



Review

Scientific principles of regenerative medicine and their application in the female reproductive system

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ABSTRACT

The goal of regenerative medicine is to repair, replace, or regenerate diseased tissues/organs in order to restore normal function. In this paper we will first discuss the general principle of regenerative medicine and the various techniques and approaches that have been used to replace or regenerate cells in diseased tissues and organs. Then, we will review different regenerative medicine approaches that have been used to treat specific diseased tissues and organs of the reproductive system in both animal and human experiments. It is clear from this article that regenerative medicine holds significant promise, and we hope that the review will serve as a platform for further development of regenerative medicine technologies for the treatment of inadequacies of the reproductive system.

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1. Introduction

Regenerative medicine represents a field in the health sciences that aims to repair, replace or regenerate human cells, tissues, or organs in order to restore normal function [1]. The field of regenerative medicine encompasses various technologies that range from tissue engineering to cloning, as illustrated in Fig. 1. Tissue engineering combines the principles of cell transplantation, material science, and bioengineering to develop new biological substitutes that may restore and maintain normal organ function. Tissue engineering strategies generally fall into two categories: (i) the use of acellular matrices, which serve as guides for proper orientation and direction of new tissue growth but depend on the body's natural ability to regenerate, and (ii) the use of the matrices seeded with cells. Acellular tissue matrices are usually prepared by manufacturing artificial scaffolds, or by removing cellular components from tissues via mechanical and chemical manipulation to produce collagen-rich matrices [2,3]. These matrices tend to slowly degrade after implantation and are generally replaced by the extracellular matrix proteins that are secreted by host cells that infiltrate the initial matrix implant.

Cells can also be used for therapy via injection, either with a carrier such as a hydrogel, or alone. In addition cells can be used for matrix-based tissue engineering strategies. For this purpose, a small piece of donor tissue is dissociated into individual cells. The cells are either implanted directly into the host, or they are expanded in culture, attached to a support matrix, and then the cell-matrix construct is implanted into the host. The source of donor tissue can be heterologous (xenogeneic source such as bovine), allogeneic (same species, different individual), or autologous. Ideally, both structural and functional tissue replacement will occur with minimal complications. Autologous cells derived from a biopsy of tissue are the preferred type to use. They are obtained from the host, dissociated from the tissue biopsy, and expanded in culture. Next, they are implanted back into the same host. The use of autologous cells, while it may cause an initial inflammatory response, avoids rejection, and thus the deleterious side effects of immunosuppressant medications can be avoided. Thus, most current strategies for tissue engineering depend upon a sample of normal autologous cells from the diseased organ of the host.

However, for many patients with extensive end-stage organ failure, a tissue biopsy may not yield enough normal cells for expansion and transplantation. In other instances primary autologous human cells cannot be expanded from a particular organ such as the pancreas. In such situations, stem cells are in vision as being an alternative source of cells from which the desired tissue can be derived. Stem cells can be derived from discarded human embryos (human embryonic stem cells), from fetal tissue, or from adult sources (bone marrow, fat, skin). Therapeutic cloning has also played a role in the development of the field of regenerative medicine. Recently the technology of induced pluripotency in which cells such as fibroblasts are "reprogrammed" to behave like stem cells has been described, and it may also provide a new source of cells for tissue-engineering strategies.

2. Biomaterials for use in tissue engineering

In many cell based tissue engineering methods, cells are obtained from the tissue, expanded *in vitro*, and then seeded onto a scaffold composed of an appropriate biomaterial. The biomaterials replicate the biological and mechanical function of the native extracellular matrix (ECM) found in tissues in the body by serving as an artificial ECM. Biomaterials also provide a three-dimensional scaffold for the cells to adhere to and form new tissues with appropriate structure and function. They also allow for the delivery of cells and appropriate bioactive factors to desired sites in the body. Bioactive factors, such as cell-adhesion peptide and growth factors, can be loaded along the cells to help regulate cellular function. As the majority of mammalian cell types are anchorage dependent and will die if no cell-adhesion substrate is available, biomaterials provide cell-adhesion substrate that can deliver cells to specific sites in the body with high loading efficiency. Biomaterials can also provide mechanical support against *in vivo* forces and ensure that the predefined three-dimensional structure of an organ is maintained during tissue development.

The ideal biomaterial should be biodegradable and bioreversible to support the placement of normal tissue without inducing inflammation. Incompatible materials that induce inflammatory or foreign body response eventually lead to cellular/tissue necrosis and/or rejection. Degradation products, if produced, should be removed from the body via metabolic pathways at an adequate rate to keep the concentration of these products in the tissues at a tolerable level [4]. The biomaterial should also provide an environment in which appropriate regulation of cell behavior (adhesion, proliferation, migration, and differentiation) can occur such that functional tissue can form. Cell behavior in the newly formed tissue has been shown to be regulated by multiple interactions of the cells with the microenvironment, including interactions with cell adhesion ligands [5] and with soluble growth factors. Since biomaterials provide temporary mechanical support while the cells undergo spatial reorganization into tissue, the properly chosen biomaterials should allow the engineered tissue to maintain sufficient mechanical integrity to support itself in early development, while in late development, it should begin to degrade so that it does not hinder further tissue growth [6].

Current biomaterials aim to mimic the role of natural extracellular matrix (ECM), which can support cell adhesion, differentiation and proliferation. ECM-mimicking biomaterial scaffolds need to be designed considering the following requirements. First, suitable biomaterials must be selected for particular applications [7–9]. Second, biomaterial scaffolds require a highly open porous structure with good interconnectivity, yet possessing sufficient mechanical strength for cellular in- or outgrowth [10]. Third, the surface of the fabricated scaffolds must be able to support cellular attachments, proliferation and differentiation [11–13]. Fourth, drug or

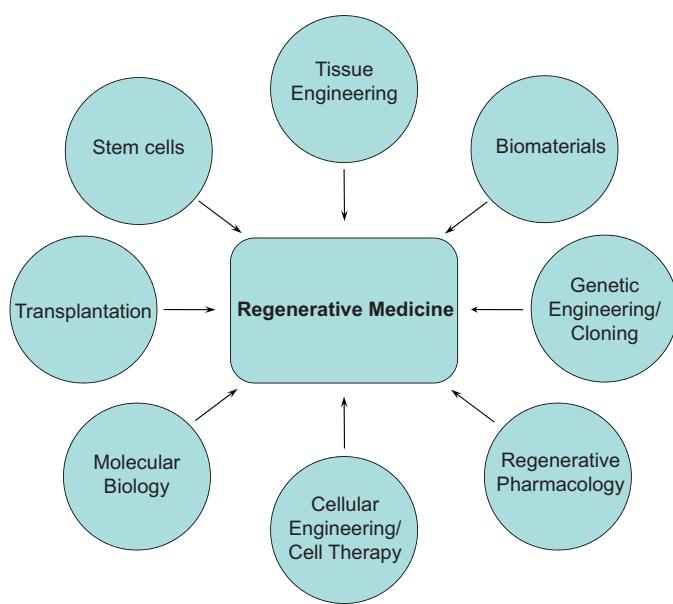


Fig. 1. Schematic representation of the various components of regenerative medicine.

cytokine releasing scaffolds are ideal for modulating tissue regeneration since cytokines such as growth factors and other small molecules have fundamental roles in growing functional living tissues [14–16].

Natural biomaterials have been extensively used for tissue engineering since they have advantages over synthetic materials such as similarity with natural ECM. For example, alginate, chitosan, collagen and its derivatives, fibrin, heparin and hyaluronic acid have been investigated for the fabrication of three-dimensional scaffolds. However difficulty in adjusting the properties and their source-related immunogenicity remains a problem with some of the natural biomaterials [17]. In contrast, synthetic biomaterials composed of artificially synthesized polymers, although most reveal poor biocompatibility, can be designed with precise control of the physio-chemical properties to give better performance when biomedically applied. Aliphatic polyesters are synthetic biomaterials approved by the Food and Drug Administration that have been widely used for biomedical applications such as surgical sutures and bone fixing screws [18,19]. They include poly-(α -hydroxyl esters) such as poly-(L-lactic acid) (PLLA), poly-(lactic-co-glycolic acid) (PLGA) and poly-caprolactone (PCL). When these synthetic polymers are implanted in the body, hydrolysis of the polymer backbone reduces the molecular weight of the polymers and their degraded products such as lactic and glycolic acids are metabolized in the body [20]. Poly-lactic acid polymers incorporated with a novel amine such as lysine (poly(lactic-co-lysine)) has been shown to have similar biocompatibility while providing additional sites for further chemical modifications. These polymers can be fabricated into macroporous biodegradable scaffolds by various techniques including molding, extrusion, solvent casting [21], phase separation techniques and gas foaming techniques [22]. Another class of degradable biopolymers is polyanhydrides. Unlike polyesters which predominantly show a bulk erosion process, polyanhydrides based on sebacic acid and p-carboxyphenoxypropane [23] exhibit a surface erosion process that is particularly useful for sustained drug delivery systems.

Recently, composite scaffolds consisting of collagen and the synthetic polymer polycaprolactone (PCL) have been fabricated using a novel electrospinning technique [24,25]. The complicated requirements of a biomaterial allowed the development of more sophisticated designs of scaffolds such as highly macro porous scaffolds for facilitating nutrient and oxygen transfer; functionalization with specific biological ligands on the surface for promoting cell attachment, proliferation and differentiation; and finally release of cytokines to manipulate the functions of encapsulated cells or host tissues. Further scientific and technological advances will envision the development of more ideal scaffolds that are specifically designed for each purpose in a wide range of applications in regenerative medicine.

3. Cell sources for regenerative medicine

3.1. Embryonic stem cells

Since cells represent one of the primary “raw materials” required for building tissues and organs, a renewable and expandable source of cells is clearly of paramount importance to the tissue engineering process. Autologous cells, because they are immune compatible, provide the gold standard for regenerative medicine applications. However, not all tissues provide a readily available source for autologous primary cell expansion (for example, brain, heart, pancreas), and moreover, as previously noted, depending on the extent of organs/tissue damage, there may not always be a sufficient pool of viable tissue or cells available for biopsy and subsequent expansion. Thus, development of alternative human

stem cell sources for regenerative medicine is required to meet the demands of these circumstances.

In this regard, stem cells are capable of self-renewal and may differentiate into multiple cell lines, making them advantageous for regenerative medicine applications. Depending on the type and maturity of tissue, stem cell potency and capacity for self-renewal can vary. Embryonic stem (ES) cells derived from the blastocyst inner cell mass of developing embryos are pluripotent, giving rise to progeny of ectoderm, endoderm, and mesodermal germ layers [26]. In addition, as further evidence of the pluripotency, embryonic stem cells can form “embryoid bodies” that recapitulate some of the events of early development, and can form teratomas *in vivo* [27]. Tumorigenicity of ES cells as well as immune response launched by the host immune system to the graft consisting of ES cell derivatives lead to a number of safety concerns surrounding the usage of the cells for therapeutic benefit. In addition, embryo-destructive derivation of human ES cells has raised significant ethical and political concerns. Thus, the use of human ES cells is not allowed in some countries, including the United States, and so their use in tissue engineering is infrequent in those countries at this time.

3.2. Alternative sources of stem cells

Somatic cloning has become widely accepted as an important tool for research [28] and can also be a source of stem cells for tissue engineering (therapeutic cloning). In this regard, somatic cloning involves the following major technical steps: (1) collection and enucleation of the recipient oocyte (2) preparation and subzonal transfer of the donor cell, (3) fusion of the two components, (4) activation of the reconstructed complex, (5) culture of the reconstructed embryo and ES cell derivation. Regeneration of histocompatible tissue using this method has been demonstrated in a bovine model [29]. Cells cloned from a patient have the advantage that they are accepted by the patient without the need for chronic immunosuppression. The concept of “therapeutic cloning” is fascinating but its application in human medicine is still in its infancy. Current knowledge suggests that reprogramming of genes expressed in the inner cell mass, from which ES cells are derived, is rather efficient. Defects in the extraembryonic lineage are a major cause of the low success rate of reproductive cloning, but these would not affect derivation of ES cells [30]. However, major practical problems include the limited availability of human oocytes for reprogramming of the donor cells, the lower efficiency of somatic nuclear transfer, the difficulty of inserting genetic modifications, the increased risk of oncogenic transformation and the epigenetic instability of embryos and cells derived from somatic cloning [31,32].

Recent research has indicated that induced pluripotent stem cells (iPS) may emerge as a viable alternative to human ES cells for therapeutic cloning. In a revolutionary experiment, Takahashi and Yamanaka [33] discovered that the genome of a differentiated somatic cell can be epigenetically reprogrammed to the pluripotent status by the expression of only four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4, resulting in the generation of induced pluripotent stem cells (iPS) that possess pluripotent features similar to those of ES cells. Since the initial report, murine iPS cells’ pluripotency, or complete reprogramming has been further confirmed by the birth of live chimeras, germline transmission, meeting the most stringent test for pluripotency, tetraploid complementation, which entails injecting pluripotent cells into engineered tetraploid embryos, allowing the iPS cells to grow into a complete mouse [34–38].

Successful derivation of human iPS cells has been recently reported [39–41]. The underlying mechanisms of the epigenetic reprogramming of somatic cells to iPS cells are not yet well

understood, but are probably similar to those required for the epigenetic reprogramming involved in somatic cloning.

The use of viral factors to transduce cells and the use of oncogenes such as c-myc and Klf4 present serious problems for the use of iPS cells in the regenerative medicine. Alternative approaches avoid integration of viral sequences in the host genome and reprogramming of somatic cells has been achieved by substituting viral factors with small molecules [21], by using non-integrating adenoviral factors [42,43], and by completely avoiding the use of viruses through delivering the reprogramming factors in the form of proteins [44]. Non-viral gene transfer using transposon technology has also been reported [45].

3.2.1. Amniotic fluid stem cells

Fetal membranes (i.e., amnion and chorion), placenta, and amniotic fluid have been extensively investigated as a potential, non-controversial source of stem cells. In the amniotic fluid (AF), two main populations of stem cells have been isolated so far: (1) amniotic fluid mesenchymal stem cells (AFMSCs) and (2) amniotic fluid stem (AFS) cells. Both can be used as primary (not transformed or immortalized) cells without further technical manipulations. AFS cells are of fetal origin, expand rapidly in culture (doubling time: 36 h) and, more interestingly, maintain a constant telomere length between the early and late passages. They express both embryonic and mesenchymal stem cell markers, are able to differentiate into lineages representative of all embryonic germ layers, and do not form tumors after implantation *in vivo*. Thus, AFS cells represent a novel class of pluripotent stem cells with intermediate characteristics between ES cells and adult stem cells [46–48]. Although further studies are needed to better understand their biologic properties and to define their therapeutic potential, AFS cells appear to be promising candidates for cell therapy and tissue engineering. In particular, they may represent an attractive source for the treatment of congenital malformations (e.g., congenital diaphragmatic hernia and required neonatal diseases requiring tissue repair/regeneration (e.g., necrotizing enterocolitis) [49].

3.2.2. Adult stem cells

Although stem cell pluripotency diminishes with development, adult tissues possess stem cells that exhibit multipotency [50–52]. The purpose of adult stem cells is to assist in repair and regeneration of postnatal tissues. Examples of such adult stem cells include mesenchymal stem cells which may differentiate in culture into a variety of cell types including chondrocytes, osteocytes, adipocytes, and hematopoietic stem cells. Additionally, adult tissues, such as skeletal muscle, liver, kidney tissue and urothelium, among others, possess progenitor cells capable of proliferating and differentiating into specific end organ cell types [53–56]. Multipotent adult stem cells also hold great promise as tools for further development of regenerative medicine technologies. For example, the use of mesenchymal stem cells derived from primarily bone marrow, but also adipose and placental tissues in animal studies has prompted the use of mesenchymal stem cells currently in more than 200 clinical trials (<http://clinicaltrials.gov>). The mechanistic basis for the beneficial effects of MS cells is not entirely understood. One possibility is that injected MS cells may directly augment host tissue function by differentiating and integrating with the relevant cell type of the injured tissue. However, given the low efficiency of injected MS cell engraftment with injured host tissue [52], a more feasible mechanism is that MS cells release soluble factors that support and enhance the endogenous regenerative capacity of the host. For example, bone marrow-and adipose tissue-derived MS cells may primarily work by immune modulation and angiogenic stimulation to improve recovery of injured or diseased tissues. The immune modulatory characteristic provides the rationale for the use of MS

cells as a potential allogeneic cell source for development of therapies in humans.

4. Tissue engineering of specific structures in the female reproductive system

4.1. Vagina

Congenital malformations such as Mayer-Rokitansky-Kuester-Hauser (MRKH) syndrome, cloaca, or, thick transverse vaginal septum, obstetric trauma and malignancy can adversely affect vaginal anatomy or result in loss of sexual function. The feasibility of engineering vaginal tissue *in vivo* has been investigated [57,58]. Vaginal epithelial and smooth muscle cells of female rabbits were biopsied, and expanded in culture. These cells were seeded onto biodegradable polymer scaffolds and the cell-seeded constructs were then implanted into nude mice for up to 6 weeks. Immunohistochemical, histologic and Western blot analyses confirmed the presence of vaginal tissue. Electric stimulation studies in the tissue-engineered constructs showed similar functional properties to those of normal vaginal tissue. When these constructs were used for autologous total vaginal replacement, patent vaginal structures were noted in the tissue-engineered specimens, while the acellular structures were noted to be stenotic [58,59]. The first case of creation of human vagina using autologous *in vitro* cultured vaginal tissue has been reported [60]. The patient had MRKH syndrome and the vaginal tissue was cultured from a 1 cm² biopsy from vaginal vestibular mucosa. Autologous reconstructed vaginal tissue reached 314 cm² in two weeks, when it was harvested from the culture plates, mounted on hyaluronic acid embedded gauze to maintain the orientation of the mucosal tissue. A McIndoe-Reed type procedure was performed to create a vaginal space to allow inlay of the cultured tissue. Clinical exam demonstrated a vagina with normal length and depth with vaginal tissue present on biopsy. Long-term results of clinical trials are awaited, in particular whether this technique avoids the risk of vaginal stenosis [60,61].

4.2. Uterine cervix

Preterm birth is a common complication of pregnancy and associated with significant perinatal morbidity and mortality. Acquired abnormalities of the cervix or its dysfunctional remodeling during gestation are implicated in a significant number of preterm births. House et al. [62] have explored the feasibility of a tissue engineering strategy to develop a three-dimensional cervical-like tissue constructs. Cervical fibroblasts were isolated from premenopausal woman and seeded on collagen-coated porous silk scaffolds. Cervical cells proliferated in three dimensions during the eight-week culture period and synthesized an extracellular matrix with biochemical constituents and morphology similar to native tissue. Compared to static culture, dynamic culture was associated with significantly improved matrix. These data demonstrate that it is possible to synthesize ECM with measurable mechanical properties and to investigate cervical remodeling *in vitro* under conditions that mimic pregnancy.

4.3. Uterus

Uterine factor infertility results from congenital anomalies or acquired causes such as hysterectomy due to malignant and benign reasons, or due to intrauterine adhesions. It affects approximately 3–5% of the general population. Gestational surrogacy is currently the only option for having genetic offspring in these patients. Recently, an allogeneic uterine transplantation was reported [16,63]. The possibility of engineering functional uterine tissue

using autologous cells has been investigated [64]. Subtotal uterine tissue replacement was performed after harvesting and expanding autologous rabbit uterine smooth muscle and epithelial cells, and seeding them onto pre-configured uterine shaped biodegradable polymer scaffolds.

In order to produce uterine grafts in the peritoneal cavity of rats and rabbits, Campbell et al. [65] implanted biomaterial templates of the appropriate shape into the peritoneal cavities of these animals. After 2–3 weeks, the templates were removed, the encapsulating myofibroblast-rich tissue harvested and grafted to replace resected segments of uterus of the same animals in which the tissue was grown. At 12 weeks after grafting, uterine graft tissue had increased in thickness and developed the morphology of normal uterus, with endometrium overlying several layers of smooth muscle cells (myometrium-like) which were interspersed with collagen fibrils; grafted uterine horns supported embryos to the late stages of gestation [66].

In another study, a basic fibroblast growth factor (bFGF) delivery system using collagen was constructed [67]. The bFGF delivery system was tested in a rat model of severe uterine damage (partial rat uterine horn excision/reconstruction). The authors found that this delivery system improved regeneration abilities of uterine endometrium and muscular cells, improved vascularization, as well as better pregnancy outcomes in rats.

4.4. Ovary

The fundamental role of the ovary is to produce oocytes capable of fertilization as well as to secrete hormones that will prepare the uterus for successful implantation. Ovarian sex steroids also play a major role in breast development, bone health and sexual function in the female. A number of pathological conditions such as premature ovarian failure and cancer chemotherapy may severely compromise reproductive function. In addition, with the decline of sex steroid hormones around natural menopause, significant pathology such as vasomotor symptoms, genital atrophy, and bone loss, ensues. In the attempt to restore a function of the ovary, several approaches have been employed at the experimental level. Some of the significant approaches include, (1) regenerating ovarian tissues from stem cells; (2) transplanting ovarian tissues; and (3) use of tissue engineering tools.

4.4.1. Regenerating ovarian tissues from stem cells

It was previously considered that the germ cell renewal (formation of oocyte and follicle) is restricted to fetal life. A decade ago Johnson et al. [68] during their investigation of follicular atresia in adult mouse, identified a group of cells in the surface epithelium of ovary (OSE) immunostained positive for germ cell-specific marker, MVH (mouse vasa homologue) and showed BrdU (5-bromodeoxyuridine) incorporation. These germ-line stem cells (GSCs) were also found to express meiosis-specific marker, SCP3 (synaptonemal complex protein 3) and these GSCs were reported to maintain the oocyte and follicle production in the postnatal ovary. Later these GSCs were commonly referred to as oogonial stem cells (OSCs). Even though there existed a debate on the existence of OSCs in adult mammalian ovaries and their potential significance, the report by Zou et al. [69] provided concrete evidence that the OSCs are capable of producing offspring. Following the initial report on neo-oogenesis, there has been a tremendous development recently in this field to regenerate ovarian tissues (especially follicles). However, the existence of OSCs in adult mammalian (mouse) ovary was reported to be exceedingly rare (0.014%) [70], as it seemed to decline with age [71]. As an alternatives to OSCs, extra-ovarian stem cells such as bone marrow-derived stem cells [72], ES and iPS have been used to produce oocyte-like cells [71]. The differentiating ES were reported to have various

limitations including their heterogeneity (reviewed in Dunlop et al. [73]), which makes the *in vitro* oogenesis from these stem cells challenging.

4.4.2. Autologous transplantation of ovarian tissues after cryopreservation or *in vitro* activation

Cryopreservation of ovarian tissues retrieved from both donors and autologous sources is another feasible approach to restore the lost ovarian function. Autologous transplantsations of cryopreserved whole ovary or partial ovarian tissues into rabbits [74,75], sheep [76–78] and monkeys [79] were reported to exhibit functions of the ovary including follicle development, resumption of regular estrus cycles and ovulation with corresponding hormone pattern. Recently, Kawamura et al. [80] reported a promising technique for autologous transplantation that recapitulates the fertility in murine ovarian failure model, where the follicular growth was induced through disruption of the Hippo signaling by fragmenting ovaries, followed by treating with Akt stimulator, which yielded retrievable and fertilizable mature oocytes. This technique is referred to as *in vitro* activation (IVA). Using this technique a 30-year-old woman in Japan was treated for infertility and she has successfully given birth to a healthy baby [81]. Nevertheless, when it comes to heterologous (allogenic or xenogenic) transplantation, the graft tissue has to be protected from the host immune system either by immune suppression or by immuno-isolation. As discussed below, the use of tissue engineering tools is considered as a viable approach to overcome the immune hurdles. Since the functional unit of the ovary (follicle) is comprised of three major cells types namely, oocyte, granulosa and theca cells and they are interdependent on each other for function it has been a challenge to come up with a tissue design to accommodate all these cells in the same construct. Moreover, the designs should also provide the option to implant the constructs in the recipient organism.

4.4.3. Tissue engineering approaches in ovarian biology

Recently, new *in vitro* culture methods involving tissue-engineered matrices have been developed to study the maturation of ovarian follicles [82–84]. Unlike the two-dimensional culture systems supporting the production of immature mouse follicles or granulosa cell-oocyte complexes where the granulosa cell is attached to the culture substrate, and migrate away from the oocyte [85,86], Shikanov et al. [87] have developed hydrogels to mimic the native ovarian environment by maintaining the 3-D follicular architecture, cell-cell interactions and paracrine signaling that direct follicular development. In this system, fibrin and alginate are gelled simultaneously and make up an interpenetrating network (FA-IPN). The degradable component of this FA-IPN may be particularly critical for clinical translation in order to support the greater than 10^6 fold increase in volume that human follicles normally undergo *in vivo*. Krotz et al. [88] developed a design which they claimed as pre-fabricated self-assembled artificial human ovary. In this design, theca cells were cultured to form a honeycomb structure using agarose molds and later cumulus-oophorus complexes (COCs) were seeded in the luminae (multiple openings of the honeycomb structure). After 72 h theca cells surrounded the COCs and formed self-assembled complex micro-tissues reported to support the development of the oocyte, which was evident from the polar body extrusion. These kinds of artificial ovarian tissues serve as useful tools for *in vitro* maturation. On the other hand, for the purpose of cell-based hormone replacement therapy, we have recently developed an engineered multilayer ovarian tissue model that secretes sex steroids and peptide hormones in response to gonadotropins *in vitro* [89]. Theca and granulosa cells placed in different layers of multilayer alginate micro-capsules that mimic the architecture of native follicles, their endocrine functions were

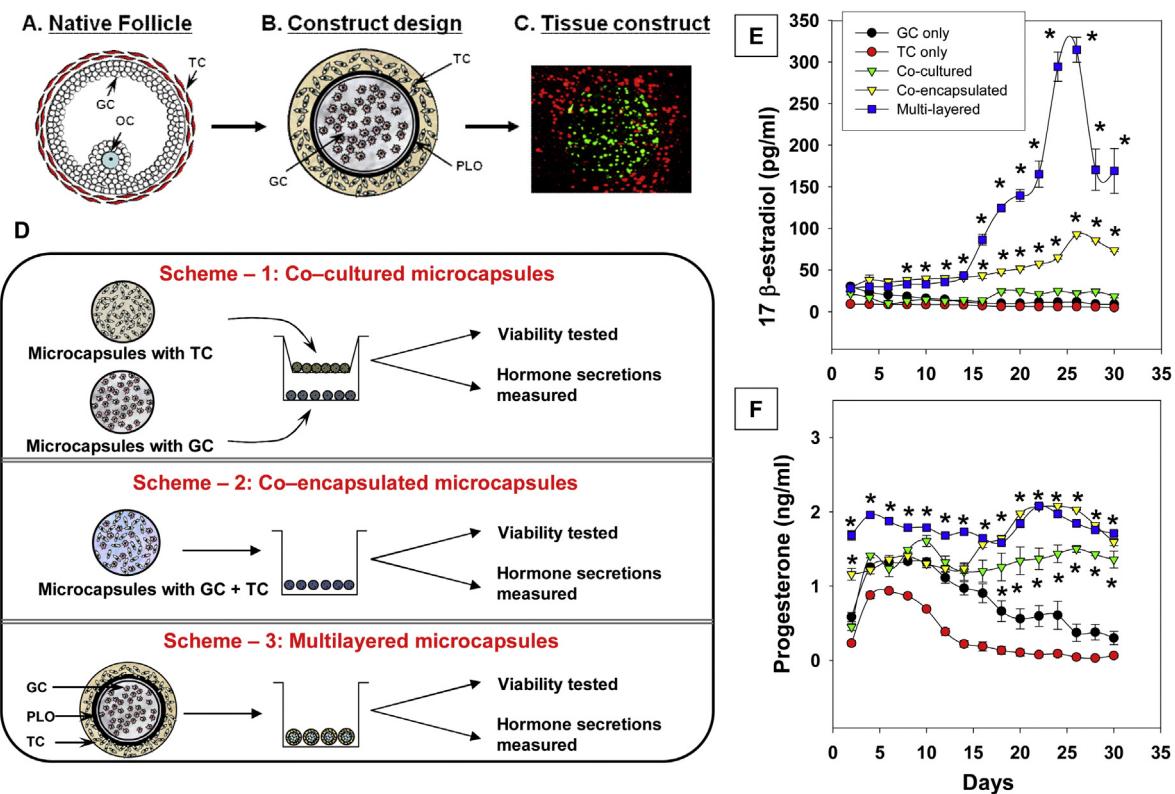


Fig. 2. Encapsulation approach for reconstructing ovarian endocrine tissue: (A) schematic diagram of an ovarian follicle. (B) Approach of using multilayered alginate microcapsule to mimic native follicular structure. (C) 3D – confocal image of microcapsules demonstrating compartmentalization of different cells – distribution of CellTracker green-labeled cells (granulosa) in the inner layer and CellTracker orange-labeled cells (theeca). (D) Outline of the study demonstrating the co-cultured/co-encapsulated/multilayer microcapsules as the three approaches used. (E) Sustained 17 β -estradiol and (F) progesterone secretion by granulosa and theca cells encapsulated using three different schemes. In contrast to 2-D cultures, E_2 levels are maintained or increased with time and the presence of both GC and TC (either through Schemes 1, 2, or 3) led to increased levels of E_2 production compared to GC or TC alone. Multi-layer cultures led to significantly increased levels of E_2 production. Each data point represents mean \pm SEM of 6 values (3 wells/group assayed in duplicates). * Denotes statistical significance at $P < 0.05$ compared to granulosa and/or theca cells alone. The figures represent data from one of two separate experiments. GC – granulosa cells; OC – oocyte; TC – theca cells; PLO – poly-L-ornithine; E_2 – 17 β -estradiol; P_4 – progesterone. Adapted from Sittadjody et al., 2013, with permission from © Elsevier.

found to be more potent than two other possible combinations (Fig. 2). Similarly, encapsulated endocrine cells implanted into the peritoneum of rodents have been reported to deliver hormones in ovariectomized animals [90,91].

5. Conclusion

In summary there is a growing interest in the development of regenerative medicine approaches for the treatment of a variety of diseases of the reproductive system. The approaches have included regenerative, replacement and/or tissue engineering techniques for various organs and tissues of the reproductive system including the vagina, the uterine cervix, the uterus, and the ovary. In the studies so far reported ES, iPS, and adult mesenchymal cells and other stem cell species have all been used either alone or in combination with biomaterials such as biopolymeric hydrogels and natural ECM scaffolds to develop a substitute tissue/organ for a diseased organ. In particular, it is of interest that during the current period of controversies surrounding the use of pharmacological approaches to hormone replacement therapy (HRT) in women, there are simultaneous efforts to develop cell-based approaches to HRT in a few laboratories. We believe that regenerative medicine holds immense promise for the treatment or cure of a variety of diseases of the reproductive system, and it is hoped that in no distant future the clinical translation of novel technologies in this emerging field of medicine will become a reality.

Contributors

TMY and SS contributed in writing the manuscript; ECO contributed in writing, reviewing and editing the manuscript.

Competing interest

The authors declare no conflict of interest.

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