pISSN 1738-2696 · eISSN 2212-5469
http://dx.doi.org/10.1007/s13770-016-9107-0
FEATURE ARTICLE

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Efficient Biomaterials for Tissue Engineering of Female Reproductive Organs

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Current investigations on the bioengineering of female reproductive tissues have created new hopes for the women suffering from reproductive organ failure including congenital anomaly of the female reproductive tract or serious injuries. There are many surgically restore forms that constitute congenital anomaly, however, to date, there is no treatment except surgical treatment of transplantation for patients who are suffering from anomaly or dysfunction organs like vagina and uterus. Restoring and maintaining the normal function of ovary and uterus require the establishment of biological substitutes that can cover the roles of structural support for cells and passage of secreting molecules. As in the case of constructing other functional organs, reproductive organ manufacturing also needs biological matrices which can provide an appropriate condition for attachment, growth, proliferation and signaling of various kinds of grafted cells. Among the organs, uterus needs special features such as plasticity due to their amazing changes in volume when they are in the state of pregnancy. Although numerous natural and synthetic biomaterials are still at the experimental stage, some biomaterials have already been evaluated their efficacy for the reconstruction of female reproductive tissues. In this review, all the biomaterials cited in recent literature that have ever been used and that have a potential for the tissue engineering of female reproductive organs were reviewed, especially focused on bioengineered ovary and uterus.

Tissue Eng Regen Med 2016;13(5):447-454

Key Words: Tissue engineering; Biomaterial; Ovary; Uterus

INTRODUCTION

Physiologic functions of female reproductive system can be lost permanently by various acquired disorders and congenital defects, which can lead to infertility or subfertility. These permanent acquired dysfunctions caused by primary and secondary factors. Reproductive organ neoplasia or their current treatment methods such as chemotherapy, radiotherapy [1,2] or surgically total or partial removal of reproductive tissues [3], adhesions or fibrosis and age-related dysfunctions [4] are the most important instances for the cessation of natural function of ovaries, uterus or vagina.

Different strategies have been developed or currently are translationally evaluated in *in-vivo* or *in-vitro* models for the treatment of infertility in women suffering the aforementioned illnesses. For the restoration of ovarian function, various hormonal therapies, assisted reproductive technologies including tissue freezing and auto- or xeno-transplantation, *in-vitro* maturation and fertilization of oocytes [5], and developing of potential artificial ovaries have been investigated in the recent decade. Due to the more complex anatomical and histological nature and extensive structural alterations in different physiological situations such as puberty, estrous or menstrual cycle, and pregnancy, uterine replacement or functional restoration seems to be complicated. Various efforts on this subject with different success rates have been done including hormone therapy [6], uterine auto- and allotransplantation [7], cell therapy of endometrial layer [8], and *in-vivo* [9] and *in-vitro* [10] production of artificial uterus.

Regarding the progress in the female infertility therapy, reproductive bioscience has opened a new window in the field of regenerative medicine and tissue engineering for designing and development of artificial or bioengineered reproductive tissues. Ovary, uterus, and cervix can be constructed by applying biomaterial scaffolds, primary or conditioned cells and pharmaceutical agents. Biomaterial matrices can be divided into two

Received: November 6, 2015
Revised: December 18, 2015
Accepted: December 21, 2015
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categories of natural and synthetic biomaterials. This review describes the recent advances in the field of searching for efficient biomaterial matrices for the construction of functional female reproductive organs.

OVARY AND ITS FUNCTION

The most intriguing part of women reproductive system is the pair of ovaries and their two main roles are ovum production and endocrine function. The main function of human ovaries after birth and then after puberty is the stationary storage of oocytes within the environment provided by follicular cells, which contributes to the development of follicles and periodical release of oocytes. After puberty, the endocrine function of ovaries start and estrogen, testosterone and progesterone are secreted [11]. A complex set of signals including endocrine hormones and paracrine factors, components of extracellular matrix and mechanical signaling are exchanged between follicles and their environment. These highly dynamic interactions lead to both folliculogenesis process which include recruitment, selection and ovulation and cyclic reproductive endocrine alterations [12]. This endocrine and cellular orchestration is established in a finely tuned microenvironment. Construction of degradable component which mimics the ovarian extracellular matrix is critical to support the growth of human follicles since their volume increase greater than 106-fold *in-vivo* [13]. During folliculogenesis, human primordial follicles (30 μm) grow to around 600 times its size to ovulate (18–24 mm) [14].

The extracellular network of ovarian tissue consists of various proteins and glycoproteins including collagens, fibronectin and laminin. The collagen distribution is consistent throughout the period of follicle maturation. Higher concentrations of collagen type I was observed in the follicular compartments and surface epithelium and collagen type IV in the theca cell compartment [15]. Throughout follicular development, fibronectin, a high-molecular weight glycoprotein, increased in the stroma and theca cell compartment and follicular fluid of antral follicles [15]. Laminin localized to the theca cell compartment and basement membrane of follicular granulosa cells [15].

BIOENGINEERED OVARY

To develop a so-called 'artificial' ovary using biomaterials, *in-vitro* and *in-vivo* evaluations should be performed, which are summarized in Figure 1. Although, tunable microenvironments are employed to investigate the basic biology of ovarian or follicular development [16], they can be used to develop therapeutic strategies for the organ failure or tissue loss in the field of reproductive regenerative medicine. For the purpose of

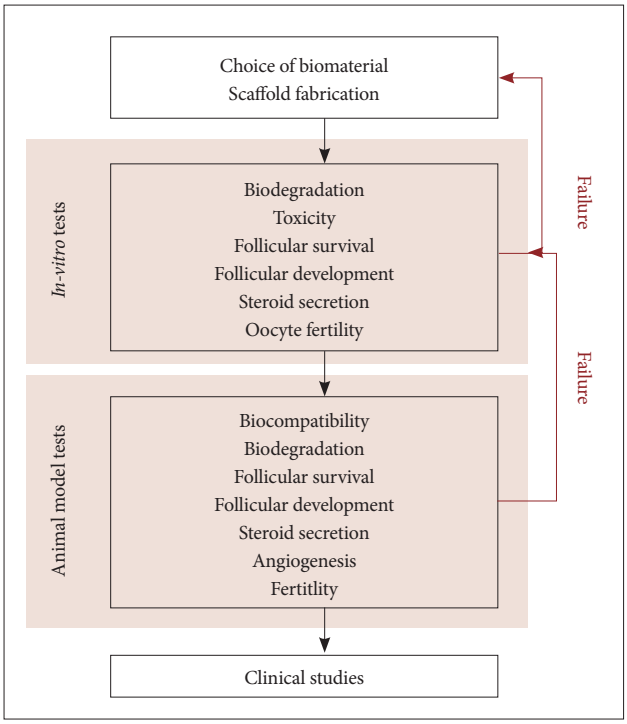

Figure 1. Stages for evaluation of ovarian tissue engineering.

providing support for growth of follicles and ovarian stromal cells and attachment within a three-dimensional structure, various biomaterials with different chemical and mechanical characteristics are introduced (Table 1). Sequestration or presentation of biological signals and mechanical strength are the properties of biomaterials can mimic the ovarian tissue [17]. Materials currently used in the field of female reproductive regenerative medicine for the repair and regeneration of ovarian function are based on either natural polymers isolated from animal or human tissues including alginate, gelatin, collagen, fibrin and hyaluronic acid, or synthetic molecules such as polyethylene glycol, polydimethylsiloxane and silica (Fig. 2). Natural biomaterials for three-dimensional bio-printing have the advantages of similarity to human extracellular matrix and inherent bioactivity while synthetic polymers have poor biocompatibility and loss of mechanical properties and toxic products during degradation [18]. However, synthetic polymers can be fit and suit with specific applications and the hydrophilic ones can be used as a hydrogel scaffold [18]. The natural and synthetic biomaterials which can be applied to the development of bioengineered ovary are introduced as follows.

Alginate

Alginate, also called algin or alginic acid, which is derived from brown algae is an anionic polysaccharide which form a hydrogel through binding with water [19]. The alginate scaffold is a

Table 1. Ideal properties of biomaterials for reproductive tissue engineering

Properties	Descriptions
Biocompatibility	Without undesirable local or systemic immune system responses
	Biological and functional contribution in the construct
Degradation kinetics	Adjusted to the extracellular matrix secretion ability of the cells
	Allow exponential growth of ovarian follicles or fetus
Degradation byproducts	Byproducts should not be toxic
Mechanical properties	Demonstrate appropriate mechanical characteristics
Biomimicry	Designing based on the compositions of specific endogenous material

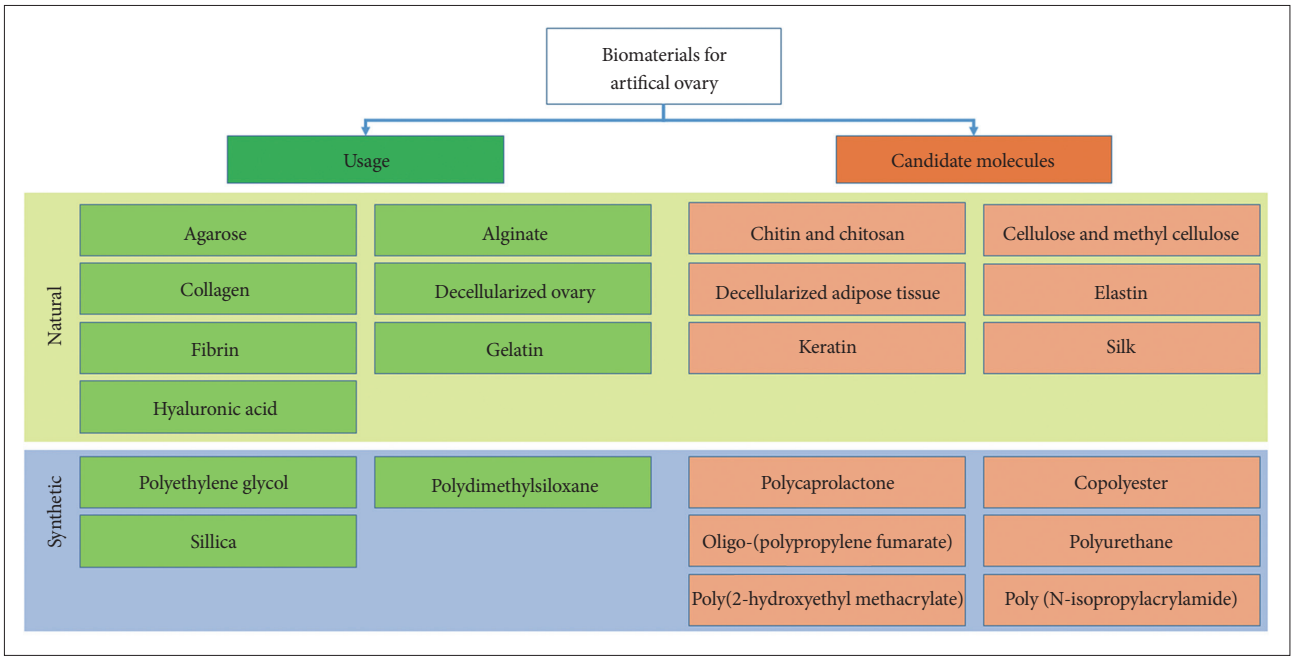

Figure 2. Appropriate biomaterials for ovarian tissue engineering.

hydrophilic, biocompatible, mechanically stable matrix and can provide better yielding human primordial follicles in organ culture than on matrigel scaffold [20,21]. Alginate provides a safe hydrogel matrix to handle and cryopreserve isolated human follicles. Primordial/primary follicles were isolated from human ovaries were embedded in alginate and *in vitro* cultured for 7 days [22]. The most permissive alginate hydrogels for follicle culture formed at a concentration of 0.25% w/v [23]. Cultured oocytes in alginate matrix fertilized *in vitro* and embryo transferred individuals were fertile [24]. Ascorbic acid combined alginate hydrogel [25] or mouse embryonic fibroblasts co-cultured in hydrogel [26] improves the survival of primary mouse ovarian follicles. Furthermore, an alginate-matrigel matrix was used in mice as a scaffold for ovarian cells and after *in vitro* culture [27] and heterotopic autografting, vascularization was observed with a low inflammatory response [14].

Fibrin

In the process of blood coagulation, a fibrous, non-globular protein is called fibrin polymerizes a network for trapping the cells. In the presence of calcium and with the enzymatic action of thrombin, fibrinogen, a soluble protein, is polymerized into fibrin by forming the covalent bonds of lysyl-glutamine [28]. The rigidity and morphology of the fibrin network depending on the proportions of fibrinogen and thrombin concentrations and can influence cell proliferation [29].

Fibrin matrix was developed for producing an 'artificial' ovary. For designing one, ovarian stromal cells were encapsulated in the fibrin clot and different concentrations of fibrinogen and thrombin were evaluated [30]. In the other study, mice pre-antral follicles were encapsulated in fibrinogen and thrombin (with proportion of 12.5/1 and 25/4) which were mixed with ovarian cells [31]. A 15-μL droplet formed a clot which contains 15 follicles after 45 min and then autotransplanted to inner side of the

mice peritoneum. After one week, angiogenesis and follicular development and recovery rate of 31% were reported. Fresh cells of medulla of human ovary are the suggested source of stromal cells for artificial ovary construction using fibrin scaffold [32]. Beside the advantages of fibrin matrix, it is shown that stromal cells produce plasminogen activators which induces fibrinolysis [33] and may lead to degradation and shrinkage of clots *in-vitro* [30]. As alginate hydrogel is not degradable, a compressive force on the follicle increase by increasing the follicle diameter leads to affect follicle growth [34]. On the other hand, a combination of fibrin and alginate provides a dynamic mechanical environment. Proteases secreted by the growing follicle degrade fibrin in the fibrin-alginate matrix leaving only alginate to provide support [35]. Matrices of fibrin-alginate and fibrin-alginate-matrigel were successfully used for the culture of ovarian cells and ovarian follicles. Aprotinin, a protease inhibitor, is used to prevent the fibrin degradation [36].

Fibrinogen and polyethylene glycol

Polyethylene glycol is a non-toxic and biologically inert material with highly hydrophilic characteristics. Polyethylene glycol can be easily functionalized and is used for modifying proteins and glycoproteins in combination with soluble fibrinogen [37]. The polyethylene glycol-fibrinogen hydrogel network can provide a structure for various cells culturing and prevents biodegradation of materials [38]. Ovarian slices were cultured in polyethylene glycol-fibrinogen hydrogel had more primordial follicles and follicular growth and less atretic follicles in comparison with ones on alginate scaffolds [39].

Collagen

Type I collagen is the normal constituent of extracellular matrix of ovarian tissue with higher concentrations in the follicular compartments and surface epithelium [15]. A three-dimensional type I collagen scaffold is used for culture of granulosa cells and after grafting into immunodeficient mice ovaries, spherical structures of granulosa cells exhibited steroidogenesis and localized within antral follicles [40].

Gelatin

Partial hydrolysis of collagen forms a mixture of peptides and proteins which is called gelatin. Gelatin is extracted from the bones, skin, and connective tissues of cattle, horses, pigs, chicken, and fish. Gelatin cryogel is used to grow human ovarian follicular fluid cells from waste follicular liquid during *in-vitro* fertilization [41]. The cells were grown on the top of biomaterial and also in the layers below 60 mm of deepness [41]. A three-dimensional printed gelatin scaffold was developed based on the extracellular matrix porosity and composition of decellularized human and bovine ovaries [42]. Ovarian follicles were seeded and somatic cells along the follicle periphery adhered to the scaffold [43]. By increasing one follicle adhesion point to the gelatin scaffold, oocyte survival increased [43]. Human chorionic gonadotropin and luteinizing hormone could

Table 2. Potential biomaterial can be used as an extracellular matrix for ovarian tissue bioengineering

Biomaterial	Description	Reference
Chitin	A glucosamine polysaccharide, made by treating crustacean shells	[65]
Chitosan	Deacetylated chitin	[66]
Cellulose	Porous bacteria polysaccharide foam with pure cellulose nanofiber mesh excreted extracellularly by the *Acetobacter xylinum* bacteria	[67]
Methyl cellulose	Cellulose with hydroxyl and methyl groups to disrupt crystallinity of cellulose and provide its solubility	[68]
Decellularized adipose tissue	Enzyme-solubilized decellularized adipose tissue	[69]
Elastin	A key structural protein found in the extracellular matrix and interacts with cells through specific biochemical mechanisms	[70]
Keratin	Intermediate filament protein of eukaryotes	[71]
Silk	A biopolymer mostly derived from silkworm and spiders	[72]
Polycaprolactone	A low melting point biodegradable polyester	[73]
Copolyester	Poly butylene/diethylene glycol succinate with elastomeric and hydrolytic properties	[74]
Oligo-(polypropylene fumarate) and oligo(poly(ethylene glycol) fumarate)	Linear polyesters based upon fumaric acid	[75]
Polyurethane	Its foam has a tunable damping properties and controllable degradation rate and supports cellular infiltration and generation	[76]
Poly(2-hydroxyethyl methacrylate)	Non-degradable polymer modifiable for degradation	[77]
Poly(N-isopropylacrylamide)	A temperature-responsive polymer	[78]

induce maturation and ovulation in primary and secondary follicles, oocytes resumed meiosis *in-vitro* and follicles secreted estradiol [42,43].

Silica

Silicon dioxide, also known as silica is used as a hydrophilic, biocompatible, mechanically strong, pore-size controllable and thermal stable and microbial resistant scaffold [44]. Based on these properties, silica matrices of tetraethoxysilane and tetrakis (2-hydroxyethyl) orthosilicate are applied for encapsulation of rat mature ovarian follicles and steroid hormones are secreted by the follicles [45].

Agarose and polydimethylsiloxane

Agarose is a polysaccharide polymer biomaterial which is extracted from seaweed [46]. Polydimethylsiloxane is a polymeric organosilicon. Theca cells were seeded into an agarose-polydimethylsiloxane mold containing honeycomb-shaped wells and spheroids of granulosa cells or cumulus oophorous complexes were seeded and cultured successfully in the openings of the theca cell honeycomb of human artificial ovary model [47].

Decellularized ovary

Decellularized ovary can be used as an organ skeleton of extracellular matrices by removing cellular material and can be reseeded with ovarian cells [48]. This biomaterial can be suggested for the patients suffered from ovarian cancers after ovariectomy. Transplantation of recellularized bovine and human extracellular matrices of ovary with primary ovarian cells into mice produced estradiol *in vivo* and caused puberty initiation [48].

Hyaluronic acid

Hyaluronic acid, an anionic, nonsulfated glycosaminoglycan, is widely distributed throughout epithelial and connective tissues [49]. It is used with tissues or cells of implants for regeneration [50]. Ovarian follicles were encapsulated and cultured three-dimensionally in a tyramine-based hyaluronic acid hydrogel [51].

Other potential biomaterials

Based on ovarian tissue structure and function other biomaterials individually or in combination with the others can be used for development of 'artificial' ovary. Table 2 introduces a group of natural and synthetic biocompatible materials which have been used for the conventional tissue engineering of other organs and have a potential for the use for bioengineered ovary as extracellular matrix.

UTERUS AND ITS FUNCTION

Most female mammals including women have a hormone-responsive reproductive organ with hollow tube shape characteristics where fetuses develop during the gestational period. Several function of uterus should also be substantiated such as providing an environment for egg fertilization, embryo implantation, fetus nourishment and development, hormonal control of luteal function and directing blood flow to the other part of reproductive system as a sexual response. During pregnancy, the human uterine weight and size change exponentially (from 60 g to 1 kg). These alterations during normal life cycle of women and the expected functional aspects restrict application of available biomaterials.

BIOENGINEERED UTERUS

Either the uterine absence or non-functional uterus is the indication of applying artificial uterus for the treatment of uterine infertility. Patients with congenital anomalies, or acquired causes such as hysterectomy due to malignant diseases or intrauterine adhesions are candidate of transplantation of artificial uterus [52,53]. However, for genetic motherhood, other methods such as uterine allotransplantation [54] or surrogacy [55] are among the approaches for solving the issue, but ethical, social, technical safety, and tissue rejection are the challenges of these methods. Theoretical devices were introduced or patented which allow for extrauterine fetal incubation or extracorporeal pregnancy. Additionally, there are limited number of studies which evaluated the function of two categories of artificial uterus, *in-vitro* and *in-vivo*. In both models, many a biomaterial was applied in their construction.

Biomaterials with biocompatibility and non-toxic property such as cellulose and its polymer derivatives, collagen polyalpha esters, fluorinated polyethylene, phenolic polymers, poly-4-methylpentene, polyacrylate, polyacrylonitrile, polyamide derivatives, polyanhydrides, polybenzoxazole, polycarbonate, polycyanoarylether, polyester derivatives, polyether derivatives, polyethylene, polyfluoroolefin, polygalactin, polyglycolic acid, polylactate acid, polyolefin, polyoxadiazole, polyphenylene derivatives, polypropylene, polystyrene, polysulfide, polysulfone, polytetrafluoroethylene, polytriazole, polyurethane, polyvinyl derivatives, silicone, and urea-formaldehyde were introduced for the construction of artificial uterus [56]. Despite many studies on the bioengineering uterine tissues, achieving to a level of complete functional uterus was very rare.

Collagen

Uterine horn of rat was reconstructed by a collagen scaffold

membrane loaded with basic fibroblast growth factor and transplanted to a severely damaged model [57]. The delivery of basic fibroblast growth factor in the scaffold improved the regenerating abilities of endometrial and myometrial cells with vascularization and a pregnancy was resulted in rats [57].

Peritoneal-derived myofibroblast-rich capsule

Peritoneal-derived myofibroblast-rich capsule was developed over a two-week period by the insertion of non-adherent boiled blood clots or polyethylene bulbs into peritoneum of rat and rabbit based on the mechanism of inflammatory response [53]. After autotransplantation of the myofibroblast-rich capsule into uteri, pregnancy occurred in the grafted horn [58].

Decellularized uterine matrix

Aortic detergent perfusion was performed for uteri decellularization of rat, which leads to the formation of an acellular, perfusable vascular architecture together with extracellular matrix of uterus [59]. Decellularization of rat uterus can be done using either sodium dodecyl sulfate or high hydrostatic pressure [9,60]. The matrix was then recellularized for 10 days *in-vitro* in a bioreactor using rat uterine cells and mesenchymal stem cells and transplanted and showed a comparable pregnancy results to those of intact uteri [59]. In the other investigations, direct and partial transplantation decellularized uterine matrix to the rat uteri revealed the regeneration of three-layer structure 30 days after engraftment [60].

Microfluidic chip

Microfluidic chip is a technique which provides both microchannels with similar sizes to those of the microvessels and micro-scale laminar flow of fluid. Artificial uterus was designed to develop mouse zygote to day 8 of implantation using a microchip [10]. For this purpose a three-layer microchip was developed which consists of the first polydimethylsiloxane layer, the second layer of a porous polycarbonate membrane which covered by gelatin and endometrial cells and the third polydimethylsiloxane layer [10].

BIOENGINEERED CERVIX

In the female reproductive tract, the cervix is the lower part of the uterus. Patients with cervical agenesis or cervical dysgenesis such as obstruction of the cervical ostium of an intact cervical body, fibrous band in cervical body or cervical fragmentation are candidate for cervical reconstruction [61]. Recently, acellular porcine small intestinal submucosa, an extracellular matrix-based collagen material, is clinically grafted in women for cervico-vaginal reconstruction during the end of menstruation [62,63]. Also using a silk protein sponge scaffold, isolated cervical cells from premenopausal women undergoing hysterectomy were seeded to construct an artificial cervix [64].

CONCLUSIONS AND FUTURE PERSPECTIVES

To date, numerous different methodology and biomaterials have been investigated for the construction of artificial ovaries in animal models and short-term recovery of ovarian function such as estradiol secretion have been evaluated. Broader category of biomaterials, especially in combination forms with different ovarian cells need to be designed and developed. The future step of ovarian tissue bioengineering should include the physiological ovarian function such as fertile ovulation, corpus luteal formation and progesterone secretion and long-term evaluation of grafted biomaterials. In comparison with ovary bioengineering, artificial uterine tissue construction is at the early stage of development. Based on the current knowledge of three-dimensional cell culture, novel materials, partial or whole uterine transplantation, new horizons can be explored for the functional uterine construction.

Acknowledgements

This research was supported by grants from the Korean Health Technology R&D Project, Ministry of Health & Welfare (HI14C2259 and HI14C2289), Republic of Korea.

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

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