Collagen and Leather

REVIEW

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Collagen-based biomaterials in organoid technology for reproductive medicine: composition, characteristics, and applications

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Abstract

Collagen-based biomaterials (CBB) are highly esteemed by researchers in materials science and biomedicine due to their extensive applications across various biomedical disciplines. In recent years, owing to advancements in developmental biology techniques, this superior biomaterial has seen increasing utilization in 3D in vitro tissue culture. Three-dimensional cell cultures, often referred to as organoids, have emerged in response to technological advancements in biomaterials and the growing need in the field of medical research. They serve as important models for simulating normal physiological activities in vivo, addressing limitations in experimental material sources, and resolving ethical issues. In this review, we discuss the material characteristics of CBBs commonly used for organoid culture, integrating aspects such as Matrigel and decellularized ECM as culture matrices. We also analyzed the development prospects and directions of various materials in the context of biology, clinical medicine, and particularly reproductive medicine. Currently, despite the FDA approval and clinical research incorporating numerous CBBs, existing challenges in multiple studies indicate a significant unmet need in the development of key tissue models for both medical research and clinical applications. In summary, CBBs are swiftly broadening their applicability in the realms of organoid nature and medical research, serving as a versatile and high-performing material for 3D in vitro tissue culture.

Keywords Collagen, Organoids, Biomaterials, Applications, Component and characteristics

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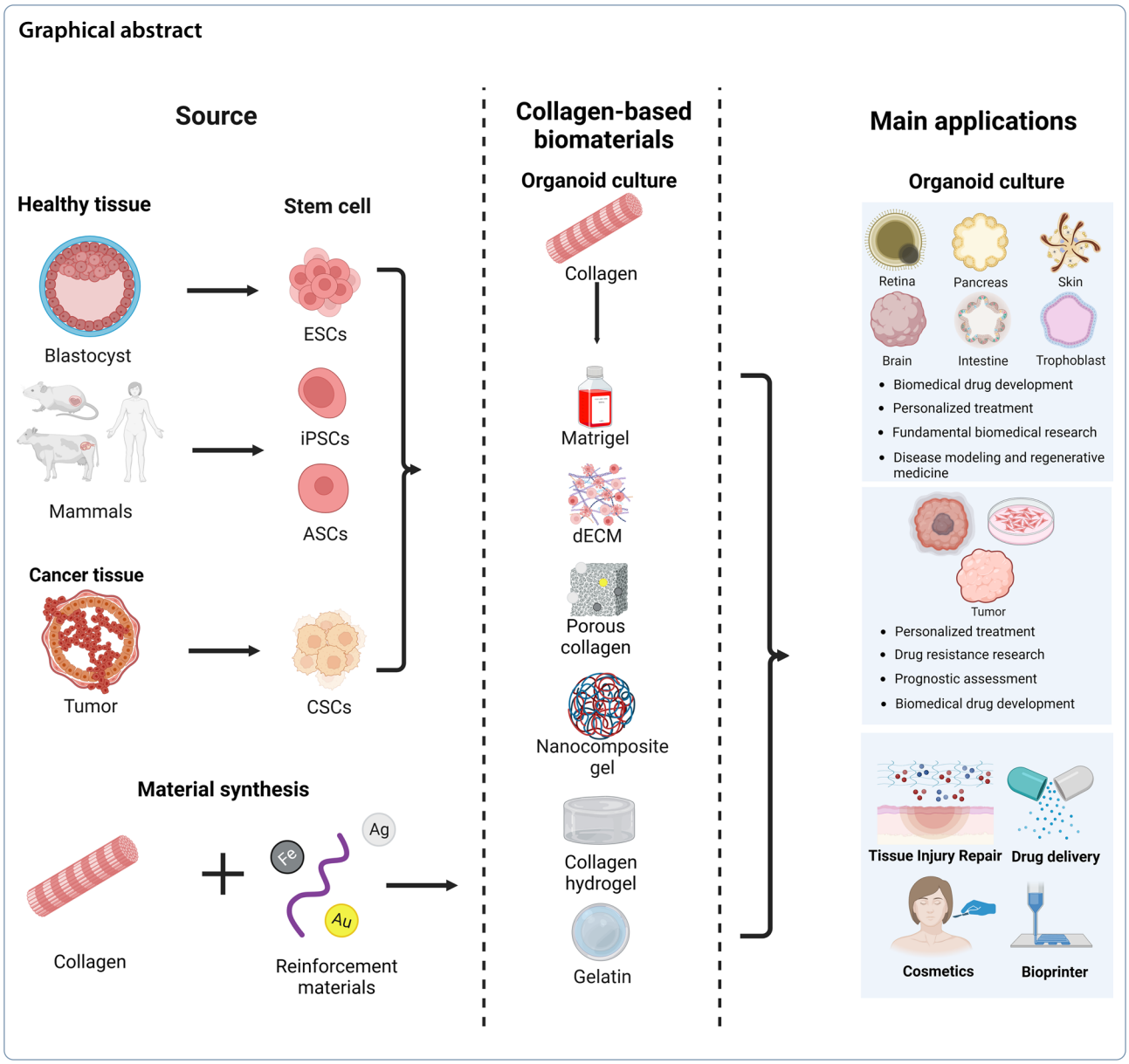


1 Introduction

Collagen is a right-handed helical protein that is highly abundant in the extracellular matrix of animal cells. It has widespread applications in biomedical engineering due to its good biocompatibility and relatively low immunogenicity [1, 2]. So far, 28 types of collagens with different structures and origins have been discovered [3–5]. They are further classified into four subtypes according to their supramolecular structure: fibril-forming collagens, fibril-associated collagens, network-forming collagens, and membrane-anchored collagens [3]. The most basic structure of collagen is a triple helix, including the repeated GlyXaaYaa sequence composed of glycine (Gly), along with interchain hydrogen bonds, proline (Pro) content, and proline positioning related to the characteristics of collagen [1]. Based on the triple-helix structure, collagen is endowed with good thermal stability, mechanical strength, and the ability for molecular interactions [6, 7]. It is precisely these structural and functional characteristics, especially the ability of collagen to interact with biological cells, that have led to the widespread application of CBBs in the fields of bioengineering and medicine [8, 9].

Organoids are complex 3D multicellular tissues that are similar to in vivo organs, self-organized from pluripotent stem cells (PSCs) or adult stem cells (ASCs)

under in vitro culture conditions [10, 11]. In recent years, with the continuous development and application of CBBs and the increasing maturity of stem cell culture technology, significant progress has been made in the in vitro culture techniques for organoids. Historically, conducting tissue and organ studies on mammals has been a contentious issue in the field of biomedicine due to ethical concerns and limitations on sample availability [11]. The emergence of organoids not only alleviate the conflict between tissue and organ research and ethics but also provides favorable conditions for biomedical research in areas such as disease modeling, personalized treatment, drug screening, and transplant therapy [12]. Figure 1 illustrates the cultivation process of organoids from different sources.

The extracellular matrix (ECM) serves as the physical scaffold of tissues, is rich in biomolecules, and plays a key role in signal transduction, cell behavior, and tissue repair [13, 14]. By simulating the ECM, experimenters could achieve cell growth and differentiation outside the organism, which is an important concept in the artificial construction of biological tissues. In recent years, although research on natural hydrogels, synthetic hydrogels, etc., for in vitro cultivation has proliferated, the gold standard for in vitro cell culture, including matrix gel cultivation, has

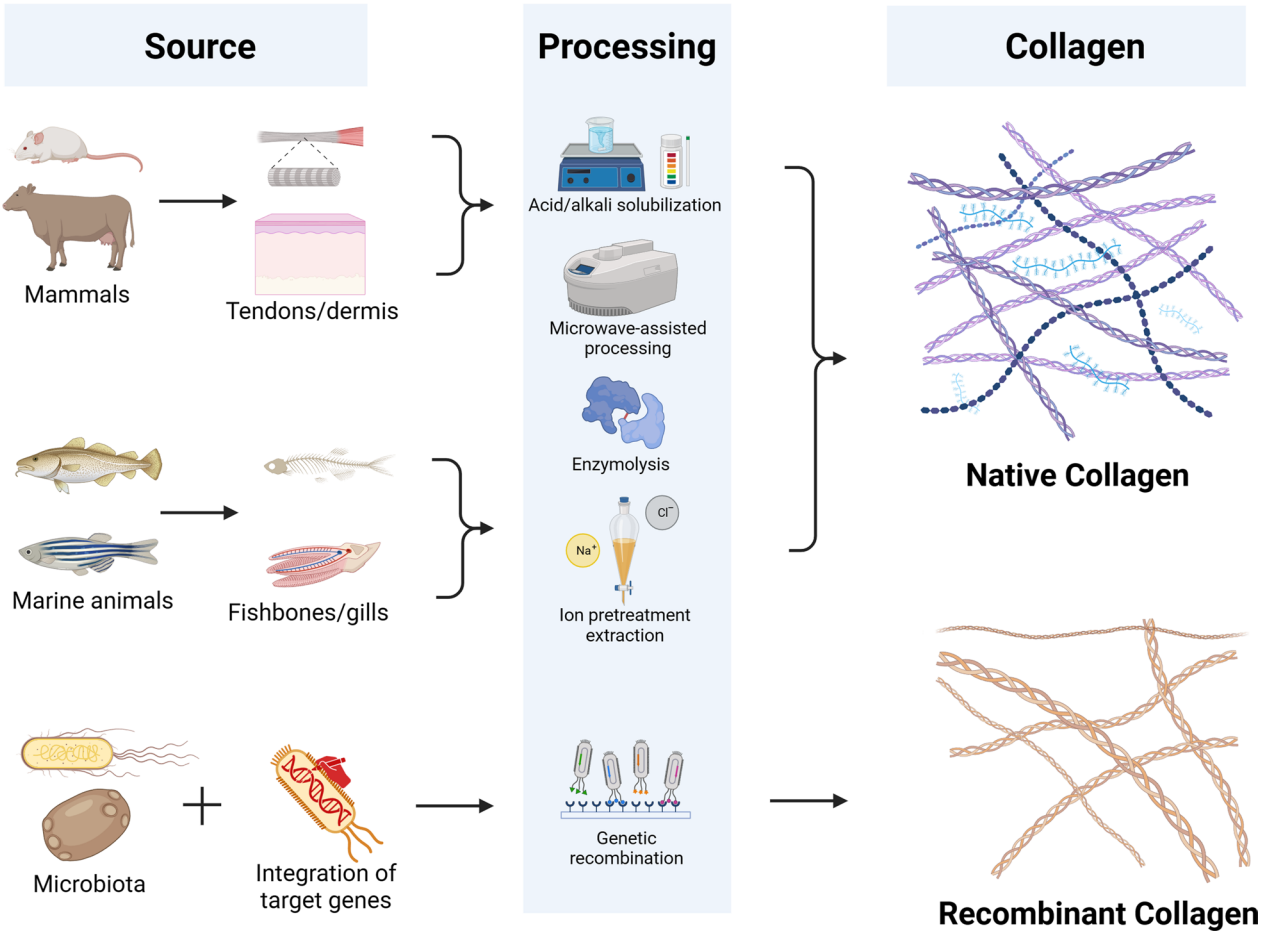


Fig. 1 Overview of different sources of collagen in biomaterials *Source* Collagen can be sourced from two primary categories: nature and recombinant. Nature collagen is predominantly extracted from mammals and marine fish. Specifically, mammalian-derived collagen is primarily harvested from skin, bones, tendons, and various connective tissues. In contrast, collagen from marine fish is chiefly sourced from skin, bones, and scales. Alternatively, recombinant collagen is synthesized using advanced genetic engineering techniques and is produced in either prokaryotic or eukaryotic microbial hosts. *Processing* The purification and processing of nature collagen commonly encompass several stages, including hydrolysis facilitated by acids or bases, microwave- or ultrasound-assisted extraction, and enzymatic breakdown. On the other hand, recombinant collagen is amenable to large-scale biosynthesis via genetic engineering in specialized microbial cultures. *Collagen* While nature collagen typically presents as a multifaceted mixture with a complex chemical composition, recombinant collagen offers a far more uniform molecular profile. This homogeneity is advantageous for the formulation and application in specialized synthetic materials

performed barely satisfactory in biomedical fields such as disease model construction. As the most abundant protein in the ECM, the role of collagen has been re-emphasized, and experiments related to biologically active hydrogels as substrates for in vitro cell culture are being continuously conducted. Accompanied by the continuous improvement of stem cell culture ecology in recent years, the field of organoid technology in in vitro cultivation has achieved systematic development and has been proven to hold a significant position in tissue and organ technology [15].

Due to genetic susceptibility, age, and iatrogenic effects of treatment, individuals with certain diseases may face reproductive or endocrine failure [16]. The prevalence of this problem was reflected in a global infertility rate study in 2010, in which 480,000 infertile couples were found in 190 countries, with 50,000 suffering from primary infertility and 190,000 diagnosed with secondary infertility [17]. This is a potential threat, especially for countries with an increasingly aging population. To address the challenges of an aging population, researchers have been working to increase fertility and birth rates and develop assisted reproductive technologies over the past 50 years. Breakthroughs in this field began with the successful creation of an embryo in vitro in 1978 [18] and subsequently achieved a series of important advances, including the successful cultivation of live animals in 2006 [19] and the realization of human in vitro blastocyst culture in 2016 [20]. Meanwhile, in the late 1980s, Matrigel was used as a basement membrane to culture mouse mammary epithelial cells [21], and in 2009, the first organoid was successfully cultivated [10], as well as the successful construction of a scaffold-free organoid model containing endometrial epithelial cells and stromal cells in 2020 [22]. In the interdisciplinary realms of bioengineering and material science, significant strides have been achieved in reproductive medicine, specifically through advancements in biomaterial technologies. These developments not only amplify the efficacy of infertility treatments but also propel the field of endometrial organoids to new scientific frontiers. CBBs stand as a cornerstone in these innovations, exerting a pivotal influence within biomedical applications. This review discusses the recent critical roles that CBBs have played in both medical science and organoid culture. The article further elucidates, from a reproductive medicine perspective, the application of organoid technology in studying in vitro blastocysts and endometrial diseases. Finally, the article also highlights the valuable contribution of CBBs to the burgeoning field of organoid nature.

2 Sources of collagen

Collagen is derived from various sources, with natural collagen mainly coming from mammals [23] and non-mammals [24]. Currently, most collagen products are extracted from the tissues and organs of mammals or marine fish [25]. Synthetic collagen, on the other hand, is primarily synthesized through industrial recombination using microbes, plants, or insects [26, 27]. Depending on the different characteristics of collagen from various sources, the manufactured biomaterials can have different physiological functions and material values. Figure 1 illustrates the different sources of collagen in biomaterials.

The primary sources of collagen are the tendons and dermis of mammals such as pigs, cattle, and rabbits, as well as the bones, scales, and skin of marine vertebrates and invertebrates [28, 29]. Conventional extraction techniques include acid dissolution, alkaline environment extraction, and enzyme-linked extraction, among others [30, 31]. However, these sources come with the risk of transmitting infectious agents and inducing immune responses [32]. Industrial-grade recombinant collagen is increasingly being employed as an alternative, produced through various expression systems such as prokaryotic and yeast expression systems [33]. Compared with traditional methods, recombinant collagen offers advantages like enhanced safety and water solubility [34]. However, certain recombinant technologies are still in the developmental stage [35], and challenges related to synthetic protein immunogenicity, yield, degradation, etc., have led to the limited biological production of collagen (I, II, III [33], IV [36], V [37], VII [38], IX [39]) to date.

Collagen finds widespread applications in tissue regeneration, drug delivery, matrix scaffolding, and cosmetics. Compared with microbial recombinant technology, recombinant sources of collagen such as plants, animals, and insects have limitations in terms of production costs and yield. In summary, collagen, whether derived from natural sources or synthesized through advanced recombinant technologies, holds an irreplaceable value in various medical and bioengineering fields. Given these considerations, in-depth research into collagen and its base materials not only advances the frontiers of tissue engineering and drug delivery but also offers new possibilities in overcoming the limitations associated with natural sources of collagen.

3 Principal collagen in CBBs

Collagen serves as a critical component in a myriad of physiological functions, including tissue structural integrity, cell migration, and signal transduction. In humans, the collagen family is predominantly comprised

of fibrillar collagens (Types I, II, III, V, and XI), FACIT collagens (Types IX, XII, XIV, XVI, XIX, and XX), short-chain collagens (Types X and VIII), and basement membrane collagen (Type IV). A hallmark of collagen is its unique triple-helix structure, which bestows it with its defining mechanical and functional characteristics, making it indispensable in various biological contexts [5]. At present, the collagen types that find extensive application in the field of biomaterials are primarily Types I, II, III, and IV. These materials modulate cell signaling pathways, offer structural scaffolds for tissue growth, and are an integral component of CBB due to their exceptional biocompatibility and capacity to mimic the cellular microenvironment. Notably, type I and type IV collagen show promising applications in tissue engineering and in vitro tissue culture.

3.1 Type I collagen

Type I collagen is the most abundant form found in the human body. Synthesized predominantly by osteoblasts and fibroblasts, this collagen type plays a pivotal role in the structural integrity of bones and teeth [40]. Recognizing the myriad of promising applications this material could offer, extensive research has been conducted to investigate its structure [41], mechanics [42], biology [43], and other characteristics.

Type I collagen serves as a cornerstone in the field of biomaterials, chiefly due to its unique structural foundation. Comprised of a heterotrimer consisting of two identical α1(I) chains and a single α2(I) chain, this molecular configuration significantly influences the characteristics of derivative biomaterials. Its helical architecture features a recurring Gly-Xaa-Yaa triplet sequence, bookended by N- and C-termini. These chains are primarily synthesized through the regulatory functions of the COL1A1 and COL1A2 genes. During the post-translational modification phase, prolyl 4-hydroxylase 1 and lysyl hydroxylase hydroxylate specific proline and lysine residues in procollagen [40], thereby enhancing the stability and solubility of the helical structure. Subsequently, monosaccharides are enzymatically grafted onto the hydroxylysine residues of α1(I) and α2(I) chains, facilitated by the catalytic action of pertinent glycosyltransferases [40], This glycosylation step further optimizes collagen folding, cross-linking, and intermolecular interactions. Finally, a triple helical structure materializes through right-handed coiling from the C-terminus to the N-terminus, orchestrated by thioredoxins and additional factors that govern collagen trimer formation. Following this, the procollagen undergoes further modification within the Golgi complex before being secreted extracellularly. Once outside the cell, Type I collagen possesses the capacity to respond to various hormonal and cytokine signals, thereby modulating the proliferation, differentiation, and migration of adjacent cells. Attributable to its intrinsic biocompatibility and biodegradability, CBBs from Type I collagen, such as nanoparticles and gels, demonstrate exceptional antibacterial, hemostatic, and anti-inflammatory characteristics, making them ideally suited for applications in wound care, notably for treating infectious and burn wounds [44]. What's more, bioengineered materials crafted from collagen's advantageous cell-interactive and biocompatible attributes are poised for significant contributions to tissue engineering, aiding in the regeneration and repair of damaged organs [45]. In the field of drug delivery, Type I collagen stands out for its beneficial characteristics, including its ease of hydration and biodegradability [46]. In conclusion, widely utilized culture materials incorporating type I collagen are primarily employed in organoid research for cultivating parenchymal organs like the liver, lung, and heart, as well as glandular organs such as the mammary gland and hair follicle. Derived from it, dECM stands out as a crucial CBBs with highly promising applications in the cultivation of diverse organoids (Fig. 2).

3.2 Type II collagen

Type II collagen is the main protein composing human elastic cartilage. It has a triple helical structure consisting of a homotrimer formed by three identical α1(II) chains. The chondrocyte-secreted collagen regulates the osteogenic differentiation of bone marrow-derived mesenchymal stem cells. This collagen is widely used to enhance ossification-like process, primarily in CBBs for regenerative repair of bone tissue [47], such as the repair of pathological cartilage [48], and the in vitro culture of human and animal cartilage [49], etc.

3.3 Type III collagen

Type III collagen is composed of three identical α1(III) helices, predominantly regulated by the COL3A1 gene. This collagen type serves as a pivotal component in various extracellular matrices, notably in the interstitium, skin, and vascular systems. It is particularly adept at forming intricate 3D networks in synergy with Type I collagen. Accumulating research highlights its indispensable role in maintaining the structural integrity of hollow organs, including but not limited to arteries, the uterus, kidneys, and the gastrointestinal tract. Previous research has emphasized the indispensable role of collagen in maintaining the structural integrity of various hollow organs, including but not limited to arteries, the uterus, kidneys, and the gastrointestinal tract. Emerging studies at the cellular level have highlighted the crucial role that Type III collagen plays in modulating key cellular behaviors in human dermal fibroblasts, such as adhesion,

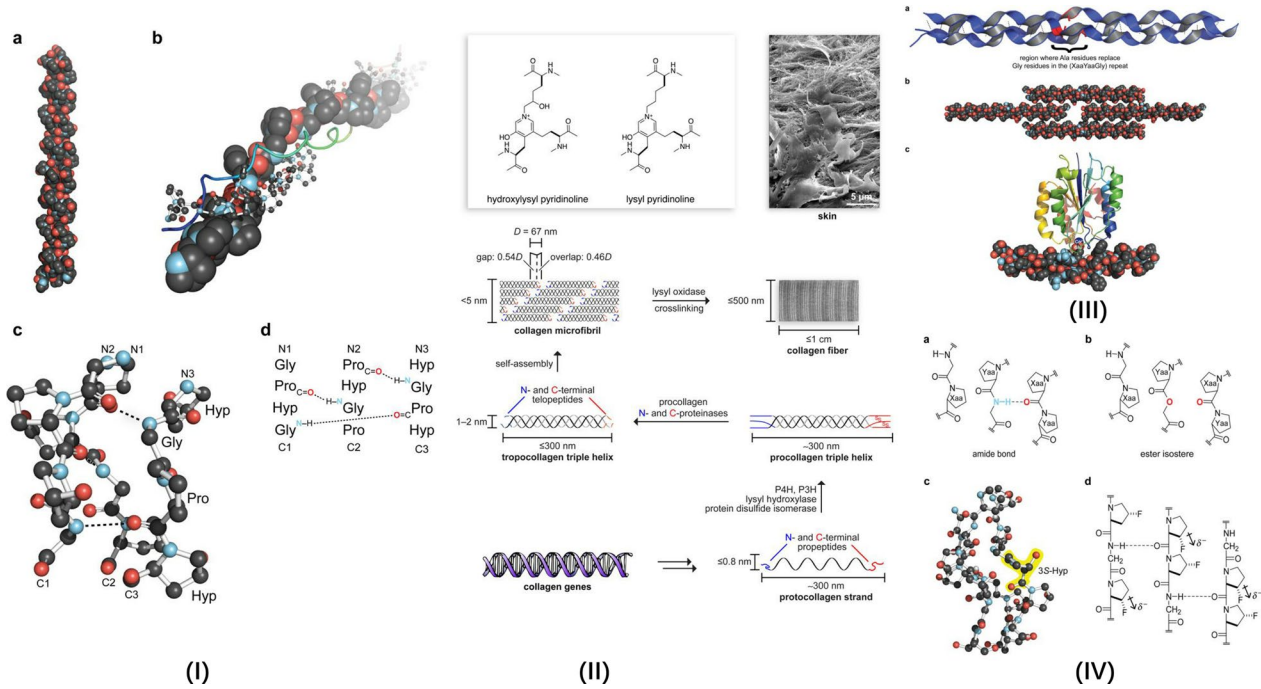


Fig. 2 Molecular structure and biosynthetic routine of collagen. Reproduced permission from [1]. (I) Overview of collagen triple helix structure; (II) Biosynthetic pathways of collagen from the skin; (III) Snapshots of crystal structures of collagen triple helices; (IV) Importance of interstrand hydrogen bonds for collagen triple-helix stability

proliferation, and migration [50]. Given these important biological functions, mechanisms involving Type III collagen have also garnered widespread attention in the field of biomaterials. For example, applications in wound dressings [51], vascular hemostasis [52], and as supplementary constituents to augment the biocompatibility and biodegradability of other biomaterials [53]. However, as an important collagen, its derived CBBs are not widely used in organoid culture, but more as a marker to verify the degree of organoid differentiation.

3.4 Type IV collagen

Type IV collagen serves as the predominant collagenous component of the extracellular matrix's basal membrane. Its structure and function are chiefly regulated by an ensemble of six genes: COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, and COL4A6. Unlike other collagen types, Type IV collagen lacks the quintessential glycine residue at every third position in its peptide chain, leading to a more relaxed molecular arrangement. This results in a sheet-like conformation within structural tissues, where it plays a pivotal role in facilitating cell adhesion. Currently, it has promising potential in the treatment of eye diseases [54], screening for kidney damage [55], bioscaffolds [56], drug delivery [57], and other areas. In organoid cultures, the addition of type IV collagen to standard culture media facilitates the in vitro cultivation of endoderm, including intestinal epithelium [58], uroepithelium [59] and mesoderm, such as kidney [60]. Moreover, collagen IV is a crucial constituent of Matrigel, the primary culture medium, playing a vital role in various organoid cultures.

In general, culture matrices incorporating type I, type II, and type IV collagen initially demonstrate the capability to culture organs in vitro. However, owing to the suboptimal mechanical properties and developmental constraints of these materials, they are primarily utilized for the fundamental structure formation of simpler organs, simulation of basic physiological functions, and disease modeling. To delve into complex signaling pathways and advance clinical research, the quest for materials with enhanced biological and mechanical properties becomes imperative to simulate a more realistic in vivo environment.

4 Commonly used CBBs in organoid research

4.1 An exemplary biological material

The meticulous construction of the tissue microenvironment stands as the linchpin in delineating the intricacies of cell and tissue culture. In comparison to other frequently employed media components, collagen, a predominant constituent within the extracellular matrix under physiological conditions, assumes a distinctive role in shaping the tissue microenvironment. Insights

gleaned from oncology-related reviews underscore collagen's involvement in the construction of tumor cell signaling pathways, exosome formation, tumor development, tumor immunity, and cancer healing by orchestrating the tumor microenvironment [3]. This highlights collagen's expansive influence in regulating cellular life activities, a realm often challenging for other components to traverse.

Within studies focused on the cultivation of normal tissues, the microenvironment established by collagen hydrogels introduces heightened negative charges, thereby amplifying the differentiation of stem cells, including bone marrow mesenchymal stem cells, both in vitro and in vivo [61]. This enhancement contributes significantly to the generation of more mature organoids. Moreover, research indicates a pivotal correlation between rational viscoelasticity and intercellular signaling, transcription factor activation, and epigenomic expression—critical design parameters for biomaterials [62]. As one of the primary molecules providing viscoelasticity to the extracellular matrix, collagen's physical properties in nanocomposites or hydrogels can be meticulously tailored to closely mimic those of the natural ECM through doping or cross-linking. This strategic approach holds great significance in emulating the microenvironment requisite for organoid growth.

In mechanistic investigations within reproductive medicine, collagen assumes a crucial role in tissue culture, leveraging its capacity to construct signaling pathways and replicate cellular physiological functions. A study has reported that ecdysis Nature Killer (NK) cells can secrete small amounts of type IV collagen [63], and related leukocytes, such as leukocyte-associated immunoglobulin-like receptor (LAIR)-1, which serves as an inhibitory receptor for various immune cells and is also a receptor for collagen, may possess collagen receptors. This implies that collagen might contribute to successful pregnancies by engaging with receptors on immune cells at the maternal–fetal interface. Such interactions may pave the way for the development of collagen-based biomaterials for restoring fertility in women with uterine adhesions. Moreover, an increased expression of type III collagen has been observed in the microhypoxic environment of the maternal–fetal interface [64]. Although the underlying mechanism remains unclear, this observation provides valuable insights for modeling the in vitro microenvironment of the maternal–fetal interface. Concerning spermatogenesis, substantial studies have demonstrated that collagen chains (type IV collagen) in the basement membrane can secrete specific bioactive peptides, promoting spermatogenesis and release [65]. Utilizing such collagen proves beneficial for the in vitro cultivation of spermatogenic tissues. In summary, collagen plays diverse and vital roles in the in vitro culture of reproductive organs. The rational utilization of these advantages in producing CBBs contributes significantly to cultivating in vitro organs that closely mimic normal physiology.

In the evolving landscape of materials science research, collagen's exceptional attributes such as degradability, viscoelasticity, negative electronegativity, biocompatibility, signal transduction capability and structural and supportive functions have been harnessed and swiftly incorporated into a broader spectrum of research and development in the field of CBBs. This surge in utilization positions collagen as a preeminent biomedical material, replete with distinct advantages that resonate prominently in contemporary biomedical research and development efforts.

4.2 Nanocomposite materials and collagen

Nanocomposite materials constitute a specialized category of multiphase materials, distinguished by having at least one dimension measuring within 100 nm. The preparation of these materials for use in CBBs is inherently complex, with various methods currently in use, primarily including solution mixing, electrospinning, in situ synthesis, and self-assembly [66]. First, the solution mixing method involves mixing the collagen solution with other components. It enables the uniform distribution of components, facilitating the creation of specific material structures, such as customizable in vitro three-dimensional extracellular matrix models [67]. Moreover, electrostatic spinning, a continuous method creating nanofibers from viscous fluids [68], is versatile for synthesizing biomimetic bio-networks that emulate collagen fiber structures [69]. These nanofibers contribute actively to organoid culture. Self-assembly involves molecules spontaneously forming organized structures through non-covalent interactions. In tissue engineering, this method has been used to create injectable hydrogels resembling collagen fibers for regenerative medicine [70]. In situ synthesis generates materials directly in a specific location or condition by introducing and converting appropriate precursor substances through chemical reactions or physical processes. Studies have utilized this approach to enhance various properties of collagen, such as innovating biomimetic tissue scaffolds with hydroxyapatite and collagen nanocomposites to improve collagen's mechanical characteristics [71].

Serving as critical constituents in biomaterials, nanocomposite materials have been employed in the realms of materials science and biomedicine for a period extending over four decades. Commencing in the early 1980s, scholarly discourse on nanocomposite materials emerged within the materials science community [72], garnering incremental recognition over the ensuing

years. Post-1990s, scholarly investigations transitioned toward functional nanocomposites, exploring materials endowed with specific optical and magnetic characteristics, as well as diverse compositions ranging from gels and metals to silicon dioxide-based nanocomposites. During this time, preliminary developments were made in biomedical applications, including DNA extraction [73], medical biomaterials development [74], etc. After 2000, the applications of nanocomposite materials in biomedicine gradually expanded, and biomimetic materials for various tissues and organs gradually emerged [75], and collagen began to be applied to the development of nanocomposite materials. From 2010 to the present day, an array of collagen-based nanocomposites has made remarkable strides in biological attributes, mechanical robustness, and electrical conductivity, delivering tangible clinical outcomes. For instance, Purwada et al. developed B-cell follicular organoids using an RGD-presenting hydrogel scaffold reinforced with silicate nanoparticles, enabling tunable parameters for individualized immunotherapy [76]. Beier, J.P.'s group demonstrated enhanced in vitro skeletal muscle proliferation using collagen-I-fibronectin matrices as opposed to conventional porous materials, broadening the potential applications in tissue engineering [77]. In reproductive medicine, Sharif and team found higher attachment and proliferation rates of human endometrial stem cells (hEnSCs) on PCL/collagen scaffolds than standard PCL scaffolds, offering new ideas for culturing human endometrial organoids in vitro. Considering hEnSCs' remarkable proliferative capacity, it could also be applied in tissue repair, especially for injuries [78]. Currently, such nanocomposites are primarily used in biomedical fields for tissue engineering, with limited use in organoid culture due to material cost and availability constraints. Increasingly, these nanocomposites are finding broader applications in organoid cultures, with expanding relevance in the field of reproductive medicine. The characteristics and applications related to nanocomposite materials are shown in Table 2.

4.3 Gelatin composite hydrogels

Gelatin is a naturally derived biopolymer resulting from the partial hydrolysis of collagen. It is extensively common in daily life and finds wide applications in the processing of foods, pharmaceuticals, and cosmetics. Simultaneously, due to its observed good biocompatibility and degradability, gelatin has been extensively used in the field of biomaterials, such as the preparation of gelatin-methacryloyl hydrogels for simulating the extracellular matrix (ECM) [79]; fabrication of gelatin/poly(lactic acid) sponge scaffolds for tissue regeneration [80]; development of cellulose nanofiber-gelatin composite scaffolds [81]; and creation of chitosan–gelatin hydrogel scaffolds [82]. It has also been optimized as a bio-ink for advancing bioprinting technologies [83].Similar to collagen, gelatin is widely sourced and versatile. Distinct extraction techniques, such as acidic or alkaline pretreatments, result in the production of anionic Gelatin A or Gelatin B, respectively. Utilizing electrospinning technology, one can fabricate tubular scaffolds that effectively emulate the extracellular matrix (ECM) [84]. This all provides a wide selection of excellent biomaterial scaffolds for cell culture.

In reproductive organoid culture, tubular scaffolds offer solutions to persistent challenges in vascular assembly. In 3D bioprinting, versatile gelatin methacryloyl (GelMA) plays a crucial role in cell-supported cultures due to its exceptional plasticity and biocompatibility. Wu, T., and colleagues aimed to create in vitro 3D artificial ovaries using GelMA, observing follicle growth and ovulation [85]. Moreover, research efforts have focused on culturing reproduction-related organoids. Sivasubramaiyan team developed a dual biomimetic platform with embryoid bodies, fibronectin, and gelatin to enhance trophoblast cell differentiation, closely mimicking blastocyst morphology [86]. Resulting trophoblast cells showed an increased capacity for secreting human chorionic gonadotropin and progesterone, impacting endometrial microenvironment modeling [86]. In recent years, gelatin-based composite culture media are increasingly used in cultivating reproduction-related organoids, including ovarian cancer and disease modeling [87], and for chemotherapeutic drug screening. However, limited diversity, research, and commercial availability of gelatin-related organoid culture materials still constrain broader utilization. The characteristics and applications related to gelatin composite hydrogels are shown in Table 1.

4.4 Collagen composite hydrogels

Collagen composite hydrogels are formed by cross-linking collagen with other polymers and are widely used in medicine. While collagen is biocompatible and promotes cell adhesion, hydrogels made solely from collagen often lack the characteristics needed for specific applications. By carefully selecting composite components, one can enhance the hydrogel's biocompatibility, mechanical strength, stability, and adaptability to biological systems. Hydrogels, as a class of water-rich soft materials, serve as the base for these composites [88]. The basic 3D structure of hydrogels is formed through cross-linking. Collagen-based hydrogels can be either physically or chemically crosslinked, depending on the method used [89]. The characteristics and functions of hydrogels depend greatly on the type and degree of cross-linking [90]. Common secondary components added to collagen-based hydrogels include hyaluronic acid, alginate, and polyethylene

Table 1 Overview of the characteristics and main biomedical applications of biocomposite materials

Biocomposite materials	Major component	General characteristics	Applications
Gelatin composite hydrogel	Gelatin/collagen is commonly combined with materials such as polyethylene glycol (PEG), alginate, hyaluronic acid, polycaprolactone (PCL), and chitosan to form composite materials	Pros: Biocompatibility; adjustability; cost-effectiveness; repeatability; mature material technology; broad clinical application prospects Cons: Difficult to control mechanical characteristics; difficult to control bioactivity	1. In vitro 3D tissue culture: ovary [85], trophoblast [86], tumor [87, 94, 95], spinal cord [96], liver [97, 98], bone/cartilage [99], tooth germ [100], alveoli [101], heart [102], nerves [103], hair follicle [104], spleen [105], muscle fiber [106], 2. Drug screening or delivery [107, 108] 3. Tissue injury repair [109, 110] 4. Disease modeling [111] 5. 3D bioprinting [112, 113]
Collagen Composite Hydrogel		Pros: Biocompatibility; Adjustability; Repeatability; Broad clinical application prospects Cons: Potential biotoxicity; Difficult to control bioactivity	1. In vitro 3D tissue culture: gastrointestinal tract [114], tumor [115, 116], cerebellum [117], nerves [118], mammary gland [119], endometrium [91, 120, 121], Placenta [93], cervix [122] 2. 3D bioprinting [123, 124]
Collagen Porous Scaffold		Pros: Biocompatibility; Repeatability; Tissue-inductivity; Good mechanical characteristics Cons: High overall cost; Limited scope of application	1. Tissue injury repair [125, 126] 2. Drug screening or delivery [127] 3. In vitro 3D tissue culture: endometrium [128], mammary gland [119, 129], mouse C3H10 cells [130], liver [131] 4. Disease treatment [132]

Due to the diverse types of materials derived from collagen, it is difficult to uniformly characterize the special characteristics endowed through compounding or specialized processing. Therefore, the table only includes the general characteristics of CBBs used in the biomedical field, while materials with other special purposes are not included

Table 2 Overview of the characteristics and main biomedical applications of nanomaterials

Nanomaterials	Major component	General characteristics	Applications
Collagen nano Composite materials	Collagen can be combined with various nanomaterials such as Metal Nanoparticles, Carbon Nanotubes, Silica Dioxide (SiO2), and Nano-Hydroxyapatite, and may also include additional growth factors, proteins, and polysaccharides	Pros: Biocompatibility; adjustability; repeatability; mimics cellular electrophysiology Cons: Relatively complex fabrication process; potential biotoxicity	1. Drug screening or delivery [134] 2. In vitro 3D tissue culture: skeletal muscle [135], epidermis [78], ligament [136] immune system [76], intestine [137], endometrium [78]

Due to the diverse types of materials derived from collagen, it is difficult to uniformly characterize the special characteristics endowed through compounding or specialized processing. Therefore, the table only includes the general characteristics of CBBs used in the biomedical field, while materials with other special purposes are not included

glycol. Collagen composite hydrogels offer both biocompatibility and enhanced physical characteristics, making them promising materials for organoid nature.

Collagen composite hydrogels serve as a versatile platform not only for applications like in vitro 3D tissue culture, regenerative medicine, targeted drug delivery, and 3D bioprinting, but also present a compelling avenue for broader interdisciplinary research and technological development, owing to collagen's exceptional processability and bio-functional characteristics.

Currently, a key focus in reproductive medicine's in vitro modeling research is the development of endometrial organoids to unravel the complexities of embryo implantation. In 2020, Rawlings and team cultivated organoids in type I collagen hydrogels, revealing insights into endometrial stromal cell-induced epithelial cell differentiation in vitro and providing a practical protocol for embryo implantation studies [91]. Gnecco et al. contributed significantly by creating a synthetic medium using polyethylene glycol and collagen derivatives, offering a versatile matrix for exploring molecular aspects of diseases [92]. This breakthrough is pivotal for advancing clinical investigations into disease mechanisms. Notably, this collagen material has been applied to develop in vitro models simulating the placental barrier. Cao and team achieved a milestone by creating a placental model with human trophoblast stem cells and endothelial cells, validating hormone expression, and associated signaling pathways [93]. This marks a successful initial step in establishing an in vitro placental model, promising advancements in placental physiology and disease connections.

In summary, collagen hydrogels emerge as exceptional materials in Cell-Based Bioengineering (CBBs) for medical research. While their use in constructing in vitro models for reproductive medicine may not be widespread, their numerous advantages for investigating molecular mechanisms in physiology and disease highlight their significant potential for future applications. The characteristics and applications related to collagen composite hydrogels are shown in Table 1.

4.5 Collagen porous scaffolds

Collagen porous scaffolds are porous structural materials composed of collagen proteins, widely used in the field of biomedicine. They mainly utilize the basic biological characteristics of collagen proteins and can achieve specific functions by combining with lactoferrin, certain polysaccharides, or cytokines. These composite scaffolds contain interconnected porous structures that can promote cell adhesion, and have good mechanical strength, structural stability, and tissue induction ability, particularly in osteoinduction. Various methods are available for preparing porous scaffolds, such as emulsion freeze-drying technology, salting-out method, and 3D printing technology. Currently, these porous scaffolds are involved in a wide range of areas and are commonly found in damaged tissue repair, such as bone injury repair [133], as well as temporary scaffolds during cell culture, and prefabricated scaffolds for drug delivery and drug release [127]. In the domain of organoid culture, collagen porous scaffolds have gained extensive recognition for their application in in vitro tissue culture, particularly for tissues like breast, liver, and tumors. These materials have been favored due to their unique material properties. However, their adoption in the realm of organoid cultures related to reproduction hasn't been as widespread as some other materials.

Notably, Abbas and collaborators advanced the field by pioneering a multicellular model, incorporating endometrial stromal and epithelial cells. This innovative approach involved the use of matrix adhesives in combination with porous collagen scaffolds. Their efforts culminated in the successful development of a hormone-responsive lumen-like epithelial layer, marking a significant achievement in the realm of reproduction-related organoid cultures [128].Depending on the selected purity, preparation method, and specific needs, the cost of these scaffolds may vary. In summary, collagen porous scaffolds, with their biological characteristics and flexible preparation techniques, have become an indispensable part of the biomedical field. Overall, the application of collagen porous scaffolds in organoid and especially reproductive medicine-related fields has yet to be expanded, but it is still one of the materials worth considering for in vitro culture. The characteristics and applications related to collagen porous scaffolds are shown in Table 2.

4.6 Matrigel

Matrigel is a tumor extract widely used for in vitro cell culture, also known as Geltrex or Cultrex BME. It was initially isolated by Dr. Swarm from a mouse tumor provided by Dr. Engelbreth-Holm (now referred to as EHS tumor). Later, Elizabeth D. Hay improved in vitro cell culture technology through 3D Matrigel, coupled with its relatively simple manufacturing process [138], making Matrigel one of the common materials for in vitro organoid construction. Matrigel is a complex mixture of basement membrane proteins, containing more than 1800 proteins [139]. It mainly includes laminin, type IV collagen, actin, sulfated acetylheparin glycoprotein, and certain enzymes, chemokines, and growth factors [140]. Among them, laminin, and type IV collagen, as the main proteins of matrix gel, play important roles in cell adhesion, signal transduction, tumor migration, angiogenesis [141], etc. Early studies have found that cells inoculated

in Matrigel rapidly adhere and exhibit significant differentiation and were then applied to in vitro culture of cells such as the retina, breast epithelium, salivary glands, Endometrium [142], and research on extracellular matrix and cell–cell interactions [143]. Currently, due to its relatively simple extraction process and commercialization, it has become the main scaffold for tissue nature. In addition, variants of Matrigel have been continuously developed to meet the special needs of some studies, such as growth factor reduced (GFR) [139], high concentration (HC) [144], phenol red free [145], and hESC-qualified [146] Matrigel commercially produced by Corning.

Matrigel formulations have been widely used for in vitro cultivation of various tissues and organs across species. Initially applied in primate and mammal research in reproductive medicine, it successfully yielded cultivated endometrial tissues from domestic cats [142] and mice [147], as well as testicular tissues from pigs, mice, and macaque [148]. Recent advancements in related technologies have enabled the establishment of effective in vitro models for human-derived reproductive organs, including the endometrium, testes, placenta, ovaries, cervix, and fallopian tubes. This progress provides a robust platform for investigating physiological processes and molecular mechanisms in reproductive system-related diseases.

For instance, Yucer et al. achieved in vitro cultivation of human fallopian tube epithelial cells (FTEC) using Matrigel, demonstrating the expression of markers associated with normal cell differentiation. [149]. This breakthrough serves as a valuable framework for the in vitro cultivation of fallopian tubes and the investigation of related diseases. As another illustrative instance, Abbas and colleagues conducted stiffness measurements on the endometrium, parietal meconium, and the placental-maternal–fetal interface using an in vitro culture model, generating data for mechanistic inquiries and enhancing the fidelity of subsequent organoid models [150]. Alves-Lopes and his team achieved a significant milestone by developing a 3D culture system with a three-layer gradient, effectively cultivating testicular organoids with intricate recombinant structures. This innovative approach marks a breakthrough in enhancing in vitro tissue culture methodologies, showcasing remarkable differentiation and experimental reproducibility with the resulting organoids [151]. In summary, Matrigel is extensively applied in organoid research within reproductive medicine, with ongoing investigations for modeling diseases associated with the reproductive system.

However, regardless of the purpose of Matrigel, it is a tumor-derived natural culture substrate, and its biosafety, scalability, and chemical composition are difficult to determine. Additionally, batch-to-batch variations make its clinical translation very challenging. Therefore, finding a safer, more chemically defined in vitro cell culture medium is one of the main directions for current research and application in the organoid field. The characteristics and applications related to Matrigel are shown in Table 3.

4.7 Decellularized ECM

The extracellular matrix (ECM) is a complex network structure composed of various collagen fibers and non-fibrous components [152], mainly containing collagen proteins, elastin, adhesion molecules, and some cytokines [153], playing an essential role in maintaining general cellular activities and the functional integrity of tissues. dECM scaffolds are natural 3D materials made by using physical, chemical, and enzymatic methods [154] to remove cells from the tissues as much as possible while retaining the integrity of the ECM. In organoid culture, due to the preservation of the complex chemical composition, 3D ultrastructure, and lower immunogenicity of the ECM in biological tissues, dECM has been considered as an ideal alternative to Matrigel. It has already been applied in the in vitro culture of various organs such as the kidney, brain, thymus, and heart, etc. The characteristics and applications related to dECM are shown in Table 3.

One of the essential application areas of organoid culture is regenerative medicine, which includes the repair of damaged tissues. A significant focus in the repair process is the compatibility of the material with the patient's tissues. The primary chemical composition of currently used dECM scaffolds is human and animal-derived collagen proteins. Extensive research has shown that these collagen proteins have only weak immunogenicity in most humans [155, 156]. This is smaller compared with the human rejection reaction caused by whole cell or partially decellularized human implants. In addition, many studies have shown that organoids cultured in dECM have higher maturity compared with traditional Matrigel culture [157]. Research by Kim, S. and others found that more core matrisomes and matrisome-related proteins, especially collagen subtype proteins, were detected in dECM scaffolds compared with Matrigel, where over 96% were glycoproteins [158]. Their results indicate that the culture environment provided by dECM scaffolds is closer to the tissue growth environment within the organism, so it can better reconstruct the natural microenvironment of extracellular culture compared with Matrigel. Alabi et al. also found that cancer cell lines cultured in dECM matrix have a stronger differentiation ability. Furthermore, it was summarized through mass spectrometry analysis that these abundantly expressed proteins in dECM matrix may be related [159]. These

Table 3 Overview of the characteristics and main biomedical applications of natural material

Natural material	Major component	General characteristics	Applications
Matrigel	Laminin, collagen type IV, actin, heparan sulfate proteoglycan, enzymes, chemokines, and growth factors	Pros: Simple extraction; Commercialized; Tissue-inductive; Broad applications Cons: Safety concerns; Low repeatability; Batch variability; Hard-to-control mechanics; Limited omics analysis	In vitro 3D tissue culture: brain [172, 173], heart [174], lung [175], endometrium [142, 176]–[178], placenta [150], fallopian tube [149], cervix [179], liver [180], stomach [181], colon [182], bile duct [183], pancreas [184], tumor [185, 186], hair follicle [187], kidney [188], lacrimal gland [189], dental pulp [190], testis [151, 191]–[193], tongue epithelium [194], inner ear [195], cornea [196], sweat gland [197], mammary gland [198], taste bud [199], prostate [200], epithelial cells [201], blastocyst [202] 2. Tissue repair and regeneration [203, 204] 3. Drug screening or delivery [205] 4. Disease modeling [206] 5. Personalized treatment [207]
dECM	Collagen, Elastin, Fibronectin, Laminin, Matricellular Proteins, Inorganic Salts, and Growth Factors	Pros: Facilitates natural microenvironment reconstruction; Biocompatible; Tissue-inductive; Broad clinical application prospects Cons: Limited material sources; Low repeatability; Safety concerns; Hard-to-control mechanical characteristics	1. In vitro 3D tissue culture: inner cell mass [208], gastrointestinal tract [209], brain [210], kidney [211], mammary gland [212], ovary [213]– [215], fallopian tube [216], tumor [217, 218], thymus [219], lymph node [220], heart [221], liver [222], bile duct [223], salivary gland [224], testis [225, 226], lung [227], pancreas [228], retina [229], endometrium [167, 230, 231] 2. Tissue repair and Regeneration [222, 229] 3. Disease modeling [232] 4. 3D Bioprinting [218]

Due to the diverse types of materials derived from collagen, it is difficult to uniformly characterize the special characteristics endowed through compounding or specialized processing. Therefore, the table only includes the general characteristics of CBBs used in the biomedical field, while materials with other special purposes are not included

two studies also explain the vital role of collagen proteins in extracellular culture. It is worth mentioning that, as the mainstream material for extracellular culture, Matrigel and its derivatives have never been approved by the U.S. Food and Drug Administration (FDA), whereas many dECM biomaterials have been commercialized, obtained FDA approval, and are gradually moving toward clinical applications [160].

Currently, dECM plays a pivotal role in advancing in vitro tissue studies related to reproductive medicine. The year 2012 marked a significant milestone with the first decellularization of bovine and human ovaries [161], initiating the use of dECM in in vitro ovarian culture. Subsequent research has explored the application of human ovary-derived dECM for cultivating organoids to assess early follicular viability [162]. Furthermore, porcine-derived dECM has been investigated as a bioprinting material for correcting premature ovarian failure [163]. These developments highlight the potential of dECM as an ideal platform for fostering follicular development and restoring ovarian function. Additionally, various protocols have been implemented for other reproductive system organoids, such as endometrial repair [164], construction of testicular bioscaffolds [165], and the use of whole uterus decellularized scaffolds [166]. These applications underscore the promise and potential of dECM as a biologically-derived class of CBBs.

However, some research also shows that although the in vitro organs cultured in dECM are structurally closer to natural organs, the organ proliferation efficiency is lower than that of Matrigel [167, 168], which may be caused by the loss of some key proteins during the decellularization process. When laminin was added to the above ECM matrix, it was found that organoids gradually formed, resembling the morphology of Matrigel [167], indicating that laminin helps in the proliferation of organoids. A comprehensive study shows that adhesive ligands, mechanical characteristics, fiber structure, and matrix degradation ability in biomaterials all affect the formation of in vitro cultured organs [169]. It can be seen that scaffolds suitable for organoid growth cannot simply come from dECM. Improving the preparation technology, artificially enhancing the microfiber structure and mechanical characteristics within the scaffold that help in stem cell proliferation and differentiation, will be the possible development direction of this biomaterial.

In practical applications, the use of dECM biological scaffolds is also restricted by the source of the material, as the collagen fiber structure of the obtained ECM scaffolds depends on the biological functional characteristics

of the raw material. That is, the dECM required for culturing organoids should be extracted from organs with the same function. For example, when Wüthrich and his colleagues were developing vascularized nerve scaffolds, they chose pig sciatic nerves as the raw material for the dECM scaffolds to ensure that the biological scaffolds used for treatment matched the nerve structure [170]. In the development of cardiac tissue engineering for organ transplantation, it was also found that the dECM scaffolds from large animals and humans are the best sources for bioartificial ECM [171]. However, it is worth noting that research has found that dECM hydrogels allow long-term storage at low temperatures (− 80 °C for 6 months, 4 °C for 1 month) and long-term passage culture of organoids, and support long-term expansion to produce a large number of organoids [158]. This may alleviate the problem of limited sources of dECM raw materials to some extent.

In summary, dECM has found widespread application in the field of biomedicine despite constraints related to material sourcing and preparation. Its appeal lies in its ability to better emulate the microenvironment of in vivo tissue growth, facilitate higher degrees of differentiation, and extend culture periods. Furthermore, it is gradually replacing Matrigel as a culture medium in organoid-related research and currently stands as the optimal choice, second only to an ideal fully synthetic medium. In the realm of reproductive-related organ construction, including the endometrium, fallopian tube, ovary, testis, and cervix (Table 3), dECM plays a crucial role. These models are frequently employed for unraveling the molecular mechanisms of physiological processes such as embryo implantation, the menstrual cycle, and spermatogenesis. Additionally, they are instrumental in simulating pathological processes like sexually transmitted diseases, mucinous ovarian cancer, infertility, and serve as platforms for personalized drug development and drug delivery in pharmacological research. In vitro modeling of relevant organs will be discussed in the application section.

5 Important advancements in reproductive medicine organoids

The maintenance of a normal endometrial microenvironment is vital for the implantation and development of mammalian embryos. Its physiological function is intricately influenced by hormone levels and the immune system in vivo, as well as by environmental factors and drug use in vitro. In recent years, constructing an in vitro model of the endometrial microenvironment and understanding the molecular mechanism of embryo implantation has proven to be a challenging task in reproductive medicine. While traditional tissue engineering materials have been extensively employed in therapeutic studies related to reproductive system diseases [233], meeting the requirements for in vitro construction of highly biomimetic reproductive system tissues and exploring molecular mechanisms has proven difficult. In contrast, CBBs harnessing the exceptional biological properties of collagen, exhibits unique advantages in constructing intercellular signaling pathways [234], simulating microenvironmental mechanical properties [235], and mediating immune-inflammatory responses [236]. This facilitates researchers in enhancing biosignal transduction ability, tissue differentiation, cellular activity, stem cell migration effects, and the organoid survival cycle of in vitro tissues. Currently, CBBs are making remarkable contributions to molecular biology research in reproductive medicine.

5.1 Endometrium

When a woman reaches reproductive age, her uterine lining undergoes cyclical changes every month. What is known is that these changes are primarily driven by cyclical fluctuations in hormone levels within the female body. However, there are many other unexplored mechanisms that influence the physiological state of the endometrium, thereby affecting female reproductive function. Therefore, to further explore the physiological and pathological mechanisms of the endometrium and to support women's physiological and reproductive health, researchers have initiated primary cultures of endometrial tissues since the 1970s [237]. Since 1988, when Rinehart et al. first cultured endometrial glandular epithelium from primary cells in serum-free Matrigel, there have been considerable advances in human techniques for the construction of endometrial organoids. Currently, there are three different types of endometrial organoids based on various stem cell sources: the most common are derived from endometrial epithelial cells, followed by endometrial stromal cells induced by iPSC, and organoids derived from CD146+ mesenchymal stem cells. Currently, there are three different types of endometrial organoids based on various stem cell sources: the most common are derived from endometrial epithelial cells, followed by endometrial stromal cells induced by iPSC, and organoids derived from CD146+ mesenchymal stem cells. In addition to the cell source, various in vitro culture environments are utilized to create organoids for diverse studies; common matrices include chemically defined media, dECM, porous collagen scaffolds, Matrigel, etc. Endometrial organoids constructed under the different culture conditions mentioned above also have different applications: currently, they are mainly focused on disease modeling, embryo implantation studies, and drug screening.

5.1.1 Embryo implantation

Research indicates that there is still a high incidence of infertility worldwide. Among these, the 12-month prevalence is 3.5% to 16.7% in more developed countries, and 6.9–9.3% in less developed countries [238]. It is known that pregnancy is closely related to the interaction between the endometrium and the blastocyst. During the secretory phase of each menstrual cycle, i.e., 6–8 days after ovulation, the receptivity of the endometrium increases, allowing the embryo to implant through alignment, adhesion, and invasion processes, a period also known as the "implantation window." During this time, abnormalities in molecules related to implantation, such as prostaglandins, integrins, immunoglobulins, and the failure to establish immune tolerance, will all lead to infertility [239]. Depending on different etiologies, drug therapy, surgical treatment, and in vitro fertilization can be used to mitigate the societal impact of human infertility. However, we still need to explore the factors causing human infertility from the root. Yet, about 15% of infertility patients with unexplored pathogenesis still exist globally [240]. In vitro models are considered to simulate to a great extent the physiological processes of organs or tissues in vivo and handle ethical issues in tissue engineering well, thus playing an important role in exploring the molecular mechanisms of embryo implantation. However, due to the limitations of human in vitro blastocyst technology, there are almost no independent human organ-like systems specifically for studying the blastocyst implantation process. In vitro studies on blastocyst implantation into the endometrium are mainly limited to animal organ-like systems (Fig. 3) and human blastocyst and endometrial organoid systems with ethical issues [241]. Encouragingly, the long-term in vitro culture model for the endometrium has been established, and with the key breakthroughs in human in vitro blastocyst technology in 2021, more and more reliable in vitro blastocyst culture techniques will be developed.

5.1.2 Disease modeling

There are many types of human endometrial-related diseases with complex pathogenesis, including endometriosis, endometrial cancer, adenomyosis, and endometrial hyperplasia. In recent years, due to a lack of

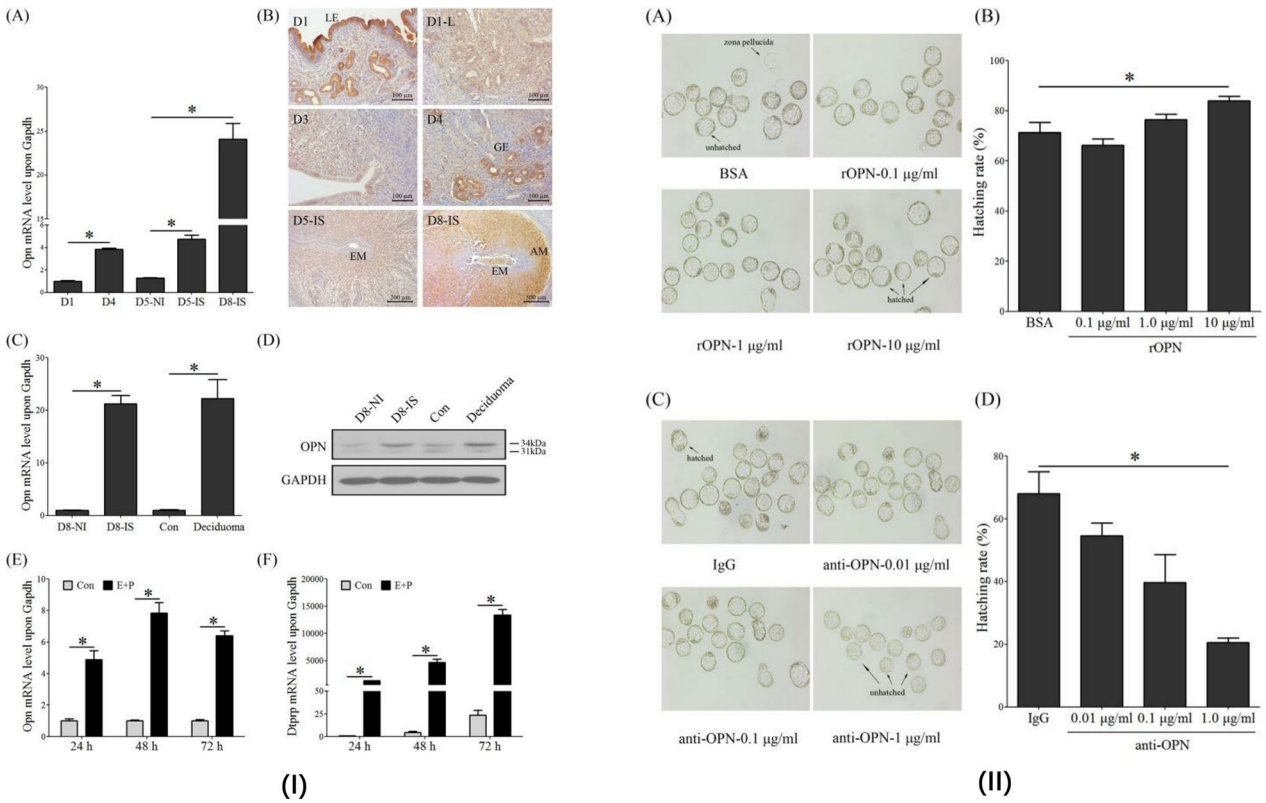


Fig. 3 Research on the process of blastocyst implantation through in vitro embryo culture in mice. Reproduced permission from [242]. The elevated levels of the secreted protein OPN promote in vitro blastocyst hatching and adhesion. (I) Early pregnancy expression of OPN in the mouse uterus; (II) Uterine-localized OPN protein facilitates blastocyst hatching: The adhesion rate of blastocysts in rOPN protein culture group (70.3%) was higher than in the control group (52.4%), whereas anti-OPN antibodies could neutralize the effects of rOPN on blastocyst adhesion (43.6%)

understanding of the disease mechanisms, there has been no significant improvement in survival rates and treatment efficiency for endometrial-related diseases. The key to solving this problem is the construction of reliable preclinical models [243]. Over the past decade, endometrial organoids from various sources have been widely used in clinical disease modeling. In the study of pathogen pathogenesis, Bishop et al. used mouse endometrium to develop animal endometrial organoids for the study of the developmental cycle of Chlamydia trachomatis in the endometrium and its interaction mechanisms with endometrial cells [244]. In human disease research, Turco et al. successfully developed endometrial organoids with genetic stability from non-pregnant human endometrium using Matrigel and certain cell factors and hormone blockers as nutritional substrates. In the ovarian hormone stimulation experiments of this model, they demonstrated its good hormone responsiveness and potential value in early pregnancy and postmenopausal research [178]. Wiwatpanit and his colleagues successfully established a new endometrial organoid system containing epithelial cells and matrix cells, without a scaffold (agarose 3D culture plate), for the study of hormone abnormalities related to polycystic ovary syndrome [22]. Up to now, a common understanding is that 3D preclinical models derived from patients are considered the best way to construct tumor microenvironments due to advantages such as retaining overall tumor structure, capturing tumor heterogeneity, and including patient-specific growth factors [245]. Researchers from the United States used Matrigel to develop an organoid system derived from endometrial cancer patients. Moreover, they established organoids from multiple ethnicities and different endometrial cancer subtypes, aiming to create an organoid bank that can respond to complex clinical conditions, to advance organoid research from the major factors influencing cancer clinical outcomes and prognosis [246]. This may provide insights for the clinical application of cancer organoid models. In addition to applications in clinical model construction and disease mechanism research, patient-derived tumor organoids are also widely used in the screening and development of chemotherapy and hormone drugs.

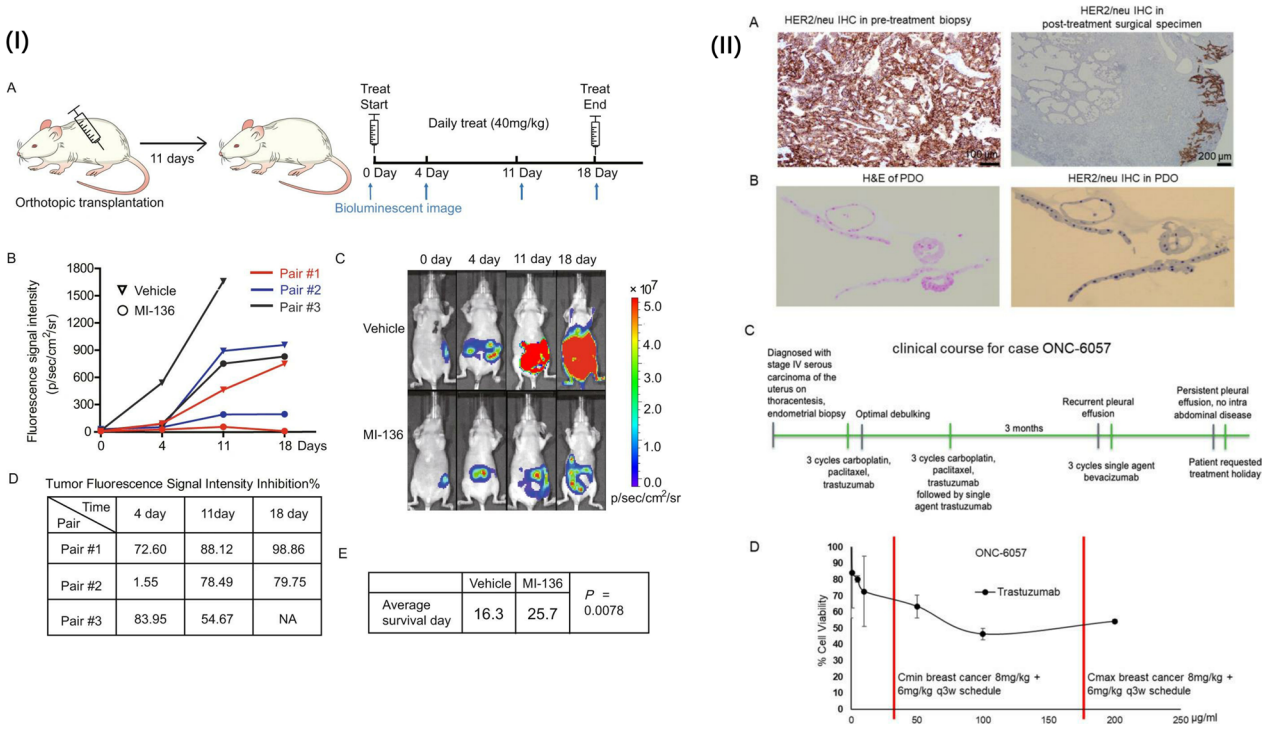
5.1.3 Drug screening

Drug screening is a common application across various types of organoids. Drug screening concerning endometrial organoids is mainly used for the prevention and treatment of common endometrial diseases, and it is also an essential means to change the treatment efficiency and survival rates of malignant endometrial diseases. Due to the differences between individual clinical patients, drug screening generally selects animal-cultured or individualized organoids derived from different patients. Chen et al. developed an organoid missing Trp53, Pten, and Pik3r1 genes by cultivating mouse endometrial tumors on Matrigel. They initially screened three targeted drugs that inhibit tumor growth and finally identified MI-136 ((Histone methyltransferases inhibitors) as a potential inhibitor of endometrial cancer, revealing the menin-HIF (hypoxia-inducible factor pathway) axis as the molecular mechanism of endometrial cancer (Fig. 4 I). Bi et al. studied the construction of endometrial cancer and ovarian cancer organoids in Matrigel. Their model successfully predicted the drug resistance produced by tumors when patients were re-exposed to platinum compounds and trastuzumab. Their research indicated the significant role of organoids in drug sensitivity experiments in cancer treatment (Fig. 4 II).

It's worth noting that, due to the potential heterogeneity of each patient's tumor in the clinic, the use of common first-line anticancer drugs may lead to missing the best treatment window. Relying on organoids to establish personalized treatment plans may be the key to unlocking personalized cancer medication. However, it should be noted that most of the materials used to construct in vitro models currently are Matrigel, and the variations between batches and the potential pathogens carried by this substrate may limit the clinical application of organoids. Therefore, the development of chemically defined culture mediums, recombinant collagen-based biological culture mediums, and improved dECM culture mediums will have enormous potential in advancing the clinical application of organoids.

5.2 Blastocyst in vitro

In recent years, with the continuous development of tissue engineering, ethical and technical issues related to blastocyst research have found ideal solutions. The successful construction of in vitro blastocysts not only helps people understand the mechanisms of early embryo development on a deeper level but also assists researchers in improving embryo in vitro cultivation and implantation techniques to help families with reproductive issues regain the opportunity for childbirth. Assisted Reproductive Technology (ART), an important technique for dealing with reproductive issues, especially infertility, has made the dream of childbirth possible for many who cannot conceive naturally. Related techniques, such as In Vitro Fertilization (IVF), Intracytoplasmic Sperm Injection (ICSI), and In Vitro Culture of Embryos (IVC) (mainly to the blastocyst stage), are widely used in developed countries for the treatment of human infertility. As one of the keys to ART, in vitro culture of blastocysts has always been a focus of attention. Animal studies have shown that improper in vitro culture conditions can lead



Pair \ Time	4 day	11day	18 day
Pair #1	72.60	88.12	98.86
Pair #2	1.55	78.49	79.75
Pair #3	83.95	54.67	NA

	Vehicle	MI-136	
Average survival day	16.3	25.7	*P* = 0.0078

Fig. 4 Application of Endometrial Organoids in Drug Screening. (I) MI-136 repressed in vivo tumor growth in an orthotopic endometrial cancer model [247]. Experimental workflow demonstrates MI-136's impact on in situ endometrial cancer in mice. Bioluminescence imaging reveals reduced tumor growth and signal intensity in MI-136 treated mice compared with controls. This treatment also extends average survival duration in the endometrial cancer mouse model. (II) The endometrial cancer organoid model successfully predicts patient resistance to platinum-based drugs and trastuzumab. Reproduced permission from [248]. **A** Positive immunostaining for HER2 in pre-treatment and negative HER2 immunostaining in post-treatment; **B** H&E staining and HER2 IHC for corresponding organ models of endometrial cancer; **C** Timeline of the patient's clinical course; **D** Cell viability profiles of organoid cells from endometrial cancer treated with increasing concentrations of trastuzumab for 72 h

to persistent changes in the embryo's epigenome, resulting in increased susceptibility to diseases [249]. Therefore, finding biomaterials that can simulate oocytes and in vivo blastocyst culture conditions might be a solution to problems with in vitro culture conditions. Furthermore, in vitro models can effectively control and screen biomolecules for tissue culture or embryo implantation, such as thyroid hormones and leptin, which might help customize biomaterials that can be used for in vitro culture [250].

Throughout history, the construction of an in vitro model of human blastocysts has been a challenge in tissue engineering due to ethical and other restrictive factors. Encouragingly, two studies from Nature have made key breakthroughs in this area. In 2021, research by Liu and others reprogrammed human dermal fibroblasts into human iPSCs and placed them in conditions such as human extracellular matrix 1/2, iBlastoid base medium (a mixed medium), collagen IV plates, successfully cultivating iBlastoids that can summarize human blastocysts [251]. This discovery has three distinct advantages: firstly, the reprogrammed cells can be widely accepted because they do not destroy human embryos; secondly, iBlastoids are similar to human blastocysts in transcription, typical structure, and certain key functions; thirdly, the lack of typical features of blastocysts under normal physiological conditions, such as the zona pellucida, may resolve ethical issues. These advantages have tremendous potential in exploring infertility and mechanisms of early pregnancy loss.

Similarly, in 2021, Yu et.al developed an effective 3D culture strategy for in vitro blastocysts—using the WIBR3 human embryonic stem cell line (ES) in human trophoblast stem cell medium (TSM) with a mixed medium of 5i/L/A (containing five kinase inhibitors and two growth factors), they cultivated blastocyst-like structures [252]. Although there may be limitations due to factors such as human PSC culture conditions, these human blastocyst-like structures obtained through sequential lineage differentiation and self-organization will still have significant potential value in the fields of embryogenesis, early pregnancy loss, contraceptive drugs, and others. These studies have proposed the first in vitro model of human blastocysts and have opened possibilities for

exploring the causes of pregnancy failure, the success rate of embryo implantation, and other aspects. With the continuous improvement of material science technology, widely used and high-performance biomaterials such as collagen-based biocomposites will gradually be applied to the improvement of PSC culture and in vitro culture environment. Although research on collagen protein and in vitro blastocyst culture is still lacking, we can still foresee the tremendous potential of CBBs in the construction of human in vitro blastocysts.

6 Discussion

Collagen, as a natural biomaterial, possesses excellent biocompatibility and biodegradability. Consequently, it holds crucial applications across various medical domains. In the realm of reproductive medicine, collagen plays a significant role in simulating extracellular matrix, cellular adhesion, proliferation, and differentiation within the in vitro uterine environment. The discussion within this paper regarding in vitro blastocyst culture and the study of endometrial organoid contributes to a profound comprehension of intricate human reproductive and early developmental processes. This holds paramount significance in terms of serving as disease models, enhancing fertility rates, and alleviating the challenges posed by an aging population.

While there is a substantial body of literature confirming the feasibility of CBBs in the applications of uterine-like organs and in vitro blastocysts, challenges persist in the clinical implementation of reproductive practices.

Firstly, although collagen has good biocompatibility, the process of producing CBBs may lead to changes in the protein's spatial structure and other characteristics, thereby potentially reducing its biocompatibility. For instance, chemical cross-linking agents in chemically cross-linked composite materials can alter the spatial structure of collagen, leading to immune responses in the body. Therefore, cell-based scaffold-free 3D cultivation might be a more suitable approach for organ-like structures in the future. In scaffold-free 3D cultivation, collagen, serving as an intercellular binder, can help avoid decreased biocompatibility caused by alterations in spatial structure.

Secondly, the issue of collagen-based degradation rate remains challenging to achieve precise control. Degradation rate refers to the speed at which a biomaterial breaks down and disappears within the body. This process is intricate and may involve mechanisms such as hydrolysis, enzymatic degradation, and biodegradation. The degradation rate is closely tied to the behavior of the material within the body and has a significant impact on therapeutic outcomes. The ideal degradation rate should align with the pace of natural tissue repair and regeneration. If the material degrades too quickly, it might lose its supportive function before the tissue has fully healed. On the other hand, if degradation is too slow, it could impede the growth of new tissue or lead to chronic inflammation.

Furthermore, excessively rapid degradation could release a large amount of degradation byproducts, causing local or systemic toxic reactions that interfere with treatment outcomes. Conversely, overly slow degradation could result in the prolonged presence of foreign substances, potentially triggering immune reactions or other adverse effects. Current solutions include incorporating molecules with potent antioxidant characteristics into biomaterials [253], as well as employing chemical cross-linking agents like PEG-diester-dithiol [254].

Thirdly, medical interventions related to the reproductive system may involve a multitude of sensitive ethical and societal issues. In the context of embryo implantation research, the Human Fertilization and Embryology Act of 1990 stipulates that it is unlawful to maintain a viable human embryo in vitro beyond 14 days of development or after the emergence of a primitive streak, whichever occurs earlier [255]. This regulation restricts the widespread clinical application of in vitro blastocyst culture techniques, currently allowing only limited utilization, primarily within animal studies.

Fourthly, beyond the lab-scale achievements in the development of CBBs, the translation of these advancements into clinically viable solutions entails a complex interplay of technological, economic, and regulatory challenges. These multifaceted barriers span issues ranging from stringent quality control and the preservation of biological activity to cost-effectiveness, technological scalability, regulatory hurdles, sustainable manufacturing, and accounting for inter-individual biological variations. While some exploratory research, such as the utilization of recombinant collagen for quality control, offers promising avenues—particularly in enabling the synthesis and purification of rare collagen isoforms [256]—the limitations of elevated production costs and suboptimal yields must be squarely addressed.

In addressing the above issues, insights could potentially be drawn from the tanning chemistry employed in the leather industry, offering potential avenues for improvement.

In fact, the tanning stage in leather production serves as a pivotal juncture where raw or animal hides are transformed into leather, principally through the stabilization of collagen proteins. This stabilization equips collagen with the resilience to withstand a variety of adverse conditions, such as thermal degradation and enzymatic activity. Tanning chemistry thus stands as the core technology facilitating this critical transformation and is commonly employed in the production of

leather and similar materials [257]. In the production of CBBs, chemical cross-linking agents used in tanning can interact with collagen to increase cross-linking density, thereby enhancing the stability and mechanical strength of the biomaterial. Chemical cross-linking agents such as glutaraldehyde, epoxy compounds, and genipin have been utilized to improve the thermal stability of collagen scaffolds. However, they possess inherent drawbacks including potential carcinogenicity, cellular toxicity, high costs, and limited functionalities. Consequently, there is a need to develop greener and non-toxic chemical cross-linking agents [258].

Current research has introduced a novel hydrophilic, biocompatible, and multifunctional carboxylic acid polymer (MCP) with multiple reaction sites, offering promise for stabilizing collagen throughout various stages of leather processing. MCP exhibits excellent water solubility and its hydroxyl groups, which are easy to modify, make aqueous synthesis straightforward and effective. Furthermore, the synthesized polymer is devoid of toxic cross-linking agents and monomers [259]. This innovation provides new avenues for the clinical application of CBBs.

Although challenges persist in the clinical application of CBBs, the prospects in this field remain remarkably expansive. For instance, in tissue engineering and regenerative medicine, collagen's biocompatibility and biodegradability contribute to its widespread use. In the future, the integration of advanced technologies such as stem cell techniques and 3D printing holds the potential for achieving more precise and personalized tissue and organ regeneration and repair.

In the context of trauma treatment and surgical applications, collagen-based materials can serve as scaffolds, fillers, or sutures, accelerating wound healing and tissue regeneration. In the realm of drug delivery, collagen can function as a specific drug delivery carrier, enabling precise control over drug release. This can enhance drug efficacy, reduce side effects, and open new avenues for treating challenging diseases.

Regarding reproductive medicine, collagen materials could improve the success rates of assisted reproductive techniques like IVF. For instance, collagen-coated culture dishes may more effectively simulate the natural environment, facilitating embryo cultivation and transplantation. Additionally, CBBs can be used to repair damaged reproductive tract tissues, such as ovaries, fallopian tubes, and endometrium. This could hold significant value in addressing fertility disorders and reproductive system diseases.

The biomimetic characteristics of collagen can also be utilized to create disease models for medical education and surgical training, providing reproductive medicine specialists with more authentic simulation experiences.

7 Conclusion

In this review, we have provided a comprehensive overview of collagen, including its sources, basic characteristics, composite biomaterials, and applications. We have focused on biomaterials such as Matrigel, dECM, and bio-inks, highlighting their significance in various applications. Specifically, we have extensively reviewed applications in endometrial organoids and in vitro blastocyst culture. We also discussed the challenges that exist in integrating CBBs into reproductive medicine. Finally, we explored the insights that challenges in the leather industry could offer to this field. This review aims to offer an integrated understanding and intriguing perspectives for the development of CBBs in reproductive medicine, particularly in the areas of endometrial organoids and in vitro blastocyst culture.

Abbreviations

CBB	Collagen-based biomaterial
3D	Three-dimensional
FDA	Food and drug administration
ASC	Adult stem cells
PSC	Pluripotent stem cells
ECS	Embryonic stem cell
CSC	Cancer stem cell
FACIT	Fibril Associated Collagens with Interrupted Triple helices
ALI	Air–liquid interface
dECM	Decellularized extracellular matrix
Gly	Glycine
Pro	Proline
ECM	Extracellular matrix
PEG	Polyethylene glycol
EDC/NHS	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide/Hydroxysuccinimide
EHS	Engelbreth-Holm-Swarm
GFR	Growth factor reduced
PBS	Phosphate buffered saline
CAD	Computer-aided design
RGD	Arginine-glycine-aspartate
AV-Alg	Aloe vera-sodium alginate
ART	Assisted Reproductive Technology
IVF	In vitro fertilization
ICSI	Intracytoplasmic sperm injection
IVC	In vitro culture of embryos
TSM	Trophoblast stem cell medium
rOPN	Recombinant OPN protein
IHC	Immunohistochemistry
HER2	Human epidermal growth factor receptor 2
MCP	Carboxylic acid polymer

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Author contributions
Conceptualization, BF and HY; investigation of available articles, MZ and JL; writing—original draft preparation, BF, HY and MZ; writing—review and editing, BF and HY; figure and table production, BF; project administration, supervision JG, HC, PCL; funding acquisition, supervision, YZ. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The datasets analyzed in this review are cited and listed in the 'References' section of the article. We have obtained permission from the copyright holders to reproduce charts previously published elsewhere.

Declarations

Competing interests
The authors declare no competing financial interests in this work.

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