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3D bioprinting technology innovation in female reproductive system

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ABSTRACT

Several diseases affect the female reproductive system, and both disease factors and treatments impact its integrity and function. Consequently, understanding the mechanisms of disease occurrence and exploring treatment methods are key research focuses in obstetrics and gynecology. However, constructing accurate disease models requires a microenvironment closely resembling the human body, and current animal models and 2D in vitro cell models fall short in this regard. Thus, innovative in vitro female reproductive system models are urgently needed. Additionally, female reproductive system diseases often cause tissue loss, yet effective tissue repair and regeneration have long been a bottleneck in the medical field. 3D bioprinting offers a solution by enabling the construction of implants with tissue repair and regeneration capabilities, promoting cell adhesion, extension, and proliferation. This helps maintain the long-term efficacy of bioactive implants and achieves both structural and functional repair of the reproductive system. By combining live cells with biomaterials, 3D bioprinting can create in vitro 3D biomimetic cellular models, facilitating in-depth studies of cell–cell and cell–extracellular microenvironment interactions, which enhances our understanding of reproductive system diseases and supports disease-specific drug screening. This article reviews 3D bioprinting methods and materials applicable to the female reproductive system, discussing their advantages and limitations to aid in selecting optimal 3D bioprinting strategies. We also summarize and critically evaluate recent advancements in 3D bioprinting applications for tissue regeneration and in vitro disease models and address the prospects and challenges for translating 3D bioprinting technology into clinical applications within the female reproductive system.

1. Introduction

The health of the female reproductive system is crucial for women's quality of life and population reproduction. Anatomically, the female reproductive system comprises internal and external genitalia. In this article, the term "female reproductive system" primarily refers to the internal genitalia, located within the true pelvis, including the ovaries, fallopian tubes, uterus, cervix, and vagina [1,2]. Diseases, medications, genetics, physical injuries, and other endogenous and exogenous factors can cause damage and dysfunction in the female reproductive system, thereby affecting women's health and fertility [3].3D bioprinting is an emerging technology that has great potential in tissue and organ construction because of its ability to precisely control the spatial,therefore, therefore,it holds the promise of facilitating breakthroughs in the treatment and research of the female reproductive system.

The female reproductive system is a dynamic structure regulated by the hypothalamic-pituitary-gonadal axis, where periodic hormonal changes drive corresponding changes, such as ovulation and the cyclic growth of the endometrium. Therefore, hormone therapy plays a significant role in treating conditions affecting the reproductive system [13]. For instance, estrogen therapy can promote endometrial repair, thus treating intrauterine adhesions caused by improper curettage [14]. However, the success of hormone therapy depends on the presence of target cells, as a substantial loss of these cells can limit therapeutic outcomes. Moreover, the reproductive system is susceptible to space-occupying lesions, including tumors, for which surgical intervention is often the primary treatment. In cases of malignancy, additional treatments like radiotherapy and chemotherapy are employed. However, these treatments may damage or remove reproductive organs, impacting both reproductive and endocrine functions [15]. As a result, there is a pressing need for new treatment strategies to repair damaged reproductive tissues and restore reproductive capacity. The rapid

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development of regenerative medicine has attracted widespread attention in female reproductive health. Current approaches include autotransplantation of cryopreserved ovarian tissue for women with complete ovarian loss and the use of stem cell perfusion for patients with severe endometrial damage to achieve endometrial repair and regeneration [16]. However, these strategies lack a three-dimensional structural framework for functional cells, resulting in significant cell loss and challenges in establishing long-term engraftment in the body [17–19]. 3D bioprinting technology can address these limitations by depositing bioinks into high-resolution scaffolds that act as temporary extracellular matrices (ECMs). This 3D structure provides a microenvironment that supports the growth and proliferation of functional cells, restricts their outward migration, and sustains therapeutic effects within the body [20]. Thus, 3D bioprinting holds broad application potential in the regenerative medicine of the female reproductive system.

Currently, research on the female reproductive system's pathology and physiology relies mainly on animal and cell studies. However, genetic differences and varying metabolic pathways limit animal models' ability to replicate specific human biological processes. For instance, less than 8 % of cancer research findings from animal models advance to clinical trials [21]. Additionally, ethical concerns restrict the use of animal models. While human cells are preferred in research, traditional 2D cell cultures cannot accurately mimic the human microenvironment, such as growth conditions, cellular signaling, and interactions with neighboring cells or ECM [22]. The application of 3D bioprinting in creating 3D in vitro models offers a promising approach for studying the female reproductive system. Compared with 2D systems, 3D models demonstrate notable differences in morphology, cell vitality, proliferation, differentiation, and gene expression profiles, providing conditions closer to in vivo [23]. In studying reproductive system tumors, traditional cell culture models often cannot simulate the later stages of tumor development due to limitations in constructing complex structures and vascular networks. 3D bioprinting technology can accurately control the composition and spatial distribution of tumor-related cells and ECM components, enabling high-resolution and high-throughput creation of tumor models with complex, multi-scale structures, multiple biomaterials, and vascular networks. This is crucial for effective, patient-specific drug screening and biomedical research in reproductive system tumors. For example, cervical tumor spheroid models produced via 3D bioprinting are used to examine cell proliferation, matrix metalloproteinases (MMPs), and the response to paclitaxel treatment. In these models, HeLa cells exhibit increased proliferation, enhanced MMP protein expression, and greater resistance to paclitaxel than traditional 2D culture models [4]. Therefore, 3D bioprinting presents valuable opportunities for constructing in vitro models of the female reproductive system, particularly for tumor modeling.

In this review, we summarize the methods and materials in 3D bioprinting relevant to the female reproductive system and discuss the current applications of 3D bioprinting in tissue regeneration and in vitro model development. Finally, we address challenges and potential solutions for the future application of 3D bioprinting technology in the female reproductive system (Fig. 1).

2. 3D bioprinting methods and biomaterials suitable for the female reproductive system

3D bioprinting technology can be understood in both broad and narrow contexts. Broadly, 3D printing that is directly related to biological applications can be classified as 3D bioprinting. In the narrow sense, however, 3D bioprinting specifically refers to the process of using 3D printing technology to manipulate living cells in order to construct biomimetic 3D tissues [24]. In this article, "3D bioprinting" refers to this narrower definition. The process of 3D bioprinting involves layering bioinks along a predefined path to form 3D tissues and organs with complex structures [25]. Therefore, 3D bioprinting is centered around two key components: bioink formulation and the selection of printing methods. To effectively apply 3D bioprinting to the female reproductive system, it is essential to understand the benefits and limitations of various 3D bioprinting methods and the characteristics of biomaterials used in bioinks. The following sections provide a summary of these aspects (see Table 1 and 2).

2.1. 3D bioprinting method

2.1.1. Extrusion-based 3D bioprinting (EBB)

EBB involves continuously extruding bioink filaments through a nozzle using an air pump or screw plunger to generate pressure [26,27]. This process builds 2D patterns layer by layer to create a 3D spatial structure [28]. The advantages of EBB include high printing speed, low cost, ease of operation, repeatability, high cell density, scalability of print models, and compatibility with a wide range of biomaterial viscosities. EBB is currently the most commonly used 3D bioprinting technology for applications related to the female reproductive system [29]. When fabricating biomimetic structures of the female reproductive

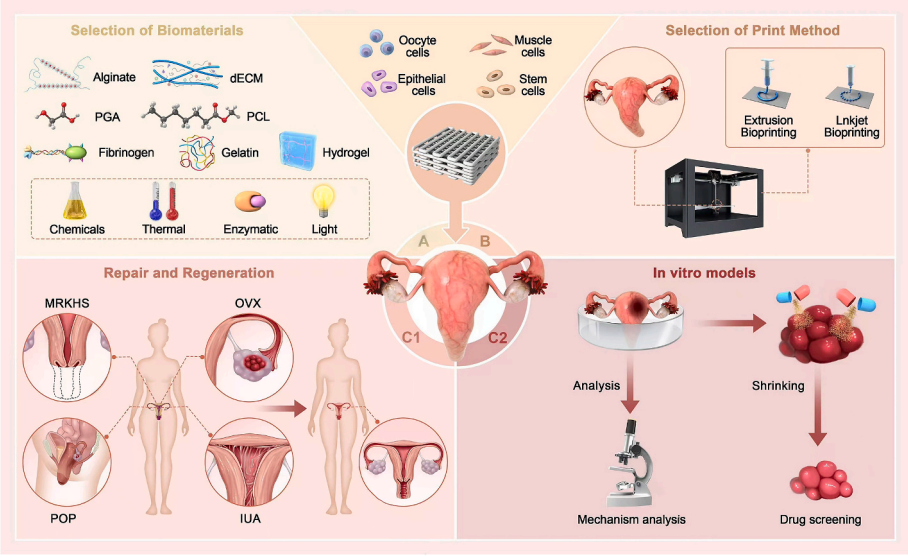

Fig. 1. The main steps of 3D printing of the female reproductive system and its main applications in the female reproductive system.

Table 1
Comprehensive overview of common bioprinting techniques and its applicability in the female reproductive system.

3D Bioprinting	Nozzle inner diameter	printing temperature	Cell type	Cell density	Cell construction method and viability	Advantages and salient achievements	Limitations and challenges	Ref
Extrusion-based 3D bioprinting	250 μm	10 °C	Hela cells	10^6 cells/ml	Cell-laden bioprinting. 94.9 % ± 2.2 % after printing	● Reveal that the printed 3D models have more simulated tumor characteristics compared with the 2D planar cell culture models.	● That increased mechanical forces cause cellular damage and thus reduce cell survival rate.	[4]
	100 μm	30 °C	Follicles of the mice	40–50 follicles	Deposited follicles onto the Scaffolds. 78.57 ± 3.57 % (30°) 75.89 ± 4.04 % (60°)by day 8 of culture	● To scale the size of the tissue to the size needed for the transplant recipient. ● Investigated how scaffold pore geometry affected the growth and maturation of ovarian murine follicles as well as developed a bioprosthetic ovary that restored ovarian function.	● Require optimizing the number of cells transferred to the scaffold and the assessment of durable function.	[5]
	100 μm	25 °C	hiMCS	10^6 cells/ml	Cell-laden bioprinting. 84 % ± 4 %	● That the 3D-printed hydrogel can support the long-term viability of hiMSCs in vivo and in vitro.	● An excessive cell concentration not only blocks the nozzle but also prevents formation of the scaffold. ● The problem of early vascularization of the 3D-printed scaffold needs to be addressed.	[6]
Extrusion-based 3D bioprinting	340 μm	25 °C (the nozzle temperature)	BMSCs	10^6 cells/ml	Cell-laden bioprinting. >95 %	● The scaffold may allow unlimited nutrition and oxygen delivery to the cells within the solid graft, which makes it possible for long-term culture in vitro.	● The cells in the printed constructs may be damaged by the applied shear force when printing. ● The cytotoxicity of residual chemicals in the bioink from AVM may affect cell viability. ● The cell density of existing 3D printing are rough estimates.	[7]
Inkjet-based 3D bioprinting	150 μm	_	OVCAR-5 cells and MRC-5 cells	10^6, 2×10^6, 5×10^6 10×10^6 cells/ml	93.8 % (OVCAR5) 90.1 %(MRC-5) at 72 h post patterning	● Micropatterned ovarian cancer cells (OVCAR-5) and fibroblasts (MRC-5) with spatial control. ● Both OVCAR-5 and MRC-5 can be ejected with controlled number of cells per droplet maintaining high viability.	The nozzle configuration restricts it to low-viscosity materials.	[8]

Table 2
Materials used as bioink components for female reproductive system.

Materials	Crosslinking strategies of materials	Crosslinking strategies of model	Printing method	Reason for selection	Application organization	Ref
Gelatin/ alginate/ fibrino-gen	Temperature-induced gelation (25 °C)	Chemically crosslinked(CaCl2 3 %)	Extrusion	To Simulate the natural extracellular matrix environment and to achieve both high cellular viability and a stable and clear structure.	Cervical cancer model	[4]
Gelatin	Temperature-induced gelation (30 °C)	Chemically crosslinked	Extrusion	Gelatin was selected because it is derived from collagen, an extracellular matrix protein abundant in both human and mouse ovaries,is degradable to allow for cellular remodel, contains cell adhesion sites and has soft, yet durable mechanical properties.	Ovary	[5]
GelMA	Blue light crosslinked	Chemically crosslinked	Extrusion	Good swelling properties, degradation kinetics and shape fidelity of GelMA scaffolds were proved.	Ovary	[9]
dECM/alginate	Chemically crosslinked(CaCl2)	Chemically crosslinked(CaCl2)	Extrusion	dECM can achieve structural remodeling by supporting the formation of specific tissue at the implanta.tion site, rather than forming poorly functional scar tissue.	Vagina	[8]
Alginate	Chemically crosslinked(CaCl2)	Chemically crosslinked(CaCl2)	Extrusion	Alginate is commonly used and highly biocompatible,low cost,and its printing technology is mature。	Cervical cancer model	[10]
dECM/gelatin/ alginate	Temperature-induced	chemically crosslinked	Extrusion	dECM support a variety of cells due to their complex tissue-specifc properties and unique composition of functional components.	Ovary	[11]
AloeVera-Sodium Alginate and PCL	In situ cross-linked	_	Extrusion	AV containing hydrogel can enhance the overall immunomodulatory effect of eMSCs and reduce the foreign body response to MES PCL meshes.	Pelvic floor tissue	[12]

system, biological scaffolds loaded with functional cells are often employed. These scaffolds serve two main functions: first, to provide growth and attachment points for cells, and to present a spatial structure that allows functional cells to establish connections with the surrounding microenvironment, thereby better supporting physiological functions [30]. For example, Laronda et al. [5] used EBB to construct an artificial 3D-printing ovary by implanting exogenous follicles into a GelMA hydrogel scaffold. EBB was selected because it facilitates precise porosity in scaffolds, which in turn supports follicle attachment, vascularization, and ovulation through the scaffold pores [9]. The biological scaffold fabricated by this extrusion method meets the requirements for constructing artificial 3D ovaries. However, the EBB method still has disadvantages, such as low printing resolution and nozzle clogging when using high-viscosity bioprinting ink [31–33]. These issues can be addressed by adjusting the nozzle diameter and bioink viscosity [34]. In the past, hot gelatin (solution phase) was extruded onto a cold stage to induce rapid gelation in EBB applications [35,36] or cooled, fully crosslinked gel was directly extruded [37,38]. The former approach often leads to filament diffusion and poor layer resolution, whereas the latter results in blocky and uneven lines, thereby forming irregular pores. Laronda et al. [5] addressed these issues by using the thermal response characteristics of gelatin. They cooled the gelatin solution to achieve a partially crosslinked gel state and extruded it through a fine-diameter nozzle (100 mm) at 30 °C, resulting in smooth and continuous filaments and precise porosity.

In addition, EBB enables the incorporation of additional injectors, allowing multiple bioinks such as methacrylate (GelMA) and polycaprolactone (PCL) loaded with various cell types to be printed, which is valuable for creating female reproductive system models with diverse cell types and complex microenvironments [39].

2.1.2. Inkjet-based 3D bioprinting

Inkjet 3D bioprinting, also known as droplet printing, is a non-contact method that differs from EBB by producing fine droplets instead of continuous filaments. In inkjet bioprinting, a single droplet acts as the basic unit, with thermal, piezoelectric, and electrohydrodynamic mechanisms being the primary methods for droplet formation [40]. Fig. 2 shows the specific classification and process. Compared with EBB, inkjet bioprinting provides greater control over biological elements, with high resolution that makes it ideal for fabricating small-scale tissues. The resolution of the printed structures depends on the droplet size passing through the nozzle. By adjusting droplet size and density, the concentration and gradient of cells and biomaterials within the scaffold can be precisely modified [41]. In research on female reproductive system diseases, such as reproductive tumors, the growth kinetics of tumor cells (e.g., cell size and density) are closely related to the spatial structure and cell distribution density. Inkjet bioprinting can modify cell and biomaterial gradients throughout the scaffold by adjusting droplet size and density, making it highly suitable for fabricating in vitro models of female reproductive system diseases with controlled and accurate cell density distribution. For example, Xu and colleagues employed inkjet 3D bioprinting to create 3D culture models containing human ovarian tumor cells (OVCAR-5) and MRF-5 cells (normal human fibroblast cell line) to investigate tumor-stromal cell regulatory feedback mechanisms and drug sensitivity [6]. However, the principles of inkjet bioprinting limit the selection of bioinks, as the nozzle configuration restricts it to low-viscosity materials [42].

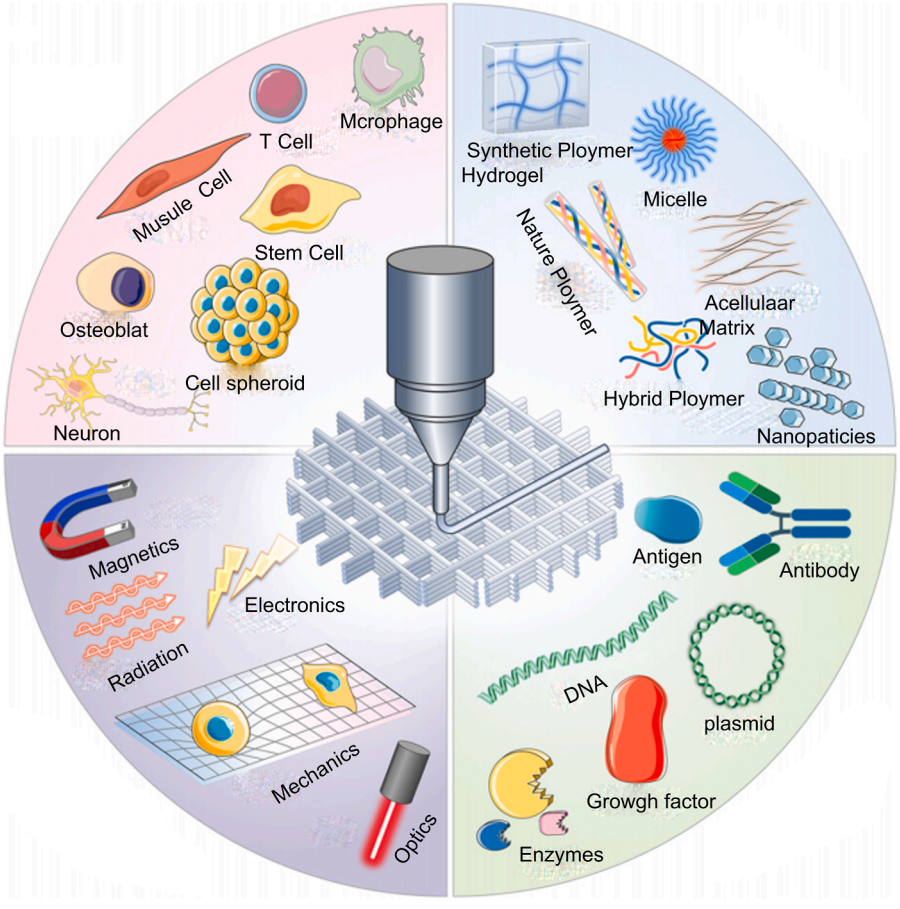

Fig. 2. The constituent elements of bioink. Reproduced with permission from Ref. [43]. Copyright © 2020, American Chemical Society.

2.2. Biomaterials

Bioink typically comprises three components: biomaterials, live cells, and bioactive factors such as growth factor. Biomaterials are deposited layer by layer into a predefined 3D biomimetic structure using 3D bioprinting technology, where they serve as an ECM supporting cell attachment, migration, proliferation, and differentiation within the printed structure (Fig. 2). Biomaterials must possess both printability and cell compatibility. Printability is crucial for maintaining the shape fidelity and mechanical stability of 3D-bioprinted structures, ensuring that biomaterials can withstand the forces applied during the printing process and retain structural integrity post-printing. The compatibility between biomaterials and cells impacts cell viability, migration, proliferation, differentiation, and the eventual formation of tissue. For effective application of 3D bioprinting in the female reproductive system, selecting biomaterials for bioink formulation should align with the characteristics of the intended printed tissues and the chosen printing methods [44]. The commonly used biomaterials for 3D bioprinting in the female reproductive system are summarized below

2.2.1. Natural high-molecular-weight polymer

Natural polymers, which are abundant in animal, plant, and microbial tissues, offer high biocompatibility and biodegradability [45]. Currently, protein- and carbohydrate-based biomaterials are among the most commonly used natural polymers for 3D bioprinting. Protein-based biomaterials, such as gelatin and fibrin, exhibit strong biocompatibility and suitable physical properties, making them ideal for 3D bioprinting. Carbohydrate-based biomaterials, including alginate, are widely used due to their abundance, ease of acquisition, biodegradability, biocompatibility, and non-toxicity, attracting significant interest in recent years [46]. These natural polymers can form hydrogels with a three-dimensional network structure via physical or chemical crosslinking. Hydrogels, due to their high water content and resemblance to the natural ECM, are well-suited for 3D bioprinting in the female reproductive system as they protect cells during the printing process and provide a conducive microenvironment for cell growth and proliferationAdditionally, they can generally be remodeled and degraded into metabolizable compounds, such as aminoacids or carbohydrates [47,48].

2.2.1.1. Gelatin. Hydrogels formed by gelatin and its derivatives are commonly used as biomaterials in women's 3D bioprinting.Laronda et al. [5]chose to implant exogenous follicles into gelatin hydrogel scaffolds because gelatin is an extracellular matrix protein rich in both human and mouse ovaries, when constructing an artificial 3D printed ovary [49],It is biodegradable, allows cell remodeling, contains cell adhesion sites, and has soft but durable mechanical properties [50,51]. The solution gel transition point of gelatin is about 28 °C, and the gelatin bio ink can be transformed from the solution state to the gelled state by heating and cooling. When printing, the nozzle needs to be heated to melt the gelatin, so that it can appear in a flowing state, which can be extruded together with other biological materials, and then the gelatin can be transformed into a gel state at a low temperature by cooling the printing platform at the bottom to achieve shaping [52]。Laronda et al. [5] used the thermal response characteristics of gelatin to change gelatin into a partially cross-linked gel state at 30 °C, and then squeezed it through a fine diameter nozzle (100 mm) to print smooth and continuous filaments, which were finally manufactured into a scaffold structure with precise porosity. After exogenous follicles were implanted into the scaffold, ovarian follicles gradually developed and mature oocytes were produced.However, due to the insufficient mechanical properties of gelatin, its printed shape fidelity is generally average. Studies have found that mixing gelatin with alginate can increase shape fidelity and improve printing resolution [53]. Therefore, gelatin is often mixed with alginate to produce biological ink. For example, gelatin/alginate/fibrin water gel has been used to 3D print HeLa cells to build an in vitro model of cervical cancer [4].In addition to mixing with other biomaterials to increase printing fidelity, gelatin can also be chemically modified to enhance its mechanical properties. T. Wu et al. chemically modified gelatin into gelatin methacryloyl (GelMA) and applied it as a biomaterial for 3D biological ovarian printing. GelMA exhibited good mechanical and biocompatibility properties, not only forming regular, smooth, and clear grid scaffolds, but also gradually developing follicles after implantation, producing mature oocytes [9].

2.2.1.2. Alginate. Alginate (ALG) is a polysaccharide polymer extracted from natural algae such as brown algae [54]. Because the induced gel process is simple and the degradation kinetics is easy to control, it is the most commonly used biomaterial in extrusion and inkjet bioprinting [55].The composition of alginate gel is similar to that of glycosaminoglycans in extracellular matrix. It can be crosslinked with calcium ions under normal temperature, pressure and mild conditions, and quickly gel to obtain calcium alginate hydrogel. The bio ink based on alginate has good formability and mechanical properties, low toxicity, low cost, no immunogenicity, good biocompatibility, and is easy to degrade. Therefore, it is widely used in biomedical engineering [56].Alginate hydrogel loaded with human induced mesenchymal stem cells (hiMSC) has been used to repair damaged endometrium in rats by micro extrusion 3D printing technology [57].

But alginate is a low viscosity fluid that requires the use of thickeners to assist in 3D bioprinting in order to maintain the integrity of its printed structure. Alginate can be combined with gelatin and hyaluronic acid to alter the printability of alginate based materials, becoming a bio ink with high viscosity and high cell viability after printing.Kallyanasis, P et al. [58] encapsulated endometrial stem cells (eMSC) with aloe sodium alginate (AV-ALG) hydrogel, and then 3D bioprinted them onto biodegradable fused melt electrospinning and polycaprolactone (PCL) grids for mechanical support for the treatment of pelvic organ prolapse (POP). There are also studies [10] indicating that the printing ability of alginate solutions depends on the molecular weight and crosslinking ratio of alginate. In order to find the optimal ion pre crosslinking process, this study tested several ratios of alginate/Acl2 and obtained a bio ink with a printable ratio less than or equal to 1 ($Pr \leq 1$), which means good shape fidelity and extrusiveness. Finally, the bio ink was successfully applied to construct an in vitro model of cervical cancer.

2.2.1.3. Fibrin. Fibrin is a natural biopolymer produced through blood clotting and is an important component in the healing process of damaged tissues. Like gelatin, fibrin has excellent biocompatibility and biodegradability and can be applied in various biomedical fields such as wound healing, microencapsulated cell delivery, tissue engineering, and 3D bioprinting [59]. Silk fibroin is a natural high-molecular-weight fibrin. In a study, researchers transplanted a silk fibroin scaffold into the vagina of rats, which induced the growth of healthy tissues after implantation, indicating that silk fibroin is a suitable biomaterial for restoring female vaginal dysfunction [60]. Fibrin is also commonly used as a biological ink thickener to promote the extrusion of materials and reduce the excessive diffusion of sedimentary materials [61,62]. Zhao et al. used fibrinogen together with gelatin and alginate to make biological hydrogels to build a 3D biological external model of cervical tumors [4].

2.2.1.4. Decellularized extracellular matrix (dECM). dECM biomaterials have recently gained attention for their use in bioprinting. dECM materials are produced by chemically removing cellular components from tissues while preserving the ECM structure [63,64]. dECM offers a biomimetic composition of structural proteins such as collagen, elastin, and laminin and provides mechanical properties similar to those of natural tissues, making it advantageous for reproductive tissue reconstruction [65]. Decellularization reduces immune response by removing cells and

antigens while preserving the ECM scaffold [66]. dECM hydrogels, formed by temperature and pH adjustments [67], offer cell growth factors and niches conducive to cell adhesion, proliferation, tissue formation, and regeneration [68]. Li et al. developed an ovarian dECM-based bioink containing adipose-derived stem cells (ADSCs) to enhance angiogenesis and reduce ischemic damage in follicular structures for premature ovarian failure (POI) models [11]. Additionally, Huo et al. [8] used a decellularized vaginal matrix to encapsulate bone marrow mesenchymal stem cells as bioink to construct three-dimensional vaginal tissue, which exhibited vascularization and epithelialization, suggesting that decellularized vaginal matrix induces the transformation of bone marrow mesenchymal stem cells into vaginal epithelial cells to a certain extent.

2.2.2. Synthetic polymer

Synthetic polymers, produced by monomeric chemical reactions, are used as main components in 3D bioprinting bioinks. Synthetic polymers offer advantages such as easy synthesis, accessibility, affordability, and resilience [69]. However, due to potential bioactivity reduction from organic solvents and toxic crosslinking agents in synthetic polymer printing processes, biocompatibility may be limited [70–72]. As implantable biomaterials, synthetic polymers pose a risk of causing foreign body reactions and adverse immune reactions and are not easily adhered to living tissues [73]. In 3D bioprinting of the female reproductive system, synthetic polymers often play a supporting role in repairing defects in the female reproductive tract.

2.2.2.1. Poly(ε-caprolactone) (PCL). PCL is a semi-crystalline, hydrophobic polymer with good solubility, a low melting point, and excellent compatibility. PCL's viscoelastic and rheological properties facilitate scaffold fabrication with tissue-compatible pore sizes and drug-release control. Modifying PCL's functional groups can improve hydrophilicity, adhesion, and biocompatibility. These advantages and characteristics make it potentially applicable in the field of 3D bioprinting [74]. Paul et al. [12] used endometrial mesenchymal stem cells encapsulated in hydrogel to print on PCL meshes, creating a pelvic floor implant. The study selected PCL as a biomaterial for 3D printing because it has good histocompatibility and degradability and low degradation rate. Such a slowly degraded mesh, together with endometrial stem cells, could promote tissue growth and promote tissue integration.

2.2.2.2. Polyglycolic acid (PGA). PGA and its derivatives are polymer materials with good biocompatibility and biodegradability, possessing high mechanical properties. Their mechanical and degradation properties can be adjusted by copolymerizing with other monomers. PGA and its copolymers are widely used in biodegradable medical surgical sutures, fracture internal fixation devices, drug controlled-release carriers, and tissue engineering scaffolds [75]. Previous studies have utilized 3D bioprinting to create PGA scaffolds, which can be loaded onto rabbit vaginal smooth muscle and epithelial cells to construct vaginal tissues with phenotype and function [76,77]. To minimize the foreign body reaction of PGA biological scaffolds and facilitate better cell adhesion, De Philippo et al. [78] used 3D printing to create PGA/PLGA biological scaffolds. The scaffolds were kept under vacuum for 2 days to remove residual solvents, disinfected with ethylene oxide gas, and wetted in Dulbecco's modified Eagle medium for 24 h before cell inoculation. Then, labeled vaginal epithelium and smooth muscle cells were sequentially inoculated onto the inner and outer surfaces of the coated scaffolds to construct an artificial vagina.

2.3. 3D bioprinting strategy for the female reproductive system

EBB is currently the most widely used technique for fabricating structures within the female reproductive system. Its key advantage is scalability, with a wide viscosity range of compatible biomaterials, such as cell-laden hydrogels and decellularized matrices, which accommodate a broad spectrum of fluid properties. High-viscosity materials provide structural support, whereas low-viscosity materials create a conducive environment for cell functionality. This broad viscosity adaptability allows EBB to incorporate high-density bioinks, achieving physiologically relevant cell densities—a principal goal in the bioprinting of reproductive tissues. However, the extrusion process introduces shear forces that can impact cell survival, particularly at high cell densities. This shear stress can be mitigated to some extent by adjusting nozzle diameter and pressure. Inkjet bioprinting technology offers high resolution and is effective for creating small-scale tissues, making it valuable for developing in vitro 3D tumor models of the female reproductive system. However, inkjet bioprinting has limitations in that it cannot print high-viscosity materials or high cell concentrations due to its lower driving force. Additionally, low-viscosity materials provide limited structural strength, reducing their suitability for subsequent in vitro culture and transplantation. Consequently, this limitation hinders the broader application of inkjet bioprinting in reproductive tissues. Both EBB and inkjet bioprinting methods also have slower print speeds and lower precision when fabricating complex structures, particularly those involving multiple biomaterials. Digital light processing (DLP) technology has recently emerged as a promising 3D printing method, offering higher resolution and faster speeds due to its surface-projection approach. This technology provides excellent uniformity and reproducibility, making it a promising candidate for developing in vitro model tissues of the female reproductive system [79]. While EBB is not yet widely applicable for reproductive tissues, it holds promise for future applications in this field. In the 3D bioprinting of the female reproductive system, hydrogels are the most commonly used biomaterial. However, hydrogels often lack sufficient mechanical strength to create tubular structures, leading to challenges with vascularization. This issue may be addressed through coaxial bioprinting, which produces core-shell structures, where the core material ensures biocompatibility, and the shell material enhances mechanical strength [80], potentially enabling vascularization within reproductive tissue models.

Several biomaterials are suitable for 3D bioprinting of the female reproductive system, such as gelatin and alginate. However, single-component biomaterials often fall short in meeting all printing requirements, leading to the common practice of using multi-material bioinks. Many biomaterials can form hydrogels with three-dimensional network structures under specific physical or chemical conditions, providing a suitable microenvironment for cell adhesion, growth, and proliferation. Thus, they are ideal for 3D bioprinting applications. In selecting materials for 3D bioprinting of reproductive tissues, key considerations include printability, biocompatibility, and mechanical properties. However, these factors can conflict—for example, high-viscosity materials may have excellent printability but poor biocompatibility. Therefore, material selection should align with specific application needs, such as bioprinting.

3. 3D bioprinting for regeneration and repair of the female reproductive system

Regenerative medicine has rapidly evolved, leveraging biological and engineering principles to repair, replace, or regenerate human tissues and organs, aiming to restore their structure and function. This field holds great potential for repairing damaged organs. However, when constructing complex tissues, regenerative medicine requires precise control of cells and biomaterials, which traditional tissue engineering approaches often cannot achieve due to the difficulty in creating complex geometric shapes and layered tissue structures, which limits the application of regenerative medicine in repairing complex tissues [81,82]. 3D bioprinting technology addresses this challenge by enabling the integration, arrangement, and combination of cells, ECM, and cytokines in specific configurations, providing precise spatial control of cells and

creating suitable microenvironments for cell function. This technology offers tremendous potential for constructing complex tissues and organs [83]. For women withy or endocrine dysfunction resulting from reproductive organ damage, 3D bioprinting offers a pathway to regenerative medicine. This approach allows the creation of biocompatible, biodegradable, and functional prosthetic tissues or organs [12], enabling repair or replacement of damaged tissues to restore fertility and ovarian endocrine function [84,85]. Below is a review of the various tissues and organs of the female reproductive system.

3.1. Ovary fabrication

The ovary is a vital organ in the female reproductive system, responsible for egg production, hormone synthesis, menstrual cycle regulation, and maintenance of female characteristics, all crucial to women's health and fertility [86]. Ovarian injury may result from various factors, including surgery, radiation, or chemical exposure, as well as genetic and autoimmune conditions, structural abnormalities, or tumors. Such injuries can lead to adverse outcomes like menstrual irregularities, premature ovarian failure, or infertility [87–89]. A

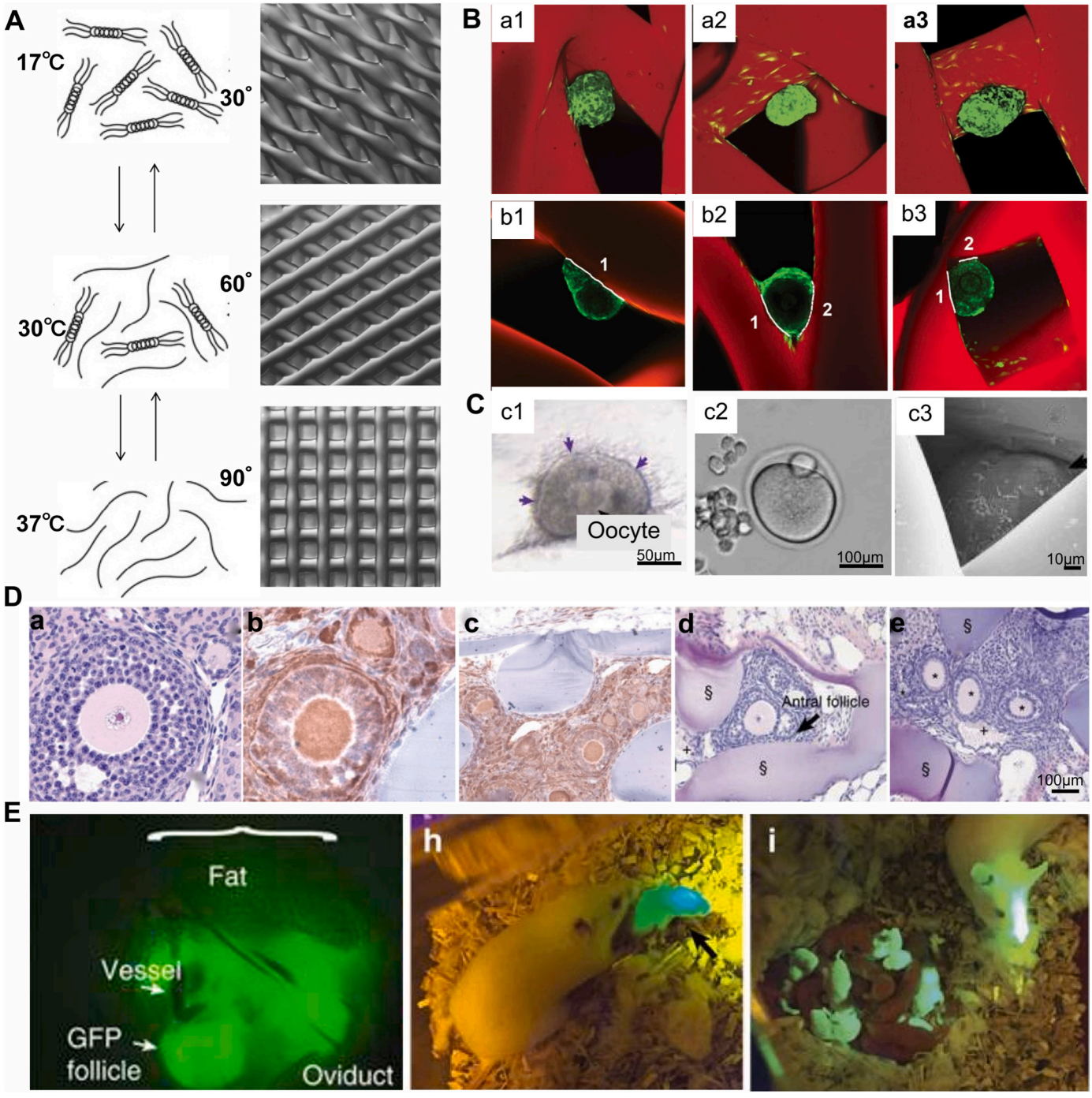

Fig. 3. Deposition of follicles into 3D-printed microporous hydrogel to create a bioprosthesis ovary. A. The state of gelatin at different temperatures and gelatin scaffolds printed with varying pore geometries. B. Follicle survival is dependent on pore geometry in vitro. C. Follicles function within 3D-printed scaffolds in vitro. D. The development and vascularization of follicles in the vivo.E. The mice with bioprosthetic ovaries restore fertility function. Reproduced with permission from Ref. [5].under the a Creative Commons Attribution 4.0 International License.

prominent approach in regenerative medicine is autotransplantation of cryopreserved ovarian tissue or whole ovaries. However, this method involves risks, such as egg loss and reintroduction of malignant cells after tissue revascularization [90,91]. Previous studies have explored tissue engineering methods for artificial ovary construction; howl primary ovarian cell types—oocytes, granulosa cells, and theca cells—in a single structure presents significant challenges due to their interdependent function [92,93]. 3D printing technology provides a viable solution by enabling precise construction of complex ovarian structures, including follicles, ovarian stroma, and vascular networks, allowing for compatibility with the patient's tissues and offering a practical solution for artificial ovary fabrication [20].

Laronda et al. [5] developed a 3D-printed microporous hydrogel scaffold, depositing follicles within a bioengineered ovary (Fig. 3). In vitro experiments showed that the follicles within the 3D-printed scaffold gradually matured and exhibited endocrine function, secreting hormones such as estrogen. In vivo experiments demonstrated that mice implanted with artificial ovaries could ovulate spontaneously and produce live offspring following mating. This study highlights 3D bioprinting's potential to closely mimic the ovarian microenvironment, supporting follicle growth and enabling artificial ovaries to perform reproductive endocrine functions. The innovation of this study lies in the scaffold's design, which provides appropriate depth and contact points for follicles, optimizing follicle survival and differentiation in vitro. The hydrogel scaffold's open micropores allow for sufficient space and nutrients for follicle maturation both in vitro and in vivo, facilitating vascular system infiltration post-implantation. This research suggests a promising approach for ovary bioprinting, proposing the use of multiple materials to construct structures with varying hardness and pore sizes. This approach could support both quiescent and growing follicles, achieving prolonged follicular cycles and extending implant lifespan.

Wu et al. [9] utilized cell-laden EBB to implant exogenous follicles into GelMA hydrogel scaffolds, creating artificial 3D-printed ovaries. Compared with Laronda et al.'s prior study, this study introduces two primary distinctions: Firstly, this study explored cell-laden EBB. Initially, ovarian cancer cells were used for EBB, with results indicating that over half of the ovarian cancer cells survived after passing through a low-temperature nozzle, confirming the feasibility of cell-laden bioprinting. However, when primary cultured ovarian somatic cells were printed using the same technique, more than 90 % of the cells died, leading to the conclusion that primary ovarian somatic cells may not be suitable for cell-laden bioprinting. Nonetheless, cell survival rates are influenced by both cell density and printing conditions, suggesting that this conclusion warrants further investigation. Secondly, the study evaluated three types of hydrogels for printing 3D artificial ovaries: alginate gel, GelMA alginate gel, and GelMA hydrogel. Results showed that alginate gel was fragile and unstable. GelMA alginate saline gel formed a well-structured scaffold but exhibited low transparency, hindering clear observation of follicles. GelMA hydrogel, on the other hand, formed a smooth, well-defined scaffold structure with high fidelity. Degradation kinetics demonstrated its good biodegradability, and after implantation, follicles developed to produce mature oocytes, indicating that GelMA hydrogel is an ideal biomaterial for constructing 3D artificial ovaries. Additionally, the study found that follicles with a diameter of 100–130 μm exhibited limited growth following in vitro scaffold implantation, whereas those with diameters between 130 and 180 μm grew rapidly by day five post-implantation. This observation suggests that a major challenge in sustaining follicular cycles lies in inducing in vitro and in vivo maturation of immature follicles. The study concludes by suggesting that adding mesenchymal stem cells to 3D artificial ovaries may extend their reproductive endocrine function.

As mentioned, stem cell therapy is a key approach in regenerative medicine for premature ovarian failure. Stem cells can restore ovarian function through mechanisms such as cell proliferation, anti-apoptosis, autocrine and paracrine signaling, anti-inflammatory effects, gene regulation, and angiogenesis promotion. However, limitations such as insufficient cell adhesion and transient organ colonization remain challenges in stem cell therapy [17–19]. The use of 3D-bioprinted biomaterial composites, such as collagen scaffolds, may help overcome these limitations. On collagen scaffolds, stem cells increase survival, restrict outward migration, and support attachment and proliferation, sustaining long-term therapeutic effects [94,95]. Li et al. [11] designed an ovarian dECM-based bioink to encapsulate ADSCs and combined them with ovarian fragments to construct artificial ovaries (see Fig. 4). This study is groundbreaking in applying 3D bioprinting and stem cell therapy to treat premature ovarian failure. The dECM scaffold effectively recreated an in vivo ovarian microenvironment, enhancing the viability of ADSCs and ovarian cells and promoting necessary cell-environment interactions. ADSCs significantly stimulated neovascularization, increased blood perfusion, and reduced primordial follicle loss. This study randomly assigned rats with a POI model into five groups: (1) POI group, (2) ovarian fragments group, (3) 3D scaffold combined with ovarian fragments group, (4) ovarian fragments with ADSCs, and (5) 3D scaffold with ADSCs combined with ovarian fragments as the engineered ovary group. Normal rats served as controls. Final results showed that CM-DiI labeled ADSCs were primarily located in the ovarian stroma, with retention rates significantly higher in the 3D-bioprinted ovary group. Some regions co-stained with CM-DiI and CD31 were identified in endothelial cells in blood vessels. Additionally, the engineered ovary group displayed substantial improvements in follicle count, granulosa cell proliferation, neovascularization, and hormone levels. Improved ovarian function and angiogenesis were achieved through PI3K/AKT pathway modulation. This research highlights the promising potential of combining 3D bioprinting with other regenerative medicine technologies for the female reproductive system.

These advancements present new horizons for 3D bioprinting in creating artificial ovaries. From this perspective [96], 3D printing holds significant promise for in vitro follicular culture, ovarian tissue transplantation, and menopausal hormone therapy [96]. However, the ovary's complex structure and function, alongside specific reproductive hormones, highlight the need for further biomaterial development to enhance structural regeneration and functional recovery in the future.

3.2. *Endometrium repair*

The endometrium is the inner tissue layer of the uterine cavity, primarily responsible for supporting zygote implantation and fertilization, and for sustaining embryo growth and development [97]. Structurally, the endometrium consists of two layers: the outer functional layer and the basal layer adjacent to the endometrium. The functional layer includes an outer luminal epithelium, which faces the uterine cavity, and numerous vertical glands close to the luminal epithelium. Basal cells include glandular stromal cells and a horizontally branching vascular network, which provides stem/progenitor cells essential for post-menstrual functional regeneration [98,99]. Typically, the endometrium undergoes periodic shedding and scar-free regeneration [100]; however, functional regeneration only occurs once repair is completed [101,102]. When endometrial repair is impaired, or if basal endometrial layers are damaged, as seen in endometriosis, endometrial cancer, or after curettage, repair and regeneration may be hindered [103]. Stem/progenitor cells play an essential role in endometrial repair and regeneration [104,105]. In regenerative medicine, stem or endothelial cell injections into damaged endometrial areas have been attempted to achieve tissue repair and regeneration [16]. Challenges include the normal expansion of stem cells, while the biochemical composition and physical properties of the cell matrix critically impact cell activity. Bioactive 3D-printed hydrogels with adjustable hardness closely simulate natural tissue due to their high water content, porosity, and soft consistency [47,48]. Additionally, they can regulate stem cell activity through modifiable biochemical composition or drug encapsulation [106].

Ding et al. [107] constructed a collagen scaffold loaded with bone

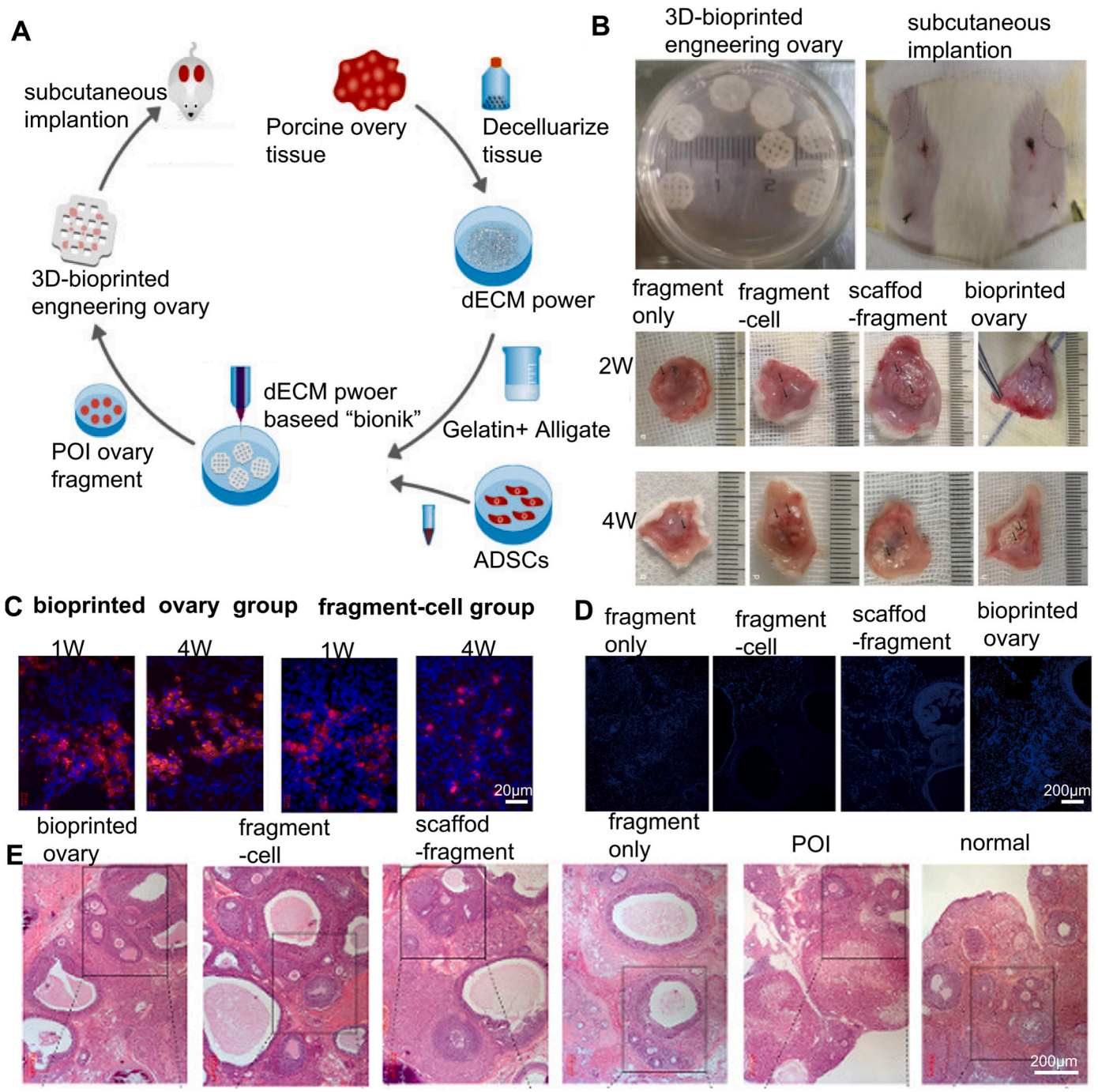

Fig. 4. DECM's bioink was used to encapsulate adipose-derived stem cells and construct artificial ovaries by combining ovarian fragments. A. Schematic representation of construction of 3D-bioprinted engineering ovary. B. 3D scaffolds and subcutaneous transplantation. C. Localization and differentiation of ADSCs in grafts. D. Assessment of graft revascularization. E. Assessment of graft revascularization. Reproduced with permission from Ref. [11]under Creative Commons license.

marrow mesenchymal stem cells (BM-MSCs) to examine its repair effects on full-thickness uterine injury in rats. Results indicated that 4 weeks post-transplantation, most BM-MSCs were located on the regenerated endometrium's basement membrane, with some cells differentiating into endometrial stromal cells. Rats receiving only collagen constructs or in spontaneous regeneration groups expressed higher levels of growth factors such as bFGF, IGF-1, TGFb1, and VEGF near damaged tissues. Endometrial tissues in rats treated with collagen/BM-MSCs also showed stronger fertility, suggesting that BM-MSCs promote the proliferation and differentiation of surrounding cells through autocrine or paracrine mechanisms, rather than directly differentiating into specific endometrial cells. This study validates the synergy of biotechnology and stem cell technology for endometrial repair. However, the collagen scaffold production method used in this study is relatively simple. Ji et al. [7] employed 3D bioprinting technology to construct a porous hydrogel scaffold loaded with hiMSC cells for endometrial repair (see Fig. 5). This study detailed the process of cell-laden EBB to create endometrial scaffolds. The results revealed that 3D-bioprinted scaffolds loaded with hiMSCs promoted endometrial tissue morphology repair (endometrial tissue and glandular regeneration), cell regeneration (stromal, epithelial, and endothelial cells), and endometrial receptivity, restoring partial embryo implantation and pregnancy maintenance functions in damaged endometria. The study suggested that active factors secreted by hiMSCs are likely critical for endometrial regeneration. However, regenerated

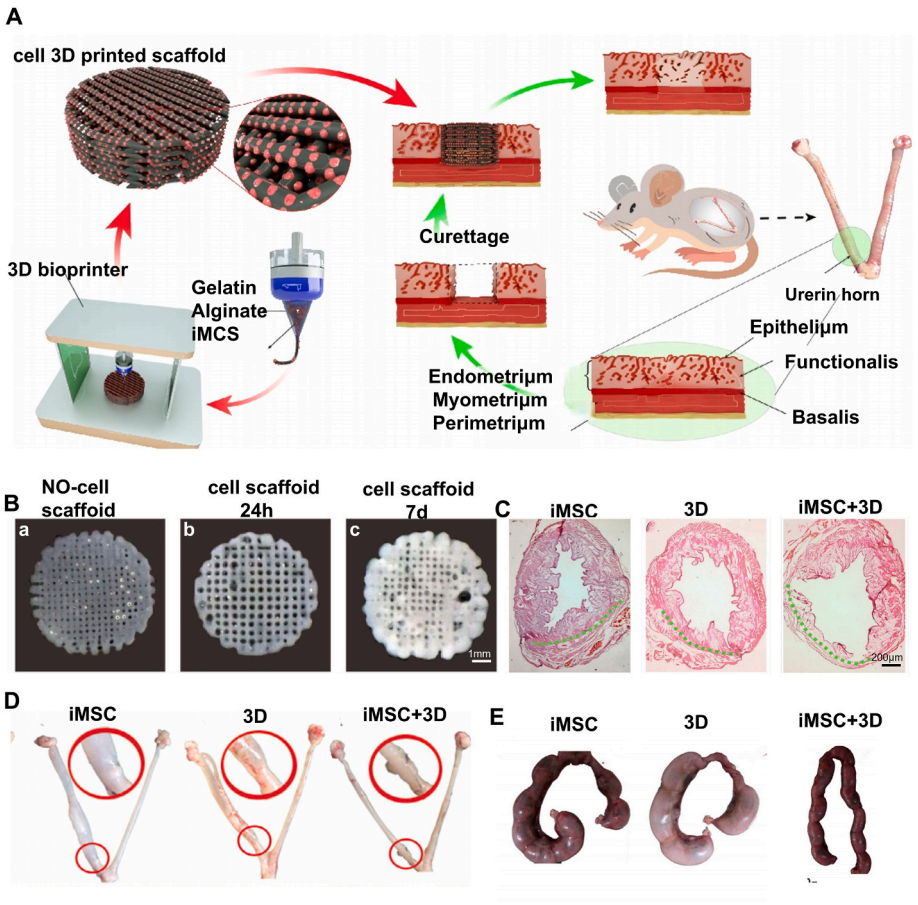

Fig. 5. Fig. 5. 3D Bioprinting a human iPSC-derived MSC-loaded scaffold for repair of the uterine endometrium. A. Overall schematic of the experiment. B. Characterization of the 3D-printed hydrogel scaffold and hiMSC-loaded hydrogel scaffold. C. Morphological assessment of the regenerated endometrium. D. Views of the damaged area of the uterine horn in each group. E. Embryo breeding status of the injured uterus (red arrows) in each group. Reproduced from Ref. [7] Copyright 2021, Elsevier Ltd.

endometrial tissue exhibited significant structural and functional differences from normal endometrial tissue. To optimize results, the study proposed methods like improving the morphology of 3D printed hydrogel, using integrin-binding agents to induce directional cell differentiation, or maturing the scaffold in a bioreactor before implantation to enhance cell survival. Early vascularization issues, often restricted by oxygen and nutrient diffusion limitations post-implantation, remain a challenge for 3D-printed scaffolds.

Wen et al. [108] attempted to combine a 3D-printed hydrogel scaffold with a granulocyte colony-stimulating factor (G-CSF) sustained-release microsphere system (SRM) to assess its effects on endometrial repair. In this study, both the hydrogel scaffold and the SRM system were manufactured using 3D printing technology. The hydrogel was mixed with G-CSF-SRMs, creating a G-CSF sustained-release system with precise drug distribution and personalized characteristics. Results indicated that, compared with a non-3D-printed G-CSF-SRM-loaded hydrogel, the 3D-printed sustained-release system not only enabled long-term G-CSF release in vivo but also increased the local release concentration of G-CSF. The 3D-printed active scaffold effectively reduced endometrial adhesions post-injury in an IUA rat model, promoting the reconstruction of endometrial structure and function. Although live cells were not incorporated into the 3D printing technology in this study, colony-stimulating factor—a hematopoietic factor produced by lymphocytes, activated macrophages, and endothelial cells—stimulates the proliferation and differentiation of bone marrow hematopoietic cells. The SRM release system emulates the continuous release of colony-stimulating factor by cells, offering a novel application of 3D bioprinting in the female reproductive system.

3.3. *Artificial vagina*

The vagina functions as the female sexual organ and serves as the passage for menstrual blood and fetal delivery. Its wall consists of three layers: mucosa, muscle, and fibrous tissue. The mucosa is lined with non-keratinized squamous epithelium and is highly extensible with many horizontal folds. Influenced by estrogen, it undergoes periodic changes. The muscle layer consists of two smooth layers of inner and outer longitudinal muscles, and the fibrous tissue membrane is closely adhered to the muscle layer [109]. Vaginal hypoplasia may be caused by various diseases, including Mayer-Rokitansky-Küster-Hauser (MRKHS), cloacal abnormalities, endocrine disorders such as adrenal hyperplasia, and other intersex abnormalities [110]. Acquired diseases such as cancer and trauma may also lead to vaginal injury or loss [111]. In regenerative medicine, there are two common approaches for vaginal reconstruction. One approach involves surgically creating potential anatomical spaces in the pelvic cavity. However, this method lacks the muscle structure of a normal vagina and can result in chronic stenosis or graft contraction, often requiring periodic dilation. Another approach involves using self-vascularized tissue segments as vaginal structures, such as tubular muscle flaps or intestinal segments [112,113]. This method entails extensive surgery and increased risk of complications, such as excessive mucus production by intestinal segments or an elevated risk of tumors in the new vagina [114].

An ideal artificial vagina should possess essential characteristics, including sufficient length, width, elasticity, and a functional vaginal axis, as well as secretion and lubrication capability to meet patients' physiological and psychological needs. It should also be minimally

invasive or non-invasive, preserving other physiological functions without requiring special postoperative care [115]. As early as 2008, De Philippo et al. [78] proposed inoculating labeled vaginal epithelium and smooth muscle cells onto the inner and outer surfaces of a PGA/PLGA-coated stent to construct an artificial vagina for full vaginal replacement in rabbits. Six months post-surgery, radiographic analysis revealed a wide and unobstructed vaginal diameter without stenosis. Histological analysis confirmed well-organized epithelial and muscle cell layers, and physiological studies showed normal responses to electrical stimulation and adrenergic agonists. This study established a foundation for using tissue engineering technology in vaginal repair. Raya-Rivera et al. [116] later applied this technology in human studies. Epithelial and muscle cells from patients were cultured, expanded, and seeded onto biodegradable scaffolds to construct an artificial vagina. The artificial vagina was implanted via a perineal approach, and over an 8-year follow-up, no serious complications occurred (Fig. 6). Annual biopsies revealed a three-layer structure—matrix, epithelial cells, and muscle. A validated female sexual function index questionnaire indicated high postoperative satisfaction. However, the study had limitations, including a small participant sample and limited information on materials and 3D scaffold manufacturing. Despite these limitations, the study's human subject focus and long follow-up period offer a practical basis for tissue engineering applications in vaginal reconstruction.

Hou et al. [8] utilized 3D bioprinting technology to load bone marrow mesenchymal stem cells onto decellularized vaginal matrix scaffolds. The 3D scaffold group and a 3D scaffold containing CM-Dil-labeled bone marrow mesenchymal stem cells were then transplanted subcutaneously into rats. Results from HE staining, immunohistochemistry, and immunofluorescence indicated that the 3D scaffold with bone marrow mesenchymal stem cells significantly promoted vascularization and epithelialization in the printed vaginal tissue. Bone marrow mesenchymal stem cells were observed to adopt phenotypes resembling vaginal epithelial cells and endothelial-like cells. This study provides a detailed overview of the materials and methods used in 3D bioprinting, offering a reference for future research. The primary innovation of this study is the use of a decellularized vaginal matrix to

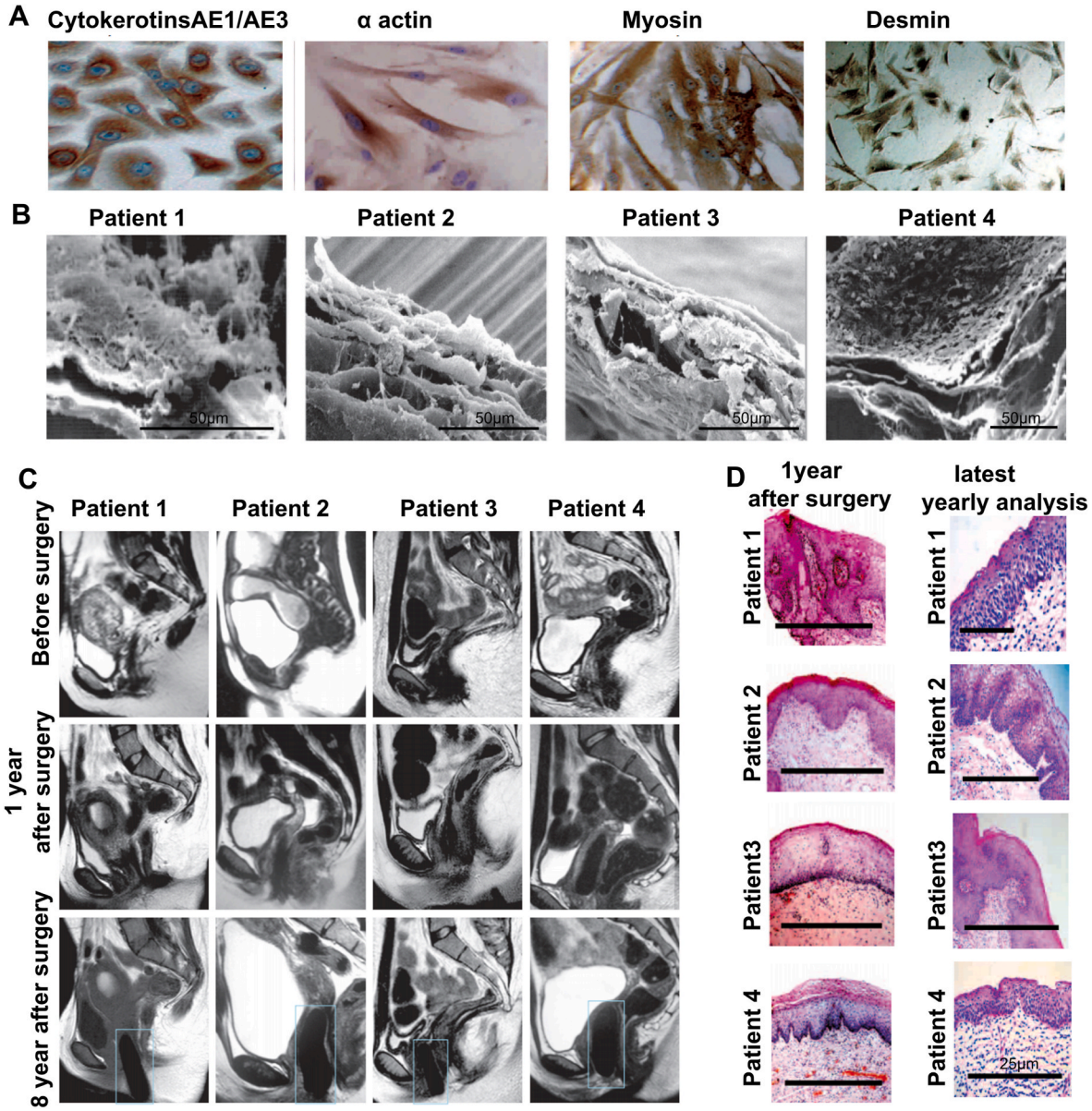

Fig. 6. Tissue-engineered autologous vaginal organs in patients.A.Vaginal smooth muscle and epithelial cells.B.Scanning electron microscopy of scaffolds 6 days after cell seeding.C.Preoperative and postoperative MRI images.D.Tissue analyses at 1 year after surgery and at the latest yearly analyses after surgery (up to 8 years). Reproduced from Ref. [116] with the permission of Copyright 2014, Elsevier Ltd.

encapsulate bone marrow mesenchymal stem cells as bioink for constructing three-dimensional vaginal tissue. While bone marrow mesenchymal stem cells are pluripotent and can differentiate in a targeted manner, inducing directed differentiation remains challenging in stem cell technology. Here, the decellularized vaginal matrix simulated the native microenvironment of bone marrow stem cells, partially inducing their differentiation into vaginal epithelium and endothelial cells. However, the regenerated epithelium was not fully mature, possibly due to an insufficient transplantation period. Another key finding was the synergistic effect of bone marrow mesenchymal stem cells and bioink on angiogenesis in the transplant structures, addressing the challenge of insufficient vascularization in 3D-bioprinted grafts.

3.4. Pelvic floor tissue reconstruction

POP and stress urinary incontinence affect 30–40 % of women worldwide [117]. Current strategies for treating these conditions include non-surgical options, such as physical therapy, and surgical options, such as synthetic vaginal mesh implants. However, synthetic vaginal mesh has been associated with complications like chronic pain, infection, and mesh erosion [118,119], possibly due to its non-degradable nature, poor tissue integration, and long-term inflammation [120]. Thus, there is an urgent need for biodegradable implants that promote tissue fusion and reduce inflammatory responses, providing reinforced support for pelvic floor tissues. With advancements in 3D bioprinting, some studies have explored using endometrial mesenchymal stem cells encapsulated in hydrogel with a poly--ε-caprolactone network for 3D-printed pelvic floor implants (Fig. 7). After implanting these constructs into mice, acute in vitro and in vivo evaluations in NSG mice showed that the endometrial mesenchymal stem cells in the implant were preserved and contributed to tissue integration. M2 macrophages were observed to exert anti-inflammatory effects on the implant. This study demonstrates that 3D-bioprinted implants loaded with endometrial mesenchymal stem cells have potential as a degradable alternative to synthetic mesh for treating pelvic prolapse disorders [12].

3.5. Cervix tissue manufacturing

The cervix is located at the lower end of the uterus. The functions of the cervix mainly include connecting the uterus to the vagina, protecting the uterus, secreting mucus, participating in the reproductive process, having immune functions, and sensing stimuli. Its tissue is primarily composed of connective tissue. However, pathological changes in the cervix, such as cervical intraepithelial neoplasia (CIN) and cervical cancer, may require the removal of part or all of the cervical tissue [121].Annachiaraet al. [122]developed competent three-dimensional connective tissue equivalents (e.g. skin, gut, cervix)(see Fig. 8).They

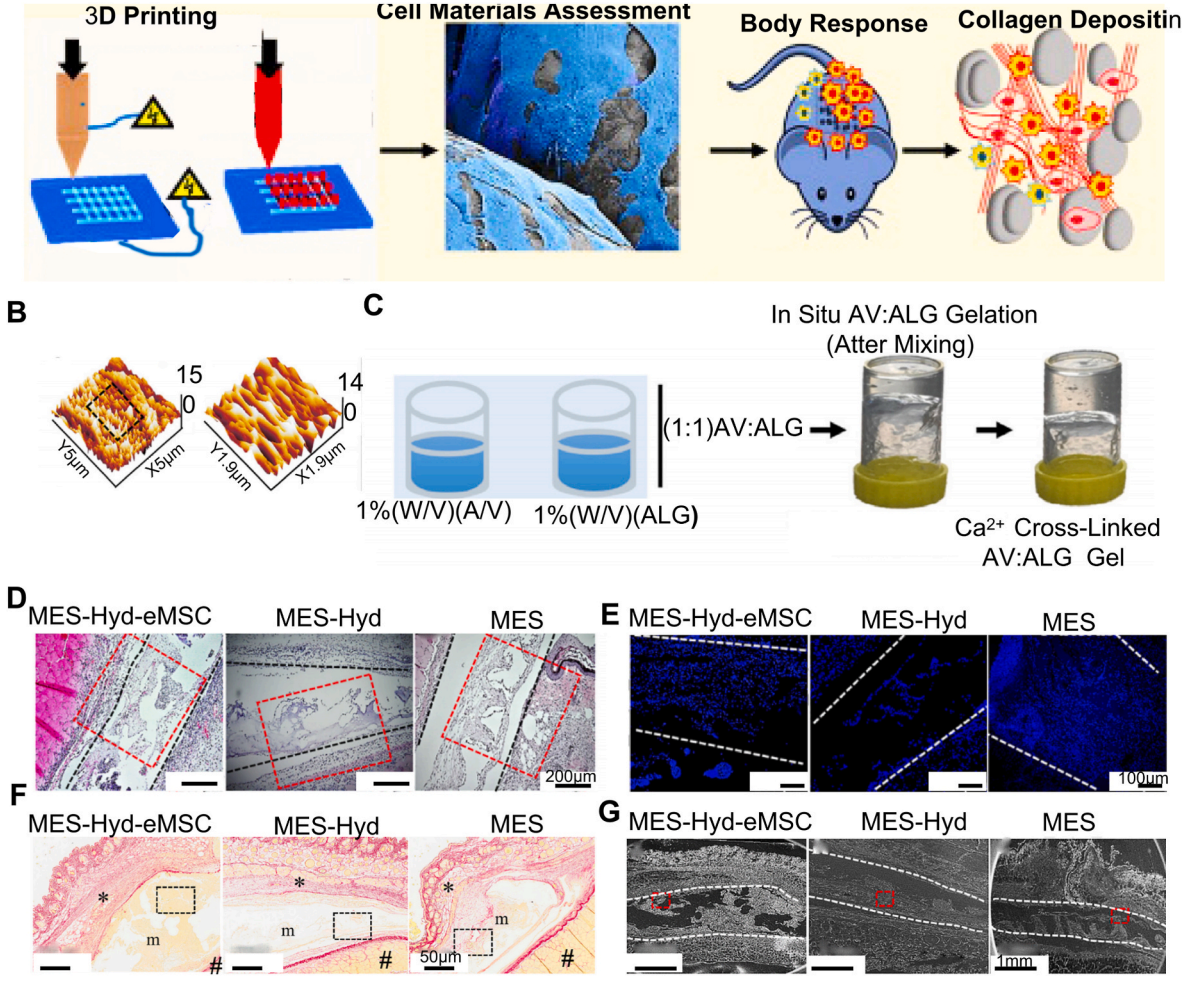

Fig. 7. 3D-bioprinted endometrial stem cells on melt electrospun poly-caprolactone mesh for pelvic floor application. A. Overall schematic of the experiment. B. Mesh characterization and assessment of eMSC attachment. C. Preparation characterization of aloe vera-alginate hydrogels. D. Acute inflammatory response to implanted meshes after 1 week implantation in NSG mice. E. M1 macrophage associated with the foreign body response to implanted meshes after 1 week. F. In vivo collagen deposition inside mesh after 1 week. G. Fate of meshes after 1 week of in vivo implantation. Reproduced from Ref. [12] with permission of Copyright 2019, Elsevier Ltd.

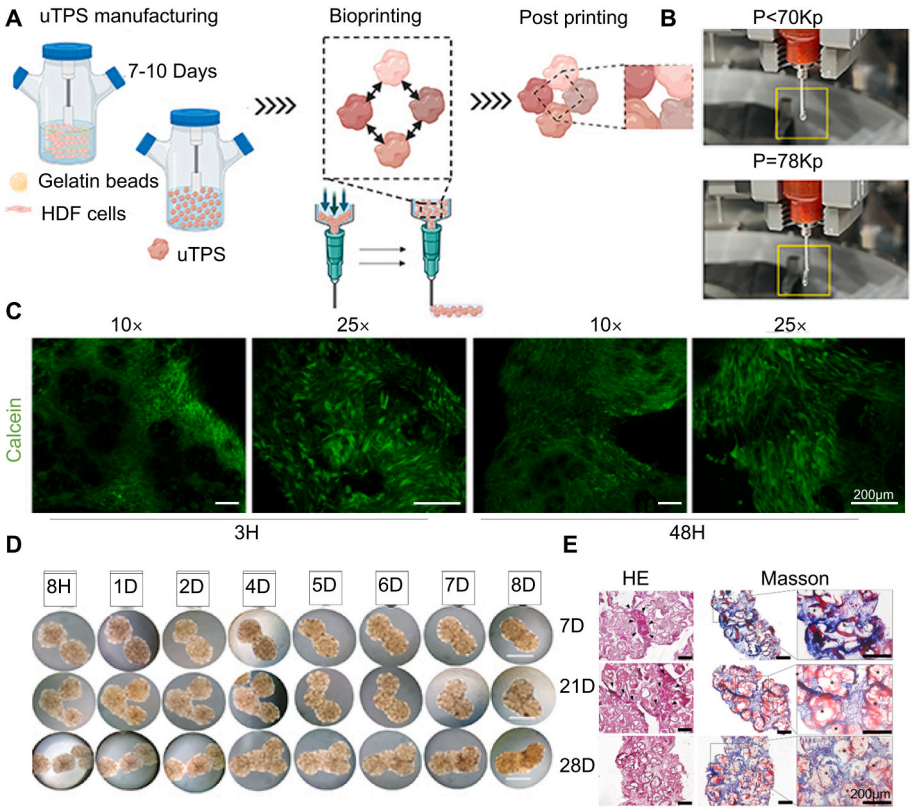

Fig. 8. Bioprinting of in vitro connective tissue. A. Schematic illustration of the whole process. B.Pressure optimization.C.Live/Dead assay results.D.Snapshots of the fusion of spheroids.D.The increase in cell-synthetized ECM over time and the fusion points between adjacent μTPs are indicated by arrows.Reproduced from Ref. [122]under the a Creative Commons Attribution 4.0 International License.

developed a two-step bottom-up strategy that involves the dynamic assembly of microtissue precursors (μTPs) to create macroscopic functional tissues composed of cell-secreted extracellular matrix (ECM). Notably, the application of μTPs in 3D bioprinting is highly innovative. μTPs fuse and self-assemble to form larger tissues more rapidly than individual cells. Additionally, the use of 'sacrificial' bioinks enables scaffold-free bioprinting. Simultaneously, they employed an extrusion-based bioprinting technique, embedding the building blocks within a matrix to dispense microtissues of various sizes using nozzles with different diameters. Histological, immunofluorescence analysis, and second harmonic generation reconstruction revealed an increase in endogenous collagen and fibronectin production within the bioprinted construct, closely mimicking the composition of native connective tissues.

4. 3D bioprinting for in vitro model construction of the female reproductive system

Due to ethical concerns surrounding the use of human subjects in biomedical research [123] most basic research utilizes in vivo animal models and 2D cell/tissue culture models to study the female reproductive system [124]. However, the complexity and species-specific differences in the female reproductive system make direct comparisons with animal models challenging. Additionally, reproductive organs in the human body do not function in isolation; they are interconnected with other organs to maintain reproductive and endocrine functions. Single-layer cells cultured on flat plastic or glass lose their three-dimensional structure and physical or biochemical interactions with other cells in the body [125]. These limitations highlight the urgent need for structural models that accurately replicate the physiological microenvironment of the human female reproductive system. The three-dimensional distribution of cells in 3D-bioprinted in vitro models more closely resembles the in vivo situation, better simulating cell–cell and cell–ECM interactions within the female reproductive system. Particularly in research on tumors of the female reproductive system, 3D-bioprinted in vitro models can replicate tumor heterogeneity and its relationship with the microenvironment, providing a foundation for studying tumor pathology and screening potential drugs [126]. Below is a review of research on 3D bioprinting for constructing models of female reproductive system tumors and other pathological or physiological conditions.

4.1. Female reproductive system tumor model

4.1.1. Cervical cancer

Cervical cancer is the third most common malignant tumor among women worldwide [125]. It can be classified into three pathological types: squamous cell carcinoma, adenocarcinoma, and adenosquamous cell carcinoma [127,128]. Persistent infection with high-risk HPV is the primary risk factor for cervical cancer, with over 90 % of cases associated with high-risk HPV infection [129]. Surgical treatment is the standard approach for early-stage cervical cancer, and synchronous radiotherapy and chemotherapy are performed for patients with high risk of recurrence pathological factors after surgery; There is no consensus on adjuvant therapy for patients with intermediate risk factors, and Sedlis criteria are commonly used in clinical practice. The standard treatment for locally advanced cervical cancer is concurrent radiotherapy and chemotherapy, with a cure rate of up to 60 %. Despite standard treatment, 30 % of patients still experience local recurrence or metastasis, resulting in a low survival rate and being the main cause of death at present. The treatment options for advanced or recurrent cervical cancer remain limited, with poor prognosis posing a significant challenge in cervical cancer

treatment [128].

In 2014, Zhao et al. [4] constructed an in vitro cervical tumor spheroid model with tumorigenic properties using a gelatin/fibrinogen/alginate hydrogel loaded with HeLa cells via EBB (Fig. 9). This model was used to study cell proliferation, matrix metalloproteinases (MMPs), and responses to paclitaxel treatment. Compared with traditional 2D culture models, HeLa cells in the 3D model showed enhanced proliferation, migration abilities, and higher paclitaxel resistance. This study pioneered the use of 3D bioprinting to construct a cervical cancer model in vitro and comprehensively compared 2D and 3D cervical cancer models concerning cell proliferation, metastasis, and drug resistance, demonstrating that the 3D model effectively mimics in vivo tumor characteristics. Additionally, the study provides detailed insights into constructing an in vitro cervical cancer model using 3D bioprinting, establishing a theoretical and practical foundation for this technology's application in cervical cancer tumor biology research.

In 2018, a study [130] based on Zhao et al.'s methods developed a model of advanced cervical cancer using a gelatin/fibrinogen/alginate hydrogel loaded with HeLa cells. Upon TGF-β supplementation, HeLa cells aggregated and began to disassemble, with some cells acquiring fibroblast-like spindle morphology, indicating induction of epithelial-mesenchymal transition (EMT). The addition of disulfuron and EMT pathway inhibitor C19 inhibited TGF-β-induced EMT in a dose-dependent manner. This study demonstrated TGF-β′s role in inducing EMT within a 3D-bioprinted cervical cancer model, offering a theoretical basis for research on cervical cancer metastasis and treatment.

In 2023, another study [10] used 3D bioprinting to investigate spatial gradients of HeLa cell concentration in high-resolution cervical tumor models. SECM was employed to quantitatively measure drug molecule diffusion over time in the 3D cervical cancer tumor structure. EBB with alginate as the bioink provided the basis for the model, with alginate's printability being influenced by its molecular weight and crosslinking ratio. The study detailed the preparation of biocompatible and printable alginate solutions and optimized conditions to ensure bioink consistency and model fidelity. Using SECM, the study spatially resolved oxygen concentrations within HeLa cell spheroids, leveraging nanoelectrodes and phase-contrast microscopy. Since oxygen levels critically regulate cellular processes and significantly influence cellular behavior under both physiological and pathological conditions, this technique provides valuable insights into how oxygen concentration affects cervical cancer cells. Additionally, SECM has the potential to investigate the spatial distribution of cellular metabolites within spheroids. By evaluating drug penetration and distribution within 3D-bioprinted models, SECM can simulate anti-cancer compound diffusion in tumor clusters, aiding in drug efficacy studies for cervical cancer.

4.1.2. *Ovarian cancer*

Ovarian cancer has **the highest mortality rate among gynecological tumors**, posing significant threats to **women's health and life** [131]. Most **cases (70 %) are diagnosed** at **late** stages **because the clinical manifestations of early ovarian cancer are relatively insidious and non-specific In recent years, although significant progress has been made in the treatment and research of ovarian cancer, the lack of early diagnosis and the occurrence of post-operative chemotherapy resistance have limited** improvements in **the 5-year survival rate for patients with ovarian cancer** [132].

The demand for individualized models in ovarian cancer treatment and the development of precision therapies have promoted the creation

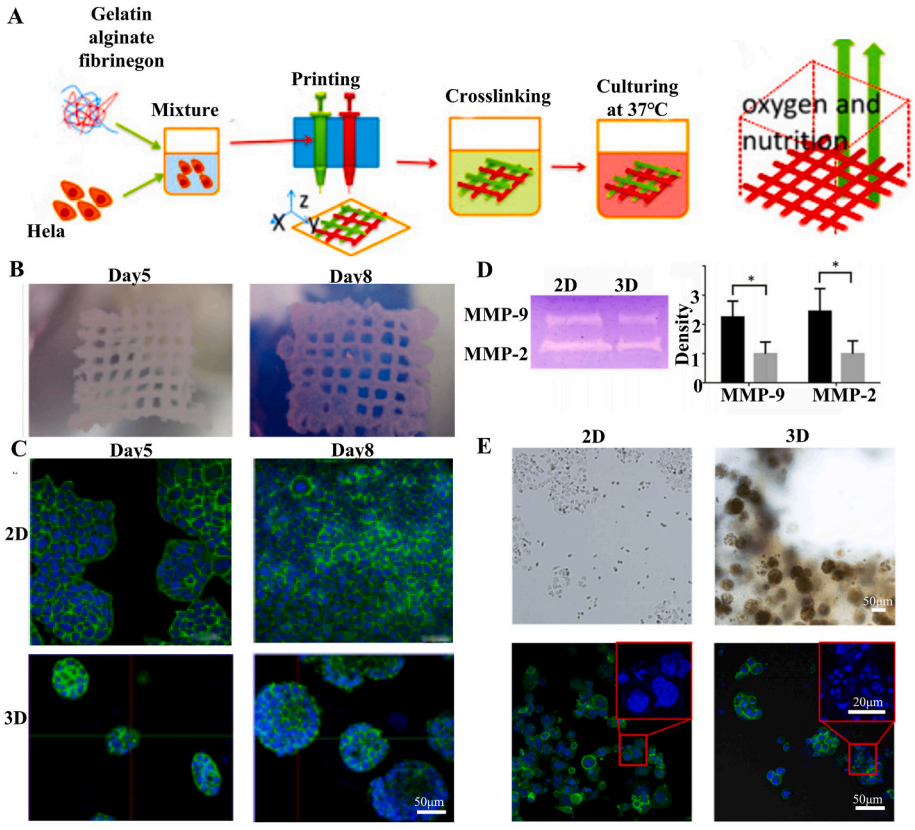

Fig. 9. Three-dimensional printing of HeLa cells for cervical tumor model in vitro. A The schematic of 3D HeLa/hydrogel constructs. B. Top view of 3D HeLa/hydrogel constructs on day 0, day 5, and day 8. C. Cellular morphological changes during 8 days of culture. D. MMP secretion of HeLa cells in 3D constructs and 2D planar culture. E. Chemoresistance of HeLa cells in 3D HeLa/hydrogel constructs and 2D planar culture. Reproduced from Ref. [4], with permission of 2014 IOP Publishing Ltd.

of 3D-bioprinted tissue models. Xu et al. employed an inkjet-based 3D bioprinting method to print human ovarian cancer cells (OVCAR-5) and MRF-5 cells (a normal human fibroblast cell line) in a controlled spatial distribution atop a Matrigel matrix scaffold, creating a 3D culture model to study the regulatory feedback mechanisms between tumors and matrix cells, as well as for drug sensitivity testing [6].

Baka et al. [133] used a gelatin-alginate saline gel loaded with ovarian cancer cells (SKOV-3) and cancer-associated fibroblasts (CAFs) for 3D bioprinting to develop an in vitro model of ovarian tumors. In this model, it was observed that cells self-assemble into heterotypic aggregates, which can be utilized to construct ovarian cancer organoids. Mekhileri et al. [134] created a macroscale ovarian model by assembling a 3D-printed hydrogel scaffold into a heterogeneous spheroid containing ovarian adenocarcinoma cells and fibroblasts (Fig. 10). Compared with a single spheroid module, the increase in size and complexity of tumors leads to a decrease in sensitivity to chemotherapy drugs. These results indicate that the planar culture model cannot accurately simulate the impact of the physiological microenvironment on drug pharmacokinetics after losing the three-dimensional structure of normal in vivo tissues. Therefore, when comparing 3D-bioprinted organoids with 2D cultures, organoid models exhibit resistance to chemotherapy drugs.

The application of 3D bioprinting in drug screening enhances physiological relevance by creating three-dimensional tissue models that closely mimic the in vivo environment. Therefore,3D-bioprinted cervical and ovarian cancer tumor models have been successfully used to study tumor occurrence and cell response to clinically relevant chemotherapy drugs. This novel in vitro bioprinted tumor model exhibits 3D biological features, making it an important tool for studying 3D tumor biology.This advancement is poised to improve the efficiency and accuracy of high-throughput drug screening. Additionally, it enables personalized drug screening, providing strong support for personalized medicine.

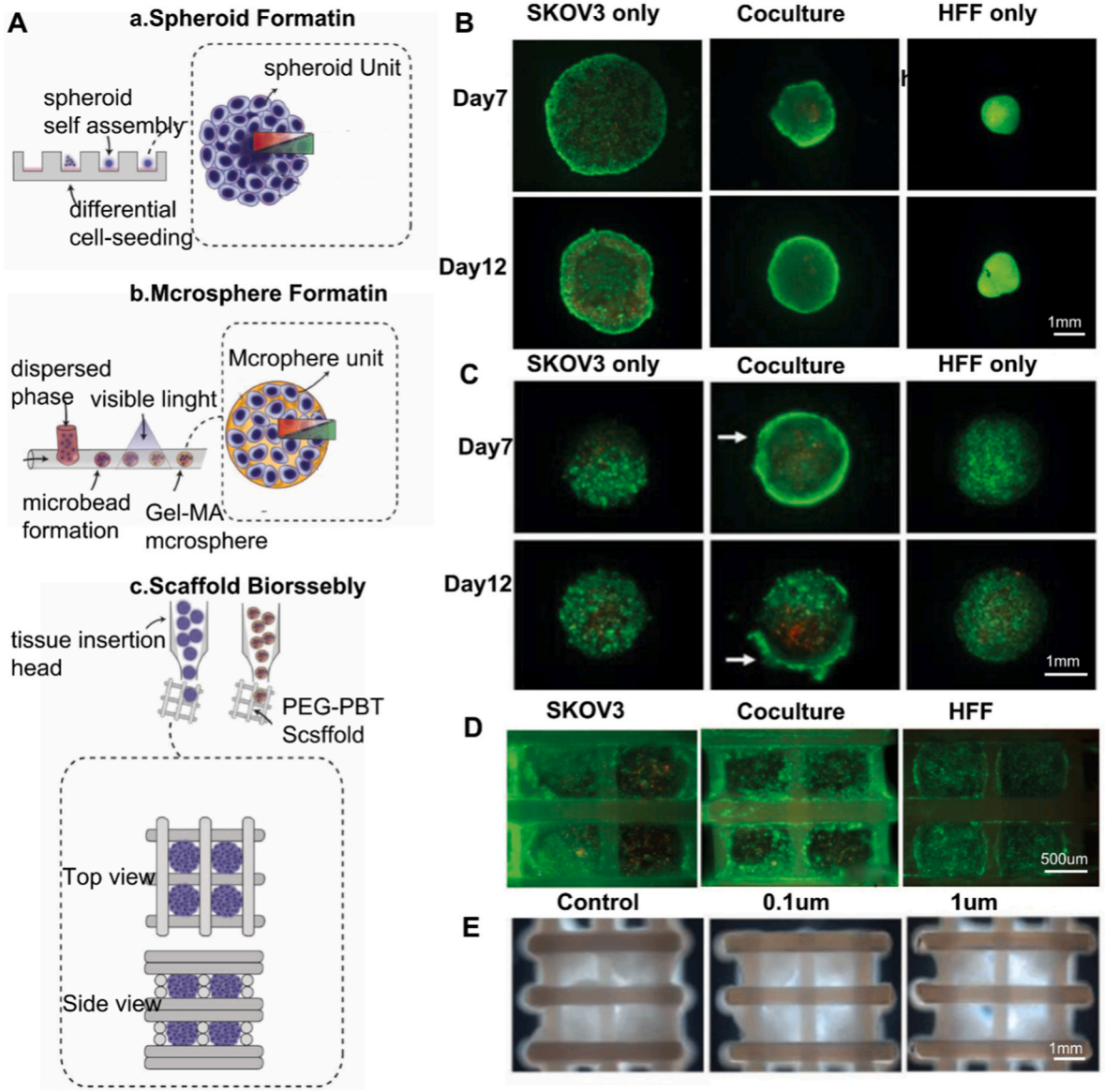

Fig. 10. Construction of 3D in vitro model of ovarian cancer. A. Cancer 3D in vitro model overview. B. Live and dead staining cells of reproducible ovarian carcinoma spheroids on day 7 using the liquid overlay method. C. Live and dead staining cells of reproducible ovarian carcinoma GelMA microspheres using a visible-light microfluidic approach. D. Ovarian carcinoma coculture construct bioassembly into PEGT:PBT scaffolds. E. Darkfield images of assembled coculture construct imaged after 4 days of exposure to doxorubicin. Reproduced with permission from Ref. [134] under Creative Commons license.

4.2. Others

4.2.1. Endometriosis

Endometriosis is a common disease that affects 178–200 million women worldwide. It refers to the growth of endometrial-like tissue outside the uterine cavity, often invading structures within the pelvic cavity, including the peritoneum, ovaries, bladder, small intestine, and colon. The main clinical manifestations of endometriosis are chronic pelvic pain and infertility [135,136].The lack of representative in vitro models of endometriosis hinders research in this field. In a 2020 study [137], 3D bioprinting was used to create a three-dimensional biological model of frameless endometriosis using the 12Z endometrial cell line and a phosphate matrix, employing the Kenzan method (Fig. 11). This model expresses high levels of estrogen-related genes and secretes a significant amount of inflammatory factors associated with endometriosis, independent of TNFα stimulation. Additionally, the study constructed a 3D construct with 12Z cells in the periphery and HeyA8 cells in the core, which can be used to investigate the pathogenesis of endometriosis-related ovarian cancer. Furthermore, this study attempts to construct a biosphere using endometrial stromal cells (T-HESCs) and 12Z cells to explore the pathogenesis of endometriosis. Although the 12Z cell line used originates from peritoneal lesions and cannot fully represent the various molecular forms of endometriosis, this biological model is still expected to serve as a conceptual validation basis for studying endometriosis and its microenvironment.

4.2.2. Uterine contraction physiology

The uterine muscle layer is the main tissue structure responsible for uterine contractions, which are crucial for various reproductive functions, such as the menstrual cycle, transportation of sperm and embryos, pregnancy, and childbirth [138,139]. Dysregulation of uterine contractility can lead to common pathological diseases, including premature birth, infertility, implantation abnormalities, and irregular menstrual cycles [140–142]. Uterine contractility is a three-dimensional coordinated phenomenon that should be studied in a three-dimensional environment. Souza et al. [143] used 3D bioprinting for the first time to print uterine muscle cells from different patient sources into 3D-bioprinted hollow rings, which can be used to study the physiological mechanisms of uterine contractility and the effects of various clinically relevant drugs, such as nifedipine and indomethacin, on uterine contractions.

5. Discussion and perspective

3D bioprinting technology holds significant potential for applications in the repair and regeneration of the female reproductive system. However, challenges remain, such as insufficient vascularization in printed tissues and structural and functional discrepancies compared with natural tissues. Addressing these issues requires feasible approaches, including optimizing 3D printing techniques, identifying biocompatible materials suitable for bioprinting, and selecting appropriate cell types and bioactive factors.

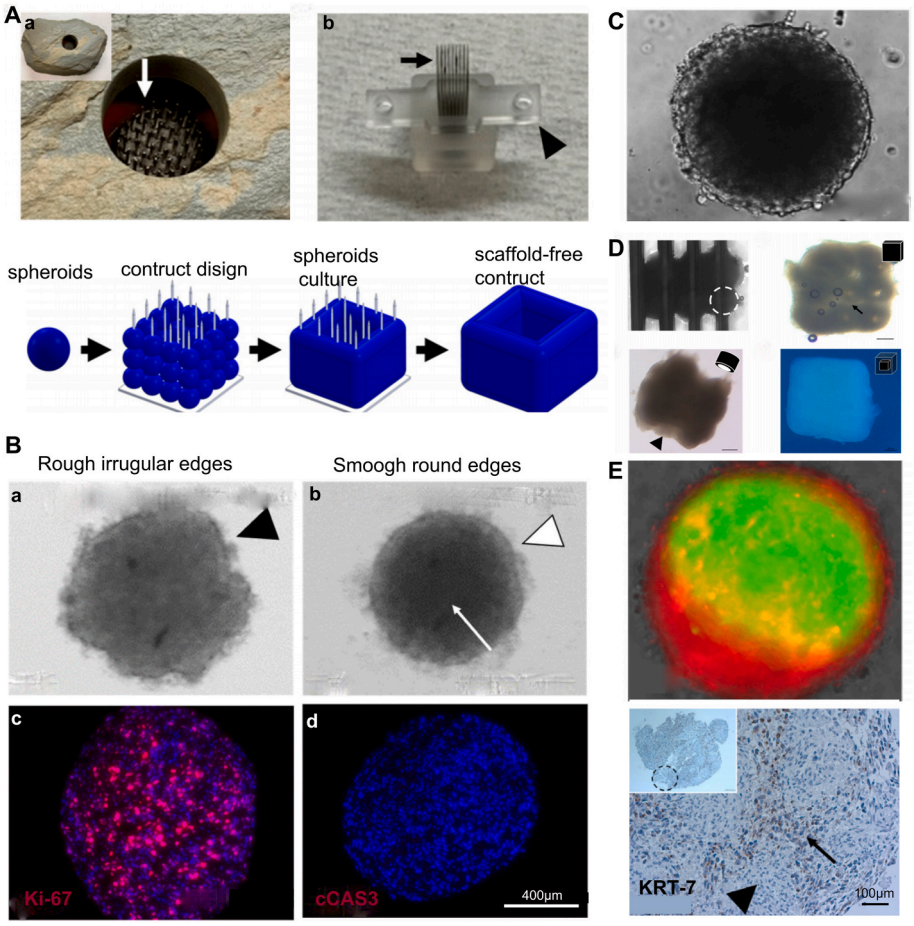

Fig. 11. Three-dimensional biofabrication models of endometriosis. A. Kenzan method of biofabrication. a-b. A Kenzan used for 3D biofabrication with the Regenova Bio 3D Printer. c. Workflow of Kenzan method biofabrication. B. Large 12Z spheroids. a-b. The change of large 12Z spheroids at 24 h and 48 h. c-d. 12Z spheroids remain alive for at least 120 h. C. Representative image of a spheroid, which fits all of the Regenova goal parameters. D. 3D biofabrication of 12Z and HEYA8 spheroids into constructs. E. Spheroids made from 12Z and T-HESCs and KRT-7 as a epithelial marker. Reproduced from Ref. [137] under Creative Commons license (CC-BY).

When integrating 3D bioprinting with stem cell technology for regenerative medicine in the female reproductive system, a key issue is how to induce the directed differentiation of stem cells. 3D bioprinting provides favorable growth conditions for stem cells, facilitating their interaction with the surrounding microenvironment. Furthermore, with advancements in bioprinting materials, such as the development of dECM, it is now possible to create specific physiological and biochemical environments for various cell lines, enriched with growth factors and stem cell niches. This environment supports cell adhesion and proliferation and can, to some extent, induce the directed differentiation of stem cells, thereby promoting tissue formation and regeneration.

3D-bioprinted tumor models for cervical and ovarian cancers have already been successfully employed in studying tumor pathogenesis and drug screening. These 3D-bioprinted tumor models exhibit unique three-dimensional biological characteristics, making them valuable tools for studying tumor biology in a 3D context. However, in vitro tumor experiments often lack the regulatory influences of other human systems. The female reproductive system, consisting of several organs interconnected through complex endocrine pathways and communication mechanisms, poses additional challenges. Consequently, simple in vitro 3D models may yield outcomes that differ from in vivo conditions. "Organ-on-a-chip" technology offers a promising solution by leveraging microfluidics and 3D cell culture techniques to create biologically active organ models on microchips. 3D bioprinting can provide precise spatial structures for three-dimensional cell cultures, while microfluidic systems can better simulate the in vivo microenvironment. Integrating different organs onto a single biochip could enable the creation of more realistic models that closely mimic the physiological or pathological conditions of the female reproductive system, providing a powerful tool for research in this field.

Given the current applications of 3D bioprinting in the female reproductive system, there is potential to explore its use in female fertility preservation. Presently, the primary techniques for fertility preservation in women include embryo freezing, oocyte freezing, and ovarian tissue freezing. Among these, embryo freezing is the most effective but is only suitable for married women. For women without a partner or for prepubescent girls, oocyte freezing and ovarian tissue freezing are the only available options. However, oocyte freezing often faces limitations due to the patient's condition, making it difficult to obtain mature oocytes. While immature oocytes can be matured in vitro, the normal development and blastocyst formation rates of embryos derived from in vitro-matured oocytes are lower than those of embryos derived from in vivo-matured oocytes. 3D bioprinting could offer a solution by constructing implants that combine immature oocytes with biomaterials, allowing these oocytes to mature in vivo and thereby increasing the chances of natural conception for such patients.

As for ovarian tissue freezing, a common issue is the significant reduction in the number of primordial follicles due to hypoxia during tissue thawing and transplantation. 3D bioprinting can address this by using biomaterials to load ovarian tissue and relevant bioactive factors to construct 3D-structured implants. These implants could facilitate better nutrient exchange between the ovarian tissue and the surrounding environment, reducing hypoxia and improving the survival rate of primordial follicles within the ovarian tissue.

3D bioprinting still faces challenges in fully meeting the demands of the female reproductive system. It is important to recognize that other areas of biological research are also advancing toward the creation of multicellular systems, such as organoids, which address the critical need for microenvironments defined by specific cellular interactions. Moreover,3D bioprinting is confronted with a host of ethical issues, including disputes over the sourcing and utilization of cells, the ethical balance in clinical applications and trials, risks of technological misuse, and challenges regarding intellectual property rights and regulation. Aditionally, bioprinting technology must achieve scalability and integrate innovatively with additional biofabrication methods. However, interdisciplinary collaboration may increase labor costs, and the materials and equipment typically used in 3D bioprinting are often expensive.Consequently, the development of low-cost hardware and improved accessibility are further necessary to advance its application in the field of the female reproductive system.

CRediT authorship contribution statement

Siyao Chen: Writing – review & editing, Software, Methodology. **Tongxin Wang:** Writing – original draft. **Jiaqi Chen:** Writing – review & editing, Resources, Data curation. **Mingxing Sui:** Supervision. **Luyao Wang:** Software. **Xueyu Zhao:** Software. **Jianqiao Sun:** Resources. **Yingli Lu:** Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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