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Biomaterial strategies to replicate gynecological tissue

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Women's health is an important and understudied area of research. The current standard of care for many gynecological diseases such as cancer or autoimmune-linked disorders such as endometriosis is surgery; however, the underlying mechanisms of action of many gynecological diseases are poorly understood. The field of tissue engineering has the potential to transform the field of women's health by developing *in vitro* models of healthy and diseased tissue that could be used to identify novel treatment strategies as well as gain a better understanding of complex signaling dynamics. Identification of the appropriate biomaterials, cell types, and stimuli (the tissue engineering triad) needed to build these *in vitro* models can be gleaned by interrogating the underlying extracellular matrix, cell organization, and soluble factors present in the tissue. In this review, we provide a general overview of the biology and components of the major tissues that make up the female reproductive system (ovaries, fallopian tubes, the uterus, and cervix) as well as a comprehensive survey of the different biomaterials that have been chosen to build *in vitro* models of these tissues. Furthermore, for each tissue, we recommend guiding principles in the design of *in vitro* models and discuss their potential to be used in drug screening and mechanistic studies.

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1. Introduction

The female reproductive system's main organs include the ovaries, fallopian tubes, uterus (cervix and corpus), and vagina, illustrated in Fig. 1. Together, they are responsible for providing hormonal support, producing ova, and maintaining a pregnancy to term, with all of these functions depending on the dynamic interactive physiology of various gynecological tissues.[1] Gynecological disorders are a source of significant suffering. In the U.S., between 2012 and 2016 approximately 94 000 women were diagnosed with gynecologic cancer, and 1 400 000 women visited the emergency department with gynecological complaints.[2] Among the most common gynecological complaints were pelvic diseases, such as endometriosis and polycystic ovarian syndrome, and gynecological cancers such as ovarian cancer, uterine sarcomas, endometrial cancer, and cervical cancer.[3] The current standard of care for the majority of gynecological diseases is surgery, chemotherapy, and radiation.[4–7] Table 1 describes the different treatment options available for the gynecological diseases covered in this review. However, the underlying mechanisms of action are often poorly understood, and there are limited personalized standards of care for patients with metastatic cancer due to its heterogeneous manifestations.[8–10] While the removal of reproductive organs may address the symptoms resulting from a gynecological diseases, this carries substantial consequences to the endocrine system.[8–10] Beyond fertility, the endocrine system has an essential role in the development and maintenance of tissue structure[11] and regulates gene expression for numerous biological processes[12] Consequentially, removing the tissue disrupts the endocrine system and can have detrimental effects on women's health. Furthermore, some diseases, such as uterine sarcomas, respond poorly to conventional chemotherapy and radiotherapy.[13] Thus, there is a need for tissue-engineered models of reproductive tissue in order to gain a better understanding of disease progression, screen potential therapies,[14,15] or restore damaged tissues.[16–20]

In vitro preclinical models are being increasingly explored as alternatives to conventional animal models as they are faster and less expensive.[21] Cells cultured in two dimensions (2D) on tissue culture plastic are widely used for *in vitro* studies. However, they fail to resemble the *in vivo* tissue.[22] In contrast, three dimensional (3D) *in vitro* models provide a better approximation of the *in vivo* tissue by providing a third dimension of biophysical cues. 3D culture models range from cancer cell spheroids, cell-seeded 3D scaffolds,[23–25] cells embedded in hydrogels,[26–28] microfluidic chips,[29] cell patterning,[16,30] and organoids.[20] The goals of 3D culture models are to mimic the microenvironment, interrogate the

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effect of the extracellular matrix (ECM), and provide an alternative to animal models of human disease.

The ECM plays a vital role in cell behavior.[31] It is responsible for providing mechanical and structural support to cells and tissues, as well as controlling different cell functions such as cell cycle, morphogenesis, apoptosis, and migration.[32] Cell–ECM and cell–cell interactions influence fundamental cell behaviors related to the function of the whole organs. Fig. 2 illustrates the most common components found in the ECM of each of the gynecological tissues discussed in this review. 3D culture systems that incorporate biomaterials are essential for studying the role of the ECM in healthy tissue homeostasis as well as disease progression.[26,33–36] Furthermore, recreating *in vitro* tissue requires biological matrices with specific characteristics and tunable properties that can provide an appropriate condition for the attachment, growth, proliferation, and signaling of different types of cells. A guiding principle in the design of 3D culture environments is the accurate presentation of the various signals (growth factors, hormones, ECM, mechanics, and others) in tunable microenvironments that will allow multiple cell types to grow. Specifically, gynecological tissues are highly dynamic, plastic, with continuously changing ECM due to hormone-responsive processes such as menstruation, pregnancy, and menopause. Thus, it is difficult to model the native tissue *in vitro*. However, numerous biomaterials have been evaluated in different *in vitro* models of female reproductive tissues to interrogate the cell response to hormone stimuli and the role of the ECM.[23,56] The selection of which biomaterial to use in an *in vitro* model depends on each tissue's characteristics, the disease, and the specific hypothesis being addressed.

In this review, we provide a general overview of the tissues that make up the female reproductive system and a description of different biomaterials used in 3D *in vitro* models to simulate healthy tissues and disorders affecting women's health. There is a specific emphasis on the guiding principles for designing 3D culture environments and their potential to be used as tools to look for alternative treatment strategies.

2. Building *in vitro* models with biomaterials

Biomaterials currently used in *in vitro* models are constructed from either natural polymers or synthetic polymers.[56,57] Natural biomaterials can provide similar biological cues to those found in the body.[34,58,59] However, they can be difficult to precisely control in terms of spatiotemporal cues or substrate stiffness.[60,61] In contrast, synthetic polymers provide more experimental control but need to be modified to provide biological cues.[26,32,62,63]

Among the most used natural biomaterials are collagen and Matrigel. Collagen is the most abundant ECM constituent; it corresponds to approximately 30% of the total mammalian protein mass.[64] The collagen family consists of 28 collagen types (I–XXVIII), where collagen type I is the main structural protein in the interstitial ECM,[65] and collagen type IV is a crucial component for observed differences in shape, structure, and function of cells.[64,66] Due to its bioavailability, role in cell–ECM interactions, and association with disease progression, collagen I is a frequently used biomaterial in 3D *in vitro* models.[32,67,68] Moreover, collagen I is hydrophilic and has a porous structure. These properties enable the diffusion of nutrients and oxygen, allowing cells to attach and grow.[67] However, collagen I hydrogels have some limitations. Collagen hydrogels are limited in protein concentration by the biological sources available, and without chemical modification, it is difficult to decouple protein concentration and substrate stiffness.[36] Additionally, collagen alone may not provide sufficient biochemical cues to induce cells to respond as they would *in vivo*.[69]

Matrigel is another commonly used natural matrix that promotes cell attachment and proliferation across a wide range of cell types.[34] Matrigel is a tumor-derived product extracted from Engelbreth–Holm–Swarm mouse sarcomas comprised of basement membrane components.[70] It is widely used for *in vitro* adhesion, invasion, and capillary formation assays as it provides cells with ECM and growth factor cues present in many tissues.[71] Moreover, Matrigel constituent proteins stimulate cell–matrix interactions and induce differentiation.[72] Matrigel hydrogels have biomimetic cues that provide a suitable environment to support cell adhesion and allow the diffusion of nutrients.[34,73–75] However, Matrigel does not contain high concentrations of some ECM components such as collagen type I and hyaluronan, limiting its ability to mimic *in vivo*

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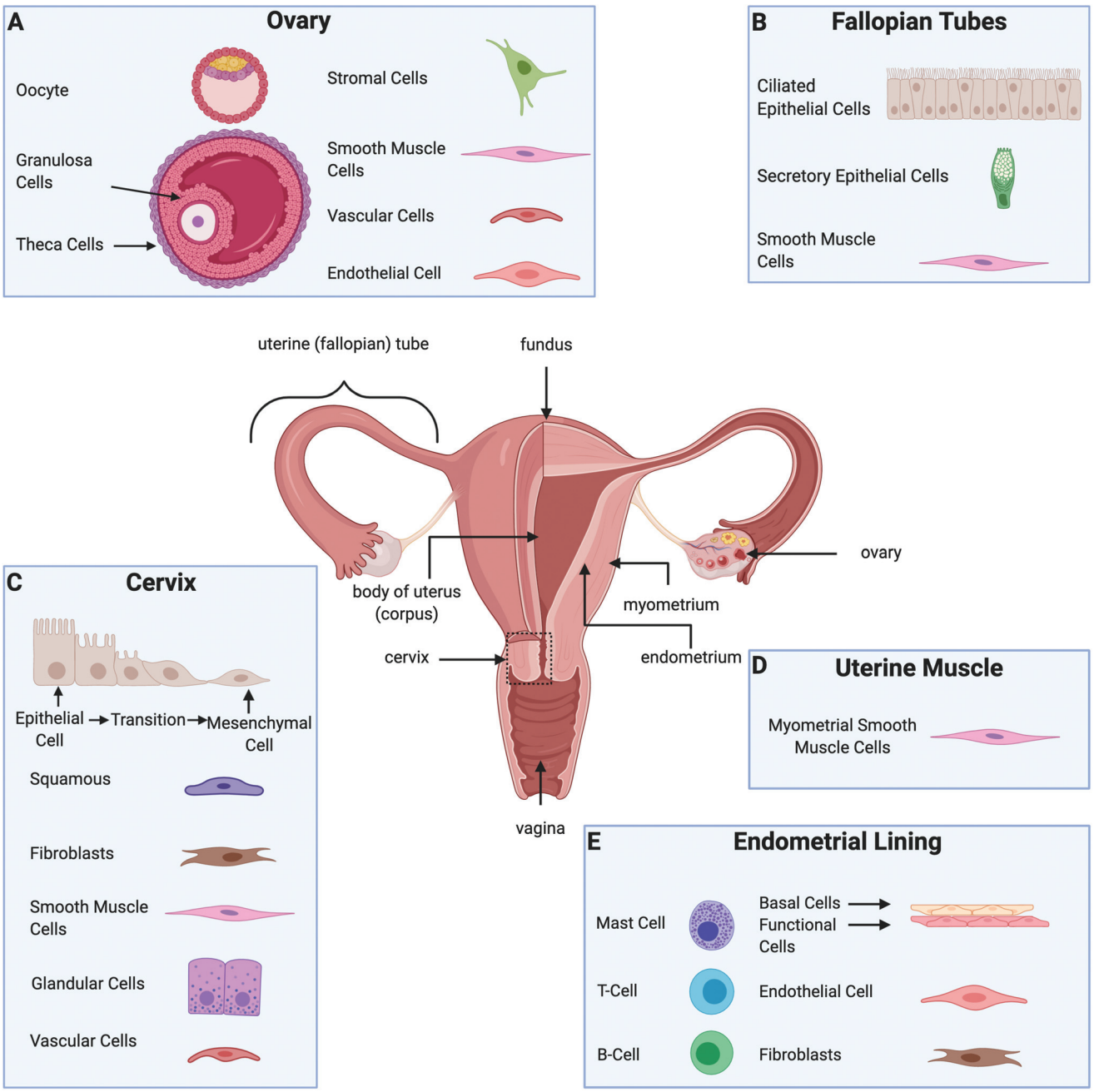

Fig. 1 Description of the gynecological tissue and predominant cells. (A) Cells found in the ovary. (B) Cells included in the fallopian tubes. (C) Cells at the cervix. (D) Predominant cells in the uterine muscle. (E) Cells in the endometrial lining. Figure created with BioRender.com.

tissue.[60] Matrigel, when combined with other biomaterials such as collagen type I, can improve the simulation of gynecological tissue and tumor models.[34] For example, Park *et al.* found that the combination of collagen type I and Matrigel simulated the architecture and physiology of the native endometrial tissue.[76] Nevertheless, Matrigel still has limitations due to its low batch-to-batch reproducibility, which creates uncertainty in cell-culture experiments.[60]

Other natural polymers include alginate,[77] gelatin,[78] chitosan,[79] fibrin,[80] hyaluronic acid,[81] and decellularized matrices.[16] These natural biomaterials are biocompatible and can be used to replicate specific types of ECM. However, they often lack mechanical integrity, and are frequently blended with other polymers. The limitations of natural biomaterials have driven the search for synthetic alternatives.[60]

Synthetic biomaterials are an alternative to natural biomaterials. Among the most common are polyethylene glycol (PEG) and PEG copolymers such as poly(L-lactide) (PLLA), poly(D,L-lactide-*co*-glycolide) (PLGA), and poly(-caprolactone) (PCL). However, synthetic polymers also have limitations due

Table 1 Current treatments for gynecological diseases

Tissue	Pathology	Current treatments available	Ref.
Ovary	Polycystic ovarian syndrome	Oral Contraceptive treatments Metformin for adolescents (weight loss, improves ovulation) Anti-androgens Estrogen-projection pills (improves hyperandrogenism) Spironolactone (decrease excessive hair growth-androgen receptor blocker)	4–7
	Ovarian cancer	Surgical resection of tumors (debulking) Chemotherapy (platinum-based-regimen) Hysterectomy and bilateral salpingo-oophorectomy Omental biopsy and/or omentectomy PARP inhibitors Lymphadenectomy	37–40
Endometrial lining	Endometriosis	Surgical excision of lesions Ablation of lesions Lysis of adhesions Hormone therapy	41 and 42
	Endometrial cancer	Bilateral salpingo-oophorectomy Lymphadenectomy Radiation Chemotherapy Hormone therapy (progestins) Hysterectomy (simple or radical)	10, 43–46
Uterine muscle	Uterine sarcomas	Surgical resection of tumors Hysterectomy (and bilateral salpingo-oophorectomy) Hormonal therapy Chemotherapy Radiation	10, 47–50
Cervix	Cervical cancer	Immunotherapy Chemoradiation Hysterectomy (simple or radical and bilateral salpingo-oophorectomy)	51–55

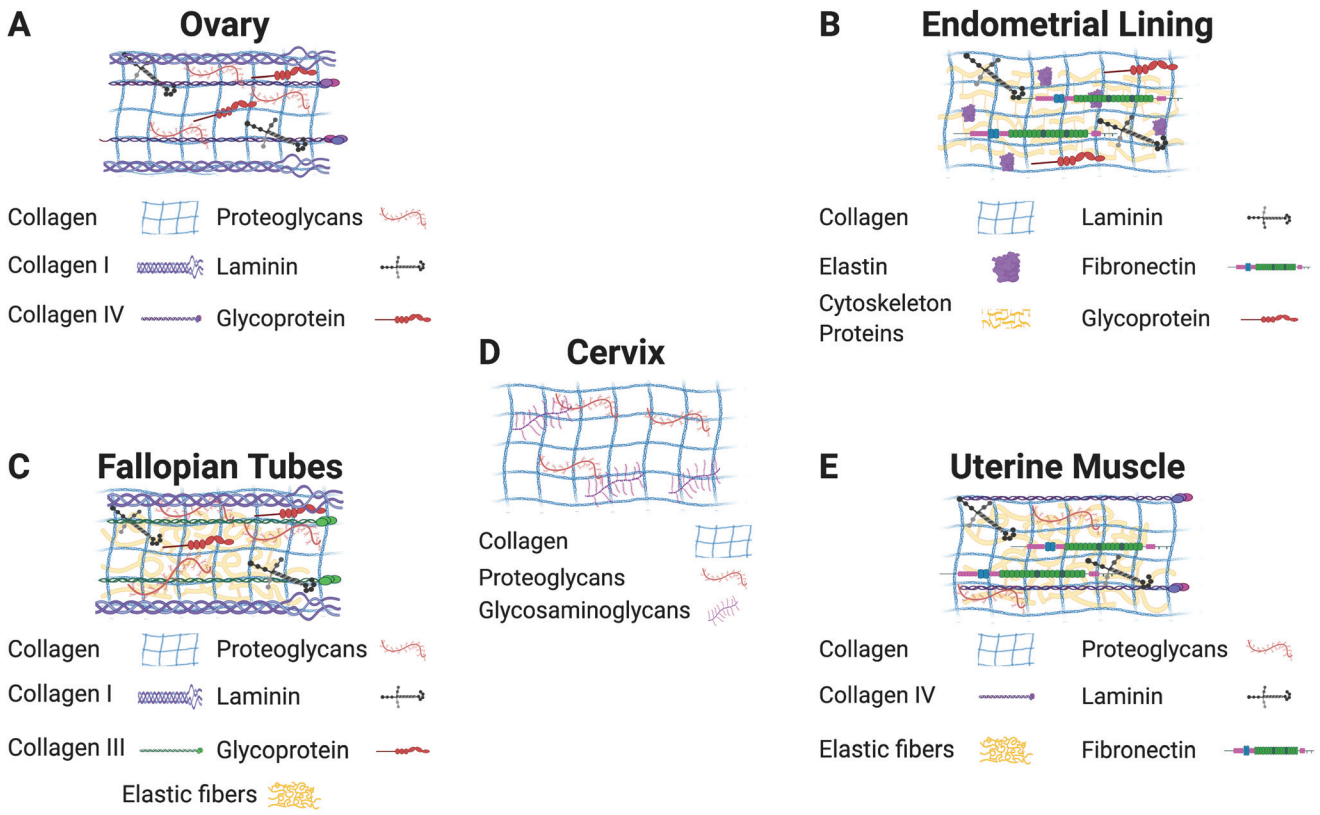

Fig. 2 Description of the major components of the extracellular matrix in each gynecological tissue. Figure created with BioRender.com.

to their bio-inert nature and without modifications often fail to support desired cell behaviors and tissue formation.[82] They can be chemically modified or combined with other natural biomaterials to design *in vitro* models and study cell–cell and cell–ECM interactions.[83] These modified synthetic biomaterials are biocompatible, biodegradable, and reproducible.[62] Furthermore, they can provide more experimental control over ECM properties in the microenvironment.[63] For example, PEG is one of the most studied and widely used synthetic polymers due to its biocompatibility and versatility.[84,85] This biomaterial also presents advantages in cell culture as it can be chemically modified and is hydrophilic, enabling cell encapsulation.[60] The use of natural and synthetic biomaterials, or a combination of the two, can lead to the formation of advanced *in vitro* models that resemble *in vivo* tissue.

As with all tissue-engineered constructs, developing 3D models of gynecological tissue requires cells, scaffold, and signal (the tissue engineering triad). The choice of cell types, biomaterials, and growth factors must be tailored to the tissue of interest as well as the disease state.[21] The size and the thickness of 3D models must take nutrient transport into account as well, as oxygen diffusion is limited to 200 μm.[86] Furthermore, if the construct is going to exceed the limit of oxygen transport or the role of vasculature in disease is being evaluated, vascularization must be considered as an added parameter.[87,88] Table 3 provides an overview of the biomaterials and cell types used to design *in vitro* models for gynecological tissue, and Fig. 3 demonstrates the properties of gynecological tissue that can inspire biomimetic *in vitro* models. As researchers have a wide array of biomaterials to choose from, this paper reviews the native function, architecture, and ECM components to be emulated.

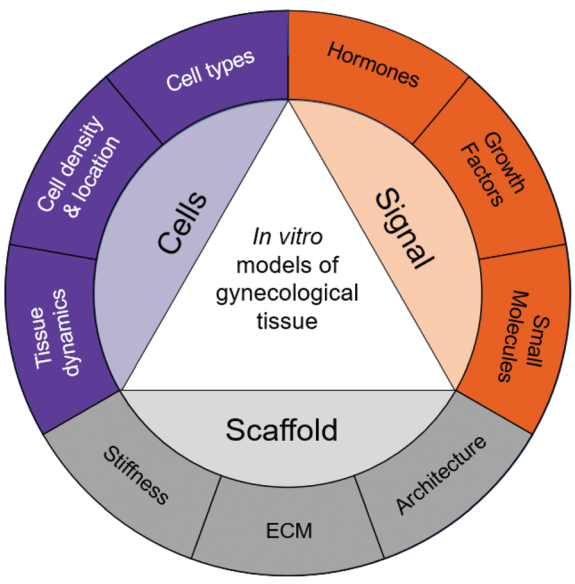

Fig. 3 Properties of native tissue can inspire biomimetic *in vitro* models of gynecological disease.

3. Biomaterials for *in vitro* models of gynecological tissues

3.1. Ovaries and fallopian tubes

3.1.1. Bioengineered models of the ovary. The ovaries are one of the most studied organs of female reproductive system; the ovarian ligaments attach them to the uterus, and the suspensory ligaments attach them to the pelvic wall (Fig. 1A). Their primary roles are ovum production and endocrine function.[77] Each ovary has two main compartments: the medulla and the cortex. The medulla is the vascular part of the ovary, and the cortex is comprised of germ cells, sex cord cells, and stromal cells. Each follicle contains an egg (oocyte), and the surrounding cells are either follicular cells or granulosa cells. Table 2 provides an overview of the cell types and ECM components of the fallopian tubes and the other gynecological tissue.[89] The ECM of ovarian tissue consists of various proteins and glycoproteins, including collagens, fibronectin, and laminin (Fig. 2A).[90] The guiding principle in the design of ovarian tissue is modeling the growth of follicles and ovarian stromal cells. Biomaterials must provide a good structure for the engraftment of follicles as well as degradability for follicle proliferation and migration.[111] Successful isolation and *in vitro* culture of follicles could serve as potential therapeutic strategies for reproductive regenerative medicine.[112] Moreover, the growth of follicular diameter in *in vitro* models requires biomaterials that can provide good architecture, diffusion of nutrients, and biocompatibility.[91,113]

In vitro studies of artificial ovaries for fertility preservation have used fibrin as a supporting biomaterial due to its physical properties and crosslinking ability (Table 3).[80] However, this biomaterial has low mechanical strength compared with other polymers, and it needs to be combined with other natural or synthetic polymers to mimic the cell microenvironment.[80] When mouse follicles were encapsulated in a fibrin alginate mixture, the hydrogels provided excellent architecture for cell attachment, proliferation, differentiation, and cell–cell signaling.[114] *In vitro* models of the ovary have also used alginate[58,90] or PEG[113,115] as supporting materials. These biomaterials also promote the survival, maturation, and autonomous function of follicles. The majority of the 3D *in vitro* models focus on the study of fertility preservation (not covered in this review, but covered in others[111,116,117]) and diseases such as polycystic ovarian syndrome and ovarian cancer.

3.1.2. Polycystic ovarian syndrome. The most common endocrine, metabolic, and menstrual disorder is Polycystic Ovary Syndrome (PCOS), affecting 4–12% of women worldwide.[4] The most common symptoms are amenorrhea, hyperandrogenism, and the presence of ovarian cysts.[7] This disorder is also associated with other health problems, such as obesity, infertility, and insulin resistance, and can increase the risk of cancer.[118] In women with PCOS, the endometrium becomes hyperplastic due to the absence of a complete menstrual cycle, which puts patients at higher risk of developing endometrial cancer.[119] *In vitro* studies that model PCOS progression and

Table 2 Gynecological tissue properties

Tissue	Prominent extracellular matrix	Cell types present	Ref.
Ovary	• Collagen ○ Type I and IV • Fibronectin • Laminin • Glycoproteins • Proteoglycans	• Germ cell ○ Oocyte • Somatic cells ○ Granulosa cells ○ Theca cells • Stromal cells • Vascular cells • Smooth muscle cells • Endothelial cells	77, 89–91
Fallopian tubes	• Fibers ○ Collagens (I and III) ○ Elastic and reticular • Nonfibrillar molecules ○ Glycoproteins ○ Proteoglycans (decorin, biglycan, fibromodulin, and versican)	• Ciliated epithelial cells • Secretory epithelial cells • Smooth muscle cells	92 and 93
Endometrial lining	• Collagen • Fibronectin • Laminin • Elastin • Cytoskeletal proteins • Glycoproteins	• Endometrial epithelial cells ○ Endometrial basalis ○ Endometrial functionalis • Endometrial Stromal cells ○ Fibroblasts • Immune cells ○ Leukocytes (T and B cells, mast cells) • Endothelial cells	28, 94–103
Uterine muscle	• Structural proteins ○ Collagen ○ Elastin • Substrate adhesion molecules ○ Fibronectin ○ Laminin ○ Collagen IV • Proteoglycans	• Myometrial smooth muscle cells	104–106
Cervix	• Structural proteins ○ Collagen fibers • Glycosaminoglycans other proteins • Proteoglycans	• Epithelial cells ○ Squamous ○ Glandular • Stromal cells ○ Fibroblasts • Smooth muscle cells ○ Cervical smooth muscle ○ Vascular smooth muscle • Endothelial cells	107–110

cell–ECM interactions focus on simulating this disease with endometrial epithelial cells and stromal cells. The major challenge of constructing an *in vitro* model of this disorder is the endometrial epithelial cells, which are the cells that become neoplastic and can further develop into endometrial cancer. These cells do not grow as well as stroma cells in monolayers and can lose their properties when expanded in 2D.[119]

There are currently multiple *in vitro* models of PCOS that have been developed to simulate the behavior of endometrial epithelial cells. Endometrial organoids of epithelial cells organized within Matrigel droplets enabled 3D passaging of normal and PCOS-derived human endometrial cells, demonstrating potential application for long-term culture.[120] Similarly, a scaffold-free endometrial organoid replaced the biomaterial with stromal cells to provide a supportive layer for the epithelial cells, much like in the native tissue. These scaffold free organoids simulated the effects of excess androgen, leading to the understanding of new mechanisms associated with PCOS and endometrial neoplasia.[119] Methods to expand endometrial epithelial cells *in vitro* are critical to enabling future development of PCOS models. Similar to cancer organoids, biomaterial strategies may help to support endometrial epithelial cells to survive and proliferate *in vitro.*[121]

3.1.3. Ovarian cancer. Ovarian cancer is the fourth leading cause of cancer-related death among women worldwide.[122] The majority of ovarian carcinomas originate in the distal end of the fallopian tube, beginning as an intraepithelial carcinoma and then spreading to the ovary.[123] It metastasizes *via* transcoelomic spread as individual cells or spheroids by detaching from the primary tumor and implanting throughout the peritoneal cavity.[124] Once the ovarian cancer cells attach to

organs in the peritoneal cavity such as the omentum, the ovarian cancer cells invade through the mesothelial lining, spread out along the underlying ECM, and remodel the surrounding tissue.[69] The tumor microenvironment is highly complex, involving cells such as cancer cells, fibroblasts, and macrophages, and ECM proteins such as collagen I, collagen III, collagen type IV and fibronectin (Fig. 2).[31] The interaction of collagen type I with collagen type IV and the accumulation of collagen type III is related to epithelial invasion and cancer progression.[64] The guiding principles in the design of 3D models of ovarian cancer are choosing which stage to investigate, selecting the appropriate ECM, growth factors, and cell types that recapitulate the microenvironment of interest (Table 3).

In vitro models have been developed to simulate the various stages of ovarian cancer progression. Ovarian cancer attachment to the omentum or peritoneal cavity is a critical step in the metastatic cascade. This stage is influenced by ECM cues, as demonstrated by studies where the ECM ligand density was varied on a 3D hydrogel matrix.[125] PEGDA/GelMA hydrogels enable changing the stiffness and ligand density independently, allowing exploration of hypotheses regarding mechanical and chemical cues in the tumor microenvironment.[125] Organotypic models can be used as a platform to evaluate cell adhesion and invasion.[126,127] A 3D co-culture model based on omentum with three layers of cell types and ECM (fibronectin and collagen type I) was used to analyze the effect of drug combinations on cell adhesion and proliferation.[128,129]

After ovarian cancer cells attach and clear through the mesothelial cell lining, they spread along the underlying ECM, remodel the ECM, and proliferate.[130,131] The underlying mechanism of these steps has also been investigated using 3D *in vitro* models. Ovarian cancer spheroid spreading in response to macrophage-derived soluble cues has been evaluated on a high density collagen I hydrogels[122] and modified gelatin hydrogels.[132] After spreading along the ECM, ovarian cancer cells proliferate, which has also been demonstrated to depend on ECM cues. Collagen I hydrogels were used as a platform to investigate the interplay of collagen I concentration, soluble cues, and ovarian cancer proliferation.[133] These studies demonstrated that increasing collagen density sensitized ovarian cancer cells to soluble cues, resulting in increased proliferation. Other platforms that have been used to investigate ovarian cancer proliferation include PLGA-PEG-PLGA tri-block copolymer hydrogels[62] and PEG-based hydrogels.[35] Both of these studies evaluated the role of ECM molecules on ovarian cancer cell proliferation, concluding that cell-integrin engagement drives responsiveness to ECM cues. Taken together, these *in vitro* models reveal significant insights into the progression of ovarian cancer.

3.1.4. Fallopian tube epithelium. The anatomy of the fallopian tubes (oviducts) is complex, starting from its origin and continuing with its vascular supply and ciliated microstructure, which is the key transporting to the egg site of fertilization.[134] They consist of three parts: the isthmus (small, narrow, thick-walled portion nearer the uterus), the ampulla (the major portion of the fallopian tube, where fertilization occurs most frequently), and the infundibulum with associated fimbriae, singular fimbria, surrounding the ostium (the widest part, nearest to the ovaries) (Fig. 1B).[93,135] Fallopian tubes are approximately 10 cm (4 in.) long extending laterally from the uterus, and are lined with ciliated and secretory epithelial cells; they secrete proteins and a nutrient rich fluid that help with the fertilization process (Table 2).[136] The secretory cells are of particular interest as they are considered precursors of ovarian cancer.[137] Type II carcinomas develop from intraepithelial carcinomas in the fallopian tube and disseminate as carcinomas that involve the ovary and, eventually, other metastatic sites in the peritoneal cavity.[138] The goal of modeling this tissue is to explore the relationship between the fallopian tubes and other health problems as infertility and ovarian cancer. However, few *in vitro* models currently exist.

The influence of ECM cues in fallopian tube epithelial cell invasion was investigated in a 3D model that captured the size and shape of cortical inclusion cysts,[139] which are thought to play a role in the initiation of ovarian cancer. Varying hydrogel concentrations of collagen I, collagen III, or both in a microfluidic lumen system suggested that increases in collagen III promote fallopian tube epithelial cell invasion.[140] The role of cortical inclusion cyst curvature was explored in a modified model in which the radius of curvature was varied, demonstrating a link between fallopian tube invasion and substrate curvature.[139]

Fallopian tube secretory cells are considered the cell of origin in the majority of ovarian cancers. When compared to 2D cultures, 3D spheroid cultures better mimicked the *in vivo* tissue and cell behavior.[92] Results showed that the 3D *in vitro* model offered a better approximation, compared to the 2D culture, of the biology of healthy tissue and malignant transformation to carcinoma.[92] In another study, 3D organoid culture was used to model the fallopian tube epithelium function. The fallopian tube is a dynamic tissue that is continuously changing in response to the stimulus of hormones, such as progesterone. Epithelial cells were embedded in Matrigel, resulting in a 3D model that closely mimicked the *in vivo* tissue and tested its ability to respond to oestradiol and progesterone, two hormones that fluctuate during the menstrual cycle.[141] *Ex vivo* models of fallopian tube epithelium have also used either collagen or alginate matrices.[142,143] A co-culture system of ciliated and secretory cells in an *ex vivo* model studied the behavior of these cells and their response to DNA damage, an initiating step in the progression of carcinoma. The model recapitulated the *in vivo* environment of the fimbria epithelium and identified a response to mutagenic injury that was confirmed with pathological samples.[142] Another *ex vivo* 3D model used human fallopian fimbriae to study the influence of specific ovulatory factors such as estradiol, oxidative stress mimetic (H_2O_2), and insulin on cell proliferation. These factors are regulators of healthy tissue physiology and were hypothesized to contribute to carcinogenesis.[143] Results showed that the alginate matrix maintained the tissue structure up to 7 days, and the presence of H_2O_2 and

Table 3 Tissue-engineered models of gynecological tissue

Tissue	General description	Biomaterial(s)	Cell(s)	Ref.
Ovary	3D culture model to assess cell matrix interactions of epithelial ovarian cancer cells, crucial in cancer progression and anti-cancer drug resistance	PEG-based hydrogel	Human epithelial ovarian cancer cell lines: OV-MZ-6, SKOV-3	35
	Thermal responsive hydrogel tri-block copolymer for 3D ovarian cancer culture	PLGA-PEG-PLGA	Ovarian cancer cell line (HO8910)	62
	Ovarian follicles for fertility preservation and follicle development	Alginate (alone or combined with fibrin)	Mouse ovarian follicles	58,90
		PEG-based hydrogel	Ovarian preantral follicles	115
			Primary follicles (isolated from B6CBAF1 mice) co-cultured with human adipose-derived stem cells (human ADSCs (Zen-Bio))	113
	3D culture of mouse follicles for Toxicity and High-Throughput (HTP) Analysis	Fibrin alginate hydrogel matrix	Two-layered secondary follicles from female F1 hybrids (C57BL/6JRccHsd inbred × CBA/J CrHsd)	114
	Human endometrial organoids constructed to study the effects of androgen levels in PCOS	Novel scaffold-free multicellular endometrial organoid	Primary endometrial epithelial and stromal cells from endometrial tissues	119
	Micro-culture device to examine the influence of macrophages on ovarian cancer spheroid spreading	Spheroid co-culture model with collagen type I hydrogel	HGSOC cell lines OVCAR3 and OV90 (ATCC), OVCA433 (NCI 60 panel (NIH, Bethesda, MD)), primary human macrophages	122
	3D organotypic model of ovarian cancer to simulate the metastatic microenvironment	Collagen type I	Ovarian cancer cell line (OVCAR4) Primary fibroblasts (NOF) Mesothelial (HPMC) cells	129
	Biomaterial-based platform of ovarian cancer spheroid growth to analyze cell–ECM interactions	GelMA-based hydrogels	Ovarian cancer cell line OV-MZ-6	132
	Collagen I hydrogels used as a platform to investigate the influence of collagen I concentration on ovarian cancer proliferation	Collagen type I	Primary human omental fibroblasts Ovarian cancer cell lines: OVCAR3, OV90 (ATCC), OVCA433 and OVCAR8 (NCI 60 panel)	133
Fallopian tubes	3D spheroid culture model of primary fallopian tube secretory epithelial cells to study the biology and etiology of fallopian tube tissues and compared with 2D cell cultures	3D spheroids coated in poly-2 hydroxyethyl methacrylate (polyHEMA, sigma)	Fallopian tube secretory epithelial cells	92
	Collagen I substrates with varying curvature to examine cell–cell and cell–substrate interactions on FTE invasion	Collagen types I and III	Mouse fallopian tube epithelial (FTE) cells	182
	Influence of ECM cues in fallopian tube epithelial cell invasion in a microfluidic lumen model	Collagen gel mixture (collagen type I and III)	Mouse fallopian tube epithelial cells expressing mutated p53	139 and 181
	3D organoid *in vitro* model to mimic the human fallopian tube epithelium	Matrigel	Fallopian tube epithelial cells	141
	3D alginate culture system to study the role of the fallopian fimbriae in serous tumorigenesis	Alginate	Fallopian fimbriae	143
Uterine muscle	Magnetic 3D bioprinting model to study uterine contractility over time	3D bioprinted human myometrium cells into rings	Human uterine smooth muscle cells (HUtSMCs): C-12575 and C-12576, PromoCell Primary human uterine smooth muscle cells (SMCs)	104
	3D *in vitro* model for myometrial smooth muscle cells studies	Fibrinogen-PEG PVA	hTERT cells PHM cells human primary myometrium cells	105
	Uterine scaffolds to repair damaged uteruses in rabbits, resulting in live births	PGA-PGLA	Rabbit autologous myometrial and endometrial cells	146
	Two forms of collagen (monomeric and fibrillar) were analyzed to show the differences in cell morphology, proliferation, and interaction of uterine fibroids	PureCol collagen solution	Leiomyoma samples (LSMCs)	152

Table 3 *(Contd.)*

Tissue	General description	Biomaterial(s)	Cell(s)	Ref.
Endometrial lining	Multicellular model to study angiogenesis and trophoblast invasion in the endometrium	GelMA	Cell lines: HUVECs: C2517A HESCs: CRL-4003 EECs: FC-0078 HTR-8/SVneo: CRL-3271	28
	3D organoid models mimic the physiology of the endometrium and endometrial stromal and epithelial cells interactions	Matrigel	Endometrial stromal cells and epithelial cells	71, 74 and 120
	Collagen/Matrigel matrix to study endometrial cancer invasion	Collagen type I–Matrigel	Human endometrial adenocarcinoma cell line KLE Human endometrial tissues	76
	Collagen scaffold-based model of the endometrium containing both epithelial and stromal cells	Bovine collagen type I	Decidual stromal cells and epithelial cells	97
	Multicellular model to analyze the influence of hormones on cell–cell interactions	PEG-VS	Ishikawa human endometrial adenocarcinoma hTERT immortalized human endometrial stromal cells Primary isolated endometrial stromal and epithelial cells	100
	3D model to analyze physiologic changes that occur in endometrial regeneration during the proliferative phase	Collagen type I-Matrigel	Human telomerase-immortalized endometrial stromal cells Ishikawa endometrial adenocarcinoma cells	162
	Developed a multicellular model of the endometrium to analyze the dissolution rate of ECM under the effect of SrtA	PEG-VS PEG-NB	Ishikawa human endometrial adenocarcinoma cells hTERT-immortalized human endometrial stromal cells	169
	3D fibrin matrix of endometrial explants reflected the environment established in the peritoneal surface as a result of the retrograde menstruation	Fibrin matrix	Fragments of human endometrium	168
Cervix	3D engineered model of the cervical ECM to study the mechanical and biochemical effects of progesterone on engineered cervical tissue	Collagen scaffolds	Human cervical fibroblasts	110
	3D organotypic culture of epithelial cell cultures from human ectocervix, transformation zone, and endocervix	Collagen type I	Human cervical tissue from the CHTN (epithelial cells, stromal cells)	109
	3D printed model with a cervical cancer cell line and gelatin/alginate/fibrinogen hydrogels simulated the tumor microenvironment	Gelatin-alginate-fibrinogen	HeLa cells	178
	Organotypic culture for analyzing the ability of human lymphocytes to infiltrate human papillomavirus (HPV)-associated (pre)neoplastic lesions of the uterine cervix	Organotypic raft cultures (collagen gel)	SiHa cell line (cervical carcinoma-derived keratinocyte cell line) Peripheral blood mononuclear cells (PBMC)	180

insulin influenced cell proliferation. These tissue models provided valuable information about the response of the fallopian tubes to the exposure to hormonal changes and ovulatory factors. Furthermore, they may provide insights into the understanding of epithelial biology and oncogenesis.

3.2. Uterine muscle

3.2.1. Bioengineered models of uterine muscle and uterine fibroids. The uterus is a muscular organ with three sections: the fundus (superior part), the body of the uterus (corpus), and the cervix (inferior section) (Fig. 1). It is responsible for nourishing and supporting the growing embryo, regulating hormones and menstruation, and providing structural integrity and support to organs (Fig. 1D).[144] The uterus has three tissue layers at the walls: the endometrium, the myometrium, and the serosa (Table 2).[145] The myometrium consists of smooth muscle fibers and is responsible for contractility.[104] The functional myometrial tissue is comprised of groups of myometrial smooth muscle cells in a 3D matrix.[105,106] A guiding principle in the design of 3D uterine models is enabling contractility of myometrial smooth muscle cells.

3D cell culture platforms can better model tissue contractility compared with 2D models and is easier to interrogate *in vitro* than in animal models. Magnetic bioprinting of myometrial cells into hollow rings was used to study uterine contractility. Abnormal uterine contractility is related to common pathological disorders, including irregular menstrual cycle, infertility, and preterm labor.[104] For the first time, this bioprinted 3D *in vitro* model of primary human uterine smooth muscle cells obtained from patients during a cesarean section showed a method for the personalization of therapies for uterine contractility disorders. Furthermore, the bioprinted rings exhibited different responses to contractility inhibitors such as indomethacin and nifedipine. In all experimental conditions, inhibitors slowed or stopped the contraction of the *in vitro* model.[104] However, modeling the human myometrium can be challenging as it is difficult to obtain primary cells from uterine biopsies, and those that are obtained often fail to proliferate in standard culture conditions. Therefore, opportunities to model human myometrium in *in vitro* culture systems are dependent on finding alternative cell sources of smooth muscle cells or developing more robust methods of expanding human myometrial cells *in vitro*.

Potential biomaterials used to design 3D *in vitro* models of the uterus provide new insights on the mechanism of cell behavior and uterus function. A recent study used a PGLA coated PGA scaffold seeded with autologous myometrial and endometrial cells from rabbits to bioengineer uterine tissue.[146] Rabbits have been frequently used in reproductive studies as they have a relatively larger uterus with a similar structure to human tissue compared with other animal models. A biodegradable cell-laden scaffold resulted in native tissue structure prior to implantation and upon implantation restored uterine function, resulting in live births. Furthermore, rabbits that received a cell-seeded scaffold were capable of responding to mechanical strains that occurred during pregnancy and developed all of the uterine tissue layers, including the myometrium and endometrium.[147] PGLA and PGA are effective scaffolds for tissue regeneration due to their high porosity. Also, these polymers are biocompatible and have tunable mechanical and chemical properties.[147] Another study used a combination of fibrinogen with PEG and PVA to build a 3D scaffold with smooth muscle cells. This modified biomaterial was biodegradable, biocompatible, had good cell adhesion, and facilitated the development of myometrial 3D structures (Table 3).[105] Overall, the development of these tissue-engineered approaches has led to a deeper understanding of dynamic mechanical and chemical cues on uterine function.

Uterine fibroids (leiomyomas or myomas) are the most common benign yet often painful tumors of the myometrium. In the U.S., they account for approximately 30% of all hysterectomies among women between 18–44 years old.[148,149] Associated symptoms include pelvic pain, excessive uterine bleeding, infertility, and pregnancy complications. Despite the frequency of these tumors, there is limited information and understanding of their mechanism and treatment. Studies suggested that the inflammatory events caused by physiological injuries in the uterus are associated with an increase in the production of ECM that results in the formation of leiomyomas.[150] Collagens are the most abundant components of the ECM in uterine fibroids (Fig. 2E) and are responsible for producing the rigid structure of leiomyomas. Furthermore, myomas present higher levels of metalloproteinases at the different stages of growth.[150,151] Therefore, a guiding principle when developing *in vitro* models of uterine fibroids is to include collagen and enable ECM variation and degradation.

Fibroid formation is dependent on the deposition of collagen I and III as well as dysregulation of signaling processes such as the mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K) pathways. An *in vitro* model of leiomyoma muscle cells embedded in collagen hydrogels evaluated their response to growth factors, including platelet-derived growth factor (PDGF), which is associated with fibroid formation and growth. This demonstrated differences in cell morphology, proliferation, and interactions with monomeric and fibrillar collagen in the presence or absence of PDGF.[152] Results showed that leiomyoma smooth muscle cells had distinct morphologies on the different collagen matrices and that proliferation depended on overall collagen concentration. Another study used collagen I to model uterine fibroids and myometrial cells to study the changes in cell behavior and gene expression in response to potential therapies. The model maintained the molecular phenotype of *in vivo* tissue and cell–ECM interaction. Furthermore, this culture system assessed the mechanism of abnormal ECM formation and the effectiveness of potential therapeutic agents.[153] Although uterine myomas are persistent tumors and often painful, it is still an understudied research area. *In vitro* 3D models have the potential to improve and understand the biology and cell behavior of uterine leiomyomas, particularly the importance of ECM in the formation of fibroids.

3.2.2. Uterine sarcomas. Uterine sarcomas are extraordinarily rare tumors with an incidence of 3–7 per 100 000 women in the U.S[154] and comprising only 3–5% of all uterine cancers.[8] These tumors are considered highly aggressive, resulting in poor patient prognosis. Uterine sarcomas are classified as leiomyosarcomas, endometrial stromal sarcomas, low grade or high-grade sarcomas, undifferentiated uterine sarcomas, or other.[8,155] Leiomyosarcomas are mesenchymal tumors and are the most common uterine sarcoma arising within the myometrium; they are malignant smooth muscle tumors and typically present with prominent necrosis.[50] Adenosarcomas are a mixture of benign epithelial cells and malignant mesenchymal cells.[48] These distinctions in cell type are critical to building 3D models that replicate the tumor microenvironment.

The only treatment available for uterine sarcomas is surgery and radiotherapy.[8] The stromal sarcomas have characteristic translocations; meanwhile, leiomyosarcomas have complex karyotypes. These characteristics make them difficult to treat and would benefit from the development of targeted therapies. There are no current 3D *in vitro* models with biomaterials for this type of tumor. However, a 2D *in vitro* model was used to interrogate the underlying molecular biology and identify biomarkers for potential treatment strategies.[156] This study evaluated a possible treatment of uterine sarcomas with PGJ2 and dasatinib, which are considered potential cancer treatments. Results showed that the combined treatment produced a synergistic effect on inhibiting cancer cells proliferation by negatively regulating the MAPK pathway. Furthermore, 3D *in vitro* models could identify potential molecular targets or evaluate currently available treatment options.

3.3. Endometrial lining

3.3.1. Bioengineered models of the endometrium. The endometrium is the lining of the uterus and the most hormone-dependent tissue in the female reproductive system. Hormone stimulation can alter its structure and thickness.[94,145] Furthermore, it undergoes dynamic remodeling to ensure a suitable microenvironment to support a pregnancy.[12,95,157] The human endometrium is a complex multicellular tissue (Table 2). It is comprised of surface epithelium (luminal) with numerous glands and surrounded by a supportive stroma with stromal, endothelial, and immune cells (Fig. 1E).[96,158] The cell–cell communication between epithelial and stromal cells maintains a normal function of the endometrium. However, cellular interactions depend on other factors, such as hormonal regulation.[97]

Endometrial lining is one of the most challenging tissues to recapitulate *in vitro* as it is highly dynamic in response to hormones produced by the ovary during the menstrual cycle. During the first days of the menstrual cycle, glandular and stromal cells proliferate due to the rising estrogen levels produced by the follicle.[98] Following ovulation, cell differentiation occurs as the corpus luteum, a dynamic endocrine gland of the ovary, synthesizes and increases progesterone and estradiol.[159] If fertilization does not occur, the corpus luteum relapses, which decreases the levels of progesterone and leads to glandular and stromal breakdown (menstruation).[99] If fertilization does occur, the changes in the endometrial blood vessels play an important role in the implantation of the blastocyst, as vascularization contributes to uterine receptivity.[160,161] Endometrium remodeling depends on different hormone changes. Thus, the guiding principle in designing 3D *in vitro* models of the endometrium is to provide scaffolding and biophysical cues such that the cells respond to stimulation with hormones.

3D *in vitro* models have been successfully developed and used to study the underlying physiology of the endometrium. The two main types of endometrial models are organoids and collagen scaffolds. Long-term expandable and stable culture organoids were achieved with the combination of Matrigel, growth and signaling factors such as EGF and WNT activators, and either human or mouse endometrial cells. This culture system showed the capacity to respond in a physiological manner to hormones and specific biomarkers in cell proliferation and maturation.[74,120] 3D endometrial organoid models allow cells to self-organize in a structure similar to stratifications observed *in vivo* and demonstrate similar molecular signatures to the *in vivo* tissue.[20] Collagen scaffolds have also been used to model the layers of endometrial epithelial cells for long term expansion.[97] A model using collagen I scaffolds with endometrial epithelial cells was responsive to stimulation with hormones, resulting in epithelial differentiation and stromal decidualization.[97] Both organoids and scaffolds present good approximations of the *in vivo* tissue as they recapitulate the original tissue (healthy or pathological) and provide a powerful tool for modeling and deciphering tissue development. By manipulating their structure, these models can be a potential tool to integrate other types of cells, such as immune or trophoblast cells, in order to gain a better understanding of the interactions between cells and their response to hormone stimulation.

Collagen and Matrigel can also be combined in order to harness the basement membrane cues from Matrigel and the physical cues from collagen. A collagen–Matrigel blend was used to create a tissue-engineered model that analyzed the communication between endometrial stromal and epithelial cells in response to endocrine signaling.[162] Using this *in vitro* model, the authors were able to elucidate the effects of cytokines present during the proliferative phase of the human endometrium. This hydrogel allowed cells to respond to endocrine effects and demonstrated that the stromal tissue did not proliferate more in response to increasing concentrations of collagen I.[162] Gelatin hydrogels, made of denatured collagen, have been used to model angiogenesis and trophoblast invasion in the endometrium, as gelatin is not fibrillar and allows for easier matrix remodeling and cell attachment compared to fibrillar collagen.[28] Trophoblast cells drove the process of the non-pathological angiogenesis, which occurs regularly in the endometrium to rebuild the vascular bed.[163] Chemically modified gelatin hydrogels have also been used to explore the relationship between biophysical, biochemical, and cellular

signals from the endometrium. Due to its crosslinking properties, methacrylamide-functionalized gelatin (GelMA) provides matrix stiffnesses relevant to the *in vivo* tissue and similar structure to the *in vivo* environment. It also promotes cell function, endometrial angiogenesis, and responsiveness to hormones. The attachment of methacrylamide groups allows gelatin to be U.V. light polymerized and relatively homogenous in composition and structure.[28] Taken together these studies further our understanding of cell behavior in the endometrium. Additionally, these *in vitro* models capture the complexity of cell–cell and cell–ECM interactions in the human endometrium.

3.3.2. Endometriosis. Endometriosis is a disease caused by endometrial tissue attaching to tissue outside the uterine corpus. It is considered one of the most common conditions affecting women worldwide, with an estimated one in ten women affected.[164] Symptoms for this condition include pelvic pain, painful menstruation, and infertility.[165] The leading cause of endometriosis is still unknown, but it is associated with interactions between endometrial cells and other cells in the uterine microenvironment.[166] In most cases, endometriosis lesions are on the peritoneum and ovaries.[164] The fundamental principle to develop a 3D *in vitro* model for endometriosis is the retrograde menstruation theory, which states endometriosis is caused by the migration of endometrial cells to the ovaries or peritoneal surfaces, ultimately leading to the formation of lesions and serosal adhesions, which may be painfully symptomatic.[167]

3D *in vitro* models have investigated the mechanism of action for endometriosis in order to identify therapeutic targets. Fibrin hydrogels were used to culture cells from a fragment of human endometrium. Fibrin is a major component of blood clots and reflects the environment established in the peritoneal surface as a result of retrograde menstruation.[168] Endometrial cells proliferated and invaded the fibrin matrix, generating new glands, stroma, and vessels similar to the *in vivo* tissue. Furthermore, the authors demonstrated that endometrial cell proliferation and angiogenesis were related to the expression of vimentin. Vimentin is an intermediate filament that crosslinks other cytoskeletal proteins and has an important role in the dynamic of mesenchymal cells.

A 3D functional co-culture model of epithelial cells with stromal cells simulated the endometrium physiology.[100] The synthetic 3D matrix of modified PEG hydrogels analyzed the response of the cells to hormones and cell–ECM and cell–cell interactions. The PEG hydrogels were modified to incorporate integrin-binding peptides and ECM-binding peptides to simulate the *in vivo* tissue. This promoted cell attachment, cell viability, remodeling of the 3D matrix, and hormone-mediated cell communication over two weeks in culture. Furthermore, this synthetic ECM model demonstrated phenotypic differences between co-culture models of patient-derived primary cells and endometrial cell lines. Another study by the same group used a modified PEG hydrogel to investigate the kinetics of gel dissolution as a function of change in concentration of enzyme and substrate as well as crosslinking parameters.[169]

3.3.3. Endometrial cancer. Endometrial cancer is caused by a disruption in hormonal balance.[78] Endometrial cancer affects approximately 61 380 women in the United States, predominantly affecting postmenopausal women.[170] There are two types of endometrial cancer tumors: low grade (type I) and high grade (type II). Type I tumors are related to unopposed estrogen stimulation and may be associated with other gynecological diseases such as polycystic ovarian syndrome.[43] Type II tumors are usually high grade and present at higher stages with associated poor prognosis.[43] Early-stage endometrial carcinoma, especially low grade, is highly curable, but advanced stage endometrial carcinomas have low survival rates.[171] *In vitro* models can lead to a better understanding of the invasion and progression of these tumors and future treatment strategies (Table 3).

The guiding principle for the design of 3D *in vitro* models for endometrial cancer is to capture the cancer-stromal cell interactions. A blend of collagen and Matrigel matrices was used to support endometrial epithelial cells cultured in a 3D *in vitro* model to understand endometrial cancer invasion. Similar to the model used to study endometriosis,[162] a mixture of collagen I and Matrigel provided a structure to the *in vivo* tissue. Collagen provided a stromal matrix and Matrigel provided an artificial basement membrane.[76] Other 3D *in vitro* models have been used to target potential treatment alternatives but did not include a biomaterial on their structure. Endometrial cancer patient-derived organoids have been used to study drug sensitivity to endocrine treatments.[130] Additionally, a study with organoids models compared 3D multicellular structures with 2D monolayer culture models to study endometrial cancer resistance to various drug treatments. This study used doxorubicin and cisplatin, which are chemotherapeutic regimes for endometrial cancer.[172] The authors demonstrated that 3D culture models displayed significant decreases in responsiveness to chemotherapies compared to 2D cell models. Overall, 3D models are a promising tool for drug screening in endometrial cancer.

3.4. Cervical tissue

3.4.1. Bioengineered models of cervical tissue. The uterine cervix is a unique organ located in the lower part of the uterus lined with epithelial cells (Table 2, Fig. 1C).[173] The cervix is a complex structure comprised of collagens, elastin, and glycosaminoglycans.[108] The human cervix contains three distinct anatomic regions, including the ectocervix, the transformation zone, and the endocervix (Fig. 1C). Modeling healthy tissue can improve the understanding of the biology of this complex system. Moreover, the development and optimization of methods for isolating culturing primary human cervical cells would be an important contribution that could lead to a better understanding of the progression of diseases and factors that can alter the cervical environment.

The ECM plays an important role in the dynamic nature of cervical tissue (Fig. 2D).[110] 3D *in vitro* models of this tissue have been used to evaluate cell–ECM interactions. Mechanical and biochemical effects of progesterone on cervical tissue were

studied in a 3D *in vitro* model with cervical fibroblasts seeded on collagen scaffolds. Using this model, the authors investigated the effects of progesterone on cervical shortening. By measuring collagen content and crosslinking in the scaffolds after stimulation with progesterone, they demonstrated that fibroblasts changed their morphology and decreased their production of collagen, leading to a decrease in cervical tissue stiffness.[110] An *in vitro* model commonly used to study skin has also been employed to study cervical cancer: organotypic raft cultures. Organotypic raft cultures are multilayered models of collagen, fibroblasts, and keratinocytes that are maintained at an air–liquid interface. Organotypic raft culture was used to observe differences between neoplastic and healthy cervical tissue in response to acetic acid, which is commonly used in clinical treatments for cervical lesions.[174] A separate study used 3D organotypic cultures with human stromal and epithelial cells from each cervical region to evaluate the invasion of epithelial cells and their interaction with stromal cells.[109] Increasing the concentration of stromal cells increased the invasion of epithelial cells from each cervical region, suggesting that stromal–epithelial interactions play an essential role in cervical tissue homeostasis.[109] The establishment of organotypic raft cultures of cervical cells represents a crucial step forward in understanding cervix biology and function.

3.4.2. Cervical cancer. Cervical cancer is the fourth most common cancer in women worldwide and is the second cause of cancer-related deaths in women under 40.[51,175] Cervical cancer derives from a persistent human papillomavirus infection (HPV), and without treatment, these neoplastic lesions may lead to carcinoma *in situ* and invasive cancer.[176] Currently, there are two types of tests for cervical cancer screening: Papanicolaou tests (commonly known as Pap smears) and HPV tests.[177] If it is caught early, stage I patients have a 92% survival rate. However, 13% percent of cervical cancer patients are diagnosed at advanced stages and have a 16.5% survival rate.[52] The study of these diseases in 3D *in vitro* models can lead to potential treatment strategies for more advanced stages of cervical cancer. The guiding principle to design 3D *in vitro* models for cervical cancer relies on modeling dynamic epithelial change due to hormone response. Understanding the response of the epithelium to HPV infection with progression to carcinoma could provide new insights into the mechanism of progression of this disease.

There are limited 3D *in vitro* models of cervical cancer and the underlying mechanism by which cervical epithelium respond to HPV infection is still under investigation. A 3D printed model with a cervical cancer cell line and a hydrogel compromised of gelatin, alginate, and fibrinogen simulated the tumor microenvironment. Chemoresistance was evaluated, and cells within the 3D *in vitro* model demonstrated increased cell viability and proliferation in the 3D model compared to the conventional 2D culture model. Furthermore, cells within the 3D printed model formed spheroids, while in the monolayer culture, cells formed sheets.[178] Similar to healthy cervix, organotypic raft cultures are useful models to study lesions of the cervix caused by HPV.[179] For example, a co-cultured organotypic raft culture was used to simulate the infiltration of human lymphocytes into HPV and cervical lesions.[180] Overall, *in vitro* models used to evaluate the progression of cervical cancer provide new insights and are a promising tool to identify potential therapies.

4. Conclusions

Tissue engineering has the potential to transform the landscape of women's health. Studies using *in vitro* models of gynecological tissues have created hope for women suffering from gynecological diseases. Furthermore, understanding the anatomy, function, and architecture of the tissue is critical for building *in vitro* models. Specifically, the concentrations and temporal nature of soluble signals such as growth factors, hormones, and small molecules should be evaluated; the scaffold supporting the cells, including ECM components, stiffness, and overall architecture, should be examined; and the cells present, importantly the types of cells, their densities, locations, and dynamics, should be assessed. These properties are summarized in Fig. 3. The biophysical cues present in native tissue can then serve guiding principles to develop 3D *in vitro* models using biomaterials, cells, and exogenous stimuli.

Building tissue-engineered constructs should be done in collaboration with clinicians, pathologists, and cell biologists. Techniques used to evaluate patient biopsies and animal models have a strong overlap with how tissue engineers strive to evaluate *in vitro* constructs. Engineering biological tissue of the female reproductive system relies on the use of biomaterials, as 3D *in vitro* models enable us to understand cell–cell and cell–matrix interactions. The *in vitro* models and biomaterials presented in this review reveal guiding principles and capture complex interactions of different gynecological tissues. There are still many questions to be answered regarding which biomaterial should be used for each model. However, the development of new models opens opportunities to better understand gynecological diseases, identify underlying signaling networks, and evaluate potential treatment strategies.

Conflicts of interest

The authors have no conflicts to declare.

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