Bioengineering trends in female reproduction: a systematic review

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Submitted on November 8, 2021; resubmitted on April 13, 2022; editorial decision on May 4, 2022

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BACKGROUND: To provide the optimal milieu for implantation and fetal development, the female reproductive system must orchestrate uterine dynamics with the appropriate hormones produced by the ovaries. Mature oocytes may be fertilized in the fallopian tubes, and the resulting zygote is transported toward the uterus, where it can implant and continue developing. The cervix acts as a physical barrier to protect the fetus throughout pregnancy, and the vagina acts as a birth canal (involving uterine and cervix mechanisms) and facilitates copulation. Fertility can be compromised by pathologies that affect any of these organs or processes, and therefore, being able to accurately model them or restore their function is of paramount importance in applied and translational research. However, innate differences in human and animal model reproductive tracts, and the static nature of 2D cell/tissue culture techniques, necessitate continued research and development of dynamic and more complex in vitro platforms, ex vivo approaches and in vivo therapies to study and support reproductive biology. To meet this need, bioengineering is propelling the research on female reproduction into a new dimension through a wide range of potential applications and preclinical models, and the burgeoning number and variety of studies makes for a rapidly changing state of the field.

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OBJECTIVE AND RATIONALE: This review aims to summarize the mounting evidence on bioengineering strategies, platforms and therapies currently available and under development in the context of female reproductive medicine, in order to further understand female reproductive biology and provide new options for fertility restoration. Specifically, techniques used in, or for, the uterus (endometrium and myometrium), ovary, fallopian tubes, cervix and vagina will be discussed.

SEARCH METHODS: A systematic search of full-text articles available in PubMed and Embase databases was conducted to identify relevant studies published between January 2000 and September 2021. The search terms included: bioengineering, reproduction, artificial, biomaterial, microfluidic, bioprinting, organoid, hydrogel, scaffold, uterus, endometrium, ovary, fallopian tubes, oviduct, cervix, vagina, endometriosis, adenomyosis, uterine fibroids, chlamydia, Asherman’s syndrome, intrauterine adhesions, uterine polyps, polycystic ovary syndrome and primary ovarian insufficiency. Additional studies were identified by manually searching the references of the selected articles and of complementary reviews. Eligibility criteria included original, rigorous and accessible peer-reviewed work, published in English, on female reproductive bioengineering techniques in preclinical (in vitro/in vivo/ex vivo) and/or clinical testing phases.

OUTCOMES: Out of the 10 390 records identified, 312 studies were included for systematic review. Owing to inconsistencies in the study measurements and designs, the findings were assessed qualitatively rather than by meta-analysis. Hydrogels and scaffolds were commonly applied in various bioengineering-related studies of the female reproductive tract. Emerging technologies, such as organoids and bioprinting, offered personalized diagnoses and alternative treatment options, respectively. Promising microfluidic systems combining various bioengineering approaches have also shown translational value.

WIDER IMPLICATIONS: The complexity of the molecular, endocrine and tissue-level interactions regulating female reproduction present challenges for bioengineering approaches to replace female reproductive organs. However, interdisciplinary work is providing valuable insight into the physicochemical properties necessary for reproductive biological processes to occur. Defining the landscape of reproductive bioengineering technologies currently available and under development for women can provide alternative models for toxicology/drug testing, ex vivo fertility options, clinical therapies and a basis for future organ regeneration studies.

Key words: bioengineering / uterus / endometrium / myometrium / ovary / fallopian tubes / cervix / vagina / female reproduction / fertility restoration

Introduction

To provide the optimal milieu for implantation and fetal development, the female reproductive system must orchestrate uterine dynamics in response to ovarian hormones. Specifically, estradiol and progesterone are produced through the processes of follicle development and luteinization in the ovary, and respectively regulate the proliferative and secretory phases in the endometrium. After ovulation, mature oocytes may be fertilized in the fallopian tubes, and the resulting zygote is transported toward the uterus, where it can implant and continue developing (if the endometrium is in an adequately receptive state). Throughout pregnancy, the cervix acts as a physical barrier to protect the fetus from external microorganisms or foreign objects that may enter through the vagina. Fertility can be compromised by pathologies that affect any of these organs or processes, and therefore, being able to accurately model them or restore their function is of paramount importance in applied and translational research.

The study of human reproduction requires multidisciplinary approaches. While animal models provide many opportunities for translational discoveries, there are inherent limitations due to differences compared to human reproductive physiology. Similarly, 2D cell or tissue culture models can provide novel insights on aspects of reproductive biology, but these models are more static and simplified and therefore do not recapitulate the dynamic, complex in vivo biology. These limitations underscore the need for continued research along with development of dynamic and more complex in vitro platforms, ex vivo approaches and in vivo therapies. This need is being filled, in part, by rapid advancements in the field of bioengineering, which applies life science and engineering principles to develop biomaterials for restoring, maintaining and/or improving tissue functions. Indeed, bioengineering is leading the way to a new dimension in the study of female reproduction by providing a wide range of potential applications and approaches for discovery.

Proposed bioengineering approaches to repair and/or improve female reproductive potential have evolved in parallel with advances in scientific knowledge and technology. Based on our systematic search, current strategies can be classified into six major categories, and these can be applied synergistically to understand reproductive biology and solve related problems: scaffold-free systems, hydrogels, decellularized extracellular matrix (dECM) or polymer scaffolds, 3D bioprinting, organoids and microfluidic approaches. Scaffold-free approaches make use of cells’ ability to self-organize and synthesize their own matrices, generating structures that can be used as functional units or regenerative blocks (Hayama et al., 2014; Orabi et al., 2017; Kuramoto et al., 2018, 2020). Hydrogels (which, for the purposes of this review, are defined by their liquid/injectable original state) can include a variety of natural and synthetic components and offer innumerable options for encapsulating or loading drugs, molecules, cells or reproductive tissues (Zhu et al., 2016; Tavana et al., 2016a; Yang et al., 2021; Zhang et al., 2021b). Selecting the most suitable hydrogel requires knowing the necessary mechanical and physicochemical properties for a given application (Kedem et al., 2011; Shikanov et al., 2011b). For example, animal-derived hydrogels include commercial mixtures of extracellular matrix (ECM) components, such as Matrigel and Cultiex, which are purified basement membrane extracts sequestered by mouse Engelbreth-Holm-Swarm tumor cells.

In contrast, dECM scaffolds derive from tissues and organs that were processed by physical, chemical and/or enzymatic methods (Hellström et al., 2014; Laronda et al., 2015; Campo et al., 2017; Pors et al., 2019; Li et al., 2021; Sargazi et al., 2021; Pennarossa et al., 2021a). These biocompatible scaffolds preserve the structure and
biochemical milieu of the tissue of origin (in terms of ECM signaling and migration), minimizing the risk of immune rejection after transplantation (Raya-Rivera et al., 2014; Daryabari et al., 2019; Yao et al., 2020b; Padma et al., 2021b). Notably, to facilitate transplantation/implementation, these scaffolds are often solubilized and used in hydrogel format (López-Martínez et al., 2021a). Scaffolds can also be produced from other natural polymers (such as collagen and bacterial cellulose) or synthetic components (Young et al., 2003; Liu et al., 2007; Edwards et al., 2015). Taking the fabrication of cell-loaded or cell-free scaffolds one step further, 3D bioprinting creates materials with precise shapes, textures and porosities, and offers vast applications in regenerative medicine (Laronda et al., 2017; Souza et al., 2017; Acién et al., 2019; Tiboni et al., 2021; Wu et al., 2022).

Among more recent developments are organoids and microfluidics. Organoids are simplified organs or organ-like structures formed in 3D culture systems, which enable recreation of the architecture and physiology of most female reproductive tissues. Organoids provide models for healthy and diseased tissue phenotypes, making them ideal platforms for personalizing bioengineering and biomedicine through both in vitro and in vivo studies (Kessler et al., 2015; Turco et al., 2017; Löhmußaar et al., 2021; Oliver et al., 2021). Microfluidic platforms, increasingly referred to as the ‘organ-on-a-chip’ concept, utilize properties of fluid dynamics in small-channelled platforms to facilitate study of the dynamic hormonal cycles and endocrine interactions that characterize the reproductive organs (Xiao et al., 2017).

The majority of bioengineering studies date from the year 2000. However, innovative works from the 20th century built the foundation of this emerging field (Fig. 1). The groundwork for scaffold-free approaches included the first bone marrow transplant between twins (Thomas et al., 1959), and the generation of cell-sheets (Yamada et al., 1990) with regenerative potential (Pellegrini et al., 1997) (Fig. 1A1). Organoids were described as early as the 1960s, when single-cell suspensions completely reconstituted whole organs (Weiss and Taylor, 1960), retinal organoids self-organized in vitro (Stefanelli et al., 1961) and later, breast (Li et al., 1987) and alveolar (Shannon et al., 1987) epithelial cells aggregated to form 3D structures in Matrigel (Fig. 1A1).

Explorations in the 1980s and 1990s produced different types of in vitro co-culture systems (Fig. 1A2 and 3). In particular, the successful combination of hydrogels with different biological products, such as pancreatic islets (Lim and Sun, 1980), prostaglandins (Embrey et al., 1980) and epithelial cells (Yannas et al., 1989), encouraged the use of different biomaterials for regenerative medicine. In this regard, studies in which embryos were cultured together with trophoblastic vesicles (Camous et al., 1984) or amputary cells (Bongo et al., 1989) inspired other co-culture systems. On the other hand, deECM scaffolds appeared after ECM was obtained from murine renal glomeruli (Hjelle et al., 1979), liver connective tissue (Rojkind et al., 1980), intact acellular matrix from porcine intestinal submucosa (Badyak et al., 1995) and bladder (Chen et al., 1999) (Fig. 1A2). Microfluidic platforms also emerged with micromachining capillary electrophoresis (Harrison et al., 1993), and microchannel networks for cell culture (Folch and Toner, 1998). Finally, bioprinting gained popularity with the first tissue-engineered ear (Cao et al., 1997), the use of 3D-printed substrates for cell adhesion (Park et al., 1998) and introduction of soft lithography (Xia and Whitesides, 1998); the latter encompasses a group of techniques for fabricating or replicating structures, channels or membranes by using soft polymeric material (usually polydimethylsiloxane) stamps or molds (Kim et al., 2018) (Fig. 1A3).

These six categories of bioengineering strategies promote four main translational and/or clinical applications: the development of next-generation in vitro platforms, or representative in vitro toxicology and drug screening models; the discovery of alternative therapies or new biomarkers; and improvement of tissue/organ regeneration and/or transplantation protocols (Fig. 1B). The establishment of a capillary system for sperm samples (Ulstein, 1972) is an excellent example of an innovative platform to improve ART, while the in vitro culture of human endometrial 3D glandular structures (Kirk and Alvarez, 1986; Rinehart et al., 1988), endometrial stromal cells embedded in a collagen matrix (and covered with epithelial cells (Bentin-Ley et al., 1994)) and ovarian epithelial organoids (Kruik and Auersperg, 1992) ensured the initial steps towards personalized in vitro screening platforms. Finally, the early development of the ESTES technique, where a portion of the ovary is transplanted into the uterus (Estes, 1909), provided a foundation for later progress in reproductive organ transplantation (Eraslan et al., 1966; Winston and Browne, 1974; Scott et al., 1981).

Since these initial discoveries paved the way, the bioengineering field has undergone rapid growth and expansion. Many engineered reproductive tissues and platforms are currently in different stages of clinical development; most models remain experimental, but others are in pre-clinical trials, and some are already being applied clinically. Given the quantity and heterogeneity of studies published within this specialty, the goal of this review was to systematically summarize the mounting evidence on bioengineering strategies, platforms and therapies, both currently available and under development, in the context of female reproductive medicine, including novel alternatives for fertility restoration.

Methods

Search strategy

PubMed and Embase were searched for relevant reports. The search strategy was limited to full-text articles, published in English, involving mammals or material derived therefrom, between January 2000 and September 2021. Combinations of the following keywords were used: bioengineering, reproduction, artificial, biomaterial, microfluidic, bioprinting, organoid, hydrogel, scaffold, uterus, endometrium, ovary, fallopian tubes, oviduct, cervix, vagina, endometriosis, adenomyosis, uterine fibroids, clarnydia, Asherman’s syndrome (AS), intrauterine adhesions, uterine polyps, polycystic ovary syndrome and primary ovarian insufficiency. Specific queries used in each database are presented in Supplementary Table S1. Additional studies were identified by manually searching the references of the selected articles and of complementary reviews.

Study selection and eligibility criteria

Literature search results were exported to an MS Excel spreadsheet and duplicates were identified using electronic and manual methods (Fig. 2). Titles, abstracts and full texts were then screened independently and in duplicate by two authors (E.F.-H. and R.L.) using the following eligibility criteria: original, rigorous and accessible peer-reviewed work published in English, on female reproductive bioengineering techniques in preclinical (in vivo/in vivo/ex vivo) and/or...
Figure 1. Key milestones during the 20th century forging the development of the bioengineering field. (A) Evidence. (A1) Advances such as the first bone marrow transplant between twins (1) (Thomas et al., 1959), the control of attachment and detachment of cultured cells (2) (Yamada et al., 1990) and the use of cell sheets (3) (Pellegrini et al., 1997) laid the groundwork for scaffold free-approaches. Concomitantly, in 1960, the reconstitution of a complete organ from single-cell suspensions (4) (Weiss and Taylor, 1960) opened an avenue to the present organoids. The in vitro self-organization of retina (5) (Stefannelli et al., 1961) and the 3D organization of breast (6) (Li et al., 1987) and alveolar (7) (Shannon et al., 1987) epithelial cells after culture with Matrigel moved this path further along. (A2) Some works from the 1980s reported the combination of hydrogels with different biological products such as pancreatic islets (8) (Lim and Sun, 1980), E2 (9) (Embrey et al., 1980) and epithelial cells (10) (Yannas et al., 1989), introducing these promising biomaterials for regenerative medicine. In parallel, obtaining ECM from renal glomeruli (11) (Hjelle et al., 1979), from liver connective tissue (12) (Rojkind et al., 1980), and a decade later, an intact acellular matrix from intestinal submucosa (13) (Badylak et al., 1995) and bladder (14) (Chen et al., 1999) provided the beginnings of the dECM scaffold approaches. (A3) The beginnings of co-culture systems are captured in two main works in which embryos were cultured together with trophoblastic vesicles (15) (Camous et al., 1984) and ampullary cells (16) (Bongso et al., 1989). Research that formed the basis of microfluidic systems was reported in the nineties; some examples are the emergence of on-chip capillary electrophoresis (17) (Harrison et al., 1993) and elastomeric microchannel networks for cell culture (18) (Folch and Toner, 1998). Works from the end of the century paved the way for bioprinting: creation of a tissue-engineered ear (19) (Cao et al., 1997), use of 3D printed substrates for cell adhesion (20) (Park et al., 1998) and introduction of soft lithography (21) (Xia and Whitesides, 1998). (B) Applications. The establishment of a capillary system for sperm samples (22) (Ulstein, 1972) and the culture of human ovarian epithelial organoids (23) (Kruk and Auersperg, 1992) were the beginnings of the development of in vitro screening platforms. The next generation in vitro platforms are based on studies like those from 1986 and 1988, which established endometrial epithelial cells were co-cultured with an ECM from glandular structures (24, 25) (Kirk and Alvarez, 1986; Rinehart et al., 1988) and similar system also containing endometrial stromal cells (26) (Bentin-Ley et al., 1994). Finally, the development of the ESTES technique for dog ovarian transplantation (27) (Estes, 1909) in the early 20th century provided an excellent basis for a later dog uterus transplantation (28) (Eraslan et al., 1966), a rabbit fallopian tube and ovary autograft transplantation (29) (Winston and Browne, 1974) and a primate ovarian transplantation (30) (Scott et al., 1981). BM, bone marrow; E2, estradiol; ECM, extracellular matrix; dECM, decellularized extracellular matrix.
clinical testing phases. Studies in which gels were developed for intravaginal delivery of hormones, bactericides, nucleic acids or contraceptive drugs were not considered in this review because of their pharmacological nature. Questions or disagreements were resolved by discussion (E.F.-H., R.L., A.P. and I.C.). The final list of included studies was approved by I.C.

Data extraction

Extracted data, including titles, authors, year of publication, reproductive organ (uterus, ovary, fallopian tube, cervix, vagina or full tract), bioengineering strategy, platform/biomaterial used, species, cell/tissue model, study type (in vitro, in/ex vivo, clinical) and main findings were compiled into a shared Google Sheets spreadsheet and revised by M.H., L.M.-G., S.H. and M.B.

Synthesis of results

Relevant findings extracted from each study are summarized in Table I. Due to the inability to completely detail the many articles comprising this systematic review in Table I, a comparison of in vivo uterine regeneration parameters (e.g. immune tolerance, recovery of thickness and muscle layer, presence of glands, angiogenesis, implantation potential and maintenance of pregnancy) is provided in Supplementary Table SIII, while specific outcomes of in vitro follicle growth (IVFG) studies (e.g. follicle survival, initial and final follicle size, steroidogenesis, oocyte maturation rates, developmental competence and/or fertility restoration) are detailed in Supplementary Table SIV.

Studies related to gynecological pathologies, both included in the initial search terms and different ones addressed by the selected articles (such as endometriosis, uterine fibroids, AS, intrauterine adhesions, polycystic ovary syndrome, primary ovarian insufficiency and Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome) were included in Table I, however owing to the extent of relevant studies applying bioengineering techniques to create novel ovarian, uterine and cervical cancer models (published between April 2014 and September 2021), the latter were grouped separately according to their application and organ in Supplementary Table SIV. Finally, throughout the entire review we classify hydrogels as originally softer/injectable materials regardless of whether they gelify afterwards (e.g. collagen solutions), and scaffolds as their more rigid counterparts (e.g. collagen membranes).

Results

Search results

The search queries yielded 10 390 results (from a total of 18 748 titles identified) after removal of duplicates. Titles and abstracts were
### Table I Main findings of bioengineering studies related to female reproductive organs.

| Strategy          | Platform/ biomaterial | Type of study (model) | Disease-related | Main findings                                                                 | Reference                  |
|-------------------|-----------------------|-----------------------|-----------------|-------------------------------------------------------------------------------|----------------------------|
| Scaffold-free approaches | Cell sheet            | In vivo (rat)         |                | Rat oral mucosal epithelial cell sheets prevented IUAs caused by endometrial damage and helped to maintain the uterine luminal structure. | Kuramoto et al. (2015)    |
|                   |                       |                       |                | Multilayered rat endometrial epithelial and stromal cell sheet transplantation regenerated endometrial tissue, supporting pregnancy similar to normal endometrial tissue. | Kuramoto et al. (2018)    |
|                   |                       |                       |                | Rat adipose-derived stem cell sheets transplanted into partially excised uteri promote regeneration of endometrial and muscle cells and stimulate angiogenesis. | Sun et al. (2018)         |
| MicroTissues 3D Petri Dish micro-mold spheroids |            | In vitro              |                | Human UC-MSC sheets improved uterine incision repair in a rat hysterotomy model. | Kuramoto et al. (2020)    |
|                   | DC endoderm cells     | In vitro + in vivo (mouse) |                | Generation of endometrial organoids with both epithelial and stromal cells of the human endometrium. | Murphy et al. (2019)      |
|                   |                       | In vivo (mouse)       |                | ECM coating from synchronous DC rabbit endometrium achieved similar results to the gold standard embryo culture conditions. | Campo et al. (2019)       |
|                   | Hydrogels             |                       |                | Porcine endometrial ECM hydrogel supports in vitro culture of human endometrial cells in 2D and 3D conditions. Improved proliferation of EnSCs with respect to collagen and Matrigel. | López-Martínez et al. (2021a) |
|                   |                       |                       |                | Porcine endometrial ECM hydrogel loaded with growth factors enhanced tissue regeneration and restored fertility in a mouse model of endometrial injury. | López-Martínez et al. (2021b) |
|                   |                       |                       |                | E2 stimulation of human Ishikawa cells induced functional changes in HUVECs within a collagen biomaterial. | Pence et al. (2015)      |
|                   |                       |                       |                | 3D collagen gel-embedded human endometrial tissue slices responded to ovarian steroid hormones over 3 weeks. | Muruganandan et al. (2020) |
|                   |                       |                       |                | A tissue-engineered human endometrial stroma manifests changes in morphology and biochemical markers of decidualization, and responds to steroid withdrawal. | Schutte and Taylor (2012) |
|                   |                       |                       |                | Human endometrial stromal cells acquired contractile ability by passive loading of cyclic tensile stretch. | Kim et al. (2020b)       |
|                   |                       |                       |                | Collagen-binding VEGF restored fertility in a full-thickness injury model of rat scarred uterus. | Lin et al. (2012)         |
|                   |                       |                       |                | Human UC-MSCs facilitated collagen scaffold degradation in rat uterine scars, promoting full-thickness wall regeneration and restoring fertility. | Xu et al. (2017c)         |
|                   |                       |                       |                | Improvement in endometrial proliferation, differentiation and neovascularization following allogeneic cell therapy using human UC-MSCs on collagen hydrogels in patients with IUAs. | Cao et al. (2018)         |
|                   |                       |                       |                | Transplantation of human UC-MSCs on collagen hydrogels improved endometrial angiogenesis, proliferation and response to hormones in patients with AS. | Zhang et al. (2021b)      |
|                   |                       |                       |                | Collagen-binding bFGF improved functional remodeling of scarred endometrium in infertile women. | Jiang et al. (2019)       |

Continued
| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|----------------------|----------------|---------------|-----------|
| Collagen-Matrigel | In vitro | 3D culture model with human stromal and epithelial cells replicates the normal endometrium physiologically and morphologically, including stromal invasion of KLE cells. | | | Park et al. (2003) |
| Matrigel | | | | | Schutte et al. (2015) |
| | | Co-culture model of human epithelial and stromal cells changed cytokine production, reducing inflammation and protease activity. | | | Stejskalová et al. (2021) |
| | | Development of a 3D spheroid human model of endometriosis where collagen I triggers directional migration, invasion and matrix remodeling of stroma cells. | | | |
| HA | In vivo (rat) | Local injection of HA-danazol gel reduced size of endometrial cysts, without disrupting the estrous cycle in a rat model of endometriosis. | | | Nomura et al. (2006) |
| | In vivo (mouse) | HA-fibrin-encapsulated murine dEMSCs repaired the damaged endometrium, with successful implantation and normal embryo development. | | | Kim et al. (2019) |
| | In vitro + ex vivo + in vivo (rat) | In situ administration of HA gel/human MSC-secretome treatment repaired endometrial injury, promoting pregnancy, in a rat model of AS. | | | Liu et al. (2019) |
| Collagen + HA + agar | In vitro + in vivo (mouse) | Three-layered artificial endometrium (made from human EnSC, stromal and vessel cells) remained functional in vitro for 28 days and restored fertility (with successful pregnancy and LBs) in endometrial ablation mouse model. | | | Park et al. (2021) |
| Dextrin | In vivo (pig) | Using a dextrin-based adhesion barrier resulted in a higher percentage of adhesion-free sites compared with the controls after laparoscopy in a pig model. | | | Kai et al. (2018) |
| Fibrin-agarose | In vitro | Human implantation is modeled by co-culturing human endometrial epithelial and stromal cells in a 3D system that allows JAR spheroid attachment. | | | Wang et al. (2012) |
| | | Human epithelial-stromal interaction enhanced prolactin expression in fibrin-agarose gel. JAR spheroids invaded the epithelium and embedded into the 3D matrix under decidualization conditions. | | | Wang et al. (2013) |
| | | PEG-based sprayable adhesion barrier reduced adhesion tenacity, extent and incidence scores in patients undergoing myomectomy. | | | Mettler et al. (2004) |
| | | Resorbable PEG-based hydrogel reduced post-operative adhesions following myomectomy. | | | Mettler et al. (2008) |
| | | Co-culture of human endometrial epithelial cells and stromal cells encapsulated in a PEG hydrogel with ECM-binding peptides remodel the synthetic matrix and display hormone-mediated differentiation. | | | Cook et al. (2017) |
| | | l-phenylalanine-loaded PEBP/PEG hydrogel suppressed uterine fibrosis and promoted embryo implantation in a rat uterine curettage model. | | | Wang et al. (2021) |
| | | Human AD-MSC exosome-hydrogel promoted neovascularization and endometrial regeneration in rats, facilitating LBs. | | | Lin et al. (2021a) |
| | | Reduced incidence, extent and severity of peritoneal adhesions following gynaecological surgery. | | | Müller et al. (2011) |
| PVA/CMC | In vivo (rabbit) | Pluronic F-12 hydrogel encapsulating vitamin C and rat bone marrow stromal cells promoted rat endometrial regeneration by restoring the endometrial membrane and reducing inflammation. | | | Yang et al. (2017) |

Continued
| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|----------------------|----------------|--------------|-----------|
| Hydrogels | Methacrylamide-functionalized gelatin | In vitro | Human endometrial epithelial and stromal cells showed proangiogenic activity in response to E2 in gelatin hydrogels. | Pence et al. (2017) |
| | | In vitro + in vivo (rat) | c-Polylysine-HP hydrogel encapsulating keratinocyte growth factor repaired the morphology of injured rat endometrium. | Xu et al. (2017a) |
| | | In vivo (rat) | HP-E2 hydrogel loaded with keratinocyte growth factor facilitates the morphologic and functional recovery of injured rat uteri. | Xu et al. (2017b) |
| | | | HP-E2 hydrogel prolongs release of E2, improving both gland numbers and fibrotic area, in a IUA rat model. | Zhang et al. (2017b) |
| | Chitosan-heparin | Clinical | Treating injured rat endometrium with a stromal cell derived factor-1α-loaded chitosan-heparin hydrogel restored endometrial thickness, gland number and reduced fibrosis. | Wenbo et al. (2020) |
| | Actamax adhesion barrier | Clinical | Spraying a degradable hydrogel adhesion barrier during gynecologic laparoscopic abdominopelvic surgery reduced postoperative adhesion development. | Trew et al. (2017) |
| | Aloe poloxamer + DC uterus nanoparticles | In vitro + in vivo (rat) | Aloe poloxamer with E2 encapsulated in DC rat uterus nanoparticles significantly recovers morphology and decreases uterine fibrosis in a IUA rat model. | Yao et al. (2020a) |
| dECM and polymer scaffolds | Proof of concept | | Comparison of three protocols for whole rat uterus decellularization. The sodium deoxycholate protocol gave rise to a scaffold that structurally and mechanically resembled native uterus. | Hellström et al. (2014) |
| | Proof of concept + in vitro | | Whole pig uterus decellularization produced a cytocompatible scaffold. Recellularization with human EnSC resulted in organoid-like structure formation. | Campo et al. (2017) |
| | In vivo (mouse) | | DC uterine matrix transplantation restored all the uterine layers and fertility. | Hiraksa et al. (2016) |
| | | | Xenogeneic crosslinked rabbit uterine ECM achieved rat uterus regeneration and was recellularized in vivo after 90 days. | Yao et al. (2020b) |
| | DC uterus | | Whole DC sheep uterus gave rise to biocompatible scaffolds with native-like biomechanical, structural and vascular properties that were recellularized in vivo. | Daryabari et al. (2019) |
| | | | Engraftment of rat MSC-recellularized DC uterine matrix on partially excised uteri yielded functional uteri with pregnancy and fetus rates comparable to the control group. | Li et al. (2021) |
| | | | Perfusion-recellularized uterine matrix is able to partially regenerate and reconstruct the damaged rat uteri. | Miyazaki and Maruyama (2014) |
| | | | Recellularized uterine ECM patches repair a partially defective uterus and support pregnancy. | Hellström et al. (2016) |
| | | | Both high hydrostatic pressure and detergent-based decellularization protocols can efficiently create rat uterine matrices for uterine regeneration. | Santos et al. (2014) |
| | | | The orientation of a DC uterine scaffold determines the tissue topology and architecture of regenerated uterus in rats, without affecting pregnancy. | Miki et al. (2019) |
| | | | Decellularization based on Triton-X 100 and deionized water generated the lowest immune response after allogeneic transplantation of DC rat uterine scaffolds. | Padma, Alsheikh, Song, et al. (2021b) |
| | | | Rat uterus decellularization with sodium deoxycholate revealed more ECM-related damage-associated molecular patterns, and resulting scaffolds induced pro-inflammatory cytokine responses. | |
| Strategy          | Platform/ biomaterial | Type of study (model) | Disease-related | Main findings                                                                 | Reference                  |
|-------------------|-----------------------|-----------------------|-----------------|-------------------------------------------------------------------------------|----------------------------|
| DC ECM and polymer scaffolds | dECM and polymer scaffolds | In vitro | Mouse uterine DC scaffolds proved to be an adequate natural niche for human MenMSCs differentiation toward uterus-specific cell lineages. | Arezoo et al. (2021) |
| | DC myometrium | In vitro | Comparison of three protocols for whole sheep uterus decellularization, generating different ECM scaffolds that supported in vitro stem cell growth and proliferation. | Tiemann et al. (2020) |
| | DC endometrium | In vitro | Enzymatic preconditioning of sheep uterine ECM scaffolds improved recellularization compared with standard culture conditions and with the use of transwells alone. | Padma et al. (2021c) |
| | DC human amniotic membrane | In vivo | Creation of allo- and xeno-neo-myometrium by culturing isolated myocytes into DC rat and human myometrial scaffolds. | Young and Goloman (2013) |
| | Urinary bladder ECM | In vivo (rat) | Human recellularized endometrium responded to a 28-day hormone treatment by expressing E2 and P4 receptors and secreting IGF binding protein-1 and prolactin. | Olalekan et al. (2017) |
| | HA/carboxymethylcellulose membrane | In vivo (rat) | Comparison of different decellularization protocols for human endometrial fragments. | Sargazi et al. (2021) |
| | HA + mitomycin C | In vivo (rat) | Engineered rat oral mucosa epithelial cells prevented progression of IUA and improved endometrial epithelium regeneration. | Chen et al. (2019) |
| | Carbylan-SX (semisynthetic glycosaminoglycan) | In vivo (rat) | Human amniotic membrane and adipose stem cells improved regeneration, angiogenesis and receptivity in a rat IUA model. | Han et al. (2020) |
| | GelMA and sodium-alginate | In vivo (rat) | Both melatonin and HA/carboxymethylcellulose membrane proved to be effective in prevention of adhesion formation in rats. | Demirbag et al. (2005) |
| | Alginate | In vitro | Micromycin C-loaded crosslinked HA films and gels reduced formation of postoperative adhesions between uterine horns and with surrounding tissues and organs. | Liu et al. (2005) |
| | Alginate-multivalent integrin α5β1 ligand | In vitro | Urinary bladder matrix scaffolds improved endometrial regeneration in a rat model of intrauterine adhesions. | Zhang et al. (2020a) |
| | Alginate | In vivo | Carbylan-SX film and gel were efficacious in reducing postoperative intra-abdominal adhesion formation in cecum-abdominal wall and uterine horn in rats. | Liu et al. (2007) |
| | Alginate-multivalent integrin α5β1 ligand scaffold | In vivo | Both melatonin and HA/carboxymethylcellulose membrane proved to be effective in prevention of adhesion formation in rats. | Demirbag et al. (2005) |
| | Alginate | In vivo | Porous scaffold from droplet microfluidics loaded with bFGF had the ability to improve neovascularization and repair rat endometrium. | Cai et al. (2019) |
| | Alginate-multivalent integrin α5β1 ligand | In vivo | Development of an embryo implantation model consisting of an alginate scaffold seeded with human epithelial cells to which JAR spheroids are able to adhere. | Stern-Tal et al. (2020) |
| | Collagen | In vivo (rat) | Collagen scaffold loaded with human UC-MSCs promoted endometrial regeneration and restored fertility in a rat model. | Xin et al. (2019) |
| | Collagen | In vivo (rat) | Collagen scaffolds with human bFGF improved regeneration of rat uterine endometrium and muscular cells, vascularization and pregnancy outcomes. | Li et al. (2011a) |
| | Collagen | In vivo (rat) | Collagen/rat BM-MSCs system increased proliferation of endometrial and myometrial cells, enhanced angiogenesis and restored fertility. | Ding et al. (2014) |
| | Collagen | In vivo (rat) | Collagen scaffold loaded with human ESC-derived endometrium-like cells regenerated the structure and function of rat uterine horns. | Song et al. (2015) |
| | Collagen | In vivo (rat) | Collagen scaffold loaded with human endometrial perivascular cells overexpressing CYR61 promoted endometrial and myometrial regeneration and induced neovascularization in injured rat uterus model. | Li et al. (2019) |
| | Collagen | In vivo (rat) | Collagen scaffold with human UC-MSCs improved IUAs in rats, by increasing endometrial glands and reducing fibrosis. | Liu et al. (2020) |
| | Clinical | In vivo (rat) | Transplantation of collagen scaffold with autologous bone marrow mononuclear cells promoted functional endometrium reconstruction in patients with AS. | Zhao et al. (2017) |
| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|----------------------|----------------|--------------|-----------|
| Bioprinting | Gelatin-coated polyamide | In vitro | Human eMSCs were differentiated into smooth muscle cells or fibroblast-like cells to simulate fascial tissue composition, using an optimized gelatin-coated polyamide scaffold. | Seeding human eMSCs in SC gelatin-coated polyamide mesh resulted in enhanced collagen growth and organization. | Su et al. (2014) |
| | | | | | |
| | PGA-PLGA | In vivo (rabbit) | | Subtotal uterine excisions were reconstructed with autologous constructs made from endometrial and myometrial cells. Fetal development supported to term and LB. | Magalhaes et al. (2020) |
| | Poly(glycerol sebacate) | In vitro + in vivo (rat) | | Directional differentiation of rat BM-MSCs and restoration of morphology and function of wounded uterus. | Xiao et al. (2019) |
| | PLA + Pluronic F68 | In vitro (rat) | | New PL:Pluronic copolymer films prevented adhesion comparable to membranes of oxidized regenerated cellulose. | Yamaoka et al. (2001) |
| | PLA-poly(del-lactone)/gelatin | In vivo (mouse) | | Degradable mesh with murine eMSCs promoted tissue integration and anti-inflammatory response after subcutaneous transplantation. | Mukherjee et al. (2019) |
| | PLA patch ("nanofilm") | Ex vivo + in vivo (rabbit) | | PLA nanofilm sealed defects smaller than 3 mm in chorion-amonion and uterine membranes allowing intrauterine development in a rabbit model. | Pensabene et al. (2015) |
| | Poly(l- lactic-co-glycolic acid) | In vitro | | 3D scaffolds enhanced differentiation of primary human endometrial epithelial and stromal cells resembling the in vivo architecture and function. | Elisa et al. (2018) |
| | Emulsion-templated porous polymers | | | Scaffolds with fibronectin improved adhesion, infiltration and function of primary human endometrial stromal cells. | Richardson et al. (2019) |
| | PTFE | | | Novel hormone responsive in vitro model of the human uterine wall by co-culturing smooth muscle cells and endometrial epithelial and stromal cells on a synthetic membrane. | Kuperman et al. (2020) |
| Organoïd | Gelatin + alginate | In vitro + in vivo (rat) | | 3D-printed hydrogel scaffold loaded with human iPSC-derived MSCs promoted the regeneration of endometrial and endothelial cells, and improved endometrial receptivity in a rat model. | Ji et al. (2020) |
| | Myometrial 3D cell rings | | | Bioprinted uterine rings created with human myometrial cells show origin-dependent patterns of contractility and respond differently to uterine contractility inhibitors. | Souza et al. (2017) |
| | DC endometrium | In vitro | | Solubilized endometrial ECM from porcine uteri enhances proliferation rates of human endometrial organoids. | Francess-Herrero et al. (2016b) |
| | Matrigel-based 3D culture platform | In vitro | | Development of a functional C. trachomatis-murine endometrial organoids infection model system. | Bishop et al. (2020) |
| | | | | Establishment of a novel organotypic culture system that models the hormonal responses of the normal human endometrium (epithelia and stroma) in vitro. | Bläuer et al. (2005) |
| | | | | Generation of long-term, hormone-responsive human endometrial organoid cultures from healthy and cancerous tissue. | Turco et al. (2017) |
| | | | | Formation of human and murine organoid structures showing long-term expansion, and reproducing the molecular and histological phenotype of the endometrial epithelium. | Boretto et al. (2017) |
| | | In vitro + in vivo (mouse) | | Establishment of human organoids from endometriotic, cancerous and pre-cancerous tissues showing disease diversity and original lesions in vivo. | Boretto et al. (2019) |
| | | | | Derivation of human endometrial gland organoids from term placenta that express typical markers of glandular epithelia. | Marinic et al. (2020) |
| | | In vitro | | Glandular organization, ultrastructural features, hormone responsiveness and glycodelin A expression make human organoids a powerful in vitro model for the endometrium-embryo cross-talk. | Luddi et al. (2020) |
| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|----------------------|----------------|---------------|-----------|
| **Organoid** | | | | | |
| | | | | Derivation of human endometrial organoids from menstrual flow, comparable to those derived from endometrial biopsies. | Cindrova-Davies et al. (2021) |
| | | | | Establishment of human endometrial organoids, consisting of gland-like organoids and primary stromal cells, to model the impact of decidual senescence on embryo implantation. | Rawlings et al. (2020) |
| | | | | Co-culture of human iPSC-ESFs with placenta-derived endometrial epithelial cells generated hormone-responsive organoids in a model of human decidua. | Cheung et al. (2021) |
| | | | | Human endometrial organoids containing hES-ESC induced into EEPs and stromal components facilitated endometrial regeneration and angiogenesis in a rat model of AS. | Jiang et al. (2021) |
| | TiO2 nanoparticles | In vitro + in vivo (rat) | | | |
| | Qdot 655 ITK carboxyl QDs | In vivo (mouse) | | Murine and rat PGCs and PGC-free gonadal cells can develop and reconstruct ovari-like tissue containing functional oocytes in an ectopic xenogenic microenvironment. | Hayama et al. (2014) |
| | PDMS | In vitro | | Development of a microfluidic model of the human endometrium, compartmentalizing culture of perivascular stroma and endothelial cells. | Gnecco et al. (2017) |
| | Porous glass | In vitro | | Human endometrium on-a-chip revealed insulin- and glucose-induced alterations in the transcriptome and proteomic secretome. | De Bem et al. (2021) |
| | PDMS + fibrin gel | | | Reconstitution of a three-layer, hormone-responsive, vascularized endometrium-on-a-chip on a 3D fibrin matrix using human HUVECs, Ishikawa and ESFs. | Ahn et al. (2021) |
| **Microfluidic** | PDMS chip | Proof of concept | Development of a microfluidic device for single mouse embryo co-culture with murine endometrial cells. | Kimura et al. (2009) |
| | Resin-based porous membrane; PDMS | | | | |
| | PDMS | In vitro | | | |
| | Porous glass | In vitro | | | |
| | PDMS + fibrin gel | | | | |

**OVARY**

| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|----------------------|----------------|---------------|-----------|
| **Scaffold-free approaches** | | | | | |
| | Micro-molded agarose gel created with PDMS cast | In vitro | Human TCs self-assembled into complex spheroid, toroid and honeycomb micro-tissues. Artificial human ovary constructed at 72 h with TCs surrounding GC spheroids or COCs without stromal invasion or disruption. | Krotz et al. (2010) |
| | Ovary-like tissue | In vivo (mouse) | Murine and rat PGCs and PGC-free gonadal cells can develop and reconstruct ovary-like tissue containing functional oocytes in an ectopic xenogenic microenvironment. | Hayama et al. (2014) |
| | Qdot 655 ITK carboxyl QDs | In vivo | QDs found in the ovaries do not affect mouse behavior or estrous cycles, but decreases IVF rate. QDs can downregulate FSH and LH receptors and decrease maturation rate. | Xu et al. (2016) |
| | PDMS | In vitro + in vivo (rat) | Spheroid human PD-MSCs likely prolonged ovarian function, produced more follicles, doubled E2 levels compared to 2D culture and increased Nanos3, Nobox and Linx8 at 1 and 2 weeks. | Kim et al. (2018) |
| | PEG-PLA versus TiO2 nanoparticles | Ex vivo | FSH/LH and IGF-1 supplementation rescued initial decrease of E2/P4 with PEG-PLA nanoparticles in rat ovaries. Neonatal exposure to TiO2 nanoparticles hindered FSH/IGF stimulation. | Scsukova et al. (2020) |
| | Chitosan-based nanoparticles | In vitro + in vivo (rat) | Treatment based on curcumin-encapsulated, self-assembled nanoparticles showed positive effects in reverting the symptoms of PCOS in rats. | Raja et al. (2021) |
| **Hydrogels** | | | | | |
| | Algnate | In vitro + in vivo (mouse) | BMP4 increased number of developing porcine follicles, E2 secretion and GDF9/AMH. After xenotransplantation, hormone levels restored in ovanectomized mice and antral follicles developed. | Felder et al. (2019) |
| | | | 1.5% algnate enhanced murine secondary follicle survival and oocyte maturation, supported normal IVF and resulted in LB after ET. | Xu et al. (2006) |
| | | | Culturing multiple murine primary follicles together promoted follicle growth, rescued follicle integrity and increased tranzyonal projections and oocyte maturation. | Hornick et al. (2013) |
| | | | Co-culturing murine primary-secondary follicles with MEFs for the whole 14-day period increased survival and growth. Primary follicles had lower oocyte maturation rates than 80-μm follicles. | Tagler et al. (2012) |
| | | | 90-μm murine follicles survived twice as much as 80-μm follicles and grew on average 29 μm more. | Tagler et al. (2013) |
### Table I Continued

| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|----------------------|-----------------|---------------|-----------|
| **Hydrogels** | | | | | |
| In vivo (mouse) | | | | | |
| | | | | 70 μm murine follicles survived much more than 60 μm follicles. Ascorbic acid supplementation improved structural integrity via expression of ECM and cell adhesion molecules. | Tagler et al. (2014) |
| | | | | 0.7% alginate resulted in visible TC layer and appropriate steroidogenesis of murine follicles, as well as enhanced size, pseudoantrum rate and GVBD. | West et al. (2007) |
| | | | | 0.5% alginate increased average murine follicle diameter. Antral follicles produced appropriate levels of E2 + P4, a 34-fold increase in aromatase expression and elevated LH receptors. | West-Farrell et al. (2009) |
| | | | | Cryopreservation (by slow freezing) produced murine follicles with similar survival, average follicle diameter, antral development, decreases in Cx-43 and Cx-37 expression and increases in P4/E2 and maturation rates. | Xu et al. (2009a) |
| | | | | Cultured murine follicles had aromatase, inhibin βa, BMP15, KIT ligand, TGFβR2 expression downregulated relative to in vivo follicles, while their COCs had increased expression of Inhibin β and βa, decreased expression of BMP15, GDF9, KIT and similar expression of Figla, JAG1, Mater. | Parrish et al. (2011) |
| | | | | Murine secondary follicles mature, ovulate and luteinize in vitro. Progesterone agents (mifepristone and ulipristal acetate) significantly inhibited rupture. | Skary et al. (2015) |
| | | | | Co-culture of mouse secondary follicles and ovarian cells in 0.5% alginate increased follicle survival, diameter and P4 production, while decreasing oocyte cortical granule abnormalities. | Jamalzadeh et al. (2020) |
| | | | | Normal OSE migrated and encapsulated wounded surfaces of mouse ovarian fragments. Direct effects of fetal bovine serum and bovine serum albumin on encapsulation and proliferation. | Jackson et al. (2009) |
| | | | | SC murine ovarian grafts with the least amount of follicles had the highest survival. SC sites produced more mature oocytes. Higher embryo development rates after IVF versus ICSI. MDA-MB-231 cells encapsulated with follicles did not produce metastatic lesions. | Rios et al. (2018) |
| | | | | 0.25% alginate increased survival of rat preantral follicles, average follicle diameter, antral development, ovulation and oocyte maturation compared to 2D culture. | Zhang et al. (2019c) |
| | | | | Pre-antral canine follicles in 0.5% alginate grew faster, but had smaller diameters, and produced 5–10× less P4 than in 1.5% alginate. LH may be required to support TC differentiation and GC function. | Songasen et al. (2011) |
| | | | | 0.25% alginate produces larger and more morphologically abnormal caprine follicles but higher E2/P4, aromatase and 3βHSD, antrum formation, growth and oocyte maturation rates. | Brito et al. (2014) |
| | | | | Ovine secondary follicles cultured in 1% alginate increased COC expansion, maturation rates, mitochondrial activity and ROS as well as upregulated TFAM, ATP6/8 and downregulated KHDC3, NLRP5. | Mastroorocco et al. (2020) |
| | | | | Collecting rhesus monkey follicles during the follicular phase (versus luteal phase) significantly increased survival, and average follicle diameter. Follicles grew significantly more with FSH alone versus FSH and LH. | Xu et al. (2009c) |
| | | | | By preserving follicle viability and growth better than ethylene glycol, dimethylsulfoxide can safely be used to cryopreserve human primordial/primary follicles encapsulated in alginate. | Camboni et al. (2013) |
| | | | | E2, P4, inhibin A/B and activin A secretion patterns of human follicles in vitro mimicked in vivo serum levels. Individually cultured human primary–secondary follicles produced AMH approximately through the time of antrum formation. | Skary et al. (2015) |
| | | | | Multilayered human secondary follicles continued to grow long term. E2/P4 positively correlated with follicle development whereas AMH transiently increased during early follicle development and then declined upon antrum formation. A total of 20% oocyte maturation and MI oocyte size was similar to germinal vesicle oocyte size. | Xiao et al. (2015) |
| | | | | 1% alginate supports survival (of oocytes and GCs) and development of small pre-antral human follicles from frozen-thawed OT for a week after enzymatic isolation. | Amonin et al. (2009) |
| | | | | Human follicles in native OT remain viable for up to 24 h whereas isolated primordial follicles did not survive in 2% alginate. Encapsulating OT fragments supported antral development and surface epithelium, but not retention of follicle organization or basement membranes. | Laronda et al. (2014) |

Continued
Table I Continued

| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|-----------------------|-----------------|---------------|-----------|
| Algnate versus collagen | Alginate versus HA | In vitro + in vivo (mouse) | Human | Primordial follicles activated in vitro via phosphorylation of FOXO3a and FOXO1. | Ding et al. (2018) |
| | Collagen | In vitro (mouse) | | Transplantation of human UC-MSCs into POF mice preserved ovarian function as well as increased E2, AMH, ovarian volume, number of antral follicles, GC proliferation and CD31 expression. | Yang et al. (2019) |
| | Algnate + PLO | In vivo (rat) | | Ovarian cornets (of rat OT, GCs, TCs) restored hormone levels, in ovariectomized rats, for 90 days after transplantation. May be used as an alternative and safe cell-based hormone replacement therapy. | Sittadpady et al. (2017) |
| | Algnate versus PEG-fibrinogen ± PTEN inhibitors | In vitro | | Algnate + bpV (pic) produced significantly more atretic follicles in human OT fragments than PEG-fibrinogen. Addition of 740Y-P (versus bpV(pic)) significantly increased follicle development and E2 levels. | Lerer-Serfaty et al. (2013) |
| | Algnate versus FA versus HA | In vitro | | FA increased survival, follicle size, antral development, oocyte maturation and embryo cleavage after fertilization but did not affect E2/P4 production. | Jin et al. (2010) |
| | Algnate in growth factor-reduced Matrigel and alginate lyase microspheres | In vitro + in vivo (mouse) | Human | After in vitro culture or grafting with murine ovarian cells, beads degraded, lost spherical shape and infiltrating blood capillaries could be observed in the grafted beads. CD34+ and CD45+ cells were found around and inside the matrix. | Vanacker et al. (2012) |
| | Algnate and/or Matrigel | In vitro | Human | Small pre-antral follicles were well preserved in both groups, but encapsulation before cryopreservation improved survival and follicle size compared to cryopreservation before encapsulation. | Vanacker et al. (2013) |
| | | | | Algnate significantly improved survival (after 1 week) and follicle development in human OT, compared to Matrigel, but did not affect E2 levels. | Kedem et al. (2011) |
| | Matrigel | In vitro + in vivo (rat) | Human | Implantation of vascularized hydrogel with ovarian spheroids (made of rat GCs and TCs) in ovariectomized rats significantly aids the recovery of endocrine function, leading to full endometrial regeneration. | Yoon et al. (2021) |
| | | | | Matrigel loaded with human UC-MSCs promote GC proliferation and ovarian vasculization in a mouse model of POI. | Zhou et al. (2021) |
| | Agar versus Matrigel | In vitro | Human | Agar substrate proved to be as suitable as Matrigel on growth and development of cryopreserved thawed human follicles in OT culture. | Ghezelayagh et al. (2021) |
| | Algnate versus VitroGel | In vitro | VitroGel improved pseudoantrum formation, E2 production, COC recovery, oocyte maturation (normal spindle and chromosome alignment and low ROS and mitochondrial membrane potential), from murine pre-antral follicles, compared to algnate. | Kim et al. (2020a) |
| | FA-IPN | | FA-IPN supports murine secondary follicle survival, GC proliferation, antral formation, growth, appropriate E2/P4/Androstenedione production and improves oocyte maturation. | Shikanov et al. (2009) |
| | | | Algnate content can be <0.25% with the IPN. Growing murine secondary follicles secrete proteases, which degrade fibrin (to reduce compressive forces), mimicking their naturally dynamic microenvironment in vivo. | Shikanov et al. (2011b) |
| Strategy | Platform/biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|----------------------|-----------------------|----------------|--------------|-----------|
| Sodium alginate | In vitro + in vivo (mouse) | Encapsulated human amniotic epithelial cells or its conditioned media can protecting GC function and enhance ovarian vascularization in chemotherapy-induced POF model. | Huang et al. (2021) |
| Sodium alginate, fibrin or fibrin-HBP-VEGF | In vivo (mouse) | Murine hemi-ovaries in the fibrin-HBP-VEGF group had more primordial follicles, allowing mice to resume cyclicity earlier and conceived more rapidly. VEGF increased blood vessels at 3 weeks. | Shikanov et al. (2011c) |
| **Hydrogels** | | | | |
| HA | In vitro | HA increased murine secondary follicle survival and accelerated antral formation. Vitrified-warmed follicles encapsulated in HA had 54% MII compared to 57% in non-embedded follicles. | Desai et al. (2012) |
| | In vivo (rat) | Rats with HA+VEGF+bFGF-encapsulated ovaries maintained primordial follicles, but had shorter first estrous cycles, lower levels of E2 and c-Myc after autotransplantation. | Tavana et al. (2016b) |
| | | OT encapsulation with HA can minimize ischemia-induced follicle loss, preserve the follicular pool, promote follicular survival, facilitate angiogenesis and restore hormone levels. | Tavana et al. (2016a) |
| | | Autotransplanted vitrified OT encapsulated with HA had less intact follicles and lower FSH levels. | Taheri et al. (2016) |
| HA gel versus PLGA/MH sponge | In vitro + in vivo (mouse) | Local delivery of human ESC-MPCs increased ovarian reserves, E2 and AMH levels, improving quality of oocytes, embryos and estrous cycle regularity in a POI model. | Shin et al. (2021) |
| **Fibrinogen-thrombin** | In vivo (mouse) | Exogenous murine endothelial cells revascularized human OT grafts, improving their viability and follicle development. Cells engineered to constitutively express AMH preserved primordial follicle reserves. | Man et al. (2017) |
| | | F25/T4 and F12.5/T1 had similar vascular surface, CD45+ cells and supported murine preantral follicle recovery, survival and development. Isolated murine ovarian cells also survived and proliferated after grafting. | Luynx et al. (2014) |
| | | More murine secondary (than primordial-primary) follicles were proliferating. After 1 week, follicles had higher viability with 5–6% of follicles reaching the next developmental stage. | Chiti et al. (2016) |
| | | Dense fibrin network encapsulated murine primary follicles, maintained physiological and morphological features, improved blood vessels around secondary follicles, but not theca parameters. | Chiti et al. (2017) |
| | | Grafting of 10 or 100 human leukemia cells with ovarian stroma (artificial ovary) was insufficient to cause leukemia after 20 weeks, while grafting with 3 x 10^6) cells produced peritoneal masses at 4 weeks and systemic disease. | Soares et al. (2015) |
| | **Human STEMPRO AD-MSCs** | Increased partial pressure of oxygen, surface area of human CD34+ vessels, follicle survival and decreased apoptosis after xenotransplantation. | Manavella et al. (2018) |
| | **Human STEMPRO AD-MSCs** | Protected follicle reserves by modulating the PI3K/Akt pathway to maintain quiescence of primordial follicles. | Cacciottola et al. (2021) |
| | | The combination of human ovarian graft embedding in fibrin clots and host treatment with simvastatin resulted in improved post-implantation outcomes in a mouse model. | Magen et al. (2020) |
| | **Fibrin** | F50/T50 best mimics native human OT (based on fiber thickness, porosity and rigidity). | Chiti et al. (2018) |
| | | F100/T4 had highest proliferation rate and least variable apoptosis, but F25/T4 and F12.5/T1 had uniform cell distribution, better homogeneity, human ovarian stromal cell density and reproducible fibrin degradation. | Luynx et al. (2013) |
| | | Initial survival of murine primordial follicles decreased but follicles developed and ovulated. Ovarian function confirmed by reduction in FSH and daily vaginal cytology. | Smith et al. (2014) |
| | | >75 μg/ml bFGF improved survival, increased proliferation and protected primordial follicles (but did not affect primary and secondary follicles), in murine hemi-ovaries, with increased revascularization. | Gao et al. (2013) |
| Strategy | Platform/ biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|-----------------------|----------------------|----------------|---------------|-----------|
| **Hydrogels** | | | | | |
| | | | Cryopreserved human preantral follicles, isolated and encapsulated in fibrin matrices (with or without HA) survive and grow for 7 days after xenografting in mouse. | Paulini et al. (2016) |
| | | | Fibrin collagen hydrogels with murine MSCs restored cyclicity earlier but delayed follicle development in mouse OT. VP-MSC increased expression of AMH, FSH receptor, GDF9 and VEGF while BM-MSC increased expression of Pch1. | Mehdiá et al. (2020) |
| Laminin versus Matrigel | In vitro | Culturing human OT using laminin components of the native ovarian ECM enhanced follicle survival and proportion of secondary follicles compared to Matrigel. | Hao et al. (2020) |
| bFGF sheet | In vivo (mouse) | Transplanting bFGF sheets (which released bFGF) with frozen-thawed human OT increased revascularization and follicle density (primordial and primary), but decreased fibrosis. | Tanaka et al. (2018) |
| | | | | | |
| Chitosan-Silk fibroin | In vitro | Development of a novel in vitro model by encapsulating human ovarian stromal cells in chitosan-silk hydrogels. | Jafri et al. (2021) |
| | | | 5% hydrogel supported murine secondary follicle survival and antral development. Follicle morphology quickly diminished and deteriorated in >10% PEG solution. The YKNR plasmin substrate degraded rapidly, but supported antral formation and oocyte maturation. | Shikanov et al. (2011a) |
| | | | >10% hydrogel supported murine antral formation, but reduced oocyte maturation (compared to 5–7.5% hydrogel). Parthenotes with highest pronuclear and blastocyst formation in 10% hydrogel. | Ahn et al. (2015) |
| | | | Antral and mature preovulatory follicles, functional blood vessels and corpora lutea (indicated successful ovulation) after endotoxine transplant of murine preantral and primary follicles. A total of 60% of follicular reserve maintained at day 60. | Kim et al. (2016) |
| | | | Although it took twice as long, murine ovaries in Dual PEG capsules produced the greatest number of cycling mice (and functional tissue), in addition to preventing sensitization and lymphocytic infiltration. | Day et al. (2019) |
| | | | Sequestered cell-secreted ECM proteins loaded in PEG hydrogel improved murine early secondary follicle survival, growth and oocyte maturation. | Tomaszewski et al. (2021) |
| | | | | | |
| **dECM and polymer scaffolds** | | | | | |
| Proof of concept | | | Comparison of three protocols of murine ovarian decellularization by agitation. | Alshakhi et al. (2019) |
| Proof of concept + in vitro | | | Comparison of protocols for de- and re-cellularization of murine ovaries proposed sodium deoxycholate as the best detergent for this application. | Alshakhi et al. (2020) |
| | | | Rapid cell adhesion and aggregation of homologous fibroblasts, consistent with porcine ovarian scaffold’s ability to sustain cell adherence, proliferation and differentiation. | Pennarossa et al. (2020) |
| | | | SDS-T A DC scaffolds had intact ECM components/microstructure, reduced residual DNA and supported fibroblast viability and recovery of murine preantral follicles. DC human cortex had smaller pores and denser collagen fibrils compared to the bovine ovary. | Nikiniaz et al. (2021) |
| DC ovary | In vitro + ex vivo + In vivo (rat) | DC porcine ovary caused minimal immunogenic response after SC xenotransplantation in rats and showed an improvement in E2 secretion ex vivo. | Liu et al. (2017) |
| | | | Comparison of protocols for decellularization of human ovary and successful recellularization with human endometrial mesenchymal cells. | Sistani et al. (2021) |
| | | | Germline stem cells (isolated through magnetic activated cell sorting) can repopulate DC porcine ovarian scaffolds and differentiate into adult mature ovarian cells when stimulated. | Pennarossa et al. (2021b) |
| | | | Porcine ovarian ECM sustained in vitro cell survival and drove epigenetically-erased cell differentiation, fate and viability. | Pennarossa et al. (2021a) |
| | | | DC bovine/human OT scaffold recellularized with murine primary ovarian cells and transplanted to initiate puberty in mice that had been ovariectomized. | Laronda et al. (2015) |
| | | | Peritoneum-derived MSCs in human OT scaffolds can produce germ cell markers (DAZL) after 1 week in vitro, and GDF9+ follicle-like structures 1 month after transplantation. | Eivazkhani et al. (2019) |

Continued
| Strategy | Bioprinting | Organoid | Microluidic |
|----------|-------------|-----------|-------------|
| dECM and polymer scaffolds | DC amniotic membrane | Bovine ovary and uterus ‘tissue papers’ | Alginate |
| | In vitro | In vivo (mouse) + clinical | In vitro |
| | | | |
| | Intact human amniotic membrane increased murine primary–secondary follicle survival, size, E2 production, survival index and expression of Cx37, GDF9 and BMP15. | DC ovarian ‘tissue paper’ supports murine follicle adhesion, viability and health in vitro, as well as maintains viability and hormonal function of primates and human OT ex vivo for 8 weeks postmortem. | Fibrin encapsulation enhanced murine primordial–primary follicle survival, integration with the host tissue and resumption of estrous cycling. LBs achieved with follicles in VEGF-loaded fibrin beads. |
| dECM and polymer scaffolds | DC SIS | Collagen versus SIS | FA versus fibrin-collagen |
| | In vivo (rabbit) | In vivo (mouse) | In vivo (mouse) |
| | Using porcine SIS to reconstruct ovarian resection reduced adhesion score and improved ovarian volume and epithelization in rabbit. | Human OT wrapped in human recombinant collagen improved grafting in mice, compared with porcine SIS. | Fibroblast development detected in human OT after 8–10 weeks and 6–8 antral follicle count achieved by 11–14 months. FSH normalized by 7 months. Embryos cryopreserved after 7–8 IVF cycles. Both patients achieved CPs after ET and one had LB. |
| Bioprinting | ORMOCER versus SUB | Porcine gelatin ‘ink’ | GelMA |
| | In vitro | In vivo (mouse) | |
| | ORMOCER did not improve doubling times or damage DNA, but forms gap junctions. Applying a two-photon polymerization to Ormocomp allows adherence to vertical/steep surfaces and layer formation after 3–4 days. | 30° and 60° scaffolds provide corners that surround murine multilayered secondary follicles on multiple sides while 90° scaffolds have an open porosity that limits follicle-scaffold interaction. Transplant restored ovarian function and LB achieved. | Cell-laden 3D printing of artificial ovaries supported follicle development and produced MII oocytes after IVM. |
| | | | |
| Organoid | Matrigel | Alginate | Alginate versus collagen |
| | Generation of organoids from dissociated human female gonad cell suspensions in a three-layered Matrigel-based system. | Static conditions produced larger primordial and supported primordial–primary follicles. | Oxidized alginate does not support murine early secondary follicle survival. More antral follicles developed in collagen (versus alginate) core. |
| | | 10 μl/min flow systems supported primordial–primary cat follicle transition and initial growth (D0–3), and dog follicle growth (but not normally). Preantral dog follicles had the highest growth rate in normal alginate beads (antral follicles grew the least). | Tp53R273H-mutated murine FTE (but not OSE) cells radially migrated out of corisal inclusion cysts. Number of invading cells and invasion distance enhanced by follicular fluid but worsened by collagen I. |
| | | | |
| | PDMS | | Germinal vesicle-stage murine COCs denuded by passing through a microchannel (without hyaluronidase). Dynamic conditions improve oocyte maturation and glutathione, developmental competence and blastocyst formation. |
| Strategy | Platform/ biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|-----------------------|-----------------------|----------------|--------------|-----------|
| **FALLOPIAN TUBE** | | | | | |
| Hydrogels | DC oviduct | In vitro | | Rabbit embryos cultured on oviductal ECM hydrogel-coated wells presented a ‘quieter’ metabolism compared to embryos cultured under standard conditions. | Francés-Herrero et al. (2021) |
| | Algnate | Ex vivo | | Co-culture of human FTE and murine secondary follicles revealed crosstalk in the reproductive cycle. | Zhu et al. (2016) |
| | Matrigel-based 3D culture platform | In vitro | | Establishment of long-term organoid cultures from mouse FTE cells. | Xie et al. (2018) |
| | Mebiol | | | Distal regions of human FTE showed increased organoid forming capacity, Wnt/inflammatory signaling and high-grade serous carcinoma signatures compared to proximal regions. | Rose et al. (2020) |
| | PDMS + Nuclepore chip | | | Co-culture of human FT-MSCs, HUVECs and FTE cells formed organoids that could be blocked by Wnt inhibitor DKK1. | Chang et al. (2020) |
| | | | | Use of human iPSCs to establish a novel in vitro 3D human FTE organoid model. | Yucer et al. (2017) |
| Organoid | | | | Establishment of long-term, stable 3D organoid cultures from human FTE that respond to E2 and P4 treatment in a physiological manner. | Kesler et al. (2015) |
| | | | | 3D human fimbriae cultures retained tissue architecture and epithelial subtypes, responding to H2O2 and insulin exposure. | Eddie et al. (2015) |
| | | | | Murine FTE stem cells formed organoid colonies in a PEG-based 3D culture system, with some cells differentiating into secretory or ciliated cells. | Lin et al. (2021) |
| CERVIX-VAGINA | | | | Bovine oviduct-on-a-chip supported more physiological (in vivo-like) zygote genetic reprogramming than conventional IVF. | Ferraz et al. (2018) |
| Scaffold free | Cell constructs | In vitro + in vivo (mouse) | Human vaginal tissue was bioengineered using a self-assembly technique, which formed mature vaginal epithelium and matrix after in vivo animal implantation. | Jakubowska et al. (2020) |
| | Self-assembly | | | Generation of a 3D human cervical model using ectocervical epithelium built on a cervical stromal equivalent (that resembles native ECM). | De Gregorio et al. (2017) |
| | Air–liquid interface | In vitro | | Generation of a 3D herpes simplex virus 2 infection model using human vaginal epithelial cells that reproduce basal and apical layers and shows pathological effects after virus inoculation. | Zhu et al. (2017) |
| Hydrogels | Silk-HA | Ex vivo | Development of an ex vivo pregnant-like tissue model (with human cervical tissue and fibroblasts) to assess silk-based hydrogels-mediated cervical augmentation. | Raia et al. (2020) |
| | Collagen derivative | In vivo (rat) | Collagen derivative T16 hydrogel improved autologous collagen arrangement, cell proliferation and vaginal epithelium thickness in ovariectomy rat model. | You et al. (2020) |
| | Chitosan-thioglycolic acid | In vivo + in vivo (rat) | Genistein-loaded chitosan-thioglycolic acid hydrogel has high mucoadhesive properties and partially recovered the epithelial thickness of atrophic murine vagina. | Yang et al. (2021) |
| | DC vagina | In vivo (rat) | Porcine acellular vagina matrix promoted tissue-engineered vagina reconstruction in a rat model of partial vaginectomy. | Zhang et al. (2017a) |
| | DC ectocervix | In vivo | Generation of a porcine vaginal ECM scaffold that allows attachment and growth of AD-MSCs and vaginal epithelial cells. | Greco et al. (2018) |
| | | | Development of three human ectocervical tissue models: (I) de- and recellularized ectocervix; (II) co-culture of ectocervical and ovarian explants; (II) cell based ectocervix construct. | McKinnon et al. (2020) |
| Strategy | Platform/ biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|-----------------------|-----------------------|-----------------|---------------|-----------|
| dECM and polymer scaffolds | | | | | |
| DC Porcine SIS | In vivo (rhesus monkey) | Clinical | | | |
| | | | | | |
| De-epidermized dermis | | Proof of concept | | | |
| RENOV | | Clinical | | | |
| Collagen | In vitro | | | | |
| | In vivo (rat) | Clinical | | | |
| | | | | | |
| PEG | Ex vivo + in vivo (mouse) | | | | |
| | | | | | |
| Alginate + chitosan | | In vitro | | Development of an alginate/chitosan membrane that is stable in a simulated human vaginal environment and with the ability of releasing metronidazole over time. | Mamel et al. (2016) |
| Alvetex | | In vitro | | Generation of 3D human endocervical model that responds to E2 and P4 during a 28-day culture. Treatment with mifepristone attenuated the inhibition of IL-1β and LIF secretion. | Arslan et al. (2015) |
| Silk | | | | Treating human cervical-like constructs with P4 decreased collagen and increased the softness of the ECM over 28 days. | House et al. (2018) |
| PLA and compact polyurethane membrane | | Proof of concept | | Fibran glue could successfully adhere a PLA and polyurethane blyer membrane to human cervical tissues. The membrane provides a fluid barrier and can be inserted through the cervix. | Roman et al. (2018) |
| Oxidized cellulose | | Clinical | | Vaginal reconstruction using oxidized cellulose proved to be a safe and effective procedure, with minimum complications and good success rates. | Dashwal et al. (2010) |
| Bioprinting | | | | | |
| PACIENA prosthesis + Interceed | In vitro + in vivo (rat) | Clinical | | Good anatomical and functional results were achieved using 3D printed PACIENA prosthesis for vaginoplasties without skin grafts. | Acién et al. (2019) |
| DC vagina bioink | | Clinical | | Human BM-MSCs could differentiate in 3D vagina tissue printed with ECM bioink of DC porcine vagina, inducing vascularization and epithelization in vivo. | Hou et al. (2021) |
| Polyetherurethane | | In vitro | | 3D-printed cervical implants supported HUVECs adhesion and growth, allowing for controlled loading and release of anti-human papillomavirus protein. | Zhao et al. (2020) |
| Polyurethane + clotrimazole | | In vitro | | 3D-printed clotrimazole-loaded vaginal ring sustained drug release over 7 days and displayed a complete C. albicans growth inhibition after 5 days. | Tiboni et al. (2021) |

Continued
screened for eligibility (based on exclusion criteria presented in Fig. 2) and 584 (5.6%) full-text papers were retrieved for detailed assessment. An additional 24 studies were retrieved from manual searching of citations. We classified studies by bioengineering strategy within each or- gan of differentiation; COC, cumulus-oocyte complex; Collplant, human recombinant virgin collagen bioengineered in tobacco plant lines; CP, clinical pregnancy; Cy37/43, connexin 37/43; Cyr61, cysteine-rich angiogenic inducer 61; DaZi, deleted in azoospermia like; DKK, Dickkopf WNT Signaling Pathway Inhibitor 1; D2, estradiol; ECM, extracellular matrix; EPFC, endometrial epithelial progenitor cell; eMSC, endometrial mesenchymal stem cell; EnSC, endometrial stem cell; ESC, embryonic stem cell; ESC-MPC, embryonic stem cell-derived mesenchymal progenitor cell; ESF, endometrial stromal cells; DKK, Dickkopf WNT Signaling Pathway Inhibitor 1; E2, estradiol; ECM, extracellular matrix; EEPC, endometrial epithelial progenitor cell; eMSC, endometrial mesenchymal stem cell.

FULL TRACT

| Strategy | Platform/ biomaterial | Type of study (model) | Disease-related | Main findings | Reference |
|----------|-----------------------|-----------------------|----------------|--------------|-----------|
| **Organoid** | Matrigel | In vitro | Development of human organoids from the squamocolumnar junction region of the uterine cervix, along with the emergence of metaplasia. | Manu et al. (2020) |
| | Cultrex RGF-BME type 2 | In vitro + in vivo (mouse) | Establishment of human ecto- and endocervical 3D organoids that stably recapitulate physiological and carcinogenic traits, growing as xenografts in mice. | Lohmussaar et al. (2021) |
| **Microfluidic** | PDMS | In vitro | Development of an organ-on-chip of the cervical epithelial layer that can recapitulate the human ecto- and endocervical epithelial regions. | Tantengco et al. (2021) |

Description of the main bioengineering findings in the female reproductive system in the last 21 years based on different platforms, biomaterials, type of study (in vivo models) and gynaecological-related diseases. To note: model in type of study column only refers to in vivo approach; studies with human cells/tissues are marked in bold, while clinical studies are highlighted in yellow; and pill icons indicate studies carried out with patients, animal models and biological samples with female reproduction-related diseases (established cell lines have not been taken into account).

Table I

| Disease-related | Reference |
|----------------|-----------|
| Derivation of human organoids from the squamocolumnar junction region of the uterine cervix, along with the emergence of metaplasia. | Manu et al. (2020) |
| Establishment of human ecto- and endocervical 3D organoids that stably recapitulate physiological and carcinogenic traits, growing as xenografts in mice. | Lohmussaar et al. (2021) |
| Development of an organ-on-chip of the cervical epithelial layer that can recapitulate the human ecto- and endocervical epithelial regions. | Tantengco et al. (2021) |

Bioengineering tools in female reproductive medicine: systematic summary of the evidence

Below we summarize studies using the six bioengineering techniques in work related to female reproductive organs.
**Scaffold-free approaches**

This section mainly contemplates studies based on the non-matrix-assisted self-organizing capacity of cells to generate multicellular entities. Six studies presented scaffold-free approaches applied to bioengineering of the uterus and its tissues, including four *in vivo* studies based on cell sheets (Kuramoto et al., 2015, 2018, 2020; Sun et al., 2018) and two *in vitro* investigations based on the generation and study of epithelial and stromal organoids (Murphy et al., 2019; Wiwatpanit et al., 2020). Of the eight studies involving scaffold-free approaches in ovary, one used a cell-based method involving primordial germ cells to generate ovarian tissue (*OT*) *in vivo* (Hayama et al., 2014), four developed self-assembled spheroids (Krotz et al., 2010; Chowanadisai et al., 2016; Kim et al., 2018; Ward Rashidi et al., 2019), while toxicity of Qdot 655 ITK carboxyl quantum dots (Xu et al., 2016) and nanoparticles made with chitosan (Raja et al., 2021), polyethylene glycol (PEG) and polylactic acid or titanium dioxide (Scsukova et al., 2020), were tested in preclinical and *ex vivo* models, respectively. Scaffold-free approaches to develop bioengineered vaginal and cervical tissues were included in four studies, which applied self-assembled vaginal constructs *in vivo* (Orabi et al., 2017; Jakubowska et al., 2020) or air–liquid interface techniques to generate vaginal and cervical *in vitro* models (De Gregorio et al., 2017; Zhu et al., 2017).

**Hydrogels**

This review unveiled a plethora of different hydrogel-based research involving uterine cells or tissues. Of the 40 studies compiled, 65%, 30% and 5% used natural, synthetic or hybrid hydrogels, respectively. The most commonly used natural hydrogels were based on collagen (46.2%), Matrigel (24.4%) and hyaluronic acid (HA; 15.4%). Synthetic hydrogels were based predominantly on PEG (38.5%) and poloxamer (30.7%). Studies applied these approaches for *in vitro* modeling of disease, tissue cross-talk and differentiation (Schutte et al., 2015; Cook et al., 2017; Pence et al., 2017; Stejskalová et al., 2021), *in vivo* regeneration of the endometrium and myometrium (Lin et al., 2012; Yang et al., 2017; Li et al., 2019; Yoon et al., 2021; Lin et al., 2021a) and the treatment of intrauterine adhesions (Müller et al., 2011; Liu et al., 2020).
Encapsulation of follicles and tissue fragments is the most exploited hydrogel-based application in ovarian bioengineering. Alginate (alone or in combination with other materials) is the most commonly implemented hydrogel, with use in 47.1% of studies evaluating hydrogels for in vitro cultures of murine (Xu et al., 2006, 2009a; West et al., 2007; West-Farrell et al., 2009; Jackson et al., 2009; Jin et al., 2010; Parrish et al., 2011; Tagler et al., 2013, 2014; Skory et al., 2015; Zhang et al., 2019c), ovine (Mastrorocco et al., 2020), and human (Amorim et al., 2014), ovine (Mastrorocco et al., 2007; West-Farrell et al., 2014; Skory et al., 2015; Xiao et al., 2015) ovarian follicles and tissue. Natural and synthetic hydrogels such as laminin (Hao et al., 2020), collagen (Joo et al., 2016), fibrin (Smith et al., 2014; Paulini et al., 2016), Matrigel (Xu et al., 2009b; Kedern et al., 2011; Ghezelayagh et al., 2021), VitroGel (Kim et al., 2020a), HA (Desai et al., 2012) or PEG (Shikanov et al., 2011a; Lerer-Serfaty et al., 2013; Tomaszewski et al., 2021) are less frequently reported.

Twenty-four studies used hydrogels in preclinical models of IVF (Xu et al., 2006), allo-/xenotransplantation of ovarian cells, follicles and fragments (Vanacker et al., 2012) or ovarian function restoration (Su et al., 2016; Ding et al., 2018; Yang et al., 2019; Yoon et al., 2021) (Table I); seven applied hydrogels in oncological modeling and drug testing (Supplementary Table SIV).

Tissue-specific hydrogels based on rabbit oviductal dECM and alginate resulted, respectively, in in vitro models of embryo culture (Francés-Herrero et al., 2021a) and crossstalk between human epithelium and murine follicles (Zhu et al., 2016), or ex vivo models of the human fallopian tube fimbriae (Eddie et al., 2015). Hydrogels based on silk-HA, collagen derivatives and chitosan could recreate an ex vivo pregnant-like human cervical model (Raia et al., 2020), or treat vaginal atrophy in vivo (You et al., 2020; Yang et al., 2021).

Decellularized extracellular matrix and polymer scaffolds

Forty-five uterus-related studies evaluated dECM and polymer scaffolds in cytocompatibility and in vitro modeling experiments, as well as in vivo regeneration and anti-adhesions tests (Table I). Those based on decellularized (DC) matrices accounted for 46.6% of reports, while 33.3% and 20% used scaffolds based on purified natural or artificial polymers, respectively. Various studies isolated and evaluated tissue-specific ECM from whole uteri of rats (Hellström et al., 2014, 2016; Miyazaki and Maruyama, 2014; Santosio et al., 2014; Miki et al., 2019; Padma et al., 2021a,b), mice (Hiraoka et al., 2016), pigs (Campos et al., 2017), rabbits (Yao et al., 2020b) and sheep (Daryabari et al., 2019; Tiemann et al., 2020; Padma et al., 2021c); from rabbit (Campos et al., 2019), pig (López-Martínez et al., 2021a,b) and human endometrium (Olalekan et al., 2017; Sargazi et al., 2021); and from rat and human myometrium (Young and Goloman, 2013). Natural polymer scaffolds included alginate (Li et al., 2011b; Stern-Tal et al., 2020), HA (Demirbag et al., 2005; Liu et al., 2005) and gelatin-coated (Su et al., 2014; Edwards et al., 2015; Cai et al., 2019) materials, while artificial polymer scaffolds were created with polyglactin (Young et al., 2003), polylactide (Pensabene et al., 2015; Mukherjee et al., 2019), polyglycolic acid (Magalhães et al., 2020), emulsion-templated highly porous materials (Eissa et al., 2018; Richardson et al., 2019) and polytetrafluoroethylene fluoropolymers (Kuperner et al., 2020).

In contrast, only dECM or natural polymers were used for 24 studies involving ovarian research. Several reports described the generation and in vitro/in vivo biocompatibility of DC murine (Alshaikh et al., 2019, 2020), porcine (Liu et al., 2017; Pennarossa et al., 2020, 2021a,b), ovine (Evazkhiani et al., 2019), bovine (Laronda et al., 2015; Nikniaz et al., 2021) and human (Hassanpour et al., 2018; Pors et al., 2019; Sistani et al., 2021) ovaries, as well as porcine small intestine submucosa (Celik et al., 2009; Abir et al., 2020) or human amniotic membrane (Motamed et al., 2017).

Of the 18 studies that assessed scaffolds for cervicovaginal applications, 33.3% were clinical studies of vaginal reconstruction using oxidized cellulose (Dadhwal et al., 2010), bovine collagen matrices (Noguchi et al., 2004; Guerette et al., 2009), human acellular dermis (Zhang et al., 2017c) or porcine small intestine submucosa (Raya-Rivera et al., 2014; Zhang et al., 2019b). The remaining studies consisted of either in vitro cervico-vagina models based on DC porcine vaginas (Greco et al., 2018), human cervical dECM (McKinnon et al., 2020) and scaffolds of collagen (Hjelm et al., 2010), alginate/chitosan (Tentor et al., 2020), silk (House et al., 2018), Alvetex (Arslan et al., 2015) and polylactide (Roman et al., 2018) or preclinical models of vaginal repair (Maiel et al., 2016; Zhang et al., 2017a; Ye et al., 2020; Ma et al., 2021).

Bioprinting

The scarcity of bioprinting implementation in reproductive studies reflects the novelty of this technique. Two reports applied 3D bioprinting technology to in vitro studies of uterine contractility (Souza et al., 2017) and in vivo endometrial regeneration using a gelatin-alginate hydrogel loaded with human induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs) (Li et al., 2020). Similarly, three studies applied bioprinting with natural polymers for in vitro ovarian modeling (Ovsianikov et al., 2007), follicle culture (Wu et al., 2022) or fertility restoration in a preclinical model (Laronda et al., 2017). Finally, five groups bioprinted bacterial vaginal rings (Tiboni et al., 2021), vaginal tissue with acellular bioink (Hou et al., 2021), PACIENA prosthesis for vaginoplasties (Acién et al., 2019), cervical implants (Zhao et al., 2020) or 3D models of cervical cancer (Gospodinova et al., 2021).

Organoids

We identified 15 studies generating endometrial organoids. Matrigel was primarily used as a supportive matrix, with (Francés-Herrero et al., 2021b) or without (78.5% of studies) endometrial dECM supplementation. Other studies used collagen (Abbas et al., 2020), or functionalized PEG matrices (Hernandez-Gordillo et al., 2020). Remarkably, in six studies, epithelial and stromal components were combined to create organoids (Murphy et al., 2019; Wivatpanit et al., 2020; Jiang et al., 2021) or assemblies (Blauer et al., 2005; Abbas et al., 2020; Rawlings et al., 2021), which simultaneously represent both endometrial populations. Ovarian spheroids or organoids were derived in 18 studies using Matrigel, Cultrex or agarose, together with diverse cell types, including ovarian epithelial cells (Kwong et al., 2009; Kopper et al., 2019), granulosa and theca cells (Yoon et al., 2021) or embryonic gonads (Oliver et al., 2021). Remarkably, 89% of the studies generated ovarian organoids, derived from patients with cancer, that reliably mimicked the original pathology (Mur et al., 2019). Similarly, in five studies, human organoids were derived from the squamocolumnar junction region of the uterine cervix (Maru et al., 2020) or ecto- and endocervical tissue using Matrigel or Cultrex (Lechanteur et al.,
et al., 2017; Chumduri et al., 2021; Löhmusaa et al., 2021; Tanaka et al., 2021). Fallopian tube organoids were generated from murine (Xie et al., 2018) and human (Kessler et al., 2015; Rose et al., 2020; Zhang et al., 2021a) fallopian tube epithelial cells used alone or co-cultured with umbilical vein endothelial cells and fallopian tube-derived MSCs (Chang et al., 2020), and from human iPSC (Yucer et al., 2017) or murine fallopian tube epithelial stem cells (Lin et al., 2021b). Culture was supported by Matrigel (in five of these six studies) or Mebiol (Lin et al., 2021b). Organoid studies accounted for 58%, 13% and 13% of bioengineering platforms reported in the fallopian tubes, ovary and uterus, respectively.

Microfluidic systems

Microfluidic platforms can be used to model static, passive (created by gravity) or active dynamic (with a determined flow rate) conditions. Six studies applied microfluidics to create advanced in vitro models of the human endometrium (Astolfi et al., 2016; Gnecco et al., 2017, 2019; Ahn et al., 2021; De Bern et al., 2021), or co-culture embryos with endometrial cells (Kimura et al., 2009). Of the 13 microfluidic-based studies in the ovarian field, two applied dynamic follicle culture in alginate and/or collagen matrices (Choi et al., 2014; Nagashima et al., 2018), while two studies evaluated the mechanical effect of flow on oocyte denudation and maturation (Sadeghzadeh Oskouei et al., 2016; Weng et al., 2018). The rest of the studies sought to recreate oncological models, evaluate drug effectiveness or elucidate therapeutic targets (as we discuss in detail in the applications sections). Finally, four studies applied microfluidics to model the human cervical epithelial layer (Lin et al., 2017; Aziz et al., 2020; Yang et al., 2020; Tantengco et al., 2021) and bovine (Ferraz et al., 2018) and canine (Ferraz et al., 2020) oviducts, while one study modeled human uterine and ovarian endocervical crosstalk (Park et al., 2020), and two studies recreated a complete female tract in microfluidic systems, combining endometrial, ovarian, oviductal, cervix and liver tissue to model the hormonal profile of a menstrual cycle (Xiao et al., 2017) or to manipulate, fertilize and culture embryos in a single device (Han et al., 2010).

Preclinical models and clinical applications: an update

Bioengineering approaches can elucidate the normo- and pathophysiology of female reproductive organs by developing next-generation in vitro/ex vivo platforms, creating representative models for toxicology/drug screening, developing alternative therapeutic strategies, discovering new biomarkers and improving tissue/organ regeneration and/or transplantation protocols.

Development of next-generation in vitro and ex vivo platforms

The creation of in vitro platforms that faithfully reproduce the physiological and pathological states of higher organisms is of paramount importance in applied and translational research. Bioengineering platforms have provided novel 3D models of follicle culture (see citations in Hydrogels section), human embryo implantation (Wang et al., 2012, 2013; Buck et al., 2015; Stern-Tal et al., 2020; Rawlings et al., 2021), a three-layered endometrium that remained functional for 28 days (Park et al., 2021), endometrial cancer invasion (Park et al., 2003) and wound healing (Stavreus-Evers et al., 2003), as well as bidirectional crosstalk between the uterus and the ovaries (Park et al., 2020). Moreover, collagen scaffolds loaded with human epithelial and endothelial cells (Pence et al., 2015) or tissue slices (Muruganandan et al., 2020) respond to ovarian hormones, while collagen-embedded human stromal cells demonstrate decidualization changes (Schutte and Taylor, 2012) and contractile ability (Kim et al., 2020b). Notably, endometrial cells encapsulated in a PEG hydrogel with ECM-binding peptides remodeled the synthetic matrix and displayed hormone-mediated differentiation (Cook et al., 2017).

In vitro follicle growth produced developmentally competent murine oocytes (Xu et al., 2006; Ahn et al., 2015) that led to live births (LBs) after embryo transfer (Xu et al., 2006). Most studies (94%) implemented individual follicle culture, while others successfully demonstrated that culturing multiple follicles together substantially improves follicle survival (>80% versus <29% with individual culture) and oocyte maturation (Hornick et al., 2013; Brito et al., 2016). Interestingly, IVFG benefits from the addition of ECM sequestering peptides (Tomaszewski et al., 2021), ascorbic acid (which increases expression of ECM and cell adhesion molecules (Tagler et al., 2014)), bone morphogenetic protein 4 (Felder et al., 2019), mouse embryonic fibroblasts (Tagler et al., 2012), ovarian cells (Jamalzadeh et al., 2020) and human menstrual blood MSCs (Rajabi et al., 2018), but not denuded oocytes, oocyte-secreted factors, granulosa cells (Hornick et al., 2013) or leukemia inhibitory factor (Youins et al., 2017). VitroGel, a novel animal-origin free hydrogel, also improves IVFG parameters, outperforming alginate and producing competent oocytes in a recent study by Kim et al. (2020a).

Furthermore, when used exclusively, alginate concentrations ranged between 0.25% and 3% (Supplementary Table SIII); however, as a fibrin-alginate interpenetrating network, the concentration of alginate can be reduced below 0.25%, providing a more realistic environment for follicle growth and improving oocyte maturation (Shikanov et al., 2009, 2011b). Notably, combinations of 25 mg/ml fibrinogen and 4 IU/ml thrombin or 12.5 mg/ml fibrinogen and 1 IU/ml thrombin, are suggested as the best scaffolds for human ovarian stromal cells in vitro (Luyckx et al., 2013) and for murine follicle development in vivo (Luyckx et al., 2014), while 50 mg/ml fibrinogen and 50 IU/ml thrombin best mimic the rigidity of the native human ovarian cortex (Chiti et al., 2018).

DC models also offer important advances by maintaining unique tissue-specific ECM milieus, not only providing the most realistic scaffold for each organ’s endogenous cell types, but also remarkably acting as a biocompatible framework for cells from other tissues/species. For example, DC mouse uterine tissue is an adequate natural niche for human menstrual blood MSC differentiation toward uterus-specific cell lineages (Arezzo et al., 2021), DC sheep uterus stimulates rat fetal dorsal root ganglion regeneration and angiogenesis during chicken embryo development (Padma et al., 2021c) and solubilized porcine endometrial decellularized ECM enhances proliferation rates of human endometrial organoids (Francés-Herrero et al., 2021b).

The generation and increasing use of organoids are revolutionizing the field of reproductive medicine. Among other limitations, the primarily epithelial nature of these structures is noteworthy. To date, several investigations have already provided models of multicompartment tissues [i.e. endometrial and stromal compartments (Murphy et al., 2019; Rawlings et al., 2021)], ecto- and endo-cervical epithelial regions (Maru et al., 2020; Chumduri et al., 2021; Löhmusaa et al., 2021) and heterogeneous tumors (see next paragraph and/or Supplementary Table SIV), in addition to research models for
chlamydia (Bishop et al., 2020) and herpes (Zhu et al., 2017) infections. Remarkably, organoids are able to reproduce specific uterine (Boretto et al., 2019; Bishop et al., 2020; Hernandez-Gordillo et al., 2020; Luddi et al., 2020; Marinin et al., 2020), ovarian (described in detail below) and cervical (Karolina Zuk et al., 2017; Maru et al., 2020; Löhnuusaaar et al., 2021) tissue phenotypes, as well as respond to hormones (Blauer et al., 2005; Boretto et al., 2017; Turco et al., 2017; Wiwatpanit et al., 2020; Cheung et al., 2021). These models can be established from patient biopsies (Maru et al., 2019; Löhnuusaaar et al., 2021), biological fluids (Cindrova-Davies et al., 2021) or cell lines (e.g. SKOV3, HO8910, OVCAR3/4/8 used in oncological studies listed in Supplementary Table SIV). Further transplantation of spheroids or organoids may restore ovarian function (Kim et al., 2018) or promote endometrial regeneration (Jiang et al., 2021).

Next-generation platforms for oncological studies include the development of 3D ovarian cancer models using scaffolds of bacterial cellulose with chitosan (UL-Islam et al., 2019), collagen (Zheng et al., 2015), poly-ε-lactide-coglycolide-PEG (Zhou et al., 2018) or RADA16-I peptide hydrogel (Song et al., 2020). Similarly, a novel 3D cervical cancer model was created with 3D-printing, using bioinks mixed with sodium alginate (Gospodinova et al., 2021). Dynamics of cancer progression can be modeled ex vivo in 3D (Ajeti et al., 2017; Fleszar et al., 2018; Loessner et al., 2019; Flont et al., 2020; Fan et al., 2021), utilizing multilayered microfluidic systems (Lin et al., 2017; Flont et al., 2020) and ovarian spheroids (to study macromolecular crowding (Bascetin et al., 2021)).

Unique ex vivo and in vitro proof of concept applications include, DC bovine ovarian and uterine ‘tissue papers’ (Jakus et al., 2017), an in vitro artificial human ovary (Krotz et al., 2010), a pregnant-like cervix (Raia et al., 2020), an endocervical model that responds to hormones during a 28-day cycle (Arslan et al., 2015), automated and reliable oocyte denudation on a chip (Weng et al., 2018) and the EVATAR platform that models the dynamics of the human menstrual cycle (Xiao et al., 2017).

Realistic in vitro toxicology and drug screening models
Bioengineered in vitro platforms enable evaluation of the biocompatibility of biomaterials (Xu et al., 2016; Scsukova et al., 2020), effects of chemical toxicants [such as doxorubicin (Zhou et al., 2015; Aziz et al., 2020), or dioxin (Park et al., 2020)] or response to cancer therapies (Supplementary Table SIV). For example, 3D tumor models in ring format currently support automated and rapid personalized drug screening (Phan et al., 2019). Other drug screening models include microdissected tumor tissues in microfluidic culture (Astolfi et al., 2016) or alginate hydrogels (Salas et al., 2020), organoids of small cell neuroendocrine carcinoma of the uterine cervix (Tanaka et al., 2021) and ovarian cancer organoids, which have proven to be excellent models to test chemotherapy drugs (Maru et al., 2019; de Witte et al., 2020; Maenhoudt et al., 2020). In fact, since endometrial and ovarian organoids can be derived from each patient’s biopsies (Kopper et al., 2019; Nanki et al., 2020; Bi et al., 2021; Chen et al., 2021; Espedal et al., 2021), they reflect specific tumor heterogeneity and are ideal for drug pre-screening and the development of personalized treatment regimens. Notably, ovarian cancer spheroids exhibited increased tumorigenicity and proportion of cancer stem cells after several passages (Ward Rashidi et al., 2019); chemoresistant cancer stem cells can also be generated with 3D culture of CD44+CD117+ cells (Chen et al., 2014). Fallopian tube organoids are similarly suitable for developing combination therapies for high-grade serous ovarian cancer (Zhang et al., 2021a) while multicellular spheroids derived from these cancer patients’ malignant effusions enable drug screening (Chen et al., 2020).

Further, recent applications of drug-loaded hydrogels (Jamal et al., 2018; Cabral-Romero et al., 2020) and microfluidic conditions (Ran et al., 2019; Saha et al., 2020; Yang et al., 2020) evaluated targeted cytotoxicity. HA-carboxymethyl cellulose spheroids facilitate the study of ovarian cancer persistence (Picaud et al., 2014), and ovarian constructs enable evaluation of metastatic potential of leukemic cells that could have infiltrated OT (Soares et al., 2015).

New therapeutic biomarkers and clinical strategies
The organs and tissues of the female reproductive system are not only arguably the application for which bioengineering is most recognized. In the reproduction field, many trials are underway to understand ovarian and uterine biology and disease, with the ultimate goal to provide a means of improving follicle survival and development (Camboni et al., 2013; Vanacker et al., 2013).

Tissue and organ regeneration or transplantation
The complete or partial regeneration of damaged tissues and organs is arguably the application for which bioengineering is most recognized. In the reproduction field, many in vivo studies have tested hydrogels and scaffolds for uterine regeneration (Supplementary Table SII). Among them, polylactide nanofilm can seal defects smaller than 3 mm in chorion-amnion and uterine membranes (Pensabene et al., 2015), while degradable polylactic acid-co-poly(e-caprolactone)-gelatin nanofiber meshes with endometrial MSCs promote tissue integration via an anti-inflammatory response (Mukherjee et al., 2019). Heparin-poxolamers hydrogels (Xu et al., 2017a;b; Zhang et al., 2017b, 2020b), collagen hydrogels or scaffolds loaded with bone marrow MSCs (Ding et al., 2014), basic fibroblast growth factor (bFGF; Li et al., 2011a), embryonic stem cell-derived endometrium-like cells (Song et al., 2014).
vascular endothelial growth factor [VEGF (Lin et al., 2012)] or human umbilical cord-derived MSCs [UC-MSCs (Xin et al., 2019; Liu et al., 2020)] and stromal cell-derived factor-1α-loaded chitosan-heparin hydrogel (Wenbo et al., 2020) repaired morphology and restored the function of injured rat uteri. Further, improved uterine regeneration, and some restoration of fertility with successful implantations, pregnancies and LBs is achievable via transplantation of DC human amniotic membrane loaded with adipose stem cells (Han et al., 2020) or oral mucosal epithelial cells (Chen et al., 2019), DC uterine matrix (Santoso et al., 2014; Hellsström et al., 2016; Hiraoka et al., 2016; Miki et al., 2019; Li et al., 2021), or DC endometrial ECM hydrogel loaded with growth factors (López-Martínez et al., 2021b) (Supplementary Table S1). Similarly, gelatin methacrylated and sodium-alginate scaffolds with bFGF (Cai et al., 2019), MSC-laden Matrigel microspheres (Xu et al., 2021), hydrogel-encapsulated decidualized endometrial stromal cells (Kim et al., 2019), HA hydrogels (Liu et al., 2019), HA-collagen hydrogels with endometrial stem cells, stromal cells and vessel cells (Park et al., 2021), PEG-based hydrogels (Wang et al., 2021) or polyglycerol sebacate) scaffolds seeded with bone marrow-MSCs (Xiao et al., 2019) also successfully regenerated a damaged endometrium.

One reproductive disorder prompting a search for an effective tissue regeneration treatment is AS, an acquired iatrogenic disorder characterized by adhesions within the uterine cavity or cervix. To date, generation 4.0 in female reproduction

one womans reproductive disorder is the development of uterine adhesions after cesarean delivery or other surgical procedures. The development of uterine adhesions can lead to infertility or recurrent miscarriages. To address this issue, researchers have investigated various approaches to prevent or treat uterine adhesions. One approach involves the use of hydrogels and scaffolds as adhesion barriers.

Hydrogels and scaffolds provide some advantages in models of premature ovarian failure (POF) or premature ovarian insufficiency (POI). For example, human amniotic epithelial cells encapsulated within sodium alginate biglass protect granulosa cell function and ovarian vascularization in a chemotheraphy-induced POF model (Huang et al., 2021). Similarly, transplant of human UC-MSCs embedded in Matrigel promotes granulosa cell proliferation and ovarian vascularization (Zhou et al., 2021), and adipose-derived stem cells in a collagen scaffold restore ovarian function in POI models (Su et al., 2016). Notably, local delivery of embryonic stem cell-derived mesenchymal progenitor cells to the injured ovarian tissue can also prevent post-operative adhesions, while hydrogels made of PEG also prevent post-operative adhesions, while hydrogels made of PEG also prevent post-operative adhesions, while hydrogels made of PEG also prevent post-operative adhesions, while hydrogels made of PEG also prevent post-operative adhesions, while hydrogels made of PEG also prevent post-operative adhesions, while hydrogels made of PEG also prevent post-operative adhesions, while hydrogels made of PEG also prevent post-operative adhesions.
in a HA gel increases the ovarian reserve, and estrogen and AMH levels, ultimately improving the quality of oocytes and embryos in mice that model POI (Shin et al., 2021).

Bioengineered materials and techniques can also be implemented during reconstructive gynecological surgeries. Recently, vaginal reconstruction was successful in a patient with MRKH syndrome, a rare congenital disorder characterized by abnormal uterine and vaginal development despite normal ovarian function and external genitalia; this approach used a DC porcine small intestine submucosa scaffold (Zhang et al., 2019b). Remarkably, this biomaterial achieves structural and functional vaginas for up to 8 years (Raya-Rivera et al., 2014). Similarly, vaginoplasty with an acellular dermal matrix (called RENOV) is safe and effective, and results in an anatomically correct vagina that provides near-normal sexual function (Zhang et al., 2017c). Neovaginas were also safely constructed using Surgicel (an oxidized cellulose scaffold) for 10 patients (Dadhwal et al., 2010), or bovine-derived dermis scaffold for another patient (Noguchi et al., 2004).

### Discussion

#### Summary of the evidence: where do we stand?

The organs of the female reproductive system—the uterus, ovaries, fallopian tubes, cervix and vagina—work together to provide the hormonal and anatomical support necessary for the generation of offspring. As such, reproductive health is susceptible to a number of negative congenital or acquired factors, restricting fertility and quality of life. These concerns prompt a large field of research into the underlying biology as well as approaches for preventing or treating various pathologies. However, ethical and technical limitations around using and/or transplanting human tissues for research purposes requires that most studies are conducted in vitro or in vivo using animal models. While valuable, these approaches face inherent limitations in translatability, such as the complexity of recreating the anatomy, physiology and interactions of reproductive organs using classical 2D in vitro models, in addition to the differences between species. Thus, bioengineering has become indispensable for creating representative and reliable 3D models (for both in vitro and in vivo uses) as well as providing alternative applications for regenerative medicine. This review’s systematic compilation of the extensive bioengineering advances in the context of the female reproductive system since 2000, provides a global overview of the different techniques, their pre-clinical testing and/or clinical applications and the anticipation of future trends.

#### Uterus

The uterus, and in particular the endometrium, is fundamental for implantation and maintenance of pregnancy (Govermin et al., 2021). As such, much research is devoted to the creation of functional endometrial models and combining endogenous endometrial cell populations in different formats and biomaterials (Table I). Notable among these are paracrine models of epithelial and stromal cell co-culture (Schutte et al., 2015; Park et al., 2021), as well as models of decidualization (Schutte and Taylor, 2012; Gnecco et al., 2019), implantation (Park et al., 2003; Wang et al., 2012, 2013; Buck et al., 2015), vascularization (Pence et al., 2017), ECM interactions (Cook et al., 2017) and uterine contractility (Kim et al., 2020b). In recent years, several groups attempted to recreate the complexity of these models with organoids or assembloids (Boretto et al., 2017; Turco et al., 2017; Murphy et al., 2019; Abbas et al., 2020; Rawlings et al., 2021), which offer an apparently unlimited potential to recreate the physiological and pathological states of the endometrium (Boretto et al., 2019). In fact, organoid technology is marking a turning point in endometrial-related research. Despite having been described only 5 years ago, more than 13% of the uterus-related articles reported in this study exploit this technology. Remarkably, although most biomaterials attempt to mimic ECM interactions in vitro, only a few studies notably implement native ECMs (Young and Goloman, 2013; Olalekan et al., 2017; Campo et al., 2019; Arezzo et al., 2021; López-Martínez et al., 2021a; Francés-Herrero et al., 2021b).

Absolute uterine factor infertility can be treated with uterine transplantation (UTx). Taking into account scientific (Brännström et al., 2021) and media reports, as well as personal communications, we currently estimate that more than 40 UTx have been achieved from over 80 UTx procedures that have been performed thus far. The surgical success rate (defined by a viable organ within 3 months, resumption of regular menstruations within a year, successful pregnancy and LB) was 78% and 64% for live and deceased donor UTx procedures, respectively, and the cumulative LB rates in surgically successful UTx procedures were estimated to be above 80%. Despite these promising success rates, this procedure involves an invasive surgery and associated risks. Bioengineering has been used to mitigate these risks by providing alternative clinical applications. Specifically, bioengineering techniques for the uterus focus predominantly on preventing/reducing adhesions, often associated with AS (Zhao et al., 2017; Cao et al., 2018; Zhang et al., 2021b) and related to uterine factor infertility. In these and other cases of endometrial damage, the main therapeutic objectives are to regenerate tissue structure (e.g. recover endometrial thickness, angiogenesis) and consequently restore function, which ultimately allows the uterus to support implantation and carry a pregnancy to term (Hellström et al., 2016; Kuramoto et al., 2018; Li et al., 2019; Liu et al., 2019; Wang et al., 2021). Toward this end, different hydrogels and scaffolds show potential in vivo, by regenerating injured uteri in rodent models (Supplementary Table SII). Emerging technologies, such as 3D bioprinting and microfluidics, remain under-utilized in research applied to uterine health, but promising possibilities exist for both in vitro modeling (Ahn et al., 2021; De Bern et al., 2021) and in vivo tissue regeneration (Ji et al., 2020).

#### Ovary

The ovaries exert two main functions, namely to tightly regulate folliculogenesis so as to avoid premature depletion of oocytes, and to produce sufficient sex hormones (e.g. estrogen and progesterone) to support decidualization, pregnancy, breast development for lactation and even bone health (Sittadjody et al., 2017). Developing new IVFG platforms opens opportunities for oncological patients who cannot benefit from current fertility preservation strategies (specifically, OT cryopreservation) due to risk of reintroducing malignancy upon autologous re-transplantation. Culturing follicles/OT in vitro ‘bypasses’ this risk and can produce mature oocytes faster than if the OT was xenografted into a murine model [usually in 8–12 days (Supplementary Table SIII) versus weeks-months (Oktay et al., 2016)], but does not have the potential to restore endocrine function. Most IVFG studies...
we included in this review successfully cultured secondary follicles to the antral stage, and some even recovered mature and competent oocytes (Supplementary Table SIII). Few groups have ventured into culturing primary follicles because these follicles tend to have lower survival and oocyte maturation rates (Tagler et al., 2012, 2014; Smith et al., 2014).

The success of IVFG is not only affected by initial follicle size, but also by the saturation of the biomaterial. Physiologically, the rigidity of the ovarian cortex and the ‘sponginess’ of the medulla play important roles in regulating folliculogenesis. In fact, the mechanical forces of the ovarian cortex ECM may maintain reserves of primordial follicles, only releasing a couple of follicles to grow in the medulla every menstrual cycle (Choi et al., 2014). Nonetheless, although softer/more flexible biomaterials, such as alginate, Matrigel and VitroGel, could facilitate follicle expansion, materials that are too soft (i.e. 1 mg/ml collagen, fibrin alone or HA-Matrigel, rapidly degrading YKNR plasmin substrate) cannot provide the necessary 3D support, causing granulosa cells to erroneously proliferate and migrate into their surroundings (Shikanov et al., 2009, 2011b; Desai et al., 2012; Joo et al., 2016). In contrast, saturated/rigid matrices [i.e. 1.5% alginate (West-Farrell et al., 2009)] hinder follicle growth. Although OT transplantation has led to more than 200 human LBs so far (Dolmans et al., 2021), encapsulating OT before transplantation may provide additional benefits by promoting revascularization, decreasing fibrosis, protecting follicles from “burn-out” (ischemia-induced death of follicles during the first couple of days after transplant), and ultimately, providing the best microenvironment for follicle development in vivo. However, in attempts to standardize OT transplantation or replacement and be able to offer these strategies to a broad population (e.g. oncological patients and/or those in need of hormone replacement therapy), the construction of an artificial ovary containing immature stimulable follicles is gaining momentum and could lead the way for the next decade. Another common ovarian bioengineering application with great potential is the development of heterogeneous and/or patient-derived organoid models to evaluate individual drug response and cancer dissemination (Supplementary Table SIV).

Fallopian tubes

Fallopian tubes (or oviducts) are the anatomical structures that connect the ovaries and the uterus, providing the space and physiological environment for fertilization and early embryo development. Few bioengineering methods exist to date to recapitulate fallopian tubes and their associated functions in vitro, despite their crucial supportive role during early embryo development. Derivation of human fallopian tube organoids from different cell types (Kessler et al., 2015; Lin et al., 2021a) provided an important breakthrough in the creation of functional in vitro models. Among the few other fallopian tube studies in the bioengineering field, some demonstrate the important cross-talk between the ovaries and the fallopian tubes (Zhu et al., 2016), or the direct effect of oviductal ECM molecules on embryonic metabolism (Francés-Herrero et al., 2021a). Microfluidic platforms, with their small channels, may be the most suitable for modeling the physiology and pathology (Ferraz et al., 2020) of this tubular organ. Indeed, the implementation of a bovine oviduct-on-a-chip led to improved IVF outcomes (Ferraz et al., 2018).

Cervix and vagina

The cervix and vagina play critical roles in reproduction by serving as an entryway for sperm during ovulation, physical barriers for infectious microorganisms and a pathway during childbirth. Bioengineering these tissues has provided novel multilayered organoid models to study herpes (Zhu et al., 2017) and cervical cancer (Tanaka et al., 2021), also enabling testing of their respective treatments. Although a functional vagina can be created by self-dilation of the vaginal dimple in a majority of patients with MRKH syndrome, vaginal sleeves are used for reconstructive surgeries (Noguchi et al., 2004; Dadhwal et al., 2010; Zhang et al., 2017c, 2019b; Acién et al., 2019). Other bioengineering alternatives may prevent premature rupture of fetal membranes and incontinence (Roman et al., 2018), or test contractility inhibitors with bioprinted uterine rings (Souza et al., 2017). Moreover, hydrogels can be used as carriers for antibiotics, antivirals, antifungals, contraceptives and other drugs (Dos Santos et al., 2020).

Full tract

Female reproductive function is orchestrated by multiple autocrine, paracrine and endocrine dialogues, which so far have only been studied in vivo in model organisms that cannot accurately reproduce the human body. To overcome the limitations of these models, there exists the need to recreate a multiorgan environment that incorporates physical, mechanical and hormonal variables. Microfluidics offers the most promising bioengineering method, having already enabled the development of an organ system-on-a-chip that combines human liver spheroids, mouse ovarian explants, human fallopian tube epithelium, human endometrium and human cervix tissues to physiologically model a 28-day menstrual cycle (Xiao et al., 2017). Recently, the endocrine crosstalk between the uterus and the ovary has been modeled on-a-chip, to be able to evaluate the effects of reproductive toxicants (Park et al., 2020). Another application rarely exploited to date is the possibility of combining, in a single microfluidic platform, a major portion of the workflow in assisted reproduction clinics, thereby minimalizing altering the environmental conditions to which gametes and embryos are exposed (Han et al., 2010).

Future perspectives

New 3D in vitro models representing multiple cell types and/or tissue layers are not only helping to elucidate the physiological dynamics of complex biological processes within the reproductive tract (e.g. those that regulate folliculogenesis, ovulation, decidualization and cancer progression), but also improving personalized medicine (Stejskalová et al., 2021). In particular, organoids generated in 3D culture can adequately mimic healthy and diseased cell-cell and cell-ECM native tissue interactions, making them ideal models for evaluating individual drug response (for cancer, endometriosis, dysmenorrhea, hormone disorders or other related issues, bacterial/viral/fungal infection, etc.) or implantation potential (Wei et al., 2021). However, organoid models, especially endometrial ones, have unresolved issues, which the scientific community has started, and should continue, to investigate. Among others, the main limitations are: the lack of expandable organoid lines with stromal and immunological components; the inaccessibility to the organoid lumen; the lack of interactions with native ECM components; and the variability associated with patient tissue origin and culture handling. Automated ‘lab-on-a-chip’ technologies that can rapidly screen various
bodily fluids (e.g., blood, ascites or pleural fluid, urine) for specific biomarkers, cancer cells; drugs or oocytes may also efficiently and reliably refine future clinical/therapeutic decisions. Since body-on-a-chip platforms have the potential to model hormone dynamics and systemic disease (e.g., PCOS, diabetes, cancer), combining them with organoid or organ culture and ECM-based environments may provide more robust 3D models for genetic/epigenetic and pharmacokinetics testing.

Much remains to be achieved for the field to create (and eventually offer) a completely artificial female reproductive system. Nevertheless, recent advances in the creation of an artificial ovary (Krotz et al., 2010; Chiti et al., 2016; Sittadjody et al., 2017; Jafari et al., 2021; Yoon et al., 2021; Wu et al., 2022), uterus (Souza et al., 2017; Ji et al., 2020; Li et al., 2021; Park et al., 2021), cervix (Arslan et al., 2015; De Gregorio et al., 2017; Zhao et al., 2020) and vagina (Orabi et al., 2017; Hou et al., 2021) have made promising headway toward this incredible goal. For example, the development of alternative, more natural options for hormone replacement therapies offers promise for mitigating menopause-associated problems (Sittadjody et al., 2017; Yoon et al., 2021). In the race to manufacture transplantable tissues and organs, 3D bioprinting has played a discreet role so far, accounting for only 3% of the studies included in this review. Specifically, its relative novelty, limited accessibility among research groups worldwide and lack of standardized protocols and technology could be slowing down its take-off, making it an attractive and necessary niche for investment. Studies focused on bioengineering of the fallopian tubes are scarce, since their functions are bypassed in assisted reproduction clinics. However, recent work demonstrates that an artificial oviduct-on-a-chip may substantially improve IVF and early embryo culture systems by providing a more realistic microenvironment (Ferraz et al., 2018). Moreover, these anatomical structures are the target of numerous studies to develop alternative contraceptive methods. Among these, artificial hydrogels based on styrene maleic anhydride (Subramanian et al., 2019) and PEG (Mclemore et al., 2005) offer promise as contraceptive approaches through successful testing in the fallopian tubes of rats and rabbits. Finally, we note the need for greater clinical translation in reproductive bioengineering. Despite the large number of proposals described at the preclinical level, only 5% of the studies compiled in this review are clinical. Advances at the legislative level, meta-analyses to establish optimal procedures, and stronger networks of collaboration between laboratories and medical centers, could be of value.

**Limitations**

This systematic review identified a wealth of bioengineering-related studies in the context of female reproduction. Nonetheless, it is possible that relevant studies were not found or were excluded because of the keyword selection, subjective nature of the filtering process or reference limit. We compiled the 312 articles that we considered the most significant and representative of the current state of the field. There is an additional limitation in terms of classification of the articles by biomaterial, since the literature lacks consensus in delineating certain hydrogels and scaffolds (e.g., collagen was reported as a hydrogel and scaffold), and some studies combined bioengineering techniques (e.g. organoid or culture with hydrogel/scaffold within a microfluidic system). Therefore, we classified articles, on a case-by-case basis, in a way we deemed most appropriate. Since the original Embase search identified numerous oncology-related studies, additional searches with keywords representing reproductive diseases were conducted to ensure appropriate coverage of the latter. Finally, due to different organs under consideration and divergences in study objectives and designs, the included studies exhibit wide heterogeneity that precluded meta-analysis of the results.

**Conclusion**

Female reproduction is regulated by complex networks of molecular, endocrine and tissue/organ interactions. As such, substituting the entire female reproductive tract will be challenging; however, interdisciplinary work provides novel insight into the physicochemical properties necessary to support and achieve these biological processes. Advances in reproductive bioengineering technologies have redefined the landscape of fertility-restoring strategies and therapeutic options that are, or soon could be, available to patients. These translational endeavors provide substantial promise for effective treatments for a wide range of reproductive system pathologies.

**Supplementary data**

Supplementary data are available at *Human Reproduction Update* online.

**Data availability**

The data underlying this article are available in the article and in its online supplementary material.

**Authors’ roles**

Conceptualization: I.C., E.F.-H., R.L., M.H., L.d.M.-G., S.H., M.B. and A.P.; systematic literature search, selection and data curation: E.F.-H. and R.L.; data review: I.C., E.F.-H. and R.L.; manuscript and figure preparation: I.C., E.F.-H., R.L. and L.d.M.-G.; manuscript review: I.C., M.H., L.d.M.-G., S.H., M.B. and A.P. All authors have agreed to the published version of the manuscript.

**Funding**

This study was supported by Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union (Fondo Social Europeo «El FSE invierte en tu futuro») [PI17/01039-PI21/00305-CP19/00149 (I.C.), CP19/00141 (S.H.)]; Spanish Ministry of Science, Innovation, and Universities [FPU18/06327 (E.F.-H.); Generalitat Valenciana [GRISOLIAP/2018/029 (R.L.), PROMETEO/2018/137 (I.C., S.H., L.d.M.-G., and A.P.)].

**Conflict of interest**

The authors declare no conflict of interest.
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