

Biomaterial-based scaffolds for ovarian tissue engineering: From extracellular matrix mimicry to artificial ovary construction for fertility preservation and endocrine restoration

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ABSTRACT

Two major causes of ovarian function and fertility loss are currently premature ovarian insufficiency (POI) and gonadotoxic cancer therapies. Tissue-engineered constructs have played an important role in this field by improving the ovarian microenvironment, supporting follicular survival, increasing vascular integrity, and improving endocrine function. This review critically compares biomaterial and biofabrication strategies in ovarian tissue engineering, emphasizing the balance between mechanical support and biological activity and the barriers to translating these technologies into clinical applications. A comparative and narrative review was conducted using PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, and related journal websites. Studies related to ovarian extracellular matrix, natural and synthetic biomaterials, decellularized matrix, composite scaffolds, advanced fabrication methods, angiogenesis, immunocompatibility, and artificial ovaries were reviewed. No preliminary experimental data were generated. Natural hydrogels have favorable bioactivity but limited mechanical tunability, while synthetic polymers offer more controllable, reproducible mechanical properties but require bioactive modifications. The decellularized matrix retains some natural biochemical signatures but suffers from variability and mechanical limitations. Composite scaffolds, 3D bioprinting, and electrospinning improve architectural control. Artificial ovary systems have largely supported follicular survival and endocrine function in preclinical models, but angiogenesis, immunocompatibility, scalability, cost, safety, and human translation remain challenging. No single material meets all requirements, and the future of ovarian tissue engineering depends on intelligently combining complementary biomaterials to achieve bioactivity, robustness, safety, and manufacturability.

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Introduction

Premature ovarian insufficiency (POI) is defined by the loss of ovarian activity, manifesting as menstrual disturbance together with biochemically confirmed hypoestrogenism and elevated gonadotropins, before the age of 40 years (1). Pooled estimates place its prevalence at roughly 3.5-3.7% of women, somewhat higher than earlier reports suggested, and this continues to be revised upward as ascertainment improves (2, 3). Its etiology is heterogeneous, spanning genetic and chromosomal abnormalities, autoimmune oophoritis, infectious and iatrogenic causes, and a substantial idiopathic fraction; this heterogeneity complicates the search for a single curative strategy (4). A largely overlapping population of women loses ovarian tissue not to idiopathic or autoimmune POI but to the gonadotoxic effects of chemotherapy, pelvic radiotherapy, or oophorectomy performed for malignant or benign indications. Because the ovarian follicle pool is fixed at birth and cannot be regenerated once depleted, both

routes converge on the same clinical problem: an ovary that can no longer support folliculogenesis, steroidogenesis, or, in younger patients, normal pubertal onset (5). Hormone replacement therapy (HRT) remains the mainstay of symptomatic management. Still, it restores neither fertility nor the pulsatile, cyclical hormone secretion of a native ovary, and it carries its own long-term risks, so it is properly regarded as palliative rather than curative (1).

Over roughly two decades, ovarian tissue cryopreservation and subsequent autotransplantation have evolved from an experimental procedure into an established fertility-preservation option, with more than 200 live births reported worldwide and documented restoration of endocrine function in most recipients (6-9). Autotransplantation of cryopreserved cortical strips nonetheless has two intrinsic limitations that no amount of surgical refinement has resolved. First, the graft must be revascularized from scratch after transplantation, during which a substantial fraction of the primordial follicle pool

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is lost to ischemia before new vessels form. Second, for patients with malignancies that carry a meaningful risk of ovarian involvement, leukemias in particular, reimplanting tissue that may harbor occult tumor cells is not considered safe (10, 11). Contamination rates as high as 8-24% have been reported for ovarian tissue harvested from leukemia patients depending on the detection method used, and the presence of even a small number of malignant cells is sufficient to cause disease relapse after reimplantation (12). These two constraints, ischemic follicle loss and the risk of tumor cell reintroduction, are the principal motivations behind the tissue-engineering strategies that are the subject of this review.

The organizing idea common to nearly all of these strategies is that the ovarian extracellular matrix (ECM) is not a passive scaffold but an active, dynamically remodeling determinant of follicle fate, a point developed in full below. Two broad translational goals have shaped the field that builds on this idea. The first is fertility preservation through what has come to be called the "artificial ovary": isolated preantral or antral follicles, together with supporting ovarian stromal or granulosa cells, are encapsulated within a biomaterial carrier and transplanted, avoiding the reintroduction of a large mass of potentially malignant tissue while still providing follicles with a three-dimensional, matrix-like environment (13, 14). The second is endocrine restoration, in which the goal is not necessarily ovulation or pregnancy but the resumption of physiological, cyclical sex-hormone secretion sufficient to support puberty, bone density, cardiovascular health, and quality of life in women who no longer require fertility but do require estrogen and progesterone exposure that is difficult to replicate with exogenous hormone therapy alone (15). Bioprosthetic and bioengineered ovary constructs that combine three-dimensionally printed or electrospun scaffolds with follicles have now been shown, in mouse models, to support both natural mating with live, nursing offspring and multi-month restoration of estrous cyclicity, and a 2026 report has extended immunoisolated human ovarian xenografts to sustained hormone cycling in mice for the first time (16-18).

A comprehensive 2021 review specifically dedicated to biomaterials for ovarian tissue engineering laid much of the conceptual groundwork this article builds upon, organizing the field around natural, synthetic, and composite scaffold classes and highlighting decellularization and follicle encapsulation as the two application areas with the clearest translational trajectory (19). The present review differs from that earlier synthesis, and from the several narrower reviews published since on individual materials or techniques, in three respects: it extends coverage to fabrication techniques and preclinical outcomes reported in the years since 2021, including the first sustained hormone cycling achieved with an immunoisolated human ovarian xenograft (17); it organizes the comparative evidence explicitly around the mechanical-bioactivity trade-off introduced above, rather than presenting materials classes as a sequential list; and it extends the discussion beyond materials science to the vascularization, immune-modulation, manufacturing, and economic-feasibility questions that will ultimately determine whether any of these constructs reaches routine clinical use. The review first summarizes the composition and stage-dependent remodeling of the native ovarian ECM. It then works through natural, synthetic,

and composite or hybrid biomaterials in a comparative framework and covers decellularized ECM scaffolds and the advanced fabrication techniques, chiefly three-dimensional bioprinting and electrospinning, that shape these materials with follicle-scale precision. It next addresses artificial ovary construction and follicle encapsulation as an application that draws on all preceding material classes. The subsequent parts review preclinical and translational outcomes, the unresolved challenges that currently separate this field from routine clinical use, vascularization, mechanical tuning, immune compatibility and its modulation, scale-up from rodent to human dimensions, outcome standardization, and regulatory and economic feasibility, and a critical discussion of what the assembled evidence does and does not show. The review concludes with priorities for the next phase of research.

Search strategy and scope of this review

This article is a narrative comparative synthesis rather than a systematic review or a report of new primary data; no new experiments, animal studies, or human participants are involved, and every quantitative or comparative claim made below is attributed to a specific primary source. The literature search was conducted in PubMed/MEDLINE, Scopus, and Web of Science, supplemented by targeted searches of Google Scholar and journal websites for the most recent (2024-2026) publications, using combinations of the search terms "ovarian tissue engineering," "artificial ovary," "biomaterial scaffold," "extracellular matrix," "decellularization," "follicle encapsulation," "fertility preservation," and "premature ovarian insufficiency," together with the name of each biomaterial or fabrication technique discussed in this review. Reference lists of retrieved reviews, including the 2021 review that anchors this field (19), were hand-searched for additional primary studies. Records were included if they reported original preclinical or clinical data, or synthesized such data, on a biomaterial scaffold applied specifically to ovarian follicle, tissue, or whole-organ engineering; non-English records, conference abstracts without a linked peer-reviewed manuscript, and studies in which the ovary was not the tissue of interest were excluded unless cited for general tissue-engineering context (for example, immunomodulatory biomaterial design or scaffold-manufacturing economics, where the ovary-specific literature is still too sparse to stand alone). Because this is a narrative rather than a systematic synthesis, no formal risk-of-bias tool was applied; instead, the later parts on preclinical outcomes, challenges, and discussion explicitly flag where conclusions rest on a single small study, an animal model with uncertain relevance to human ovarian scale, or a level of evidence lower than a matched preclinical comparison, so that readers can weigh the strength of the underlying data for themselves.

Ovarian extracellular matrix structure and rationale for biomimicry

Composition and stage-dependent remodeling of the native ovarian ECM

The ovarian ECM is not a single, uniform material but a mosaic of compartment-specific matrices that differ between the follicular basal lamina, the theca and stromal compartments, and the cortex versus medulla (20). Rodgers and colleagues, in a series of studies that remain

the reference point for the field, showed that the follicular basal lamina of bovine follicles doubles in surface area some nineteen times over the course of follicular growth and, in doing so, systematically changes its molecular composition: primordial and primary follicles are surrounded by a basal lamina containing all six alpha chains of collagen type IV, whereas growing follicles retain principally the alpha-1 and alpha-2 chains, with perlecan and nidogen-1 appearing as new components and the laminin alpha-1, beta-2, and gamma-1 chains increasing in abundance (10, 11). This remodeling is not a passive byproduct of growth; the matrix diverges compositionally from region to region, and follicle and stromal cells behave differently depending on the local matrix they encounter, which is why a bioengineered scaffold presenting a single, invariant matrix formulation is, at best, a coarse approximation of the follicular niche.

Comparable stage-dependent patterns have been documented in the mouse and in the ewe (21). Using immunohistochemistry and quantitative PCR, Grosbois and colleagues, together with Félix and Silva, found that type I collagen is distributed broadly across the ovarian stroma and follicular compartments, while type IV collagen is concentrated in the theca layer with comparatively little expression in granulosa cells, and that laminin forms a consistent ring-like structure immediately surrounding the granulosa cell layer across all follicle stages (21, 22). A related study of mouse ovaries collected across the periovulatory window showed that nidogens 1 and 2 and perlecan display fibrous expression patterns within the membrana granulosa, while a related aggregate structure termed focimatrix appears specifically in preantral and antral follicles (23). Beyond the structural collagens and laminins, proteoglycans including versican, decorin, and various chondroitin sulfate species are present in the follicular matrix. Hyaluronic acid becomes especially prominent in the follicular fluid of antral follicles, where it contributes to cumulus expansion and to the biomechanical environment that the oocyte and its surrounding cumulus cells experience just before ovulation (21).

The functional importance of this matrix diversity is best illustrated by what happens when it is perturbed. Extracellular matrix signaling participates in essentially every stage-transition decision a follicle makes: exiting primordial dormancy, initiating granulosa cell proliferation, forming the antrum, and eventually choosing between ovulation and atresia; matrix stiffness itself has also been shown to regulate follicle behavior independent of its biochemical composition (24). Whole-ovary imaging using magnetic resonance elastography has confirmed that ovarian tissue rigidity changes across the reproductive lifespan and correlates with the density of the follicular reserve, giving a non-invasive readout of the biomechanical state that engineered scaffolds are attempting to replicate; the reported storage modulus of native human ovarian cortex is on the order of a few kilopascals, a benchmark used repeatedly in Sections 3 through 9 below (25). This body of work provides the rationale, restated throughout this review, for treating scaffold stiffness as a design variable comparable in importance to scaffold chemistry, and for why the two are difficult to optimize independently.

From matrix biology to biomimetic design principles, and where each strategy falls short

Given this compartmentalized, dynamically remodeling

native matrix, four broad biomimetic strategies have emerged, each representing a different wager about which feature of the native ECM matters most to reproduce (26). The first reconstitutes individual purified ECM components, including collagen, hyaluronic acid, and laminin, into a simplified hydrogel that captures one or two features of the native matrix, such as fibrillar architecture, viscoelasticity, or a specific integrin-binding motif, without attempting to reproduce its full compositional complexity; this strategy bets that bulk mechanical and adhesive cues matter more than exact biochemical fidelity (27-29). The second decellularizes intact ovarian tissue, removing cellular and nuclear material while retaining the native three-dimensional architecture and much of its biochemical signature, on the premise that no synthetic reconstruction can currently match the fidelity of the tissue's own matrix; this strategy bets on biochemical authenticity at the cost of manufacturing reproducibility (30). The third abandons compositional biomimicry altogether in favor of synthetic polymers engineered for reproducible, tunable mechanical properties, sacrificing native bioactivity for manufacturing consistency and regulatory tractability (31). The fourth, increasingly the dominant approach, is hybridization: combining a synthetic or decellularized scaffold's mechanical backbone with a natural or bioactive coating that supplies the adhesion and signaling cues the backbone lacks, an explicit attempt to buy back some of what each pure strategy sacrifices (32).

None of these four approaches fully reproduces the compartmentalized, dynamically remodeling matrix described above, and this gap, rather than any single materials failure, is the recurring theme of the sections that follow. Chiti and colleagues, working with hydrogels derived from decellularized human ovarian ECM, found that even tissue-derived matrix required alginate supplementation to achieve mechanical properties sufficient to support follicle survival *in vivo*, since the native storage modulus of ovarian cortex proved difficult to recapitulate from decellularized material alone without additional reinforcement (33). This single observation captures the central engineering tension addressed throughout this review more directly than any broader materials comparison could: biochemical fidelity and mechanical adequacy are not automatically compatible, and essentially every scaffold design described below represents a negotiated compromise between the two, whichever of the four strategies above it starts from.

Natural biomaterials for ovarian scaffold construction

Natural biomaterials were, almost by definition, the first place the field looked for ovarian scaffold materials, since they are derived from ECM components or ECM-adjacent biopolymers and therefore already present at least some of the adhesion motifs and degradation pathways that ovarian cells recognize (34, 35). Recent syntheses of the broader regenerative-medicine literature for POI similarly emphasize that biomaterial scaffolds and stem-cell-based approaches are increasingly viewed as complementary rather than competing strategies, with scaffolds providing structural and biomechanical support that cell-based therapies alone cannot supply (36). Their principal advantages, biocompatibility, biodegradability by native enzymes, and in most cases a demonstrated safety record in other tissue-engineering contexts, are counterbalanced by batch-to-batch variability, comparatively weak and difficult-

to-tune mechanical properties, and, for animal-derived materials, a theoretical risk of immunogenicity or pathogen transmission. This section reviews the natural biomaterials most extensively investigated for ovarian follicle and tissue engineering in a comparative rather than sequential framework; Table 1 summarizes their properties, including approximate mechanical and degradation data where the primary literature reports them.

Collagen

Collagen is the most abundant ECM protein in the body, accounting for roughly a third of total protein mass, and types I, III, and IV are the dominant collagen species within the ovary itself, where they help maintain primordial follicle dormancy and support the structural transitions of follicular growth (9). Its twenty-eight family members share a triple-helical architecture that confers good biocompatibility, low antigenicity in purified form, and the capacity to be chemically cross-linked to tune degradation and mechanical properties (9, 47). For ovarian applications specifically, collagen has most often been used not as a standalone scaffold but as a cell-adhesive substrate for stem-cell or follicle delivery, a role already documented in the context of mesenchymal stem cell-based POI therapy, where collagen scaffolds seeded with umbilical cord-derived mesenchymal stem cells improved follicle activation through FOXO3a and FOXO1 phosphorylation and, in a small clinical series, restored ovarian function sufficiently for two patients to achieve pregnancy (9). For the artificial ovary application discussed later in this review, collagen hydrogels have similarly been evaluated as a follicle-encapsulation matrix. A direct comparison against Wharton's jelly-derived hydrogel in human follicle xenotransplantation found that

both natural matrices supported comparable short-term follicle survival, underscoring that collagen's principal contribution is as an adhesive, remodelable substrate rather than a mechanically load-bearing scaffold in its own right (48).

Alginate

Alginate, a linear polysaccharide extracted from brown algae, occupies a somewhat paradoxical position in ovarian tissue engineering: it is simultaneously the single most extensively validated biomaterial for *in vitro* follicle culture and one of the most consistently criticized for its biological inertness *in vivo*. The Woodruff and Shea laboratories established, through a long and internally consistent series of studies beginning in the mid-2000s, that alginate hydrogels formed by ionic gelation with calcium can support follicle growth from the secondary stage through oocyte maturation while preserving the three-dimensional architecture, cell-cell contacts, and paracrine signaling that a follicle requires to develop normally (37, 49, 50). A critical early finding was that follicle behavior in alginate depends strongly on both alginate concentration and molecular weight: lowering the solids concentration and using a lower molar mass polymer reduces matrix stiffness and, counterintuitively, improves follicle growth, antral cavity formation, theca cell differentiation, and hormone output relative to stiffer formulations (38), a principle summarized alongside other follicle-microenvironment engineering considerations in a widely cited review from the same research collaboration (51). Standardized methodological protocols for follicle isolation and encapsulation within these degradable three-dimensional systems have since been published to improve reproducibility across laboratories (52). This

Table 1. Comparative overview of natural biomaterials used in ovarian tissue engineering

Material	Source	Approx. mechanical/degradation data	Key advantages	Key limitations	Representative ovarian application	Key references
Collagen (types I, III, IV)	Animal/recombinant	Weak alone (low kPa range); tunable via chemical cross-linking	Native ECM component; excellent cell adhesion; low antigenicity when purified	Weak mechanics unless cross-linked; batch variability	Follicle/stem-cell delivery substrate; xenograft comparator matrix	(10)
Alginate	Brown algae	Storage modulus tunable roughly 1–10+ kPa via concentration/MW; degrades unpredictably <i>in vivo</i> (no mammalian alginase)	Highly tunable stiffness via concentration/molecular weight; gold-standard <i>in vitro</i> follicle culture matrix	Biologically inert; poor <i>in vivo</i> revascularization; unpredictable degradation	<i>In vitro</i> follicle encapsulation and maturation; artificial ovary composite component	(37–40)
Hyaluronic acid	Bacterial fermentation/animal	Weak mechanical integrity unsupplemented; intrinsically pro-angiogenic	FDA-approved; native follicular-fluid component; intrinsically pro-angiogenic	Weak mechanical integrity alone; optimal dose/route not established	Autotransplantation encapsulation; preantral follicle culture	(41, 42)
Fibrin	Autologous/allogeneic plasma	Rigidity tunable via fibrinogen:thrombin ratio; degrades on a physiological (days-weeks) timescale	Pro-angiogenic; native cell-adhesion receptors; tunable via fibrinogen/thrombin ratio	Rapid, variable fibrinolytic degradation; donor-dependent variability	Primary matrix for artificial ovary follicle/stromal cell encapsulation	(13, 43, 44)
Silk fibroin	Bombyx mori cocoon	Stiffness and degradation rate independently tunable via beta-sheet crystallization	Tunable mechanics independent of degradation rate; versatile processing (gel, film, fiber)	Requires processing to remove sericin/immunogenic residues	Composite hydrogel reinforcement; electrospun fiber blends	(45)
Chitosan/chitin	Crustacean exoskeleton	Enzymatically biodegradable (unlike alginate); mechanical/degradation properties tunable via crosslinking or inorganic modification (cf. Table 6)	Antibacterial; enzymatically biodegradable (unlike alginate); pro-angiogenic porosity	Solubility limited to acidic conditions; mechanical properties variable	Alginate-alternative artificial ovary hydrogel	(46)

ECM, Extracellular matrix; FDA, U.S. Food and Drug Administration; MW, Molecular weight; kPa, Kilopascal; M, Molar; cf., Compare

dose-dependent relationship between matrix rigidity and follicle developmental competence has become one of the field's more durable design principles, echoing the stiffness sensitivity of native ovarian tissue described above.

Follicles encapsulated in alginate and matured to the point of ovulation have produced live, fertile mouse offspring, one of the clearest proof-of-concept demonstrations that a fully synthetic-derived hydrogel culture system can support the entire arc of folliculogenesis outside the body (40). Encapsulation protocols have since been extended to nonhuman primate secondary follicles and to follicles isolated from the ovaries of human cancer patients, both of which showed continued growth and oocyte development in alginate culture, suggesting the approach generalizes beyond rodent biology (53, 54). Cryopreserved ovarian tissue and individually cryopreserved follicles have also been successfully cultured in alginate after thawing, extending the system's utility to banked clinical material (55). A comprehensive review of alginate-based matrices for follicle culture, and a further review focused specifically on alginate as an encapsulation material, together document dozens of these variations across species (39, 56).

Despite this strong *in vitro* track record, alginate's principal shortcoming for *in vivo* grafting applications is its biological inertness. As a plant-derived polysaccharide with no mammalian cell-adhesion motifs, it supports minimal cell attachment, degrades unpredictably *in vivo* because alginate lacks mammalian enzymes capable of controlled degradation, and, most importantly for a transplanted graft, is poorly revascularized (33). This limitation has motivated a large secondary literature on alginate composites and blends, with fibrin, gelatin, decellularized ECM, or Wharton's jelly-derived matrix, discussed below. The same reinforcement logic recurs outside the ovarian literature: alginate hydrogels combined with a bioceramic filler and graphene nanosheet reinforcement have been shown, in a bone tissue-engineering context, to allow tensile strength to be tuned systematically with filler loading, and a related alginate-hyaluronic acid composite for orthopedic use likewise achieved measurable gains in tensile strength and hardness after inorganic nanoparticle modification, illustrating that the same alginate-plus-reinforcement strategy used to overcome the material's mechanical inertness in ovarian applications is a general one across tissue-engineering context, rather than an ovary-specific workaround (57, 58). Effects of alginate concentration on mouse preantral follicle development have been further quantified in factorial experimental designs, providing dose-response data that inform current culture protocols (59), and a comprehensive review of human oocyte health and *in vitro* development situates alginate-based culture within the broader landscape of assisted reproduction (60). Interpenetrating fibrin-alginate networks, discussed further below, and multifunctional macromer-based hydrogel network designs represent two of the more successful strategies for retaining alginate's favorable culture performance while addressing its degradation and revascularization limitations (61, 62). A comprehensive consensus review of ovarian tissue culture, *in vitro* follicle development, and transplantation approaches situates alginate-based encapsulation within the broader landscape of fertility-preservation techniques and identifies scaffold-mediated follicle culture as one of the more promising near-

term extensions of existing cryopreservation programs (63).

Hyaluronic acid

Hyaluronic acid (HA), or hyaluronan, is a non-sulfated glycosaminoglycan and a major structural component of the mammalian ECM, distinguished among natural biomaterials by FDA approval for various clinical uses, excellent biocompatibility, and inherent non-immunogenicity (41). Within the ovary, HA is concentrated in the cumulus matrix and follicular fluid of antral follicles and, to a lesser degree, in the theca layer of growing follicles, where it contributes to the biomechanical and biochemical microenvironment that governs follicle expansion and eventual ovulation (41, 64). Because HA levels are measurably reduced in the serum of women with POI relative to healthy controls, and because HA has been shown experimentally to counteract immunosuppressant-induced damage to granulosa cells, HA supplementation has been investigated both as a therapeutic agent in its own right and as a scaffold component for follicle and whole-ovary grafting (65).

In autotransplantation models, encapsulating ovarian tissue in an HA-based hydrogel scaffold, even without the addition of exogenous angiogenic growth factors such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor, reduced ischemia-related follicle loss, increased the number of follicles recovered across developmental stages, lowered post-transplant follicle-stimulating hormone (FSH), and up-regulated VEGF gene expression within the graft itself, suggesting that HA encapsulation promotes an intrinsically pro-angiogenic local environment (42). Comparable protective effects have been reported when HA hydrogel is used to encapsulate vitrified ovarian tissue before intramuscular autotransplantation, improving restoration of the estrous cycle and follicular reserve relative to unencapsulated grafts (66). For isolated follicle culture, a self-gelling hydrogel formed from thiolated HA has been shown to outperform standard alginate in supporting ovine preantral follicle growth, antrum formation, and expression of folliculogenesis-related genes, offering a natural-material alternative to alginate that more closely echoes the native follicular fluid microenvironment.

The principal translational caveat, echoed across nearly every HA study in this space, is that the safety, optimal concentration, and delivery route of HA-based grafts have not yet been established with sufficient rigor to move from small-animal proof-of-concept toward clinical application (41). HA has also been combined with more mechanically robust materials, most often alginate or a decellularized ECM backbone, to offset its own comparatively weak mechanical integrity. This strategy closely parallels the alginate-HA composite already validated for mechanical reinforcement in a non-ovarian orthopedic context (57) and is considered further in below.

Fibrin

Fibrin, formed by thrombin-catalyzed polymerization of fibrinogen, has emerged as one of the two most extensively validated natural scaffolds, alongside alginate, specifically for artificial ovary applications that encapsulate isolated follicles and stromal cells for autotransplantation, a use case discussed in detail below. Its principal advantages over alginate are biological rather than purely mechanical: fibrin is a natural clotting product recognized by mammalian cell-

adhesion receptors, it is intrinsically pro-angiogenic, and its degradation by host fibrinolytic enzymes proceeds on a physiologically relevant timescale that can, within limits, be tuned by adjusting fibrinogen and thrombin concentrations (43).

The Louvain group led by Dolmans and Amorim has built a systematic, stepwise body of evidence around fibrin as an artificial ovary matrix. Their first study established the feasibility of a three-dimensional biodegradable fibrin scaffold for follicle and ovarian cell encapsulation (44), and a subsequent study directly demonstrated that isolated murine follicles encapsulated in low-concentration fibrin matrices, specifically the F12.5/T1 and F25/T4 fibrinogen-thrombin formulations, survived and proliferated after autologous transplantation, establishing proof of concept for the entire artificial ovary strategy (13). Because fibrin matrix composition strongly influences the mechanical environment experienced by encapsulated cells, later work systematically varied fibrinogen and thrombin concentrations and found that formulations with higher concentrations of both components better supported follicle survival after xenotransplantation, plausibly because their ultrastructure and rigidity more closely approximate the native human ovarian cortex (67). This concentration dependence was further explored by optimizing fibrin composition specifically to mimic the architecture and rigidity of human ovarian tissue, yielding a fibrin-based artificial ovary prototype tailored to human dimensions and biomechanics (68).

Follicle-stage dependence has also been characterized directly: outcomes after fibrin-based artificial ovary transplantation differ according to whether primordial, primary, or secondary follicles are encapsulated, information directly relevant to selecting which follicle population to prioritize for a given clinical indication (69). Beyond the encapsulation matrix itself, the choice of stromal cell source used alongside encapsulated follicles has been shown to matter: comparing stromal cells isolated from the ovarian medulla versus cortex, and from cryopreserved versus fresh tissue, identified meaningful differences in the cells' subsequent support of the artificial ovary construct (70). Co-transplantation strategies incorporating adipose tissue-derived stem cells within the fibrin implant have been shown to enhance neovascularization at the peritoneal grafting site, and a two-step transplantation protocol using these stem cells further increased follicle survival in xenografted human ovarian tissue by improving early vascularization (71, 72). Short-term xenografting studies confirm that human preantral follicles survive and continue growing after fibrin-based cryopreservation, isolation, and grafting (73). Short-term transplantation of isolated human follicles and cortical tissue into immunodeficient mice, one of the foundational demonstrations that isolated human follicles can survive xenotransplantation at all, together with the subsequent demonstration that antral follicles can develop after xenografting of small human preantral follicles, established the experimental groundwork on which the fibrin-based artificial ovary program was built (74, 75). Interpenetrating fibrin-alginate networks that combine fibrin's bioactivity with alginate's mechanical tunability have likewise supported *in vitro* ovarian follicle development, illustrating the composite strategies discussed further below (61). Fibrin-based encapsulation has also been proposed

as a companion technology to standard ovarian tissue cryopreservation, with fibrin-embedded human ovarian tissue fragments showing preserved viability through the freeze-thaw cycle, suggesting that the same matrix that supports artificial ovary construction may also improve cryopreservation outcomes for conventional cortical tissue banking (76).

Silk fibroin

Silk fibroin, derived from the cocoon protein of *Bombyx mori* and other silkworm species, offers a combination of mechanical robustness, slow and tunable biodegradation, and processing versatility; it can be formed into hydrogels, films, sponges, or electrospun fibers, which distinguishes it from the softer, more rapidly degrading natural polymers discussed above (45). For ovarian applications, enzymatically crosslinked silk-based hydrogels have been engineered with tunable mechanical properties specifically to encapsulate ovarian cells while preserving the spherical, cell-cell contact-dependent architecture follicles require for normal folliculogenesis (77). Blending silk with chitosan, for example, has been shown to slow hydrogel degradation relative to chitosan alone, illustrating the material's utility as a mechanical reinforcing agent in composite scaffold systems, even when it is not the principal bioactive component (77, 78). Because silk fibroin's degradation rate can be adjusted independently of its stiffness by controlling the degree of beta-sheet crystallization, it offers a route to decoupling two design variables, rigidity and degradation kinetics, that are often confounded in purely polysaccharide-based systems such as alginate (78).

Chitosan and chitin

Chitosan, a deacetylated derivative of chitin obtained from crustacean exoskeletons, provides antibacterial activity, controllable porosity favorable to angiogenesis, and, notably, biodegradability that alginate lacks, since host lysozyme can enzymatically degrade chitosan (79). Xiang and colleagues directly tested its promise as an alginate alternative for artificial ovary construction by synthesizing a biodegradable chitin-based hydrogel to address the low angiogenic potential, excessive rigidity, and non-degradability that limit alginate-based artificial ovaries (46). *In vitro*, follicles cultured in the chitin-based hydrogel showed growth, hormone secretion, and folliculogenesis-related gene expression comparable to or exceeding two-dimensional and alginate controls. After transplantation into the peritoneal pockets of ovariectomized mice, both alginate and chitin-based grafts restored ovarian endocrine function, though neither fully normalized ovariectomy-induced bone loss relative to sham controls. This outcome illustrates both the promise and the current ceiling of hydrogel-based artificial ovaries for systemic, non-reproductive endpoints such as skeletal health (46, 80).

The same chitosan-modification strategy that improves this material's angiogenic and antibacterial performance in the ovary has been validated repeatedly outside reproductive tissue engineering, which strengthens confidence that it generalizes rather than being an artifact of any single ovarian study. Chitosan or its carboxymethylated derivative, blended with polyvinyl alcohol and modified with inorganic agents such as magnesium chloride and silicon-based crosslinkers, or loaded with silver-titanium or zinc-oxide

nanoparticles, has been shown across several independent wound-healing and bone-scaffold studies to allow porosity, swelling capacity, degradation rate, and antibacterial activity to be tuned largely independently of one another by adjusting the inorganic loading, exactly the kind of decoupled, quantitatively tunable design space that remains comparatively under-documented for the ovary-specific chitosan hydrogels reviewed above, and a plausible route to closing that gap (81-83).

Synthetic biomaterials

Where natural biomaterials offer inherent bioactivity at the cost of batch variability and comparatively weak mechanics, synthetic polymers invert that trade-off: they can be manufactured with precise, reproducible control over molecular weight, degradation rate, and mechanical stiffness, but they generally lack native cell-adhesion motifs and must be chemically functionalized, most often with arginine-glycine-aspartate (RGD) peptides or similar integrin-binding sequences, to support meaningful cell attachment. Three synthetic polymer families dominate the ovarian tissue-engineering literature: poly(epsilon-caprolactone)(PCL), poly(ethylene glycol)(PEG), and poly(lactic-co-glycolic acid)(PLGA), each occupying a distinct mechanical and functional niche; Table 2 summarizes their comparative properties.

Poly(epsilon-caprolactone)

PCL is a slowly degrading, semicrystalline polyester whose relatively high mechanical stiffness and slow hydrolytic degradation make it particularly well suited to electrospinning into fibrous scaffolds that can bear mechanical load over the multi-week timescale that follicle maturation requires (89). Di Berardino and colleagues validated a patterned, electrospun PCL scaffold specifically for long-term culture of multiple preantral follicles simultaneously, reporting that the ovarian-bio-inspired fiber architecture adequately replaced the native ovarian matrix during the transition of follicles from the preantral to antral stage, while maintaining biocompatibility over an extended culture period sufficient to guide follicles through the successive stages of meiotic and developmental competence acquisition (84). A related study using patterned versus

randomly oriented PCL electrospun fibers to support long-term ovine preantral follicle development found that fiber alignment itself, independent of chemical composition, measurably affects follicular and oocyte growth outcomes, underscoring that in fibrous synthetic scaffolds, architecture is as much a design variable as material chemistry.

PCL's principal limitation for ovarian applications mirrors its principal strength: the same slow degradation that provides sustained mechanical support during multi-week follicle culture can, in a transplantation setting, outlast the tissue remodeling process the graft is meant to support, and PCL's intrinsic hydrophobicity and lack of adhesion motifs generally necessitate blending with a natural polymer or surface functionalization before follicle attachment is adequate, a strategy discussed further under composite scaffolds below.

Poly(ethylene glycol)

PEG hydrogels occupy a distinctive position in the synthetic biomaterials landscape because their network structure, crosslink density, degradability, and the presence or absence of cell adhesive and protease-sensitive peptide sequences can be specified with a precision that is difficult to achieve with any natural polymer (90). This tunability has been exploited most directly in two related but distinct applications: as an encapsulation matrix for isolated follicles, and as an immunoisolating capsule for whole donor ovarian tissue.

For follicle encapsulation, Kim and colleagues engineered isolated preantral mouse follicles within a 7% poly(ethylene glycol)-vinyl sulfone (PEG-VS) hydrogel modified with RGD adhesion peptide and crosslinked with a trifunctional matrix metalloproteinase (MMP)-sensitive peptide, allowing the gel to be enzymatically degraded by the same proteases follicles naturally deploy during growth (15). When orthotopically implanted into ovariectomized mice, these PEG-VS grafts developed multiple fully mature antral follicles and corpora lutea by day 30, restored regular ovulatory cycling, normalized elevated FSH for at least sixty days, and, notably, preserved 60% of the follicular pool in the dormant primordial and primary stages, demonstrating selective rather than wholesale recruitment into the growing pool. Functioning blood vessels were observed

Table 2. Comparative overview of synthetic biomaterials used in ovarian tissue engineering

Material	Degradation profile	Key advantages	Key limitations	Representative ovarian application	Key references
Poly(epsilon-caprolactone) (PCL)	Slow (months)	High mechanical stiffness; excellent electrospinnability; long-term structural support	Hydrophobic; lacks native adhesion motifs; degradation may outlast remodeling	Electrospun patterned fiber scaffolds for long-term preantral follicle culture	(84)
Poly(ethylene glycol) (PEG/PEG-VS)	Tunable (weeks-months) via crosslink chemistry	Precisely tunable network properties; can be rendered essentially non-immunogenic; supports immunoisolation	Requires RGD/peptide functionalization for adhesion; balance of degradability vs. immune protection unresolved	Isolated-follicle encapsulation; immunoisolating capsules for allogeneic/xenogeneic ovarian tissue	(15-18, 85)
Poly(lactic-co-glycolic acid) (PLGA)	Tunable (weeks-months) via lactide:glycolide ratio	FDA-approved; tunable degradation; suitable for microsphere drug delivery	Prone to shrinkage/distortion as pure nanofiber mats; acidic degradation byproducts	Composite electrospun scaffolds; controlled-release follicle-activator carriers	(86, 87)
Polytetrafluoroethylene (PTFE), porous	Non-degradable	Cell-impermeable but molecule-permeable; supports peri-implant neovascularization	Permanent implant; no biodegradation or remodeling	Endocrine-restoration membrane graft	(88)

FDA, U.S. Food and Drug Administration; PEG, polyethylene glycol; PEG-VS, poly(ethylene glycol)-vinyl sulfone; PCL, poly(epsilon-caprolactone); PLGA, poly(lactic-co-glycolic acid); PTFE, polytetrafluoroethylene; RGD, arginine-glycine-aspartic acid

within the grafts at both thirty and sixty days, evidence that a fully synthetic hydrogel, appropriately functionalized, can support graft revascularization despite lacking any native ECM component. Earlier work using PEG-based hydrogels for preantral follicle culture had already established a quantitative relationship between hydrogel swelling ratio and follicular developmental outcomes, providing some of the design parameters exploited in these later *in vivo* studies (91, 92).

The Shikanov laboratory at the University of Michigan has since extended PEG-based encapsulation toward whole-tissue immunoisolation rather than isolated-follicle culture. Encapsulating donor ovarian tissue within a dual-layer PEG-VS capsule, a proteolytically degradable core surrounded by a non-degradable outer shell, was shown to support long-term ovarian tissue survival and restore endocrine function in ovariectomized mice while providing a physical barrier against immune cell infiltration (85, 93). This immunoisolation strategy was subsequently tested against a considerably more demanding immunological challenge: encapsulated allogeneic ovarian tissue transplanted into immune-competent, sensitized recipient mice avoided the roughly twenty-six-fold rise in donor-specific IgG and the CD8-positive T-cell graft infiltration observed in non-encapsulated allografts, indicating that the PEG-Dual capsule can prevent both humoral and cellular rejection of a mismatched graft (18). Human ovarian follicles encapsulated within similar immunoisolating capsules have been shown to survive long-term implantation in mice, extending the approach from allogeneic to xenogeneic grafting (94). Most recently, and after initial dissemination as a preprint, a peer-reviewed 2026 report demonstrated that human ovarian cortex from young donors, encapsulated within an immune-isolating PEG-based capsule and implanted in ovariectomized immunodeficient mice, sustained folliculogenesis and steroidogenesis comparable to non-encapsulated control grafts, with regular estrous cyclicity resuming roughly twelve weeks after implantation and antral follicles up to three millimeters in diameter observed within the encapsulated tissue (17). Because this study used immunodeficient rather than fully immunocompetent hosts, it demonstrates biological feasibility of the encapsulated human xenograft rather than definitive proof of immune protection in an intact human recipient. The authors frame it as a proof-of-concept step rather than a clinically ready platform, an important caveat given how frequently PEG immunoisolation results are cited as evidence that the immune-rejection barrier to donor ovarian tissue use has already been solved.

Poly (lactic-co-glycolic acid) and related polyesters

PLGA, an FDA-approved biodegradable copolymer whose degradation rate can be tuned across a wide range by adjusting the lactide-to-glycolide ratio, has been used in ovarian tissue engineering both as a standalone electrospun scaffold material and, more commonly, as a blend component with silk fibroin or PCL to improve dimensional stability and reduce the shrinkage and distortion that pure PLGA nanofiber mats can exhibit in aqueous biological media (95, 96). Composite PLGA/PCL/silk fibroin nanofiber scaffolds fabricated by single-step electrospinning have demonstrated improved dimensional stability, hygroscopicity, and cytocompatibility relative to PLGA alone, illustrating how

blending synthetic polyesters with a natural structural protein can offset the shrinkage problem without sacrificing the manufacturing reproducibility that makes PLGA attractive in the first place (96, 97). PLGA-based nanoparticle and microsphere formulations have also been used, outside the scaffold context per se, as drug-delivery vehicles for controlled release of small-molecule follicle activators; triptolide-loaded PEG-b-PLA and Mal-PEG-b-PLA amphiphilic diblock copolymer nanoparticles, for example, have been shown to increase serum anti-Mullerian hormone (AMH) and restore fertility in POI rat models with low toxicity, illustrating that synthetic polyesters contribute to ovarian regenerative medicine not only as structural scaffolds but also as controlled-release platforms embedded within or alongside a scaffold construct (98).

Porous polytetrafluoroethylene (PTFE) membranes, though chemically distinct from the polyester family, share the essential design logic of the synthetic biomaterials discussed in this section: a cell-impermeable but molecule-permeable synthetic barrier, grafted onto ovarian tissue, allowed new blood vessel formation around the implant, reduced FSH levels, and increased follicle numbers in a mouse POI model, demonstrating that even a chemically inert synthetic membrane can support functional endocrine restoration when its porosity is tuned to permit soluble-factor diffusion while excluding immune cells (88).

Composite and hybrid scaffolds

The individual limitations documented above and alginate's biological inertness, HA's mechanical fragility, PCL's hydrophobicity, and PEG's lack of intrinsic bioactivity, have driven a substantial and growing literature on composite scaffolds that combine two or more materials to offset one another's weaknesses (38). The general design logic is consistent across most examples: a polysaccharide or synthetic polymer contributes mechanical stability and processability, while a protein or ECM-derived component contributes bioactivity and cell-adhesion capacity, echoing the broader tissue-engineering observation that polysaccharide-protein composites tend to outperform either constituent alone (99). This same logic is visible outside ovarian tissue engineering wherever a mechanically weak polysaccharide is paired with a stiffer reinforcing phase, for example alginate combined with a bioceramic and graphene nanosheet filler, or alginate combined with HA and an inorganic nanoparticle, both of which allow tensile strength and hardness to be tuned upward from the unmodified polysaccharide baseline in a dose-dependent way (57, 58), reinforcing the expectation that comparable composite strategies should be transferable to ovarian scaffold design, an expectation revisited below when native tissue benchmarks are considered.

Alginate-matrigel composites were among the earliest approaches applied specifically to the artificial ovary problem: encapsulating isolated ovarian cells within an alginate-matrigel matrix represented a first step toward a biodegradable scaffold for preantral follicle and ovarian cell transplantation, combining alginate's gelation properties with matrigel's basement-membrane-derived bioactive components (100). Interpenetrating fibrin-alginate networks similarly combine fibrin's pro-angiogenic, cell-adhesive properties with alginate's tunable, sustained mechanical support, and this interpenetrating architecture has been

shown to support *in vitro* ovarian follicle development more effectively than either polymer alone (61). Human amniotic epithelial cells embedded within a sodium alginate-bioglass hydrogel and transplanted alongside ovarian tissue into mice with chemically induced premature ovarian failure showed both improved cell viability within the ovary and enhanced follicle growth and angiogenesis in the treated ovaries, illustrating that bioglass, more typically associated with bone tissue engineering, can contribute favorable angiogenic properties when incorporated into a soft-tissue composite. Human amniotic membrane itself, applied directly as a natural composite substrate rather than as a bioglass hybrid, has separately been shown to support mouse ovarian follicular culture, extending the range of placental- and fetal-membrane-derived materials available for ovarian composite scaffold design beyond the amniotic mesenchymal stem cell applications already well described in the stem-cell therapy literature (101).

Graphene oxide/poly-L-lactic acid (GO/PLLA) nanofiber scaffolds represent a further hybridization strategy, in this case combining a synthetic polyester backbone with graphene oxide to improve hydrophilicity and create a porous network conducive to cell penetration; when used to restore ovarian function and reserve in mice with POI, this composite scaffold supported improvements in follicle number, angiogenesis, and overall ovarian function, with granulosa cells shown to migrate readily onto the nanofiber surfaces (102). Decellularized ECM has itself become a frequent composite partner rather than a standalone material: because native ovarian decellularized ECM

(dECM) hydrogels, as noted above, generally lack sufficient mechanical rigidity to support follicle culture on their own, supplementing dECM with alginate has become a standard strategy for reconciling the biochemical authenticity of decellularized matrix with the mechanical robustness that a functional scaffold requires (33, 103).

Silk-chitosan hydrogels follow the same logic in reverse: adding silk fibroin to a chitosan-based hydrogel slows degradation and improves mechanical stability relative to chitosan alone, again pairing a mechanically robust natural protein with a more rapidly degrading polysaccharide to tune the composite's overall performance envelope (104). Taken together, and as summarized schematically in Figure 1, the composite and hybrid scaffolds reviewed in this section suggest that no single natural or synthetic material currently satisfies all of the design requirements- bioactivity, tunable mechanics, controlled degradation, and manufacturability- simultaneously, and that combinatorial materials engineering, rather than the discovery of a single superior polymer, is likely to remain the dominant strategy for the foreseeable future.

Decellularized ovarian ECM and advanced fabrication techniques

Decellularization of ovarian tissue

Decellularization removes cellular and nuclear material from donor tissue through combined physical, chemical, and enzymatic treatment while aiming to preserve the native three-dimensional architecture and biochemical composition of the ECM, on the premise that no synthetic

Composite Scaffolds: Combining Strengths

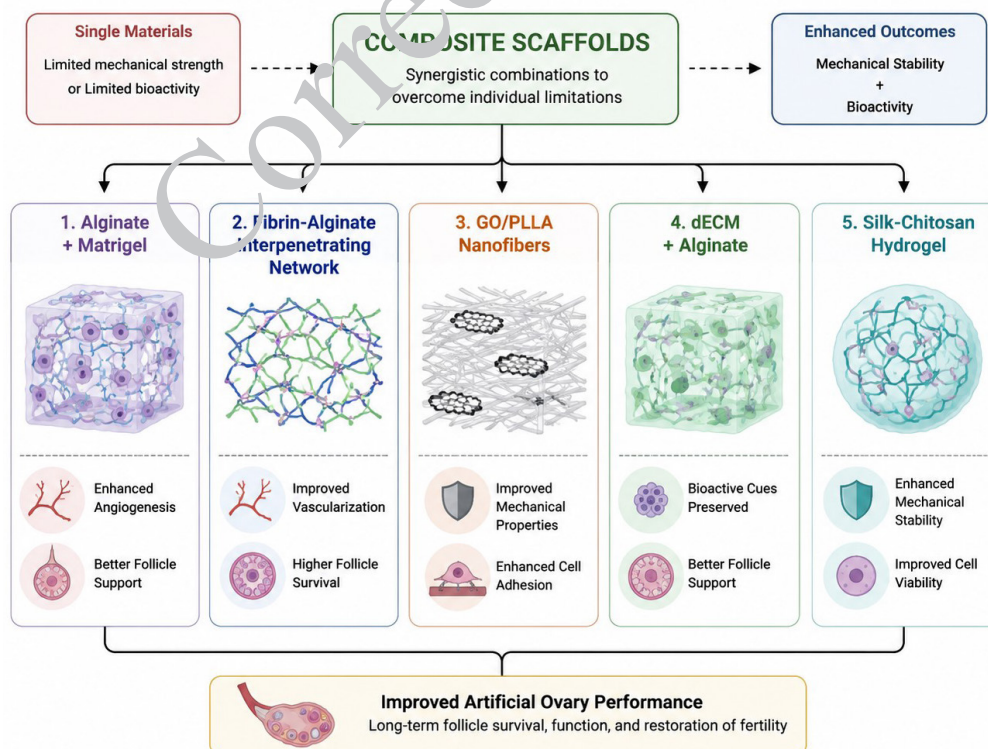


Figure 1. Composite biomaterial scaffolds for artificial ovary applications

Schematic comparison of representative hybrid strategies, alginate-protein, alginate-bioceramic, synthetic-natural, and decellularized-ECM composites, showing how each combination trades mechanical reinforcement against bioactivity, angiogenic potential, cell adhesion, and follicle support relative to either constituent material used alone. GO, graphene oxide; PLLA, poly(L-lactic acid); dECM, decellularized extracellular matrix.

or reconstituted natural material can currently match the compositional fidelity of a tissue's own matrix (30). Proteomic profiling of decellularized bovine ovarian tissue confirms that a substantial fraction of the native matrix, the structural and regulatory ECM proteins summarized above, is retained after decellularization, providing direct molecular evidence for the biochemical-fidelity claim that motivates this approach in the first place, and reinforcing why decellularization is treated throughout this review as the biomimetic strategy of first resort wherever donor tissue is available and mechanical reinforcement can be added separately (105). Table 3 summarizes representative decellularization protocols applied specifically to ovarian tissue, organized by species and detergent or method, and compares them on the same biochemical-fidelity, architectural-control, and scalability axes used for the fabrication techniques compared below. Nikniaz and colleagues systematically compared decellularization protocols applied to both human and bovine ovarian tissue. They combined the resulting decellularized ECM with alginate to create a composite scaffold shown to better support *in vitro* follicle development than either material alone (103). Hassanpour and colleagues developed a sodium lauryl ester sulfate (SLES)-based decellularization protocol for human ovarian tissue, producing a scaffold that retained key ECM constituents, type I and IV collagen, fibronectin, laminin, and glycosaminoglycans, known to be important in regulating oocyte maturation, follicle fate, and steroidogenesis, and confirmed that recellularized scaffolds were biocompatible and functional after implantation (106).

Pors and colleagues extended decellularization to a specifically clinical-translation-oriented endpoint, showing that human ovarian stromal cells and pre-antral follicles seeded onto SDS-decellularized human ovarian scaffold remained viable in culture, representing an initial step toward reconstructing a human ovary from decellularized starting material (107). Whole-organ rather than fragment-level decellularization has also been pursued: a combined perfusion and ultrasonication protocol applied to intact porcine ovaries produced a decellularized whole-ovary scaffold with preserved microarchitecture, extending decellularization from the tissue-fragment scale toward an entire, anatomically organized organ (108), and a more recent protocol employing supercritical carbon dioxide, a technique whose density and diffusion properties are intermediate between those of liquids and gases, has been proposed as an alternative decellularization route for human

ovarian scaffolds with potentially improved preservation of matrix ultrastructure (109).

A 2023 systematic methodological review of decellularized ECM scaffolds specifically applied to artificial ovary construction identified twelve eligible studies published between 2015 and 2022, most originating from a small number of research groups concentrated in Iran, and found that while decellularized scaffolds loaded with ovarian cells reliably produced estradiol and progesterone and supported growth of follicles at various stages, hormone output was highly variable across studies. No serious adverse events had yet been reported, a summary that captures both the promise and the still-preliminary state of the decellularization literature as applied specifically to the ovary (110). As already noted above, one of the most consistent findings across this body of work is that decellularized ovarian ECM alone, once processed into a hydrogel, generally lacks sufficient mechanical rigidity to reliably support follicle survival, motivating the alginate- and other polymer-supplementation strategies discussed above (33).

Three-dimensional bioprinting

Three-dimensional printing allows spatial control over scaffold pore geometry, follicle placement, and, in principle, the construction of anatomically scaled implants that would be difficult or impossible to achieve with bulk hydrogel casting. A broader review of 3D bioprinting strategies across tissue-engineering applications situates this pore-scale architectural control as the technology's central advantage over casting-based fabrication regardless of target tissue (112). The foundational demonstration in this space came from Laronda and colleagues, who 3D-printed microporous gelatin hydrogel scaffolds and systematically varied the advancing angle between printed layers, comparing 30-, 60-, and 90-degree architectures to determine how pore geometry affects follicle survival (16). Scaffolds printed with 30-degree and 60-degree angles created corner geometries that contacted follicles on multiple sides and thereby limited follicle spreading, which correlated with improved survival, whereas 90-degree scaffolds produced a more open porosity that allowed follicles to spread excessively and fared worse; follicles maintained within scaffolds providing two or more points of contact showed an 82.8% survival rate over eight days *in vitro*. When seeded with follicles and implanted into surgically sterilized mice, these bioprosthetic ovary constructs became highly vascularized, fully restored ovarian

Table 3. Representative decellularization protocols applied to ovarian tissue

Species	Decellularization method	Key reported findings	Reference
Human and bovine	Comparative detergent-based protocols, combined with alginate	Improved <i>in vitro</i> follicle development versus decellularized ECM or alginate alone	(103)
Human	Sodium lauryl ester sulfate (SLES)-based protocol	Retention of collagen I/IV, fibronectin, laminin, and glycosaminoglycans; biocompatible after recellularization	(106)
Human	SDS-based decellularization	Human stromal cells and pre-antral follicles remained viable on scaffold	(111)
Porcine (whole ovary)	Combined perfusion and ultrasonication	Preserved whole-organ microarchitecture at anatomical scale	(108)
Human	Supercritical carbon dioxide (scCO ₂) protocol	Proposed as alternative route with potentially improved matrix ultrastructure preservation	(109)

ECM, extracellular matrix; SLES, sodium lauryl ester sulfate; SDS, sodium dodecyl sulfate; scCO₂, supercritical carbon dioxide; I/IV, collagen types I and IV

endocrine function, and, remarkably, supported natural mating with live pups subsequently nursed by the recipient mothers, one of the clearest demonstrations to date that a synthetic, 3D-printed scaffold can support the complete physiological arc from follicle implantation through successful reproduction. This bioprosthetic ovary work built directly on the same group's earlier demonstration that decellularized ovarian tissue, used as a transplant substrate, could initiate puberty onset in juvenile mice (113).

Subsequent work has extended 3D bioprinting from gelatin toward gelatin methacryloyl (GelMA) and GelMA-alginate bioinks, which offer the advantage of photo-crosslinking for improved shape fidelity during the printing process itself. Wu and colleagues compared the printability of GelMA, alginate, and GelMA-alginate bioinks. They selected GelMA for further development based on superior swelling, degradation, and shape-fidelity characteristics, successfully printing three-dimensional ovarian constructs that supported murine ovarian follicle growth and diameter expansion in culture, followed by *in vitro* maturation and oocyte collection (114). A stereological approach to quantifying mouse ovarian tissue architecture has been developed specifically to inform the design parameters- pore size, wall thickness, and follicle density- used in subsequent 3D bioprinting protocols, providing a more rigorous anatomical basis for scaffold design than earlier trial-and-error approaches (115). A recent synthesis of strategies for developing 3D-printed ovarian models specifically for fertility restoration situates these anatomically informed printing approaches within the broader trajectory toward clinically scaled, patient-specific constructs, while cautioning that print-resolution and bioink-biocompatibility constraints still limit how closely any printed scaffold can approximate native follicular density (116). Designer biomaterial grafts incorporating primordial follicles, engineered with specific attention to graft architecture, have likewise produced live births in a mouse model of infertility, reinforcing that pore geometry and follicle placement, not simply bulk material choice, are primary determinants of functional outcome in printed and patterned scaffolds (117).

Electrospinning

Electrospinning generates nanoscale-to-microscale polymer fibers by applying a high-voltage electric field to a polymer solution or melt, producing non-woven fibrous mats with a high surface-area-to-volume ratio and interconnected porosity that structurally resembles native fibrillar ECM more closely than a bulk hydrogel does (118). For ovarian applications, this technique has been most extensively developed using PCL, as detailed above, where patterned electrospun fiber architecture, the specific arrangement and alignment of fibers, independent of chemistry, measurably influences follicle growth trajectories and oocyte developmental competence over extended *in vitro* culture (84). Electrospun composite scaffolds blending PLGA, PCL, and silk fibroin have similarly been developed to combine the dimensional stability of synthetic polyesters with the improved cytocompatibility contributed by a natural structural protein. The principal practical limitations of electrospinning for ovarian scaffold applications mirror those documented for the technique across tissue engineering more broadly: relatively poor cell penetration

into densely packed fiber mats, and comparatively low mechanical strength of the resulting scaffold unless reinforced or combined with a second material, both of which have motivated the composite electrospun systems discussed above (118).

Comparing decellularization, bioprinting, and electrospinning as fabrication strategies

These three fabrication approaches are not mutually exclusive and are increasingly used in combination; decellularized ECM can be solubilized and used as a bioink component within a 3D-printed construct, or electrospun fibers can be layered around a bioprinted or decellularized core. Current evidence suggests that hybrid combinations of this kind are more likely to close the remaining gaps than any single technique used alone. Decellularization offers the closest available approximation of native ovarian biochemistry but the least architectural control and the most significant mechanical limitations. Three-dimensional bioprinting offers precise, reproducible control over macro-scale pore architecture and follicle placement. Still, it requires a bioink whose rheological properties are compatible with extrusion or photopolymerization, generally at the cost of some biochemical complexity, and print resolution currently sits well above the scale of an individual primordial follicle (112). Electrospinning offers the closest structural mimicry of fibrillar ECM at the nanoscale. Still, it is the least straightforward route for embedding cells during fabrication, since most electrospinning protocols require seeding cells onto a pre-formed fiber mat rather than encapsulating them during processing. Table 4 summarizes the comparative strengths, limitations, and representative ovarian applications of these three fabrication strategies alongside the materials classes discussed above, scored explicitly on biochemical fidelity, architectural control, and mechanical control so that the trade-offs can be compared directly across rows rather than read as a simple descriptive list.

Artificial ovary construction and follicle encapsulation

Rationale and general design

The artificial ovary concept addresses a specific and clinically important gap left open by conventional ovarian tissue cryopreservation and autotransplantation: patients whose underlying malignancy carries a meaningful risk of ovarian micrometastasis, leukemias being the paradigm case, cannot safely be reimplanted with cryopreserved cortical tissue fragments, because doing so risks reintroducing malignant cells along with the follicles being rescued (105). The artificial ovary strategy circumvents this risk by isolating individual follicles, together with a defined population of supporting stromal or granulosa cells, away from the bulk of the potentially contaminated tissue, and then encapsulating this purified follicle-cell population within a biomaterial carrier that is transplanted in place of the intact cortical strip, an approach reviewed comprehensively as "a new possibility in fertility preservation" by Cho and colleagues (119). A more recent synthesis directed specifically at female cancer survivors situates artificial ovary construction alongside stem-cell-derived neo-folliculogenesis as one of two complementary bioengineering strategies most likely to expand fertility-preservation options for this population over the coming decade (119, 120).

Table 4. Comparison of advanced fabrication strategies for ovarian scaffold construction

Fabrication strategy	Architectural control	Biochemical fidelity	Mechanical control	Principal limitation	Representative studies
Decellularization	Low-moderate (constrained by donor tissue geometry)	High (native compartmentalized ECM largely retained)	Low (typically requires reinforcement, e.g., with alginate)	Batch variability; residual antigenicity; weak mechanics as hydrogel	(33, 106, 108, 111)
3D bioprinting	High (pore geometry, follicle placement, macro-scale architecture programmable)	Moderate (depends on bioink; can incorporate decellularized ECM component)	High (tunable via bioink formulation and print parameters)	Bioink rheology constraints; resolution limits at single-follicle scale	(16, 112, 114, 115)
Electrospinning	High at nanofiber scale (fiber alignment, diameter, density)	Low-moderate (depends on polymer; often requires natural-polymer blending)	Moderate (fiber mats often weak unless layered/reinforced)	Poor cell penetration into dense mats; typically requires post-fabrication seeding	(84, 118)

3D, three-dimensional; ECM, extracellular matrix

The residual-risk question is worth stating precisely rather than in general terms, since it is the entire clinical rationale for the artificial ovary approach. Reported contamination rates for ovarian tissue harvested from patients with hematologic malignancy vary widely with the detection method used, from infrequent detection by conventional histology up to roughly a quarter of samples when sensitive molecular assays such as polymerase chain reaction (PCR)-based minimal residual disease testing are applied, and even a small number of surviving malignant cells has been sufficient to cause disease relapse in animal models (12). Because the encapsulated material in an artificial ovary construct has been isolated at the single-follicle or small-cluster level rather than reimplanted as an intact tissue fragment, the malignant-cell-carryover risk is substantially reduced relative to whole-tissue autotransplantation. Still, it is not eliminated entirely: isolation and encapsulation protocols have generally been validated against artificially contaminated cell suspensions rather than against the more heterogeneous pattern of *in vivo* tissue invasion and dedicated purging methods, for example photodynamic therapies targeted at residual malignant cells within a tissue fragment, are a complementary and still-early-stage safety strategy rather than a substitute for follicle isolation itself (12). Residual risk assessment therefore remains an active area of investigation. It is one of the clearer examples in this field of a safety question that preclinical follicle-survival data alone cannot answer.

Successful artificial ovary construction requires the scaffold to meet several simultaneous, partially competing design requirements: sufficient porosity and biodegradability to permit follicle growth, vascular ingrowth, and eventual host-tissue integration; sufficient initial mechanical integrity to protect encapsulated follicles from compressive damage during surgical handling and the early post-implantation period, before host remodeling begins; and biochemical properties that support follicle attachment, paracrine signaling between follicles and their surrounding stromal cells, and, ultimately, either follicle growth toward ovulation or, for endocrine-restoration applications, sustained steroidogenic activity without necessarily requiring full folliculogenesis.

Fibrin- and alginate-based artificial ovary constructs

As detailed above, fibrin and alginate remain the two most extensively validated matrices specifically for artificial ovary construction. The stepwise body of work

from the Louvain group (Luyckx, Dolmans, Vanacker, Chiti, Amorim, and colleagues) represents the most complete translational trajectory currently available for any single biomaterial system in this application: from initial feasibility (44), through autologous transplantation with demonstrated follicle survival (15), through systematic optimization of fibrinogen-thrombin composition, follicle-stage selection (121) and stromal cell source (70), to composite refinement incorporating adipose-derived stem cells for enhanced vascularization (71, 72) and human-tissue-scale prototypes engineered to mimic native cortical architecture and rigidity. This trajectory illustrates the kind of incremental, parameter-by-parameter optimization that is likely required before any artificial ovary construct is ready for first-in-human testing, and it stands in useful contrast to the more recent single-study demonstrations of novel materials, chitin-based hydrogels, decellularized ECM composites, that have not yet undergone comparably extensive iterative refinement.

Alginate-based artificial ovary constructs have followed a parallel but methodologically distinct path, emphasizing precise control over follicle-scale culture parameters, alginate concentration, molecular weight, and encapsulation geometry, established originally for *in vitro* culture (37, 38, 122) and subsequently adapted toward *in vivo* grafting applications, most often as a composite with a second, more bioactive material such as Matrigel (100), fibrin (61), or decellularized ECM (33, 103), reflecting the general conclusion from the discussion of composite scaffolds that alginate alone rarely provides sufficient bioactivity for successful *in vivo* artificial ovary performance despite its excellent *in vitro* culture track record.

Synthetic and hybrid artificial ovary platforms

PEG-based artificial ovary constructs occupy a distinct niche within this application, emphasizing immune compatibility over biochemical mimicry: because PEG hydrogels can be engineered to be essentially non-immunogenic while still permitting selective molecular exchange with the host, they are particularly well suited to allogeneic or xenogeneic artificial ovary applications where fibrin or decellularized ECM, both of which retain donor-derived biological material, would carry a higher inherent immunogenicity risk (15, 18). The dual-layer immunoisolating capsule design discussed above, combining a degradable inner core with a non-degradable outer shell, offers a specific engineering solution to the problem of

reconciling follicle-supportive matrix remodeling, which requires degradability, with durable immune protection, which favors non-degradability, within a single construct (85).

Chitin-based hydrogels are the newest entrant in this comparative landscape, explicitly designed to combine alginate's favorable *in vitro* culture performance with improved angiogenic potential and biodegradability. Early *in vivo* results in ovariectomized mice are broadly encouraging, though no direct head-to-head comparison against fibrin- and PEG-based systems using matched outcome measures has been reported (46).

From follicle survival to functional outcomes

Across nearly all of the artificial ovary studies reviewed above, outcome measures cluster around a common set of endpoints: follicle survival rate over a defined post-transplantation interval, resumption of estrous cyclicity, normalization of FSH and restoration of estradiol to physiological levels, follicle and antral cavity counts at necropsy, and, in the small subset of studies designed to test it, successful mating and live birth (123). Relatively few studies report all of these endpoints together, which complicates direct cross-study comparison and is a limitation discussed further below. Table 5 summarizes representative preclinical outcomes across the principal artificial ovary platforms discussed in this section, organized by scaffold material, species, and reported functional endpoints.

Technology readiness, manufacturing scalability, and the limits of mouse-to-human translation

It is worth being explicit about how far along the translational pipeline each of the platforms in Table 5 actually sits, since the language used to describe preclinical

results in this field, “restored,” “supported,” “achieved,” is frequently more definitive than the underlying stage of development would justify. Borrowing the technology readiness level (TRL) framework increasingly advocated for bioprinting and other regenerative-medicine technologies, in which a numeric scale distinguishes basic proof-of-concept from validated, scaled, and clinically qualified processes, every artificial ovary platform reviewed in this section sits at an early- to mid-range readiness level: strong evidence of biological function in small-animal models, but no platform has yet been manufactured to a reproducible, quality-controlled specification, validated in a large-animal or human-scale model, or evaluated for the manufacturing and regulatory pathway it would need to reach clinical use (123, 124). Distinguishing this stage explicitly from the sociology-of-expectations problem that affects adjacent fields, where preclinical progress is regularly, and prematurely, described as being close to clinical translation, is itself part of the discipline this review has tried to maintain throughout (124).

Manufacturing scalability is a related but distinct problem from biological proof of concept. A fibrin or alginate construct that performs well when hand-fabricated in small batches for a mouse study does not automatically translate into a process that can be manufactured reproducibly, under Good Manufacturing Practice (GMP) conditions, at the scale and consistency a clinical trial or approved product would require; batch-to-batch variability, already flagged above as a limitation of natural and decellularized materials specifically, is as much a manufacturing problem as a biological one, and is addressed further from an economic perspective in below. Safety evaluation for artificial ovary constructs intended for patients with a history of malignancy must additionally incorporate the malignant-

Table 5. Representative preclinical outcomes of biomaterial-based artificial ovary and endocrine-restoration constructs

Scaffold material	Species/model	Follicle/tissue outcome	Endocrine/functional outcome	Reference
Fibrin (F12.5/T1, F25/T4)	Mouse, autologous follicle transplantation	Follicle survival and proliferation after transplantation	Not primary endpoint	(13)
Alginate (low concentration/molecular weight)	Mouse, <i>in vitro</i> culture to ovulation	Full follicle growth and maturation	Live, fertile offspring produced	(40)
3D-printed gelatin (30-60 degree pore angle)	Mouse, orthotopic implantation in sterilized hosts	82.8% follicle survival at 8 days (multi-contact pore geometry); graft vascularization	Restored ovarian endocrine function; natural mating; live, nursed pups	(16)
PEG-VS hydrogel (RGD/MMP-sensitive)	Mouse, orthotopic implantation	Multiple mature antral follicles/corpora lutea by day 30; 60% of pool retained as dormant primordial/primary follicles	Restored ovulatory cycling; normalized FSH for at least 60 days; graft vascularization	(15)
PEG-Dual immunisolating capsule	Mouse, allogeneic graft in immunocompetent, sensitized hosts	No lymphocytic graft infiltration	Prevented donor-specific IgG sensitization (vs. approximately 26-fold rise in unencapsulated controls); sustained endocrine function	(18)
Chitin-based (CTP) hydrogel	Mouse, ovariectomized peritoneal implantation	Follicle growth comparable to/exceeding alginate controls <i>in vitro</i>	Restored ovarian endocrine function; partial protection against ovariectomy-induced bone loss	(46)
Immunisolating PEG capsule, human ovarian cortex	Immunodeficient mouse xenograft	Antral follicles up to approximately 3 mm observed in graft	Estrous cyclicity resumed approximately 12 weeks post-implantation; estradiol rose to levels comparable to non-encapsulated controls	(17)
Hyaluronic acid-based hydrogel (no exogenous growth factor)	Rat, autotransplantation	Increased follicle counts across developmental stages	Reduced FSH; increased graft VEGF expression	(42)
Decellularized human ovarian scaffold (SLES protocol)	Human tissue, <i>in vitro</i> /xenograft	Retained key ECM constituents; biocompatible, functional after implantation	Not primary endpoint	(106)

3D, three-dimensional; CTP, chitin-based polymer; ECM, extracellular matrix; FSH, follicle-stimulating hormone; IgG, immunoglobulin G; MMP, matrix metalloproteinase; PEG, polyethylene glycol; PEG-VS, poly(ethylene glycol)-vinyl sulfone; RGD, arginine-glycine-aspartic acid; SLES, sodium lauryl ester sulfate; VEGF, vascular endothelial growth factor

cell-contamination question introduced above, ideally with a validated, sensitive detection assay applied to the isolated follicle-cell population before encapsulation rather than relying on the encapsulation step itself as a safety barrier (12).

Finally, nearly every functional outcome summarized in Table 5 was obtained in mice, and translating scaffold designs validated at mouse scale to the considerably larger human ovary is not simply a matter of proportional scaling, as developed in greater length below. Diffusion distances, vascularization timescales, and the mechanical loads a construct must withstand during surgical handling all change disproportionately as construct size increases, so a pore geometry, fibrinogen-thrombin ratio, or crosslink density optimized in a mouse-scale construct cannot be assumed to transfer directly to a human-sized implant without independent re-optimization, even where the underlying biological mechanism is conserved across species.

Preclinical and translational applications for fertility and endocrine restoration

Endpoints relevant to fertility preservation versus endocrine restoration

It is worth distinguishing explicitly between two translational goals that are often discussed together, but that impose different design priorities and, correspondingly, different evidentiary bars. Fertility preservation requires a scaffold to support follicle growth through to a stage compatible with either natural ovulation and fertilization or retrieval for *in vitro* maturation and assisted reproduction; the demonstration of live, fertile offspring after alginate-encapsulated follicle culture (40) and after 3D-printed bioprosthetic ovary implantation (16) remain the clearest available proofs of concept for this endpoint. However, both were achieved in mice, and neither has yet been approached at human scale. Endocrine restoration, by contrast, requires only that a graft support sustained, physiologically regulated steroidogenesis, a substantially lower bar that has been met by a wider range of scaffold platforms, including PEG-based immunoisolated grafts (15, 16, 18) and HA- and chitin-based hydrogel grafts that were not designed with fertility as a primary endpoint (42, 46). Because these two goals sit at different points on the same translational pipeline, a platform that has only demonstrated endocrine restoration should not be read as one step away from demonstrating fertility preservation; the additional biological requirements, sustained folliculogenesis through to a fertilizable oocyte rather than steroidogenesis alone, are substantial and have so far only been met by a small subset of the platforms reviewed above.

This distinction matters clinically because the patient populations served by each goal differ. Endocrine restoration alone is relevant to a large population of women who have completed childbearing or do not desire fertility but would nonetheless benefit substantially from physiological rather than exogenous hormone exposure, given the well-documented cardiovascular, skeletal, cognitive, and quality-of-life consequences of premature estrogen deficiency (1). By contrast, fertility preservation is the primary concern for prepubertal and reproductive-age patients facing gonadotoxic cancer therapy. For this population, particularly those for whom malignant-cell contamination

precludes conventional autotransplantation, the artificial ovary strategies discussed above are most directly relevant.

Comparative outcomes across species

The overwhelming majority of the preclinical evidence reviewed in this article derives from murine models, reflecting the practical advantages of mouse studies, short reproductive cycles, extensive genetic tools, and low cost, rather than any inherent suitability of the mouse ovary as a model for human ovarian biomechanics or follicle biology. Where larger-animal or human-tissue data exist, they generally corroborate the broad conclusions drawn from mouse studies while also revealing scale-dependent complications that mouse models cannot fully anticipate. Nonhuman primate secondary follicles have been successfully matured in alginate-based encapsulated culture, extending the fundamental biological findings established in mice to a species with reproductive physiology considerably closer to that of humans (53, 125). Human ovarian follicles obtained from cancer patients have likewise been shown to grow and support oocyte development when cultured *in vitro* using the same encapsulated alginate systems (54). Short-term xenografting of human follicle and cortical tissue into immunodeficient mice, an experimental design used repeatedly in the fibrin-based artificial ovary literature, has shown that isolated human follicles survive and, in some cases, progress to the antral stage after xenotransplantation (74, 126). Most recently, human ovarian cortex from young donors has been shown to sustain folliculogenesis and cyclical steroidogenesis for months after encapsulation within an immunoisolating PEG capsule and xenotransplantation into immunodeficient mice, the most direct evidence to date that a synthetic biomaterial platform can support human ovarian tissue function at a level approaching the murine benchmark (17). Even these human-tissue studies, however, are xenografts in an immunodeficient mouse host rather than transplants into an intact human recipient, and the mouse-to-human translation limitations discussed above apply to the graft's physical scale and vascularization requirements regardless of tissue origin.

Positioning tissue-engineered constructs relative to conventional ovarian tissue cryopreservation

Any discussion of the translational status of biomaterial-based ovarian constructs must be situated against the considerable and continuing success of conventional ovarian tissue cryopreservation and autotransplantation, which, as noted in the Introduction, has now produced more than two hundred live births worldwide and reliably restores endocrine function in the substantial majority of recipients (127). A systematic review and individual patient data meta-analysis of both fresh and cryopreserved ovarian tissue transplantation confirms that this approach, despite the ischemic follicle loss discussed in the Introduction, remains clinically effective for most appropriately selected patients (128). None of the biomaterial-based constructs reviewed in this article has yet been tested in a comparably large or rigorously designed human cohort. The honest translational summary is that tissue-engineered scaffolds currently represent a complementary strategy targeted specifically at the subset of patients for whom conventional autotransplantation is contraindicated, chiefly those with a meaningful risk of ovarian malignant involvement,

rather than a wholesale replacement for cryopreserved tissue transplantation in the broader fertility-preservation population (88).

Notably, the literature on stem-cell and biomaterial-based therapeutic strategies for POI, cataloged alongside this review, identified only a single completed clinical trial that combined tissue engineering and biomaterials for POI treatment, despite dozens of registered stem-cell-alone trials. This underscores that the translational lag for scaffold-based approaches, specifically as distinct from cell-therapy approaches broadly, remains substantial. This lag reflects, in part, the additional regulatory complexity of combination products (cells plus a novel biomaterial device) relative to cell therapy alone, and, in part, the genuine scientific immaturity of the vascularization, immune-compatibility, manufacturing, and scale-up problems discussed in the following section.

Challenges, limitations, and future perspectives

Vascularization

Inadequate vascularization is arguably the single most consistently cited barrier to translating any of the scaffold systems reviewed above from small-animal proof-of-concept toward clinical utility. Because tissue-engineered constructs, whether cell-seeded scaffolds or acellular grafts intended for host cell infiltration, cannot survive on diffusion alone beyond a few hundred micrometers, rapid establishment of a functional vascular supply is prerequisite for follicle survival during the critical early post-implantation window (129). This requirement has motivated the co-transplantation strategies discussed in Section 7, most notably the incorporation of adipose-derived stem cells within fibrin-based artificial ovary implants specifically to accelerate neovascularization at the graft site (71, 72), as well as the selection of intrinsically pro-angiogenic matrix components such as fibrin and HA over comparatively inert alternatives such as unmodified alginate. Even scaffolds engineered with vascularization explicitly in mind, however, generally still show a substantial early loss

of the follicle pool, and no biomaterial system reviewed here has yet eliminated the ischemic-loss problem that originally motivated the search for tissue-engineered alternatives to whole-tissue autotransplantation.

Mechanical tuning and the stiffness-bioactivity trade-off

The relationship between scaffold stiffness and follicle developmental competence, introduced earlier and echoed across nearly every materials class reviewed above, remains only partially resolved. Lower-stiffness alginate formulations improve follicle growth and hormone output relative to stiffer formulations *in vitro* (38), yet sufficient mechanical integrity is simultaneously required to protect follicles during surgical handling and to withstand the compressive forces at the *in vivo* grafting site. This tension is made explicit by findings that decellularized ovarian ECM hydrogels, despite their biochemical authenticity, are generally too soft to use without mechanical reinforcement (33). No consensus target stiffness range has yet been established across the field, in part because the relevant native benchmark—the storage modulus of the ovarian cortex, on the order of a few kilopascals as measured by magnetic resonance elastography and rheological testing, itself varies with age, reproductive stage, and possibly ethnicity, none of which has been systematically incorporated into scaffold design criteria to date (25). Table 6 compares approximate mechanical properties of native ovarian tissue with the principal engineered scaffold systems discussed in this review, along with two illustrative composite systems from outside the ovarian literature that demonstrate the achievable tuning range for the same base polymers.

Immune compatibility and strategies for immune modulation

For any artificial ovary or endocrine-restoration construct intended to use allogeneic or xenogeneic tissue, donor ovarian cortex, decellularized xenogeneic ECM, or third-party cell sources, immune rejection represents a second major barrier, distinct from but related to, the vascularization problem discussed above. Implanted biomaterials of

Table 6. Approximate mechanical properties of native ovarian tissue compared with representative engineered scaffolds

Material/tissue	Approximate stiffness/storage modulus	Notes	Reference
Native human ovarian cortex	approximately 3.5 kPa (order of magnitude)	Benchmark for engineered scaffold design; varies with age and reproductive stage	(25, 33)
Decellularized ovarian ECM hydrogel (unsupplemented)	Lower than native cortex	Generally insufficient alone to support follicle survival <i>in vivo</i>	(33)
Decellularized ECM+alginate composite	Tunable toward native range via alginate content	Standard strategy to reconcile bioactivity with mechanical adequacy	(33, 103)
Alginate hydrogel, low concentration/low molecular weight	Softer than high-concentration formulations	Favors follicle growth, antrum formation, and hormone output <i>in vitro</i>	(38)
PEG-VS hydrogel (7%, RGD/MMP-sensitive)	Tunable via crosslink density	Engineered specifically to balance mechanical support against enzymatic degradability	(15)
Electrospun PCL fiber mat	Higher stiffness, fiber-architecture-dependent	Mechanical properties additionally modulated by fiber alignment (patterned vs. random)	(84)
Alginate-bioceramic-graphene composite (non-ovarian, bone context)	Tensile strength increases systematically with filler loading (0–3 wt%)	Illustrates the achievable tuning range for alginate-based composites when a stiff filler is added; not yet tested in an ovarian construct	(58)
Alginate-hyaluronic acid-nanoparticle composite (non-ovarian, orthopedic context)	Measurable gains in tensile strength and hardness versus unmodified polymer	Illustrates that pairing alginate with the same hyaluronic acid used in ovarian scaffolds can add mechanical robustness without abandoning either material	(57)

ECM, extracellular matrix; HA, hyaluronic acid; MMP, matrix metalloproteinase; PCL, poly(ϵ -caprolactone); PEG, polyethylene glycol; PEG-VS, poly(ethylene glycol)-vinyl sulfone; RGD, arginine-glycine-aspartic acid; wt%, weight percent

essentially any composition provoke a foreign body response in which innate immune cells, principally macrophages, are recruited to the implant surface. The balance between pro-inflammatory (M1-like) and pro-healing (M2-like) macrophage phenotypes at that interface substantially determines whether the outcome is fibrous encapsulation and eventual graft failure or productive tissue integration (130). This foreign body response operates in parallel with, and is mechanistically distinct from, the adaptive, antigen-specific rejection of allogeneic or xenogeneic donor tissue that the PEG immunoisolation strategies discussed below are designed to prevent. A scaffold that solves one of these two immune problems does not automatically solve the other.

Two broad strategies for immune modulation have emerged in the wider biomaterials literature and are, to varying degrees, already reflected in the ovarian tissue-engineering constructs reviewed above. The first is immunoisolation, physically excluding host immune cells from the graft while permitting the diffusion of small molecules such as nutrients, oxygen, and hormones; this is the strategy underlying the PEG-based dual-layer capsules reviewed above, and it is the most extensively validated immune-modulation approach specifically within the ovarian literature, having been shown to prevent both antibody-mediated sensitization and CD8-positive T-cell infiltration of an allogeneic graft in immunocompetent mice (18). The second, more recently developed in the broader tissue-engineering field but not yet widely applied to ovarian scaffolds specifically, is active immunomodulation: engineering the scaffold's surface chemistry, topography, or degradation products to actively bias local macrophages and other immune cells toward a pro-regenerative rather than a pro-fibrotic phenotype, for example through controlled surface roughness or wettability, through the textured or released presentation of anti-inflammatory cytokines such as interleukin-4, or through peptide- or growth-factor-functionalized coatings that recruit regenerative rather than inflammatory cell populations (130, 131). Because most of this active-immunomodulation evidence base comes from bone, cardiovascular, and general implant contexts rather than from ovarian tissue specifically, it is best read as a set of design principles the field has not yet tested on an ovarian scaffold, rather than as an established solution.

Immunoisolation and active immunomodulation are not mutually exclusive, and combining them is a plausible near-term direction: a PEG-based capsule already engineered for immune exclusion could, in principle, be further functionalized with a pro-regenerative surface coating to encourage productive host integration at the capsule-tissue interface rather than fibrous walling-off, which is itself a form of foreign body response that could compromise nutrient and hormone exchange even where adaptive rejection is successfully prevented. However, immunoisolation of this kind necessarily limits the graft's exposure to host vasculature and immune-mediated remodeling signals, potentially working against the vascularization goals discussed above, and the balance between adequate immune protection, adequate host integration, and adequate long-term graft function has not yet been optimized for any single ovarian construct. Decellularization, discussed above, offers a partial and materially different solution to the same problem by removing the cellular and nuclear antigens most

responsible for graft rejection while retaining matrix-level bioactivity. Still, residual matrix-associated antigenicity and the theoretical risk of incomplete decellularization, residual DNA, or cellular debris, remain incompletely characterized for ovarian-specific protocols (30).

Scale-up from rodent to human dimensions

Nearly all mechanistic and functional data reviewed in this article come from murine models, and translating scaffold designs validated at mouse scale to the considerably larger human ovary raises problems that are not simply a matter of proportional scaling. Diffusion distances adequate in a mouse-scale construct become inadequate as construct size increases; vascularization must proceed over correspondingly longer distances and timescales; and the mechanical loads an implant experiences scale non-linearly with tissue volume. The relatively small number of studies using nonhuman primate tissue (53) or human tissue in xenograft models (17, 54, 74) represent important but still preliminary steps toward addressing this gap. The field would benefit from a more systematic intermediate-scale testing program, potentially using large-animal models with ovarian anatomy closer to human scale, before committing to first-in-human trials of any specific scaffold platform.

Standardization and outcome reporting

A cross-cutting methodological limitation, noted already above, is the absence of standardized outcome reporting across the artificial ovary and endocrine-restoration literature. Studies vary in which combination of follicle survival, hormone levels, estrous cycle resumption, histological follicle staging, and reproductive outcomes they report, and in the post-implantation time points at which they assess these measures, making direct quantitative comparison across scaffold platforms difficult even when the underlying biology is closely related. This heterogeneity compounds a related but distinct evidentiary problem: the studies compiled in this review are overwhelmingly small, single-laboratory, short-duration animal experiments, and they should not be weighted as though they carried the same evidentiary strength as a matched, adequately powered comparative study. Where this review has drawn a comparative conclusion, for example that fibrin outperforms alginate on bioactivity or that PEG immunoisolation prevents sensitization, that conclusion generally rests on one or a small number of studies from a single research group rather than on independent replication. Readers should weight the strength of each claim accordingly rather than assuming uniform evidentiary quality across the comparative tables in this review. The 2023 systematic methodological review of decellularized ECM scaffolds explicitly identified this heterogeneity as a limitation of the existing evidence base and called for more consistent methodology and outcome reporting in future studies (110). Establishing a minimum common outcome set, analogous to core outcome sets now used in many areas of clinical trial design, would materially improve the field's ability to compare scaffold platforms head-to-head and to prioritize the most promising candidates for further development.

Regulatory and manufacturing considerations

Essentially, every construct reviewed in this article that combines a biomaterial scaffold with living cells or tissue

constitutes a combination product from a regulatory standpoint, subject to a more complex approval pathway than either a cell therapy or a medical device alone, an added layer of complexity increasingly recognized across regenerative medicine generally and not unique to the ovary (124). Manufacturing reproducibility is a particular concern for the natural and decellularized materials discussed above, given their inherent batch-to-batch biological variability, a challenge documented outside the ovarian literature as well, where reproducible ECM-based biomaterials for organoid and tissue culture face comparable batch-consistency and characterization obstacles (132). In contrast, the synthetic materials discussed above offer better manufacturing consistency but, as discussed throughout, at some cost to bioactivity. Addressing this tension, through, for example, more rigorously characterized and quality-controlled natural biomaterial sourcing, or through synthetic materials functionalized to a standardized, reproducible degree of bioactivity, will likely be as important to eventual clinical translation as any purely biological advance in scaffold design. Table 7 summarizes the translational status, principal remaining challenges, and representative supporting evidence for each major scaffold platform discussed in this review.

Economic feasibility and cost-effectiveness of scaffold fabrication approaches

Cost-effectiveness has received comparatively little explicit attention anywhere in the ovarian tissue-engineering literature reviewed above. Yet, it will be a decisive factor in whether any biomaterial-based construct reaches routine clinical use, and the fabrication techniques reviewed above differ substantially on this axis. A broader review of scaffold-fabrication economics across tissue engineering, comparing techniques including solvent casting-particulate-leaching, gas foaming, freeze-drying, electrospinning, and additive-manufacturing methods such as 3D printing, concludes that simpler melt-processing and particulate-leaching routes remain the most cost-effective and reproducible options where their comparatively limited architectural control is acceptable. In contrast, additive-manufacturing techniques trade higher per-unit cost and equipment investment for architectural precision that casting-based methods cannot match (133). Mapped onto the ovarian-

specific techniques reviewed above, this trade-off recurs directly: bulk alginate or fibrin encapsulation is inexpensive and technically undemanding, which is part of why it remains the most widely used approach for *in vitro* follicle culture; 3D bioprinting offers the precise, follicle-scale pore geometry shown by Laronda and colleagues to determine survival outcomes (16), but at meaningfully higher per-construct cost and equipment and validation overhead; and decellularization sits between the two, inexpensive in raw material terms but labor- and quality-control-intensive because of the need to verify complete cell removal and to characterize batch-to-batch matrix composition.

Beyond the fabrication step itself, any construct that combines a biomaterial with living cells or tissue, which describes essentially every platform in Table 5, will also need to absorb the cost of GMP-compliant cell handling and, for allogeneic or xenogeneic material, of the immunoisolation or decellularization processing discussed above, costs that are not fully captured by comparing raw fabrication techniques alone. No published cost-effectiveness analysis specific to ovarian functional constructs currently exists, a notable gap given how often manufacturing cost is cited in the broader regenerative-medicine literature as a reason scientifically promising tissue-engineered products have failed to reach, or remain on, the market after regulatory approval. Extending the kind of formal cost-effectiveness modeling already applied to other tissue-engineered products toward the ovarian-specific platforms reviewed here, comparing, for example, projected per-patient cost of a fibrin-based artificial ovary against the cost of extended HRT or against the cost of conventional cryopreservation and autotransplantation where that option is available, would materially strengthen the case for translational investment in the platforms that perform best on both biological and economic grounds.

Future of ovarian tissue engineering

Extrapolating from the convergent design principles documented throughout this review, several directions appear best positioned to narrow the remaining translational gap over the next five to ten years. However, the timelines below should be read as plausible research milestones rather than firm commercial forecasts, given that much of this field's history, documented throughout this review, consists

Table 7. Translational status, principal remaining challenges, and representative supporting evidence by scaffold platform

Platform	Furthest demonstrated endpoint	Principal remaining challenge(s)	Representative evidence
Fibrin-based artificial ovary	Follicle survival/proliferation after autologous transplantation (mouse)	Vascularization speed; manufacturing scale-up to human cortical dimensions under GMP conditions	(13, 72)
Alginate-based follicle culture	Live, fertile offspring (mouse, <i>in vitro</i> culture)	<i>In vivo</i> revascularization and degradation control	(39, 40)
3D-printed/bioprosthetic scaffolds	Natural mating and live, nursed offspring after <i>in vivo</i> implantation (mouse)	Translation of pore-geometry principles to human-scale, reproducibly manufactured constructs	(16)
PEG immunoisolation	Sustained folliculogenesis/steroidogenesis with human tissue xenograft (immunodeficient mouse)	Validation in immunocompetent, human-relevant setting; balance of immune protection vs. vascularization	(15, 18)
Decellularized ECM composites	<i>In vitro</i> follicle development; early-stage recellularization	Mechanical reinforcement; standardized outcome reporting	(33, 110)
Chitin/chitosan hydrogels	Endocrine restoration in ovariectomized mice	Head-to-head comparison against fibrin/PEG systems; skeletal-endpoint efficacy	(46)

3D, three-dimensional; ECM, extracellular matrix; GMP, good manufacturing practice; PEG, polyethylene glycol

of proof-of-concept results that took considerably longer than initially anticipated to replicate at larger scale.

In the near term (roughly one to three years), the most tractable priority is methodological: adopting a minimum common outcome set across artificial ovary studies, as argued above, and initiating systematic, quantitative head-to-head comparisons of the leading platforms, including fibrin, alginate composites, PEG immunoisolation, and chitin-based hydrogels, using matched follicle-stage, species, and endpoint criteria. This is deliberately the least biologically ambitious of the priorities listed here, but it is also the one most within reach, using materials and techniques that already exist.

In the medium term (roughly three to seven years), the field is likely to see continued convergence toward hybrid, multi-material constructs that combine a decellularized or synthetic mechanical backbone with an actively immunomodulatory surface coating of the kind discussed above, alongside the first systematic large-animal validation studies bridging the mouse-to-human gap identified above. Organ-on-a-chip and microfluidic ovarian and broader female-reproductive-tract platforms, which integrate microfluidics, three-dimensional culture, and biomimetic scaffolds to reproduce hormonal regulation and follicle development with a degree of dynamic physiological control that static culture cannot provide, are a particularly active area of this convergence and are likely to serve, in the near term, primarily as higher-fidelity preclinical testing platforms for candidate scaffolds rather than as implantable constructs in their own right (134). Advanced biofabrication approaches that integrate bioprinting with decellularized-ECM bioinks using the printer for architectural control while relying on the decellularized component for biochemical fidelity represent a related medium-term convergence already anticipated by the fabrication comparison above.

In the longer term (beyond seven years), realistic clinical roadmaps for any of these platforms will depend less on further biological refinement than on resolving the manufacturing, regulatory, and economic questions raised above, together with the large-animal and, eventually, early-phase human safety and feasibility data that no platform reviewed in this article yet has. A construct intended purely for endocrine restoration, given its lower biological bar relative to fertility preservation, as discussed above, is plausibly the first to reach that stage.

Discussion

Reviewing the biomaterial-based ovarian tissue-engineering literature as a whole, several patterns emerge that are easy to miss when each materials class is considered in isolation. First, the field has converged, largely independently across natural, synthetic, and decellularized materials, on the same basic design tension between mechanical adequacy and biochemical bioactivity. Alginate is bioactive-poor but mechanically tunable, decellularized ECM is bioactive-rich but mechanically inadequate on its own, and PEG is mechanically programmable but essentially bioinert until functionalized (19). That three methodologically distinct research traditions arrived at structurally similar composite solutions, alginate-fibrin, alginate-decellularized ECM, RGD-functionalized PEG, and that the same reinforcement logic reappears in entirely non-ovarian composite systems built from the same base

polymers (57, 58), suggests this trade-off is a genuine feature of the underlying biology and materials science rather than an artifact of any single laboratory's approach. It is likely to remain a defining constraint on scaffold design going forward (135, 136).

Second, the evidence base is more mature for *in vitro* follicle culture than for *in vivo* grafting, and more mature for endocrine restoration than for fertility preservation as an *in vivo* endpoint. This is not a criticism of the field so much as a reflection of the practical difficulty of each successive step: an *in vitro* culture system need only support follicle growth under controlled, monitorable conditions, whereas an *in vivo* graft must additionally survive ischemia, evade or tolerate immune surveillance, and integrate with host vasculature, and a construct intended for fertility preservation must further support the full developmental arc through to a fertilizable oocyte rather than sustained hormone secretion (137). Readers should calibrate their expectations for near-term clinical translation accordingly: the demonstrated feasibility of alginate-encapsulated follicle culture producing live offspring, for example, is a landmark proof of concept, but it was achieved under laboratory conditions considerably more controlled than any human clinical scenario would permit, and scaling this specific result to a human application remains, at present, aspirational rather than imminent (138).

Third, direct head-to-head comparisons between competing scaffold platforms, fibrin versus PEG versus decellularized ECM versus chitin-based hydrogels, for example, are conspicuously rare in the literature reviewed here, largely because most research groups have specialized in one or two material systems and optimized within that system rather than benchmarking systematically against competitors (139). This is a reasonable research strategy at an early stage of a field's development. Still, it leaves clinicians and translational scientists without a clear, evidence-based basis for selecting among platforms for a specific patient population or indication, and coordinated, multi-site comparative studies could usefully address this evidence gap as the field matures (88). The comparative tables in this review (Tables 1 through 7) attempt to organize existing, non-comparative evidence into a structure that at least facilitates qualitative cross-platform comparison, but, as flagged above, they draw on studies with substantially uneven methodological rigor and sample size. They cannot substitute for genuine head-to-head experimental data.

Fourth, several scientific questions remain genuinely unresolved rather than simply understudied, and it is worth naming them explicitly rather than leaving them implicit in the challenges cataloged above. It is not yet established what combination of stiffness, degradation rate, and bioactive ligand density is jointly optimal for a human-scale construct, as opposed to the mouse-scale constructs from which nearly all current design parameters are derived. It is not yet established whether active immunomodulation, as opposed to passive immunoisolation, can be layered onto an already follicle-supportive scaffold without compromising the vascularization and nutrient exchange that follicle survival also requires. It is not yet established how much malignant-cell-contamination risk in artificial ovary constructs follicle isolation alone eliminates, rather than requiring a dedicated purging step, given that essentially all decontamination data to date come from artificially spiked tissue rather than

patients' own contaminated material (12). It is also not yet established which, if any, of the platforms reviewed here can be manufactured reproducibly and cheaply enough to be commercially viable, even if every remaining biological question were solved. These four gaps, more than any single materials comparison, define the research agenda this review points toward.

Finally, it is worth stating plainly that the translational gap between the most advanced preclinical results reviewed here and routine clinical availability remains substantial. This review has tried throughout to flag that gap rather than overstate how close any given platform is to clinical use (140). Encouraging mouse-model outcomes, including live births after alginate or 3D-printed scaffold encapsulation, sustained endocrine function after PEG immunoisolation, and restored ovarian function after chitin-based hydrogel grafting, are genuine and important scientific advances. Still, none of them yet constitutes evidence that a comparable outcome would be achieved in a human patient. The field would be better served by continued, honest emphasis on this distinction than by premature optimism (33, 141).

Conclusion

Biomaterial-based scaffolds for ovarian tissue engineering have evolved over roughly two decades from simple alginate encapsulation systems designed solely for *in vitro* follicle culture to a diverse ecosystem of natural, synthetic, composite, decellularized, and advanced-fabrication platforms capable of supporting follicle survival, hormone secretion, and, in the most advanced mouse-model demonstrations, natural reproduction after *in vivo* grafting. This review's central conceptual claim is that these achievements should be read through a single recurring constraint rather than as a list of independent materials successes: no single material or fabrication strategy currently satisfies bioactivity, mechanical adequacy, controlled degradation, immune compatibility, and manufacturability simultaneously, and the stiffness-bioactivity trade-off documented throughout this review is likely to continue shaping scaffold design for the foreseeable future rather than being solved by any single forthcoming material.

Judged against that standard, the most consequential recent developments are not necessarily the ones most often described as breakthroughs. Dual-layer immunoisolating PEG capsules capable of protecting allogeneic and xenogeneic ovarian tissue from immune rejection matter less for the specific hormone-cycling outcome they have achieved than for demonstrating that passive immune protection and follicle-supportive matrix remodeling can coexist within a single engineered construct. Decellularized-alginate composites matter less for any single follicle-culture outcome than for confirming, across multiple independent groups, that biochemical authenticity and mechanical adequacy genuinely trade off against each other rather than being separable design axes. Patterned electrospun and 3D-printed constructs matter less for the specific pore angles tested to date than for establishing that architecture, independent of chemistry, is itself a primary determinant of follicle survival. Read this way, convergent, combinatorial materials engineering, rather than the emergence of a single superior biomaterial, is the mechanism most likely to close the remaining gap between mouse-model proof of concept and clinical application. Future work that continues

to search for a single best material is, on the evidence assembled in this review, less likely to succeed than work that treats combination and hybridization as the goal.

Priorities for the next phase of research follow directly from the unresolved questions named in the discussion and the near-, medium-, and longer-term roadmap set out above: standardized, multi-endpoint outcome reporting to enable genuine cross-platform comparison rather than the qualitative synthesis this review has had to rely on; systematic intermediate-scale, large-animal validation before further first-in-human attempts, given how much of the existing evidence base rests on murine models whose relevance to human ovarian scale remains unproven; continued refinement of vascularization strategies and of active, not merely passive, immune-modulation approaches, since inadequate revascularization and unresolved immune compatibility remain the most consistently cited causes of early graft failure across nearly every platform reviewed; validated, sensitive malignant-cell-decontamination protocols wherever artificial ovary constructs are intended for patients with a history of hematologic malignancy; and sustained, quantitative attention to the manufacturing cost and regulatory pathway that will ultimately determine whether a scientifically promising scaffold can be produced reproducibly and affordably enough for clinical use, a question this field has so far addressed far less rigorously than the underlying biology. With these priorities addressed, biomaterial-based ovarian tissue engineering is well positioned to become a clinically meaningful complement to existing fertility-preservation and hormone-replacement strategies for women affected by POI and gonadotoxic cancer therapy: first for endocrine restoration and, on a longer horizon, for fertility preservation in the specific patient populations for whom conventional autotransplantation is not a safe option.

Highlights

- The native ovarian extracellular matrix is compartmentalized and stage-dependent, changing systematically in collagen IV, laminin, perlecan, and proteoglycan composition as follicles progress from the primordial to the antral stage, providing the biomechanical rationale for scaffold design.
- Every biomaterial and fabrication strategy reviewed here negotiates the same underlying trade-off between mechanical adequacy and biochemical bioactivity; this trade-off, rather than any single material's limitations, is the organizing constraint of the field.
- Natural biomaterials (collagen, alginate, HA, fibrin, silk fibroin, chitosan/chitin) offer strong bioactivity and biocompatibility but generally require composite reinforcement, a strategy with quantitative precedent in non-ovarian tissue-engineering composites, to achieve adequate *in vivo* mechanical performance.
- Synthetic polymers, particularly PCL, PEG, and PLGA, provide tunable, reproducible mechanical and degradation properties and, in the case of PEG, a platform for immunoisolation of allogeneic and xenogeneic ovarian tissue. Active immunomodulation of scaffold surfaces is a complementary, largely untested strategy to improve long-term graft integration.
- Decellularized ovarian ECM preserves native biochemical composition but typically requires mechanical reinforcement, most often with alginate, to support follicle

survival.

- Three-dimensional bioprinting and electrospinning enable follicle-scale architectural control, and pore geometry and fiber alignment independently affect follicle survival and developmental competence.
- The artificial ovary concept, built primarily on fibrin-, alginate-, and PEG-based platforms, offers a route to fertility preservation for patients for whom conventional ovarian tissue autotransplantation is contraindicated due to malignancy risk; residual malignant-cell contamination and technology-readiness gaps remain open safety and manufacturing questions.
- Vascularization, immune compatibility, mechanical tuning, manufacturing cost, and rodent-to-human scale-up remain the principal unresolved challenges separating current preclinical constructs from clinical application.

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Ethics Approval and Consent to Participate

Not applicable. This article does not report any new studies with human participants or animals performed by any of the authors.

Authors' Contributions

The author conceived and conducted the literature review, drafted and critically revised this manuscript, and approved the final version for submission.

Conflicts of Interest

The authors declare no competing interests.

Declaration

For full transparency, the authors declare that artificial intelligence tools were used during the preparation of this article; specifically, the Claude model (developed by Anthropic) was used for grammatical corrections and polishing the draft text.

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Corrected Proof