bone marrow and mononuclear cells (BMNCs) were isolated. Five patients with Asherman’s syndrome received a uterine transplant of a collagen scaffold seeded with autologous BMNCs. Over three menstrual cycles post-surgery, hysteroscopy and biopsy were performed to evaluate the endometrial status, and all of the patients achieved pregnancy and gave birth to a living child. Moreover, implantation of the BMNC-collagen scaffold onto the uterine lining downregulated ΔNp63 expression, reversed the associated pathological changes, normalized the stemness alterations and restored endometrial regeneration. Cao et al. proved the validity of allogenic cell therapy for recurrent intrauterine adhesion (IUA) patients using umbilical cord-derived mesenchymal stromal cells (UC-MSCs) loaded onto a collagen scaffold [45]. Twenty-six patients were enrolled in this clinical trial and 10 out of the patients achieved pregnancy, leading to 8 live births with no obvious birth defects and no placental complications. Spontaneous abortions were observed in 1 patient in the third trimester of pregnancy and another at 7 weeks.

Thus, there have been numerous basic and clinical studies exploring the use of synthetic materials and natural materials. Future analyses should determine which of the materials is optimal for bioengineering of the uterus. Critical parameters include in vivo characteristics rather than those in vitro, properties of the biomaterials and support of pregnancy. Furthermore, the use of larger animals, ideally primates, would enhance the characterization of the materials and methods used. In this context, the study conducted by Magalhaes et al. may provide useful information. They used a polyglycolic acid (PGA)/PLGA scaffold seeded with autologous cells to restore uterine structure and function in rabbits [76]. Rabbits underwent a subtotal uterine excision and were reconstructed with a scaffold seeded with autologous endometrial and myometral cells. At 6 months post-implantation, the
| Target species | Target tissue | Size of graft | Scaffold material | Cells used | Cell culture time in vitro | Histological tests in vivo | Pregnancy test | References |
|----------------|---------------|---------------|-------------------|------------|---------------------------|---------------------------|---------------|------------|
| Human          | Myometrium    | 1 × 1.5 cm    | Polyglactin-910 (Vicryl) mesh scaffold | Human myometrial cells | 3 weeks | N/A | N/A | Young et al., 2003 [36] |
| Rabbit         | Full thickness | 1 mL/well (12-well plate) | Collagen/Matrigel | Rabbit uterine cells as filled cells and mouse embryo | 14 days | N/A | N/A | Lu et al., 2009 [37] |
| Rabbit         | Endometrium   | 4, 12, 96-well plates | Collagen | Rabbit endometrial stromal and epithelial cells | 14 days | N/A | N/A | Wang et al., 2010 [38] |
| Rat            | Full thickness | 1.5 (length) × 0.5 (width) × 0.1 (thickness) cm | Collagen | No (only basic fibroblast growth factor [bFGF]) | N/A | 90 days | 90 days post transplantation | Li et al., 2011 [39] |
| Rat            | Full thickness | 1.5 (length) × 0.5 (width) × 0.1 (thickness) cm | Collagen | No (vascular endothelial growth factor [VEGF]) | N/A | 90 days | 90 days post transplantation | Lin et al., 2012 [40] |
| Rat            | Full thickness | 1.5 (length) × 0.5 (width) × -0.04 (thickness) cm | Collagen | Rat bone marrow-derived MSCs | 3 days | 90 days | 90 days post transplantation | Ding et al., 2014 [41] |
| Human          | Endometrium   | 6 (diameter, circular shape) × 3 (thickness) mm | Collagen | Endometrium-like cells differentiated from human ESCs | N/A | 12 weeks | 12 weeks post transplantation | Song et al., 2015 [34] |
| Rat            | Full thickness | Injected fibers | Collagen | Human umbilical cord-derived MSCs | N/A | 60 days | 60 days post transplantation | Xu et al., 2017 [43] |
| Human          | Endometrium   | 4 × 6 cm      | Collagen | Human autologous bone marrow mononuclear cells | 24 hours | 3 menstrual cycles | 5/5 patients gave birth | Zhao et al., 2017 [44] |
| Human          | Endometrium   | 4 × 6 cm      | Collagen | Human autologous umbilical cord-derived MSCs | N/A | 3 months | 10/26 patients became pregnant 8 weeks post transplantation | Cao et al., 2018 [45] |
| Rat            | Full thickness | 1.5 (length) × 0.5 (width) cm | Collagen | No (leukemia inhibitory factor [LIF]) | N/A | 12 weeks | 8 weeks post transplantation | Xue et al., 2019 [46] |
| Rat            | Endometrium   | 2.5 × 0.5 cm  | Collagen | Human umbilical cord-derived MSCs | 3 days | 60 days | 60 days post transplantation | Xin et al., 2019 [47] |
| Rat            | Full thickness | 1.5 × 0.5 cm  | Collagen | Human endometrial perivascular cells | N/A | 90 days | 90 days post transplantation | Li et al., 2019 [48] |
| Human          | Endometrium   | 8 (punch biopsy) × 0.75 (thickness) mm | Collagen | Human stromal cells and endometrial organoids | 10 days | N/A | N/A | Abbas et al., 2020 [49] |
| Human          | Cervix        | 10 × 3.5 × 1 (thickness) mm | Silk sponge | Human cervical cells | 8 weeks | N/A | N/A | House et al., 2010 [50] |
| Human          | Cervix        | 8 (diameter, circular shape) × 4 (thickness) mm | Silk sponge | Human cervical cells | 12 weeks | N/A | N/A | House et al., 2012 [51] |
| Target species | Target tissue | Size of graft | Scaffold material | Cells used | Cell culture time in vitro | Histological tests in vivo | Pregnancy test | References |
|----------------|---------------|---------------|-------------------|------------|--------------------------|--------------------------|---------------|------------|
| Human Cervix   | 6 (diameter, circular shape) × 4 (thickness) mm | Silk sponge | Human cervical cells | 4 weeks | N/A | N/A | House et al., 2014 [52] |
| Human Cervix   | 8 (diameter, circular shape) × 6 (thickness) mm | Silk sponge | Human cervical cells | 4 weeks | N/A | N/A | House et al., 2018 [53] |
| Human Stromal cells | 24-well plate | Hydrogel | Human endometrial stromal cells | 7 days | N/A | N/A | Li et al., 2011 [54] |
| Human Endometrium | 4, 6, 12-well plates | Fibrin-agarose | 1. Human endometrial epithelial and stromal cells 2. Human endometrial adenocarcinoma cell line and immortalized human endometrial stromal cell line | 7 days | N/A | N/A | Wang et al., 2012 [55] |
| Human Endometrium | 4, 12-well plates | Fibrin-agarose | Human endometrial adenocarcinoma cell line and immortalized human endometrial stromal cell line | 10 days | N/A | N/A | Wang et al., 2013 [56] |
| Human | 24, 96-well plates | Gelatin | Human endometrial stem cells | 28 days | N/A | N/A | Azami et al., 2013 [57] |
| Human | - | Collagen/carbon nanotubes composite | Human decidua parietalis stem cells | 6 days | N/A | N/A | Sridharan et al., 2013 [58] |
| Human | 1.5 × 1.5 cm | Gelatin/polyamide | Human endometrial MSCs | 28 days | N/A | N/A | Su et al., 2014 [59] |
| Human | 2.5 × 1 cm | Gelatin/polyamide | Human endometrial MSCs | N/A | 90 days | N/A | Edwards et al., 2015 [60] |
| Human Cervix | 12-well plate | Polystyrene | Human endocervical cells (stroma + mucosal epithelium) | 28 days | N/A | N/A | Arslan et al., 2015 [61] |
| Bovine Endometrium | 13 mm (diameter) | Electrospun polyglycolic acid (PGA) | Cattle endometrial stromal and epithelial cells | 14 days | N/A | N/A | MacKintosh et al., 2015 [62] |
| Human Cervix | 5 (diameter, circular shape) × 1 (thickness) mm | Free | Human cervical cells | 10 days | N/A | N/A | Gregorio et al., 2017 [63] |
| Human Endometrium | 12-well plate | Collagen/Matrix | Human endometrial CD146+ cells | 10 days | N/A | N/A | Fayazi et al., 2017 [64] |
| Rat Endometrium | 96-well plate | Heparin-poloxamer | Mouse endometrial epithelial cells (in vitro test) | 3 days | 14 days | N/A | Zhang et al., 2017 [65] |
| Rat Endometrium | 6-well plate | Heparin-modified poloxamer | Mouse endometrial epithelial cells (in vitro test) | 4 hours | 7 days | 90 days post transplantation | Xu et al., 2017 [66] |
| Target species | Target tissue | Size of graft | Scaffold material | Cells used | Cell culture time in vitro | Histological tests in vivo | Pregnancy test | References |
|----------------|---------------|---------------|-------------------|------------|---------------------------|----------------------------|----------------|------------|
| Rat            | Endometrium   | 6, 96-well plates | Heparin-modified poloxamer/ε-polylysine | Human endometrial carcinoma cell line (in vitro test) | 4 hours | 3 days | N/A | Xu et al., 2017 [67] |
| Rat            | Endometrium   | 24-well plate  | Pluronic F-127    | Rat bone marrow stromal cells | 7 days | 2 weeks | N/A | Yang et al., 2017 [68] |
| Human          | Endometrium   | 15 (diameter, circular shape) × 0.4 (thickness) mm | Polymerizable high internal phase emulsion | 1: Human endometrial epithelial and stromal cells 2: Human endometrial adenocarcinoma cell line | 15 days | N/A | N/A | Eissa et al., 2018 [69] |
| Human          | Endometrium   | 10 (diameter, circular shape) × 0.2 (thickness) mm | Polymerizable high internal phase emulsion/fibronectin | Human endometrial stromal cells | 9 days | N/A | N/A | Richardson et al., 2018 [70] |
| Rat            | Endometrium   | 15 (length, capillary tube) × 1.2 (inner diameter) mm (in vivo test) | Gelatin methacryloylalginate | No (in vivo test), HepG2 (in vivo test) | 10 days | 6 weeks | N/A | Cai Y et al., 2018 [71] |
| Rat            | Endometrium   | 1.5 (length) × 0.5 (width) × 0.1 (thickness) cm | Poly(glycerol sebacate) (PGS), Poly(lactic-co-glycolic acid) (PLGA), Collagen | Rat bone marrow–derived MSCs | N/A | 90 days | 90 days post transplantation | Xiao et al., 2019 [72] |
| Rat            | Full thickness| 2 × 1 cm       | Silk fibroin-bacterial cellulose | Human endometrial cells, rat uterine cells | 7 days | 90 days | 90 days post transplantation | Cai H et al., 2019 [73] |
| Mouse          | Endometrium   | 96-well plate  | Hyaluronic acid (HA) Hydrogel/fibrinogen/thrombin | Mouse endometrial stromal cells | 24 hours | 14 days | 14 days post transplantation | Kim et al., 2019 [74] |
| Rat            | Endometrium   | 2, 24, 96-well plates | HA hydrogel | Human bone marrow–derived MSCs | 3 days | 7 days | 7 days post transplantation | Liu et al., 2019 [75] |
| Rabbit         | Full thickness| 6–8 (length) × 2.5 (width) × 0.2 (thickness) cm | PGA/PLGA | Rabbit endometrial and myometrial cells | N/A | 6 months | 6 months post transplantation | Magalhaes et al., 2020 [76] |
| Rat            | Full thickness| 0.5 (diameter, tubular shape) × 2.5 (length) cm | Boiled blood clots molded into tubular shapes | No | N/A | 12 weeks | 4, 8, 12 weeks post transplantation | Campbell et al., 2008 [77] |
| Rat            | Endometrium   | Circumferentially full length of the uterus from the cervix to the fallopian tube | Cell sheet | Rat oral mucosal epithelial cells | N/A | 8 days | N/A | Kuramoto, et al., 2015 [78] |
| Rat            | Endometrium   | Circumferentially 10 mm (length) | Cell sheet | Rat endometrial cells | N/A | 4 weeks | 6 weeks post transplantation | Kuramoto, et al., 2018 [79] |
| Rat            | Endometrium   | 1.5 (length) × 0.5 (width) cm | Cell sheet | Rat adipose-derived stem cells | N/A | 60 days | 60 days post transplantation | Sun et al., 2018 [80] |
### Table 2: Studies of uterine tissue engineering using decellularized scaffolds

| Scaffold material | Scaffold size | Decellularized method | Decellularized reagent | Recellularization cells | Recellularization method | Cell culture time | Histological tests in vivo |
|-------------------|---------------|-----------------------|------------------------|------------------------|--------------------------|-------------------|---------------------------|
| Human myometrium  | 2×2×10 mm (human), 15×20 mm (rat) | Immersion with shaking | Ethanol and trypsin | Human and rat myocytes | Cultured on scaffold with shaking | 51 days | N/A |
| Rat full thickness uterus | Whole uterus | Perfusion via the aorta | SDS | Rat neonatal uterine cells + rat adult uterine cells + rat MSCs | Injected to whole uterine wall | 10 days | 90 days |
| Rat full thickness uterus | Whole uterus | Perfusion via the aorta | SDS | Rat endometrial and myometrial cells + rat MSCs | Injected to 20×5 mm pitch | 3 days | 3 months |
| Rat small intestine | 15 mm (length) (intestine) | Immersion with shaking | SDS | Sheep fetal bone marrow stem cells | Injected to the ring shape scaffold | 14 days (0.3–0.5 mm in thickness, ring shape) | N/A |
| Mouse full thickness uterus | 10×2 mm, 5×2 mm | Perfusion via the aorta, only perfusion | DMSO + triton X-100, SDC | Rabbit esophageal smooth muscle cells (in vitro only) | Cultured on the scaffold | 10 days (6.4 mm in diameter, punched) | 10 months |
| Sheep full thickness uterus | Whole uterus | Perfusion via the uterine artery | SDS + DNase, SDC + DNase, SDC + Triton X-100 + DNase | Human endometrial stromal and epithelial side population cells | Cultured on the scaffold | 12 days (5 mm in diameter, punched) | N/A |
| Human amniotic membrane + poly (ester urethane) | N/A | Immersion | Triton X-100 + DNase Hypertonic saline + DNase, Lipase + DNase, Triton X-100 + lipase + DNase | Rabbit embryo (as an implantation model) | Cultured on the scaffold | 48 hours | N/A |
| Porcine full thickness uterus | Whole uterus | Perfusion via the uterine artery | SDS | Human endometrial cells | Cultured on the scaffold | 28 days (8 mm in diameter, punched) | N/A |
| Rabbit uterus | Whole uterus | Perfusion via the uterine artery | SDC + Triton X-100 + DNase | Human endometrial cells | Cultured on the scaffold with insert | 28 days (8 mm in diameter, punched) | N/A |
| Scaffold material                  | Target species | Target tissue     | Target size                          | Graft size                    | Pregnancy test | References                  |
|----------------------------------|----------------|-------------------|--------------------------------------|-------------------------------|----------------|------------------------------|
| Human amniotic membrane          | N/A            | N/A               | N/A                                  | N/A                           | N/A            | Young et al., 2013 [81]     |
| Rat full thickness uterus        | Rat            | Full thickness    | 1.5 cm (length) × 1/2 of the total circumference | 1.5 × 0.5 cm                  | 28 days        | Miyazaki et al., 2014 [82]  |
| Rat full thickness uterus        | Rat            | Full thickness    | 1.5 cm (length) × 1/2 of the total circumference | 1.5 × 0.5 cm                  | 8 weeks        | Miki et al., 2019 [83]      |
| Rat full thickness uterus        | Rat            | Full thickness    | 15 × 5 mm                            | 15 × 5 mm                     | 30 days        | Santoso et al., 2014 [84]   |
| Rat full thickness uterus        | Rat            | Full thickness    | 15 × 5 mm                            | 15 × 5 mm                     | 30 days        | Santoso et al., 2014 [84]   |
| Mouse full thickness uterus      | Mouse          | Full thickness    | 5 × 2 mm                             | 5 × 2,10 × 2 mm (pregnancy test) | 30 days        | Hinoka et al., 2016 [85]    |
| Rat full thickness uterus        | N/A            | N/A               | N/A                                  | N/A                           | N/A            | Hellström et al., 2014 [86] |
| Rat full thickness uterus        | N/A            | N/A               | N/A                                  | N/A                           | N/A            | Hellström et al., 2014 [86] |
| Rat full thickness uterus        | Rat            | Full thickness    | 10 × 5 mm                            | 10 × 5 mm                     | 6 weeks        | Hellström et al., 2016 [87] |
| Sheep full thickness uterus      | N/A            | N/A               | N/A                                  | N/A                           | N/A            | Timann et al., 2020 [88]    |
| Human amniotic membrane + poly (ester urethane) | Rabbit  | Uterus (thickness is unknown) | N/A                                  | 1 × 1 cm                      | N/A            | Shi et al., 2015 [89]       |
| Porcine full thickness uterus    | N/A            | N/A               | N/A                                  | N/A                           | N/A            | Campo et al., 2017 [93]     |
| Rabbit uterus                    | N/A            | N/A               | N/A                                  | N/A                           | N/A            | Campo et al., 2019 [94]     |
| Human endometrium                | N/A            | N/A               | N/A                                  | N/A                           | N/A            | Olalekan et al., 2017 [95]  |
| Human amniotic membrane          | Rat            | Endometrium       | Scraped (N/A)                        | N/A                           | N/A            | Chen et al., 2018 [90]      |
| Human amniotic membrane          | Rat            | Endometrium       | Scraped (N/A)                        | N/A                           | N/A            | Chen et al., 2019 [91]      |
| Human amniotic membrane          | Rat            | Endometrium       | Scraped (N/A)                        | N/A                           | 28 days        | Chen et al., 2019 [92]      |
cell-seeded engineered uteri developed native tissue-like structures, including organized luminal/glandular epithelium, stroma, vascularized mucosa, and a two-layered myometrium. The rabbits had normal pregnancies (4 in 10) in the reconstructed segment of the uterus and supported fetal development to term and live birth.

As a unique alternative to a scaffold-based OTE, cell sheet tissue engineering has been used for uterine endometrial repair [78–80]. In 2015, Kuramoto et al. showed that the transplantation of oral mucosal epithelial cell sheets prevented IUA in rats [78]. Moreover, the same group reported in 2018 that rat endometrial cell sheets could repair IUA leading to successful pregnancies in the regenerated endometrium [79]. In 2018, Sun et al. showed that cell sheet engineering using adipose-derived stem cells (ADSCs) repaired IUA in rats and that pregnancy could be achieved 60 days after transplantation [80]. They also found that ADSCs were mainly detected in the basal layer of the regenerating endometrium and that some ADSCs differentiated into endometrial stromal-like cells and muscle cells and also stimulated angiogenesis. Given the encouraging results obtained in the 3 studies, cell sheet therapy for OTE is being explored in clinical settings [19]. Cell sheet technologies are promising as a new therapeutic strategy for endometrial damage. However, those technologies still have limitations. For example, it is difficult to achieve multilayered cell sheets in vitro. Moreover, large scale production of differentiated cells with vascularized thick tissues is difficult [19]. Thus, there are considerable obstacles to be overcome in the regeneration and reconstruction of large portions of the uterus.

Decellularization and Recellularization Strategy

Decellularization and recellularization techniques for regeneration of the uterus have emerged since 2013 as shown in Table 2 [81–98].

In 2013, Young et al. were the first to use a decellularized matrix prepared from rat and human myometrium for in vitro tissue engineering in 2013 [81]. Miyazaki and Maruyama demonstrated for the first time that the decellularized scaffold prepared from rat uterus had the potential for use as a supportive material to regenerate functional uterine tissue both in vitro and in vivo [82]. An acellular ECM scaffold together with a perfusible vascular architecture was prepared from rat uteri through decellularization by aortic perfusion with detergents such as SDS. Uterine-like tissues were then regenerated and maintained in vitro for up to 10 days through in vitro recellularization of the scaffold with adult and neonatal rat uterine cells and rat MSCs followed by aortic perfusion in a bioreactor. Moreover, placement of an acellular scaffold onto a partially excised rat uterus promoted recellularization and regeneration of uterine tissues and achievement of pregnancy nearly comparable to that in an
intact uterus [82]. The same group showed that disoriented placement of the scaffold onto a partially excised rat uterus resulted in regeneration of the uterine tissue but with aberrant structures including ectopic location of glands and an abnormal lining of smooth muscle layers [83]. They also prepared an ECM scaffold from rat small intestine, but, unlike the uterine scaffold, it had no supportive capacity. These results collectively indicate that the ECM and architecture of the uterine scaffold retain functionality and determine the orientation and topology of regenerated uterine tissue [83], Santoso et al. [84] independently demonstrated that uterine scaffolds prepared by different protocols had similar capacities as supportive materials to regenerate uterine tissue in rats. To prepare the decellularized uterine scaffold, Santoso et al. employed SDS or high hydrostatic pressure [84]. Hellström et al. used 3 different protocols—DMSO plus Triton-X100 followed by washing with PBS or distilled water, or SDS. They found that DMSO plus Triton-X100-generated scaffolds were preferable [87]. Tiemann et al., in the same group headed by Hellström, showed that perfusion with SDC is a favorable treatment for preparation of decellularized sheep uterine scaffold capable of supporting stem cells for 2 weeks in vitro [88].

Several groups prepared decellularized uterus-related or unrelated scaffolds from humans or from animals larger than rodents and used them for in vitro or in vivo uterine tissue engineering. Shi et al. and Chen et al. used human amniotic membrane as a xenograft and ectopic scaffold to repair the injured endometrium of rats or rabbits [89–92]. Campo et al. prepared decellularized porcine uterine scaffolds and recellularized them with only human endometrial stromal and epithelial SP cells (stem-like cells) for in vitro study [93]. They also decellularized whole rabbit uterus by a perfusion procedure via the uterine artery, followed by microdissection, lyophilization, milling, partial digestion and freezing [94]. A rabbit embryo was cultured in vitro on a hydrogel derived from powdered decellularized endometrium as an implantation model [94]. Olalekan et al. prepared decellularized human endometrial tissue for a novel 3D endometrium in vitro model [95]. It was repopulated with primary endometrial cells. Daryabari et al. found that perfusion with SDS and preservation in formalin could be used for preparation of a decellularized ovine uterine scaffold that was capable of regenerating the uterus when grafted into the uterus of rats [96]. Yao et al. decellularized whole rabbit uteri for xenografting to rat full thickness uterine walls [97]. They also decellularized a segment of rat uterus by an immersion procedure, pulverized it into a powder, and mixed it with aloe-polloxamer hydrogel and estradiol [98]. They injected the hydrogel into the injured uterine wall to prevent IUAI in a rat model.

Overall, most studies have employed chemical treatments using ionic detergents such as SDS to isolate decellularized uterine scaffolds (Table 2). However, Padma et al. pointed out that non-ionic detergents such as Trion X-100 were milder than ionic detergents and therefore minimized the denaturing of ECM proteins [99]. Thus, it remains to be determined which protocol and which biomaterial should be employed for the bioengineering of the uterus. Like synthetic and natural materials, in vivo characteristics of decellularized scaffolds rather than those in vitro are critical in the choice of protocol and biomaterial. Furthermore, the use of animals larger than rats and mice, ideally primates, would be better for the characterization and validation of a decellularized scaffold in terms of clinical applications. On the other hand, compared to synthetic or natural materials, the ECM and architecture preserved in a decellularized scaffold may determine the orientation and topology of the regenerated uterine tissue [83]. Therefore, in clinical testing of bioengineering methods, it would be preferable if the decellularized scaffold were prepared from a human uterus followed by transplantation in a proper orientation to fabricate the complex structure of the uterus.

**Perspectives**

Bioengineering studies of the uterus have relied upon a variety of scaffolds materials, including natural, synthetic and decellularized ECM. These studies are promising, suggesting clinical approaches to the repair of defective uteri. Nevertheless, several obstacles remain. One of them is the difficulty of in vitro repopulation of the (whole) uterine scaffold, a process that is absolutely required for regeneration of a whole uterus. As mentioned previously, many types of stem/progenitor cells, including endometrial SP cells, need an appropriate microenvironment (a niche) to exhibit maximal stem cell functions such as self-renewal, expansion and production of daughter cells that differentiate into one or multiple lineages [100, 101]. Thus, the full repopulation of the scaffold and maintenance of the resultant regenerated uterus requires a large and sufficient number of mature and/or differentiated uterine cells capable of supporting stem/progenitor cells. To obtain a sufficient amount of such cells, iPSCs and ESCs, especially the former, are needed as a cell source. A proper method of differentiation of ESCs and iPSCs into uterine cells, however, is largely unknown, although a few studies have addressed this issue [33–35].

In addition to selecting a cell source and a differentiation-inducing protocol, efficient methods of in vitro repopulation of the uterine scaffolds remains to be established. Several studies have reported successful repopulation of uterine scaffolds. However, their sizes have been relatively small (Tables 1 and 2). A few studies have attempted to repopulate whole rat uterine decellularized scaffolds through direct cell injection and/or perfusion, but the repopulation required a
huge number of cells, including stem/progenitor cells [82, 87, 88]. It appears that the repopulation efficiency is low. Furthermore, the repopulated scaffolds were difficult to maintain in vitro for a long period [82, 87, 88]. Although repopulation with iPSCs has been accomplished up to human scale for several other organs including the heart, there remain limitations in obtaining a sufficient number of different types of cells for repopulation [102]. Given that repopulation depends on perfusion and/or injection, the precise spatial positioning of different types of repopulating cells is challenging to achieve [102]. Recently, a 3D bioprinting technique has been developed as a manufacturing process [102]. In this approach, bio-compatible materials such as cells and growth factors are used as “inks” to print living tissue-like structures layer-by-layer. This approach has emerged as a new strategy for fabrication of complex biological constructs in the field of tissue engineering and regenerative medicine. Bioprinting has the potential to overcome some of the repopulation-related limitations and to substantiate the merit of the scaffold-based uterine tissue engineering.

Conclusions

We here provide an overview and perspectives of uterus bioengineering, emphasizing the type, preparation and characteristics of the currently available scaffolds. There remain many obstacles rendering bioengineering of the whole uterus quite difficult. However, partial regeneration of the uterus through scaffold-based uterine tissue engineering is feasible because bare uterine scaffolds have the potential to at least partially regenerate the uterus through their support for the migration, proliferation and differentiation of primitive cells present in the neighboring uterine tissues. Initially, the bioengineering of the uterus will be clinically applied to treatment of partial defects of the endometrium due to Asherman’s syndrome, partial or whole defects of the cervix due to conization and trachelectomy and partial defects of the myometrium due to segmental resection of the uterus.

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Declarations

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