

Biomaterial strategies for the application of reproductive tissue engineering

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ABSTRACT

Human reproductive organs are of vital importance to the life of an individual and the reproduction of human populations. So far, traditional methods have a limited effect in recovering the function and fertility of reproductive organs and tissues. Thus, aim to replace and facilitate the regrowth of damaged or diseased tissue, various biomaterials are developed to offer hope to overcome these difficulties and help gain further research progress in reproductive tissue engineering. In this review, we focus on the biomaterials and their four main applications in reproductive tissue engineering: in vitro generation and culture of reproductive cells; development of reproductive organoids and models; in vivo transplantation of reproductive cells or tissues; and regeneration of reproductive tissue. In reproductive tissue engineering, designing biomaterials for different applications with different mechanical properties, structure, function, and microenvironment is challenging and important, and deserves more attention.

1. Introduction

Human reproductive organs and tissues comprise male and female reproductive organs or tissues, including the ovaries, uterus, vagina, penis, and testes, that are vital importance to the life of an individual and the reproduction of human populations [1]. Inflammation, aggressive tumors, trauma, infectious disease, and acquired or congenital abnormalities may cause degenerative damage to reproductive organs or loss of reproductive capacity [2–4]. Traditional repair methods mainly revolve around hormone therapy, surgical methods and organ transplantation [5–7]. These methods have a limited effect in recovering the function and fertility due to the lack of regenerative ability and the risk of serious rejection in reproductive organs and tissues [8]. Therefore, new therapeutical strategies to recover the reproductive capacity and repair the defect of reproductive tissues need to be developed.

Aim to replace and facilitate the regrowth of damaged or diseased tissue, biomaterials are designed and synthesized to offer a hope to

overcome the difficulties in reproductive tissue engineering. The biomaterials are required to be non-toxic and biocompatible to the organs and tissues in the body at first. In tissue engineering, some biomaterials can not only directly combine and interact with biological organisms but also guide the reconstruction of the basic structure and morphology of organs and accelerate tissue regeneration. In addition, biomaterials are usually loaded with cells and collaborated with growth factors and drugs in most researches rather than act alone. They can markedly improve the local and durable cell retention rates in the body and also served as a carrier for drugs and growth factors [9–11].

For different organs and tissues in reproductive tissue engineering, biomaterials need to be designed so that can be harmonious with their specific mechanical performance, structure, degradation, function, and physiological environment [12]. For example, the testis and ovary have complex structures and precise systems as the organs produce germ cells and mature oocytes in individual organisms. The age-related and environmental stimuli-associated organ decline and infertility occurred

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frequently in humans with uncertain mechanisms and was hard to reverse [5,8]. Thus, *in vitro* spermatogenesis and oocyte maturation are significant treatments for fertility restoration [13]. The complex physiological processes of spermatogenesis and follicular development and ovulation have involved the interaction of stratified epithelium and gametogenic cells, and cyclical changes of sex hormone levels [14–16]. Therefore, some special requirements are imposed on the biomaterials to simulate the microenvironment of testis and ovary. In this case, biomaterials often require to have the low internal pressure and mechanical rigidity, a microporous structure that support sufficient contact between the developing cells and surrounding cell, as well as a highly hydrophilic environment that provides efficient mass transfer capabilities for the diffusion and transfer of hormones and cytokines. In contrast to testis and ovary, male penis and female vagina and endometrium might have a trauma caused by sexual intercourse or surgery [17–19]. Biomaterials applied for *in-situ* regeneration are more likely to have better mechanical strength and ability to promote vascular regeneration and resist inflammation [20,21].

In this review, we mainly focus on the biomaterials and their reproductive tissue engineering, including *in vitro* generation and culture of reproductive cells, development of reproductive organoids, *in vivo* transplantation of reproductive cells or tissues, and reproductive tissue regeneration. (Fig. 1).

2. Classification of biomaterials in reproductive tissue engineering

The main biomaterials used in reproductive tissue engineering can be divided into synthetic polymers and natural polymers (hydrogels and scaffolds), and decellularized matrices [1]. Here, we summarize the advantages and shortfalls of different biomaterials and the main typical application cases. Table 1 outlines the biomaterials that are discussed in this review.

2.1. Synthetic polymers

Synthetic polymers are inexpensive and can be easily manipulated to satisfy specific structural requirements of organs and tissues. In reproductive tissue engineering, they are usually used to repair the defect of male penis and female reproductive tract, and endometrium caused by sexual intercourse or surgery. For instance, the rods, films, and textile materials formed by biodegradable synthetic polymers, including poly(lactic-co-glycolic acid) (PLGA), polyglycolic acid (PGA), and polylactic acid (PLA), can be used in penile reconstruction [22,23]. However, the disadvantages of synthetic polymers as implant biomaterials are associated with the risk of a foreign body response, adverse immunologic reactions, and lack of adhesion to living tissues [24]. During degradation and reabsorption, some polymers such as PGA may activate macrophages, reactive oxygen intermediates, and dehydrogenases at the interface between cells and biomaterials [25]. Most synthetic polymers cannot provide an advantageous microenvironment for the culture and development of gametogenic cells and Sertoli cells compared with natural polymers and acellular matrices. However, the surface of polydimethylsiloxane (PDMS) substrates after being treated with oxygen plasma to increase surface wettability and cell adhesion can also support the culture and testicular cord formation of testicular cells [26]. Also, PLGA-based hydrogel provides a favorable environment for spermatic germ cells to proliferate and differentiate into mature spermatids [27].

2.2. Natural polymers

In contrast to synthetic polymers, natural polymers have excellent biodegradability and biocompatibility and can be advantageous biomaterials in the reproductive tissue engineering field [28]. These natural polymers mainly include hyaluronan [29], gelatin [30,31], alginate [32,33], fibrin [24,34–36], collagen [37–39], soft agar [13], and Matrigel [40–45]. Natural polymers can form natural hydrogels and scaffolds by

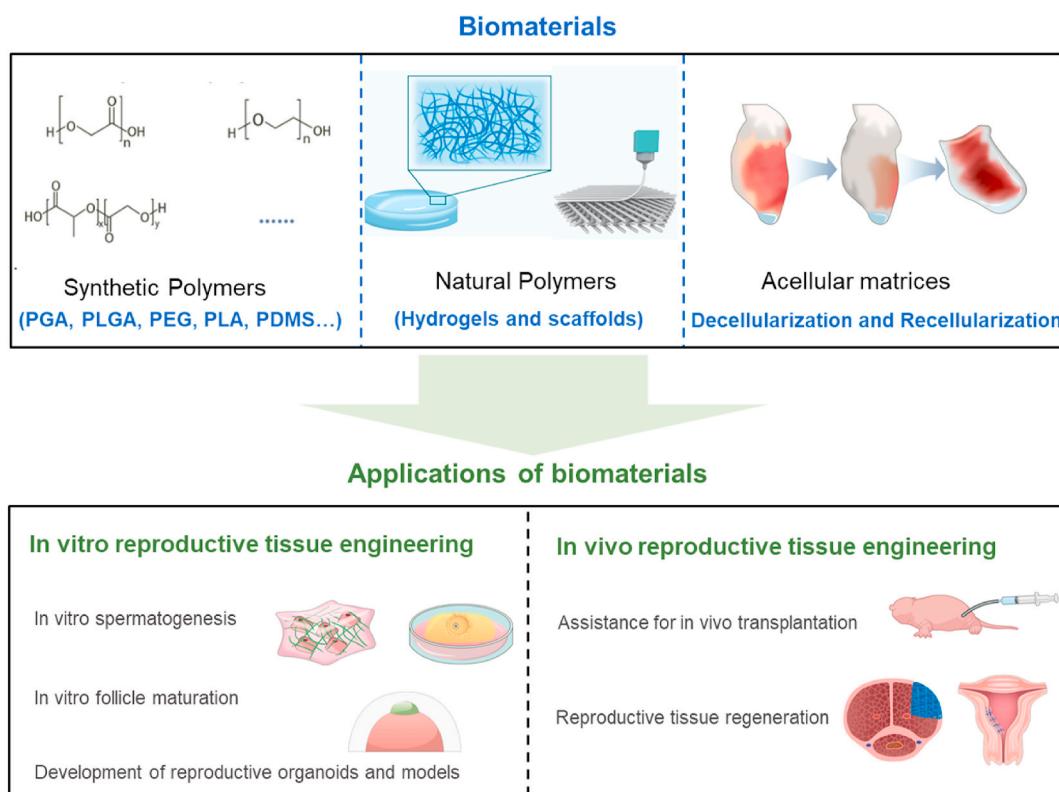


Fig. 1. Diagram of biomaterial for the applications of reproductive tissue engineering.

Table 1

Biomaterials in reproductive tissue engineering and their applications.

Classification	Merits and demerits	Biomaterials	Applications	Ref.
Synthetic polymers	Inexpensive and can be manipulated easily; tunable mechanical properties and high malleability; lack of or limited biocompatibility	PLA	Combined with autologous chondrocytes to construct penile prostheses	[22]
	Foreign body response; adverse immunologic reactions; lack of adhesion to living tissues	PGA	Combined with cells to reconstruct the smooth muscle tissue of the cavernous body	[23]
		PDMS	Coculture of embryonic stem cells and testicular cells; Microfluidic devices	[26,90]
		PLGA	In vitro spermatogenesis of immature spermatogenic germ cells	[27]
		PEG	3D follicular culture	[92]
Natural polymers (hydrogels)	High-water content; excellent biodegradability and biocompatibility; environmental stresses similar to tissue; can be easily loaded with other factors; maintain the 3D culture environment; ensure the effect of cell-cell interactions; Insufficient mechanical strength	Hyaluronan-based hydrogels Gelatin-based hydrogel Soft agar hydrogel Alginate-based hydrogels	In vitro maturation of follicles Constructing placental barrier models Coculture of spermatogonia and somatic cells In vitro follicular culture; in vivo transplantation of isolated preantral follicles and ovarian cells; 3D culture system for testicular cells Primordial follicle transplantation	[29] [31] [81] [32,54] [57,58]
		Fibrin hydrogels; fibrin-alginate hydrogels; fibrin-collagen composites Collagen-based hydrogel Matrigels	In vitro oocyte maturation of ovary follicles Generation of functional spermatids from human SSCs in vitro; testicular, ovarian, and endometrial organoids	[51] [40–45, 55]
		Collagen scaffolds	Loaded with human umbilical cord-derived mesenchymal stem cells/bone marrow mesenchymal stem cells for endometrial regeneration Culture and growth of primitive follicles	[28,117]
		Alginate-based macroporous scaffolds Gelatin-based scaffolds	Create a bioprosthetic ovary; endometrial repair; functional reconstruction of injured corpus cavernosa	[126] [30,59, 119]
			Cervicovaginal reconstruction	[127]
Acellular matrices	Retention of the bioactive matrix; Structural integrity with better mechanical performance Need improvements in terms of the morphology and precise structure of the original tissue after decellularization; cell filling rate during the recellularization process needs to be improved	Acellular porcine small intestinal submucosa graft Corpora collagen matrices Autologous cartilage rods Amniotic membrane	Functional restoration of the penis Penile prostheses Penile reconstruction (treatment of Peyronie's disease) Recellularized in vitro with primary uterine cells (Bioengineered uterine tissue) Potential scaffold for testicular repair Artificial ovaries 3D dynamic culture of mouse embryonic fibroblasts Testicular organoid construction	[62,66] [67] [128] [68–71] [72] [63,64] [70] [73–75]
		Acellular uterus		
		Bovine pericardium Ovarian scaffolds Decellularized placental scaffold Acellular testis		

chemical cross-linking, and physical cross-linking based on their formation methods. The natural polymers that have a lot of hydroxyl groups on the molecules (e.g. hyaluronan, gelatin, collagen) can be modified with photopolymerizable methacrylate (MA) groups to allow the natural matrix to be chemical covalently cross-linked by UV light [46]. As a linear anion polysaccharide, alginate, chitosan and agar can be cross-linked by Ca^{2+} to form the hydrogels, films and macroparticles [47]. Besides, enzyme reactions have been used to drive the polymerization of gelation and collagen-based hydrogels by generating no cytotoxic byproducts [48,49].

Because of the similar mechanical environment and similar components to human tissue, natural hydrogels and scaffolds have been widely used for 3D cell culture systems of cells and tissues in vitro. 3D hydrogels culture systems can ensure the effect of cell-cell interactions among different cells, which is vital for reproductive tissue engineering [50–53]. For example, an alginate-based 3D culture system can support the interactions between germ cells and somatic cells to promote the self-organization of testicular cells into seminiferous tubules [54]. In reproductive tissue engineering, hydrogel culture systems like Matrigel, which is composed of ECM proteins and growth factors, have been used to mimic the 3D environment of testis and ovary [40–45,55]. However, inherent shortcomings such as uncertainty of biosafety, mixed components, and lack of physical and chemical versatility hinder the

widespread use of Matrigel [56]. Other natural hydrogels can supply the required biological and mechanical signals by loading other factors (cytokines, growth factors, proteins or collagen components) [57,58]. Moreover, the natural scaffolds with the porous structure can be obtained by 3D printing or lyophilized preparation method. The natural scaffolds have a good tissue integration and can load some cell growth factors (e.g. VEGF) and drugs to improve angiogenesis and tissue regeneration when implanted into the body [59,60]. Nevertheless, the insufficient mechanical strength of natural hydrogels and scaffolds, especially the poor tensile strength, limits their application in reproductive tissue reconstruction.

2.3. Acellular matrices

Acellular matrices are obtained by chemically removing all cells while retaining the bioactive matrix. It can provide a bionic structure consisting of collagens, elastin, and bioactive proteins and has a similar mechanical performance to native tissue [61,62]. Acellular matrices are usually obtained via sodium lauryl ester sulfate (SLES)-treated or sodium dodecyl sulfate (SDS)-treated processes [63–65].

To date, acellular matrices, including corpora collagen matrices [62, 66], autologous cartilage rods [67], acellular uterine tissue [68–71], and bovine pericardium [72], have been used as the main methods to

regenerate the penis, uterus, and vagina. Acellular ovarian scaffolds [63, 64] and acellular matrix testes [73–75] can provide a suitable environment to culture ovarian and testicular tissue fragments and related cells for follicle growth and spermatogenesis after recellularization. For example, the acellular matrices of the ovary can maintain the specific orientation and structure of collagen fibers and biological activity after decellularization, and the natural elasticity of natural collagen can protect the formation of follicles [63]. Moreover, the composition and nanostructure of the biomaterials affect the morphological and physiological behavior of testicular cells. Artificial scaffolds usually lack or have limited biocompatibility and tissue specificity and result in disorganized testicular cells. Since the testicular matrix is rich in tissue-specific ECM proteins, it has been considered the best scaffold for testicular recellularization [73,76]. They have the functional structure to separate seminiferous tubules, provide a closed environment for spermatogenesis and are responsible for mediating the intercellular communication and transportation of biologically active molecules.

At present, the developed acellular matrix materials must be improved in terms of the morphology and precise structure of the original tissue after decellularization, and the subsequent recellularization strategy must be improved to preserve the ultrastructural characteristics of the matrix and increase the cell filling rate during the recellularization process. To improve the cellular content, a dynamic seeding approach was reported. The biomaterials were placed in the center of the perfusion bioreactors with a stirring speed of 40 rpm to keep the cells in suspension, seed on the biomaterials and continuously penetrate into the scaffold pores, and the flow perfusion rate can modulate the cell deposition [77,78].

3. Biomaterials of in vitro reproductive tissue engineering

3.1. In vitro spermatogenesis

In vitro spermatogenesis is a significant treatment for sperm deformity and azoospermia for male fertility. This technique is crucial in many clinical applications, hopefully including exploring the cellular mechanisms during the generation of male germ cells and developing new strategies to produce male germ cells in vitro [13]. Spermatogenesis can be achieved by culturing testicular tissue fragments, prepubertal testicular cells, or ESCs in vitro (Table 2). In 2011, Sato et al. placed testicular tissue fragments in the gas-liquid interphase of agarose gel and successfully reconstituted mouse sperm in vitro [79,80] (Fig. 2). Also, Stukenborg et al. developed a soft agar culture system to provide the physical support and environmental features that enable the clonal expansion of male germ cells in coculture of spermatogonia and somatic cells [81]. Spermatogonial stem cells (SSCs) are located in seminiferous tubules and have close contact with Sertoli cells in the testis [50,82]. The success of spermatogenesis depends on the correct spatial arrangement of SSCs and Sertoli cells, which organize in seminiferous tubules in the testis [50]. Based on previous studies, 2D culture systems may lose the effect of cell–cell interactions between SSCs and Sertoli cells and also have a limited potential in the formation of testicular microtubules [39, 83]. Therefore, 3D culture systems have been developed with biomaterials to support the cell–cell interactions among different cells in the process of in vitro spermatogenesis (Table 2). Natural hydrogels and scaffolds are the main choice. For instance, Baert et al. achieved mouse in vitro spermatogenesis in weeks following prepubertal testicular cells seeding on alginate-based 3D-bioprinted scaffolds to produce testicular constructs [84]. An in vitro 3D culture system of testicular cells formed by collagen hydrogel enable the spermatocytes from patients with nonobstructive azoospermia to differentiate into spermatids [39]. Moreover, the hydrogel microspheres also can serve as a 3D culture model to encapsulate mouse ESCs and improve the differentiation process to primordial germ cells [85]. As we know, the mechanical environment of hydrogels for sperm generation are important and usually be very soft in those previous works [43,86,87]. And suggested that

Table 2
3D culture systems of reproductive cells and tissue fragments in vitro.

Reproductive cell culture and transplantation	Biomaterials and cells/tissue fragments	Highlights	Ref.
In vitro spermatogenesis	PLGA hydrogel (SD rat testicular cells)	Provide a favorable environment for spermatogenic germ cells to proliferate and differentiate into mature spermatids	[27]
	Collagen gel matrix (human testicular cells)	Culture of spermatogenic cells from nonobstructive azoospermic patients to produce germ cells	[39]
	Agarose gels (mouse testicular tissue fragments, gas-liquid interphase)	Successfully reconstituted mouse sperm in vitro maintained over 2 months	[79, 80]
	Alginate-based bioprinted scaffolds (mouse prepubertal testicular cells)	Successful in-vitro spermatogenesis in 80% of cultivated prepubertal tissue fragments in 3D-printed hydrogel scaffold	[84]
	Collagen IV-alginate microspheres (mouse ESCs)	Producing mature germ cells from mouse ESCs differentiation	[85]
	Matrigel (Sprague Dawley rat testicular cells)	Generate testicular organoids with a functional BTB	[41, 42]
In vitro follicle culture	Hyaluronic acid-based hydrogel (mouse preantral follicles)	Support follicle growth, estradiol secretion and resumption of meiosis in 3D culture	[29]
	3D-printed microporous gelatin scaffolds (secondary follicles from 16-day-old mice)	Producing normal levels of hormones	[30]
	Alginate-based hydrogel (microencapsulation of rat ovarian cells)	Achieve stable secretion of hormones during 90 days	[34]
	Collagen-based hydrogel (ovarian follicles from rats)	Type I collagen hydrogel under different environmental stresses affect development of ovarian follicles	[51]
	Fibrin-based hydrogel (mouse primordial and primary ovarian follicles)	Transplantation into adult mice to obtain live births	[57]
	Fibrin-based hydrogel (ovarian tissue from young mice)	VEGF-loaded hydrogel promoted angiogenesis and enhanced engraftment and function of the tissue	[58]
	Cross-linked hydrogel of 4-arm PEG and difunctional peptide (immature secondary follicles from 14- to 15-day-old mice)	Synthetic hydrogels with tunable properties; support a 17-fold volumetric expansion of follicles	[92]
	Fibrin-alginate matrices (two-layered secondary follicles from young mice)	The rate of producing competent oocytes raised to 82%	[129, 130]

testicular cells embedded into the very soft decellularized testicular dECM gel (storage modulus <20 Pa) undergo better seminiferous tubule organization, which is superior to the reference culture in collagen hydrogels (storage modulus 75–100 Pa) [88]. However, the effect of the mechanical environment on sperm generation has not been

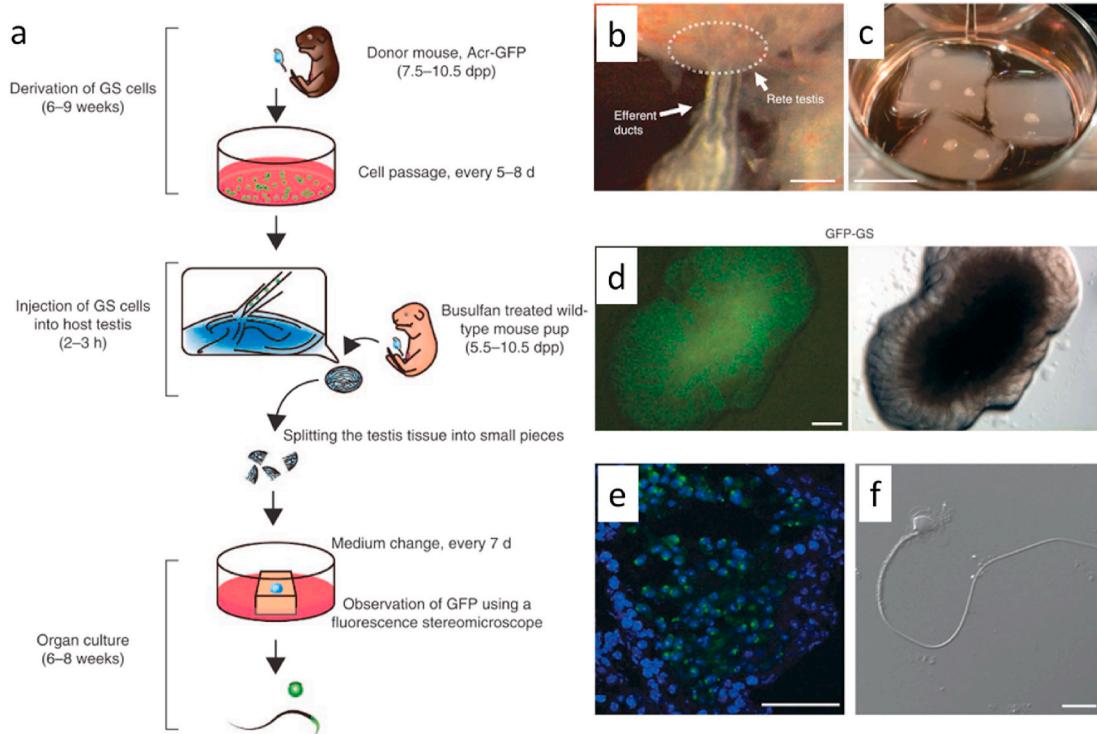


Fig. 2. Testicular tissue fragments cultured in the gas–liquid interphase of an agarose gel to reconstitute mouse sperm in vitro. (a) Schematic diagram of the protocols of the gas–liquid interphase culture method. (b) Efferent ducts and rete testis, Scale bars: 0.5 cm. (c) The testis tissue fragments were placed on agarose gel and half-soaked in the medium. (Scale bars: 1 cm) (d) GFP-expressing germline stem cells (GFP-GS). (e) Immunostaining of the host testis tissue. GFP (green), Hoechst 33342 dye (blue). Scale bars: 50 µm. (f) The flagellated sperm were found after 57 days of culture of mouse testicular tissue fragments. Scale bars: 10 µm. Reproduced with permission [80]. Copyright 2013, Nature Protocols.

systematically studied.

Still, the efficiency and duration of in vitro spermatogenesis were much lower than those in vivo [89]. To improve the efficiency and duration of sperm cell production, a microfluidic device has been adopted to culture testis tissues and ensure that nutrients and oxygen are efficiently and evenly provided [89–91].

3.2. In vitro follicle maturation

In vitro production of oocytes and fertilization can be an appropriate treatment for infertility in females and an advanced method to study the mechanism of follicle maturation in vivo [4,51]. The biophysical microenvironment can determine the cell fate and development in the process of ovarian follicle maturation [51]. A mature follicle is enclosed by theca and granulosa cells as supporting cells in the ovarian follicle. Thus, remaining in the spheroid shape of ovarian follicles and maintaining the cell-cell communication between support cells and oocytes are critical in the proliferation and maturation process [34,53]. Similar to in vitro spermatogenesis, 3D culture systems that formed by hydrogels and scaffolds also have the advantages of maintaining the cell-cell interactions and the normal shape of follicles (Table 2). Previous studies have shown that the physical environment and scaffold architectures of biomaterials and other loaded factors (cytokines, growth factors, proteins or collagen components) in hydrogels can impact the survival, phenotype and functional maintenance of follicles [30,51,53]. First, hydrogels or scaffolds can modulate the environmental stresses for the oocyte development by adjusting the concentration of the matrix. For example, Joo et al. altered the concentration of type I collagen hydrogel from 1% to 7% (weight/volume) to generate a 3D culture system for oocyte maturation of ovary follicles under different environmental stresses [51]. Ariella et al. designed the peptides crosslinked PEG hydrogels with a tunable physical properties that can support

17-fold volumetric expansion of follicles in the hydrogel network (Fig. 3a and b) [92]. The two studies demonstrated that suitable mechanical environment in ovarian follicle culture is vital for the survival, phenotype and functional maintenance of follicles. In addition, Laronda et al. reported a 3D-printed gelatin-based microporous scaffold to create a bioprosthetic ovary to restore the ovarian function of sterile mice (Fig. 3c–f) [30]. In their work, the 3D-printed microporous scaffold can support the adhesion of isolated follicles, and optimizing the porosity and pore geometry can improve the angiogenesis, survival and function of mouse follicles. Additionally, cytokines and growth factors can be introduced to improve the hydrogel culture environment for maintaining their respective phenotypes of follicles and oocyte generation [34, 58]. The development of biomaterials for in vitro oocyte maturation will be a new direction to change the current solutions for fertility preservation [93,94].

3.3. Construction of reproductive organoids and models

Organoids are an in vitro culture model of 3D source tissue (from cells to tissue fragments) and have some level of tissue structure and function [95,96]. In reproductive organ tissue engineering, the study of in vitro organoids can be programmed and provide a support matrix and appropriate physical and biochemical cues to highly simulate the natural environment of cells in living organisms to study the mechanism and interactions in reproductive organs [53,97–99]. Here, we will introduce the biomaterials to construct various reproductive organoids and models, including testicular organoids, ovarian organoids, and other organoids and models.

3.3.1. Testicular organoids

Testicular organoids are an in vitro model of functional testicles to study the mechanisms of spermatogenesis and are a predictive first-tier

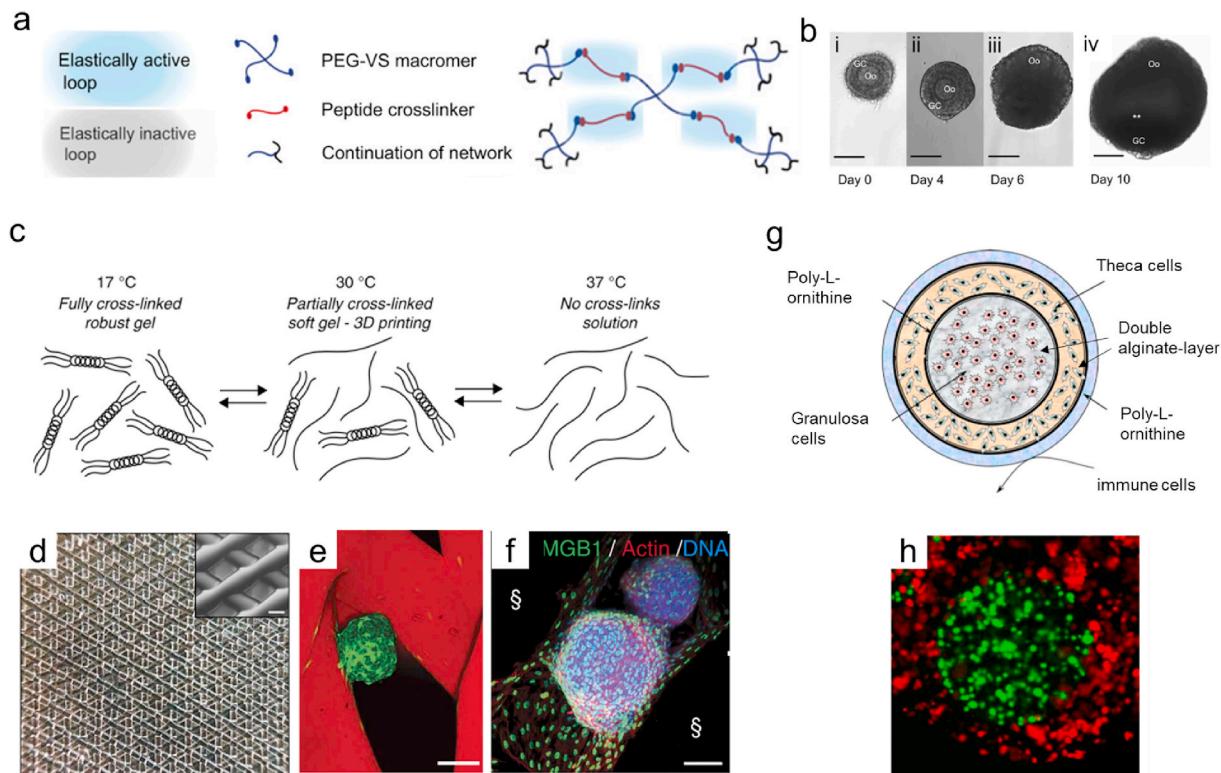


Fig. 3. Hydrogels and scaffolds designed for a 3D follicle culture. (a–b) PEG-based hydrogels with a difunctional peptide as the cross-linker for an ovarian follicle culture. (b) i–iii Morphology of ovarian follicles cultured in PEG hydrogel within 10 days. Scale bars: 100 mm. Reproduced with permission [92]. 2011, Biomaterials. (c–f) 3D-printed microporous gelatin scaffolds seeded with follicles. (c) Schematic of the thermoreversible properties of gelatin. (d) Photographs of a five-layered 3D-printed scaffold. Scale bar: 250 mm. (e) Follicles were seeded in a 60° scaffold, and confocal fluorescence images (f) of follicles cultured for 2 days. Scale bars: 100 mm. Reproduced with permission [30]. Copyright 2017, Nature Communication. (g) Schematic of the ovarian constructs. The ovarian constructs were fabricated using a cross-linked alginate layer and poly-L-ornithine (PLO) layer encapsulated with granulosa and theca cells to replicate the key structure of ovarian follicles. (h) Different cells and granulosa cells were labeled with CellTracker Green, and theca cells were labeled with CellTracker orange within the constructs. Reproduced with permission [34]. Copyright 2012, Reproductive Biology and Endocrinology.

drug-screening tool for the treatment of human spermatogenic failure [50,76]. The construction of a complete male microenvironment involves reaggregating testicular cells, performing testicular tubulogenesis, and forming the blood-testis barrier.

3D testicular culture systems can provide complex spatial conditions and microenvironments similar to the testis *in vivo*, which enables germ cell tissue to form dense cellular clusters, provides and maintains potentially important contact between germ cells and Sertoli cells during sperm formation [50,85,100]. 3D biohydrogels such as soft agar [13], methylcellulose, collagen, and Matrigel [41] are the main 3D matrices to mimic the microenvironment of testicular cells to construct testicular organoids. In the testis, the blood-testis barrier (BTB) is a vital part of male reproductive function to defend the testis against foreign pathogens and environmental alien organisms and separate apical (adluminal) compartments of the seminiferous epithelium to provide a specialized microenvironment for hormonal regulation and spermatogenesis [101,102]. João Lee and colleagues reported a three-layer gradient system; specifically, a layer of Matrigel/testicular cells was placed between two layers of acellular Matrigel to generate testicular organoids with a functional BTB [41,42] (Fig. 4a–c). These testicular organoids are robust and reproducible models that can be applied to track and quantify cell migration in testicular organoids, study the role of the growth factors and study the toxic effects of drugs and environmental pollutants on the integrity of the BTB and maintenance of germ cells [42]. BTB formation can support cell epithelialization in these testicular organoids [41]. In addition, reducing the matrix size [103] is beneficial to the nutrient and oxygen supply for organoid culture, which improves the spermatogenic function of the testis and promotes the

differentiation of germ cells in tubule-like structures.

3.3.2. Ovarian organoids

Ovarian follicles function as globular multicellular aggregates. The oocytes in ovarian follicles must maintain contact with the supporting cells until maturation and preparation for ovulation. The development of female ovarian 3D organoid technology has opened up a new approach to study its biology, pathology, and pregnancy physiology, and it has great potential in ovarian cancer drug screening. In the construction of ovarian organoids, the function of the tissue (support follicular growth, maturation, and release into the fallopian tubes) and basic morphological characteristics are required [104].

The combination of Matrigel and tissue fragments or isolated cells can construct a 3D cell culture model system as ovarian organoids [44, 45]. For example, Koppen et al. constructed ovarian cancer organoids by isolating tumor cells from tumor pieces suspended in basement membrane extract for drug screening and investigation of the subtype responses of different tumors [44,105]. In exploring the relationship between ovarian cancer and chronic inflammation, as the origin of ovarian epithelial tumors, spheroids of human ovarian surface epithelial (HOSE) cells were harvested and seeded in ovarian organoids formed by the growth factor-reduced Matrigel. Then, whether the protumor inflammatory cytokine tumor necrosis factor α would introduce a malignant phenotype in normal HOSE cells was determined [45] (Fig. 4d–f).

3.3.3. Other organoids and models

Other organoids and models that have adopted biomaterials in reproductive tissue engineering are limited. Firstly, the placental barrier

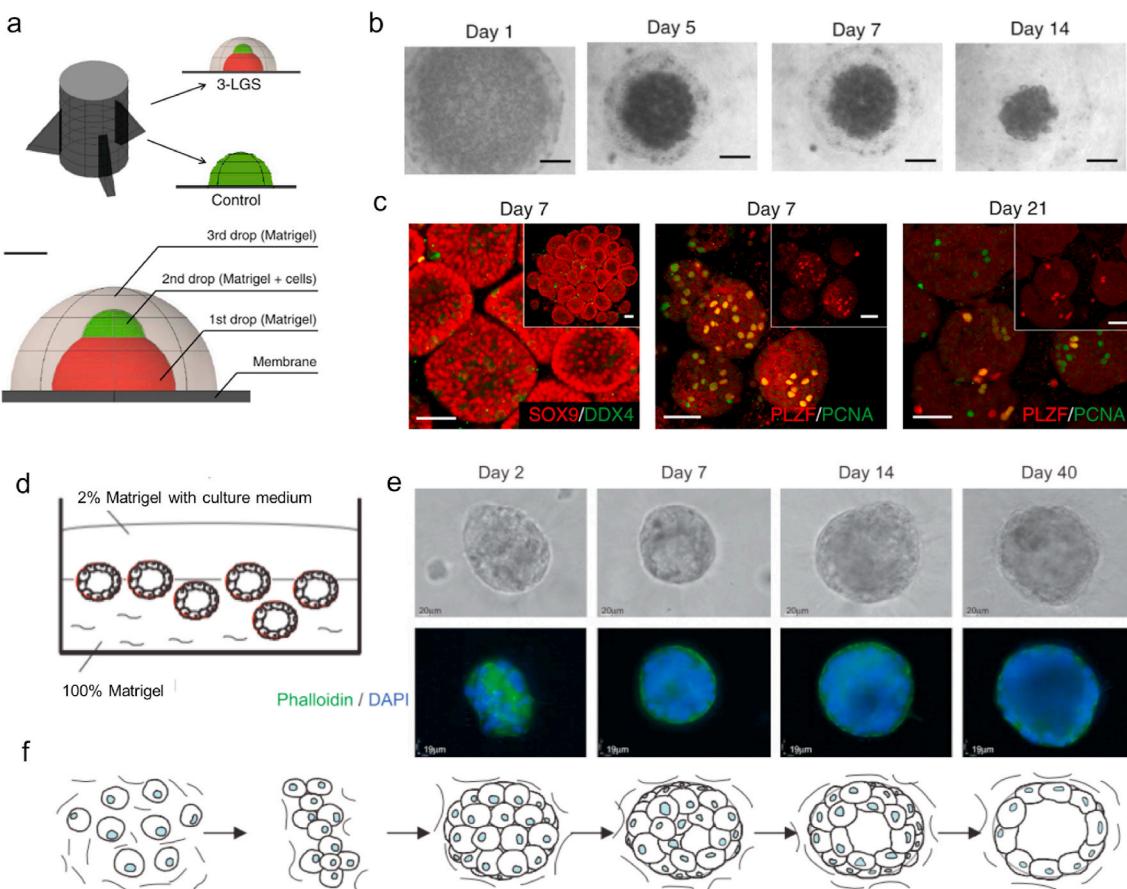


Fig. 4. Formation of testicular and ovarian organoids using Matrigel. (a–c) Scheme of testicular organoids using a three-layer gradient system (3-LGS). (b) Formation of 3-LGS testicular organoids after days 1, 5, 7, and 21 by bright-field microscopy. (c) Cells that were positive for SOX9 (Sertoli cell marker) and DDX4 (germ cell marker) in spherical-tubular structures of the testicular organoids after days 7 and 21 in culture. Reproduced with permission [42]. Copyright 2018, Nature Protocol. (d) Schematic diagram of the in vitro ovarian organoid model with spheroids of normal HOSE cells cultured in 3D matrix. (e) Phase-contrast images and fluorescence staining of F-actin and nuclei in the spheroids of normal HOSE cells. (f) Schematic diagram of morphogenesis in the spheroids of normal HOSE cells. Reproduced with permission [45]. Copyright 2009, Neoplasia.

regulates the maternofetal exchange of endogenous and exogenous substances, which plays an essential role in fetal development [106]. Therefore, many scientists have focused on constructing placental barrier models [107] with great potential for testing interventions for diseases, improving the pregnancy process, identifying better treatment options, and achieving more accurate diagnoses. Kuo et al. reported a dynamic 3D placental model constructed by bioprinted gelatin hydrogels with a patent central lumen to model the spiral arteriole and study interactions between trophoblasts and endothelial cells and investigate the trophoblast–endothelium signaling involving angiogenesis and apoptosis [31]. Nanofilm biomarkers have also been used to construct a human placental barrier model with bilayer structures similar to the placental barrier in early pregnancy [106]. In this work, collagen and laminin nanofilms were used to mimic the basement membrane and encapsulate primary fibroblasts and endothelial cells. The nanofilms could support cell adhesion and function, cell communication, and gap junction formation [106]. The endometrial model is a good platform for studying embryo gestation and dynamic changes during the menstrual cycle. In the work of Boretto and his co-workers, processed tissue fragments of uterine horns have been cultured in 70% Matrigel, as a 3D extracellular matrix, to develop an endometrial organoid [55] and Bishop et al. then followed this research to model chlamydia infection in endometrium [108]. Besides, Matrigel is also the main biomaterials used in other organoids to study the human female reproductive tract like fallopian tubes and cervix [104,108,109].

4. Biomaterials of in vivo reproductive tissue engineering

In vivo reproductive tissue engineering includes the in vivo transplantation and functional regeneration of reproductive organs. Biomaterials for in vivo reproductive tissue engineering must provide similar mechanical properties to those of original tissue with biodegradability and nontoxic byproduct generation in vivo and bionomic structures.

4.1. Biomaterials for in vivo transplantation of reproductive tissue

In previous studies, in vivo transplantation of artificial ovaries, primordial follicles or testicular tissue cells can recover the normal levels of hormones in experimental animals, while some biomaterials can be adapted to assist in vivo transplantation to enhance the success rates [30,37,38,57].

Hydrogels based on natural polymers can also be a potential matrix for in vivo transplantation of isolated preantral follicles and ovarian cells to construct transplantable artificial ovaries [32–38,57,58,65]. For example, isolated mouse follicles were inserted into microporous gelatin scaffolds and embedded into ovary-removed mice, which results in the production of normal levels of hormones [30]. Kniazeva et al. encapsulated the primordial and primary ovarian follicles into fibrin-alginate, fibrin-collagen, and fibrin for subsequent transplantation into adult mice [57]. In a further study, the results showed that VEGF highly increased the probability of live births among women with ovarian tissue

transplantation [58]. Acellular ovarian scaffolds, which maintains the monodisperse pores of the matrix and ovarian vascular structures, have been implanted into pubertal mice that have been ovariectomized and increased the levels of serum estradiol and inhibin A [65].

Moreover, testicular tissue has limited survival after *in vivo* transplantation as an ectopic xenograft [110]. Hui Gao et al. used different hydrogels to encapsulate mouse testicular cells and transplanted them into the dorsal region of recipient nude mice to study self-organized seminiferous tubule processes [86]. Among them, collagen hydrogels and Matrigel can promote the formation of a seminiferous tubule structure and sustained for 12 weeks. Conversely, the encapsulation of CELLINK hydrogel that based on cellulose-alginate, would prevent the proliferation of embedded testicular cells [86].

4.2. Biomaterials for reproductive tissue regeneration

Biomaterials have also been widely used in reproductive tissue regeneration. The majority of research cases on regenerative repair are related to the male penis and female uterus and endometrium. Biomaterials applied for *in-situ* regeneration and repair of penis and vagina are more likely to have better mechanical strength and the ability to promote vascular regeneration [21,60].

Traditional penile repair treatments, including prosthesis replacement and allogeneic transplantation, cannot meet the requirements of functional penile restoration. Synthetic polymer-based biomaterials are used for penile repair in the early years [22,23]. PGA mesh, combined with cells, can be used to reconstruct the smooth muscle tissue of the cavernous body [23]. To date, with good mechanical strength, acellular matrices have been used as the main method of repairing corpus cavernosum, including autologous cartilage rods [67], acellular corporal collagen matrices [62,66], and other acellular matrices, such as the porous bladder acellular matrix [111]. To address the challenges associated with the functional recovery of corpus cavernosum, decellularized matrices seeded with cells led to a more effective approach [61]. It has been demonstrated that corpora collagen matrices loaded with smooth muscle cells (SMCs) and endothelial cells (ECs) can recover a short segment of the penile corporal body and achieve structural and functional restoration of the penis [62]. As for burgeoning 3D bio-scaffold, we recently developed a gelatin-based hydrogel scaffold by 3D printing. Seeded with stem cells to improve neovascularization, the scaffold achieved a positive effect on penile reconstruction and recovered the reproductive capability of males [59] (Fig. 5a).

To reconstruct uterine tissue, the acellular uterus can serve as a bioengineered scaffold that has been recellularized by primary uterine cells or other MSCs [68–71]. Miyazaki et al. recellularized and

regenerated uterine tissue by placing acellular uterine stroma on a partially resected uterus [112]. Thermal dehydration cross-linked collagen scaffolds can maintain the normal cavity structure and induce the proliferation of inherent endometrial cells and epithelial cells, thereby improving the ability of the regenerated endometrium to receive embryos [28]. However, no effective method or corresponding biological material can be used to treat patients with complete loss of uterine structure or function.

Hydrogels [113–115], films [116], and scaffolds [117–119] based on natural or synthetic polymers are used as biological supports and cell or drug delivery systems to resist inflammation and promote regeneration of vascular and endometrial [120]. For example, a synthetic pluronic-F127 hydrogel delivered with BMSCs and vitamin C (Vc) molecules can be used to regenerate the endometrium in an intrauterine adhesion rat model [114]. Additionally, Yunlang et al. developed drug-loaded porous gelatin-based scaffolds by droplet microfluidics for endometrial repair, which can repair damaged endometrium and improve neovascularization [119] (Fig. 5b).

However, there is no successful case of using biomaterials to repair the testis and ovary because of their complex structures, components and functions. Nonetheless, with the development of biomaterials and reproductive tissue engineering, acellular matrix and bioscaffolds are potential biomaterials for testis repair [73–75]. In the future, the technology to print complex living-tissue constructs within seconds has led to hopes for future use in regenerating ovarian and testicular tissues [121,122].

5. Outlook

Although there are relatively few cases of developed biomaterials being introduced into the research of reproductive tissue engineering compared with other tissues. The development of biomaterials still help gain further research progress in reproductive organ tissue engineering. We believe that designing biomaterials for reproductive tissue engineering is an important research direction and deserves more attention. Also, due to the complex structure and function of organs containing specific reproductive hormones, especially the ovary and testis, reliable biomaterials have not been realized to promote the structural regeneration and functional recovery of these reproductive glands and have numerous obstacles.

Fortunately, various advanced biomaterial biomanufacturing technologies are rising and have a great potential in reproductive tissue engineering: 3D bioprinting technology, material modification and microfluidic technologies [14,123–125]. The emerging 3D bioprinting (printing with cells) has obvious strengths in developing complex 3D

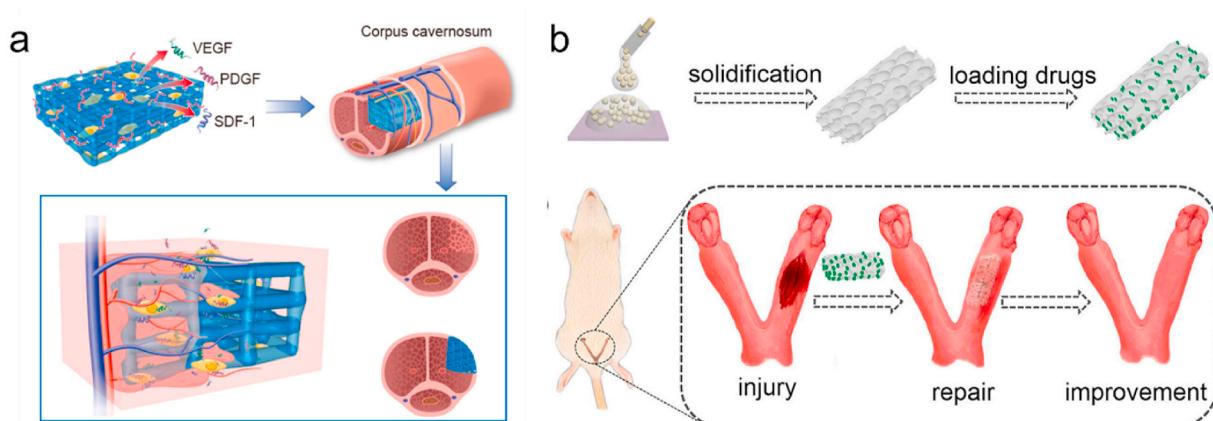


Fig. 5. 3D scaffolds were implanted for reproductive tissue regeneration *in vivo*. (a) 3D-printed methacrylated gelatin hydrogels seeded with stem cells for penile reconstruction [59]. Copyright 2020, Nature Communications. (b) Drug-loaded porous scaffolds (prepared from methacrylated gelatin and Na-alginate) were developed by the droplet microfluidics method for endometrial repair [119]. Copyright 2019, Acta Biomaterialia.

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