Functional Maintenance of Differentiated Embryoid Bodies in Microfluidic Systems: A Platform for Personalized Medicine

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INTRODUCTION

Ovaries have two distinct functions that are critical to a woman’s reproductive health: hormone synthesis and gametogenesis. A significant population of reproductive-age patients experience premature ovarian failure (POF) and lose regular hormone synthesis owing to either iatrogenic causes, such as chemotherapy, or idiopathic, presumably genetic, causes. The number of female cancers diagnosed in reproductive-age women has approached 9% of all diagnoses [1], and survival will continue to increase as treatment options and novel biotechnological advances emerge [2]. The loss of ovarian function has physiologic and considerable psycho-social repercussions on patients that negatively affect their quality of life. Currently, gonadal failure and the associated loss of hormone synthesis in patients with POF, or menopausal women, is treated by hormone replacement therapy (HRT) using synthetically produced steroids [3]. However, the Women’s Health Initiative raised several outcome concerns related to this approach for two specific types of conjugated estrogens of hormones, Premarin and Prempro, which increase the risk of stroke, blood clot, myocardial infarction, and neoplasia [4–11]. These reported observations have since been clinically expanded by healthcare providers to include all synthetically generated hormones used in HRT. In contrast, recent reports have suggested that bioidentical hormones could be a safer alternative for HRT [10]. The presumed risks associated with the current HRT regimen necessitate improved therapeutic options. In the present study, we propose a novel approach for HRT using stem cells in a cell-based therapy. On a larger scope, we present evidence supporting the use of a microfluidic chip system with continuous flow for the differentiation and extended culture of functional steroidogenic stem cell-derived EBs, the differentiation of EBs into cells expressing ovarian antigens in a microfluidic system, and the ability to cryopreserve this system with restoration of growth and functionality on thawing. These results present a platform for the development of a new therapeutic system for personalized medicine. 

ABSTRACT

Hormone replacement therapies have become important for treating diseases such as premature ovarian failure or menopausal complications. The clinical use of bioidentical hormones might significantly reduce some of the potential risks reportedly associated with the use of synthetic hormones. In the present study, we demonstrate the utility and advantage of a microfluidic chip system to enhance the development of personalized, on-demand, treatment modules using embryoid bodies (EBs). Functional EBs cultured on microfluidic chips represent a platform for personalized, patient-specific treatment cas- settes that can be cryopreserved until required for treatment. We assessed the viability, differentiation, and functionality of EBs cultured and cryopreserved in this system. During extended microfluidic culture, estradiol, progesterone, testosterone, and anti-müllerian hormone levels were measured, and the expression of differentiated steroidogenic cells was confirmed by immunocytochemistry assay for the ovarian tissue markers anti-müllerian hormone receptor type II, follicle-stimulating hormone receptor, and inhibin β-A and the estrogen biosynthesis enzyme aromatase. Our studies showed that under microfluidic conditions, differentiated steroidogenic EBs continued to secrete estradiol and progesterone at physiologically relevant concentrations (30–120 pg/ml and 150–450 pg/ml, respectively) for up to 21 days. Collectively, we have demonstrated for the first time the feasibility of using a microfluidic chip system with continuous flow for the differentiation and extended culture of functional steroidogenic stem cell-derived EBs, the differentiation of EBs into cells expressing ovarian antigens in a microfluidic system, and the ability to cryopreserve this system with restoration of growth and functionality on thawing. These results present a platform for the development of a new therapeutic system for personalized medicine. 

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both researchers and clinicians to be able to generate any cell or tissue type through directed differentiation protocols. Nondirected differentiation of ESCs seeded on nonadhesive plates in suspension can lead to formation of an embryoid body (EB), a densely packed spheroid of embryonic stem cells that differentiate into cell types from all three developmental germ layers: endoderm, ectoderm, and mesoderm. More recent studies in our laboratory have suggested that EBs derived from G4 mouse ESCs can differentiate under specific culture conditions into ovarian tissue, a primary steroidogenic organ of the female reproductive system [13] and that these differentiated G4 EBs synthesize physiologically relevant levels of estradiol [14]. Estradiol is the primary female hormone, important for women’s health and development, and is used in a wide range of medical treatments, in particular, in postmenopausal women and infertility patients.

The limitations of long-term in vitro culture of EBs for therapeutic purposes using the current standard tissue culture approaches include the high cost, risk of contamination, dependency on the operator, labor intensity, and necessity for large volumes of reagents. For example, during the interval between culture media changes, toxins and waste accumulation and the depletion of nutrients can interfere with the metabolism of the EBs. Moreover, with the increasing size of the cultured EBs, we have encountered concerns regarding insufficient gas and nutrient exchange at the core regions of the EB, which, in turn, can result in cell death within the EB inner mass [15]. By developing a microfluidic system with a continuous flow of fresh media, this limitation has been partially addressed. Using a dynamic continuous flow system of microfluidic chips, not only will the accumulation of toxins and waste be decreased, but it will also allow improved control of the culture parameters, enabling standardized microenvironments and a sustainable supply of fresh nutrients within a closed system in experiments [16–20]. In the present study, we propose a method in which we immobilize EBs in a closed microfluidic system that provides fresh media and simultaneously collects the steroid hormone from the supernatant from the terminal port. Using this approach, the cells can be kept in a contained system and survive prolonged culture durations without requiring exposure to air or other sources of contamination. Furthermore, the differentiated EBs in individual chips can be cryopreserved and thawed on demand at a later time.

**Materials and Methods**

**Generation of EBs**

Mouse embryonic fibroblast (MEF) medium was prepared using Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% L-glutamine 200 mM (100×) (Life Technologies, Carlsbad, CA, http://www.lifetech.com). The 5 × 10⁶ MEF feeder cells were mitotically inactivated using mitomycin C (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com) and seeded on a 100-mm tissue culture plate coated with 0.1% gelatin (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com) and 200 mM (100×), 0.2 mM 2-mercaptoethanol, and 5 ng/ml of basic fibroblast growth factor (R&D Systems, Minneapolis, MN, http://www.rndsystems.com). Next, 2 × 10⁶ mESCs were seeded on a 100-mm Petri dish or 96-well plate coated with 1.5% agarose to generate EBs in a low-adhesion environment. Using a simple decantation method, at least 50% of the medium was replaced with fresh EB medium every day. The same culture conditions were used for static culture of EBs.

**Microfluidic Chip Fabrication**

The microfluidic devices were designed and fabricated using 1.5-mm-thick poly(methyl methacrylate) (PMMA; McMaster-Carr, Elmhurst, IL, http://www.mcmaster.com) and 80-μm-thick, double-sided adhesive film (DSA) (ITapestore, Scotch Plains, NJ, http://www.itapestore.com), as described in previous studies [16]. In brief, three 4-mm × 28-mm parallel channels separated by a gap of 3 mm were cut onto a 24-mm × 40-mm DSA film and PMMA plate using a laser cutter (VersaLaser, Scottsdale, AZ, http://www.versalaser.com). The surface of a 24-mm × 40-mm glass coverslip (150 μm thick) or polystyrene plate (1 mm thick) was plasma treated for 90 seconds and adhered to the DSA film, forming the base and middle layer of the microfluidic device, respectively. The channels were covered with 24 mm × 40 mm PMMA with 3 inlet and 3 outlet openings of 0.78 mm in diameter, serving as the top layer of the microfluidic device. The openings in this layer were aligned to the end point of the DSA channels to be used as inlets and outlets during the fluid flow. Finally, PMMA channels with the inlet and outlet openings were assembled into the DSA-polystyrene plate combination to make a three-layer microfluidic device with microchannels of 4 mm × 28 mm × 1.5 mm in dimension. All components used in assembly were cleaned with detergent and ethanol and UV sterilized for 15 minutes under a laminar flow hood before assembly.

**Dynamic Culture of EBs in Microfluidic Chip**

Approximately 5 × 10⁶ EB cells per milliliter were mixed uniformly with ice cold Matrigel (growth factor reduced; BD Biosciences, San Diego, CA, http://www.bdbiosciences.com). Next, 70–100 μl of this EB-Matrigel mixture was carefully pipetted into each 4-mm × 28-mm × 1.5-mm channel of the microfluidic chip. The assembled, cell-laden, microfluidic chip was then transferred to a 37°C incubator for 15 minutes to produce a uniform layer of hydrogel on gelation. After gelation of Matrigel, the third layer of the microchip (PMMA layer with the inlet and outlet openings) was carefully aligned and assembled onto the body of the chip. Silicon tubes (inner diameter 0.25 mm; catalog no. EW-06419-00; Cole-Parmer, Vernon Hills, IL, http://www.coleparmer.com) were inserted into the inlet and outlet openings for unidirectional flow.
through the microchannels. The microchip with encapsulated EB cells was transferred into the cell culture incubator providing continuous flow of fresh EB media at the rate of 2 μL/min using 10-mL syringes (BD Biosciences) and a syringe pump (NE-1600; New Era Pump Systems, Farmingdale, NY, http://www.syringepump.com) (supplemental online Fig. 1). The terminal ends of the channels were connected to 15-mL tubes to collect the drained conditioned medium of 24 hours at days 1, 5, 11, 15, and 21 for detection and quantification of the secreted steroid hormones using enzyme-linked immunosorbent assay (ELISA).

**Cryopreservation of EB Immobilized Microfluidic Chips**

After 24 hours of dynamic culture, the EB immobilized microfluidic chips were washed with PBS, and the channels were filled with cryoprotecting solution (80% FBS, 20% dimethyl sulfoxide). After blocking the inlets and outlets, the microfluidic chips were sealed, immersed in isopropanol (Sigma-Aldrich), frozen at −80°C overnight, and then transferred into liquid nitrogen. After 48 hours, the cryopreserved chips were thawed in a 37°C water bath and rinsed 3 times with fresh culture media.

**Viability and Proliferation Assays**

The viability of the cells within the EB was assessed after 21 days of microfluidic chip culture and after thawing with calcein-AM/ethidium homodimer-1, LIVE/DEAD assay (Life Technologies). The assay was performed directly within the microfluidic chip without harvesting the EBs by incorporating the LIVE/DEAD kit reagents and subsequent washing steps. The samples were imaged using the Zeiss Axio fluorescence microscope (Carl Zeiss, Jena, Germany, http://www.zeiss.com). The proliferation of cells was determined using the bromodeoxyuridine (BrdU) proliferation assay kit (Sigma-Aldrich) according to the manufacturer’s instructions.

**Immunocytochemical Analysis**

Mouse ESC colonies, EBs in suspension, and EBs in the microfluidic chip were harvested and fixed with 1% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, http://www.emsdiasum.com). The samples were blocked with 1% bovine serum albumin (Sigma-Aldrich), permeabilized with 0.3% TritonX 100 (Sigma-Aldrich), and stained for stem cell markers Oct4 (ab18976; Abcam, Cambridge, U.K., http://www.abcam.com), SSEA-4 (330410; BioLegend, San Diego, CA, http://www.biolegend.com), and Nanog (ab80892; Abcam), germ layer markers α-fetoprotein (sc-8108, Santa Cruz Biotechnology, Santa Cruz, CA, http://www.scbt.com), α-smooth muscle actin (ab5694; Abcam), and neurofilament (ab7794; Abcam) and ovarian tissue markers anti-müllerian hormone receptor (AMHR) type II (ab64762; Abcam), inhibin B-A (sc-166503; Santa Cruz Biotechnology), follicle-stimulating hormone receptor (FSH-R; sc-7798; Santa Cruz Biotechnology), and anti- aromatase (CYP19A1) (ab35604; Abcam) primary antibodies overnight at 4°C. Alexa Fluor 488 and Alexa Fluor 568 were used as secondary antibodies, and the cell nuclei were stained with 4′,6-diamidino-2-phenylindole (Life Technologies). The stained samples were analyzed using a Zeiss LSM 510 META confocal microscope.

**Enzyme-Linked Immunosorbent Assay**

Conditioned medium from EBs cultured in a 96-well plate under static conditions and conditioned medium collected from the terminal end of the EB-immobilized microfluidic channels before and after cryopreservation were collected for a 24-hour period and analyzed for the presence of the sex hormones estradiol, progesterone, and testosterone. The levels of secreted steroid hormones were detected using ELISA with a specific kit for estradiol, progesterone, androstenedione, and anti-müllerian hormone (AMH) according to protocols of the Wisconsin National Primate Research center, University of Wisconsin, Madison (Madison, WI). The antibodies for progesterone and testosterone were provided by Coralie Munro from University of California, Davis (Davis, CA); the antibody for estradiol was supplied by Holly Hill Biologicals (Hillsboro, OR). ELISA analysis for the basal levels of the sex hormones in EB media was done in-house according to manufacturer’s instructions (estradiol and progesterone from Calbiotech, Spring Valley, CA, http://www.calbiotech.com; testosterone from Enzo Life Sciences, Farmingdale, NY, http://enzo lifesciences.com).

**Statistical Analysis**

The experimental results were analyzed using analysis of variance with Tukey’s post hoc test for multiple comparisons and Student’s two-tailed t-test for single comparisons, with statistical significance set at p < .05. Unless otherwise stated, the mean values represent three experiments with two or three channels per experiment and the error bars represent the SEM. Statistical analyses were performed using GraphPad Prism, version 5 (GraphPad Software, Inc., San Diego, CA, http://www.graphpad.com).

**RESULTS**

We fabricated a simple microfluidic device to physically stimulate the generated EBs with continuous laminar flow and shear stress. Dynamic culture introduces mechanical stimulation on cells in their native environment [16]. The bottom of the device is designed as a 150-μm-thick glass coverslip, enabling a sufficient penetration depth to monitor the EBs using confocal microscopy. To immobilize the EBs within a microfluidic channel and provide ECM-like support, we plated the EBs within a Matrigel depth of 500 μm, avoiding total encapsulation. After immobilization of the EBs, the microfluidic channel allowed 1.5 mm of depth for the flow of the media (Fig. 1). The cell culture media were perfused with a syringe pump with flow rate of 2 μL/min. We used silicon tubing that allows gas exchange for oxygenation of the media. The contained microfluidic system developed in the present study provides advantages over classic two-dimensional culture because it uses smaller amounts of reagents and multiplying the test conditions for high-throughput analyses. The designed chip also allows in situ tracking and staining platform without the removal of the EBs from the channels (supplemental online Fig. 1).

**Microfluidic System Supported Long-Term Culture of Mouse ESC-Derived Embryoid Bodies**

In the present study, we generated EBs from mouse embryonic stem cells and incorporated them into microfluidic channels with culture under continuous flow (supplemental online Fig. 2). The EBs used in the present study ranged from 70 to 200 μm in diameter. After culture under continuous laminar flow in the microfluidic channels for 21 days, we observed that the EBs were highly viable (Fig. 2A) comparable to that observed in standard tissue culture plates. Minimal cell death was detected within the EBs (Fig. 2A), demonstrating that the microenvironment and the physiological conditions supported the viability of the cells. We
also demonstrated the preservation of the metabolic activity of the cells within the microfluidic culture. We investigated the proliferation of long-term cultured EBs using the BrdU assay. The newly formed cells within the EBs were detected using an anti-BrdU assay (Fig. 2C) showing that the cells were metabolically active and pursuing proliferation.

**mESCs and EBs Cultured in Microfluidic Chips Continued to Grow and Differentiate**

The characterization of the germ layers within the EBs after static and microfluidic culture was assessed using immunocytochemistry for stem cell markers and germ layer-specific cell surface markers. The stemness properties of the mESC colonies, EBs grown in static conditions, and EBs in the microfluidic chips were assessed by staining for anti-Nanog, anti-Oct4, and anti-SSEA-1 markers. The mESC colonies and EBs in microfluidic channels expressed these ESC antigens after 21 days comparable to that of the mESCs and EBs grown in tissue culture plates (Fig. 3). The EBs also demonstrated cell differentiation into the three major germ layers mesoderm (α-smooth muscle actin), ectoderm (neurofilament), and endoderm (α-fetoprotein). This immunocytochemistry assay showed that the EBs under laminar flow conditions were able to differentiate into the three germ layers.

**EB-Microfluidic Chips Can Be Cryopreserved With Recovery of Function**

The fabricated microfluidic cassettes are designed to resist the low temperatures (−196°C) of cryopreservation by replacing the glass coverslip with a 1-mm-thick polystyrene plate. EB-loaded microfluidic chips were cultured for 24 hours under continuous laminar flow and later cryopreserved according to the adopted cryopreservation technique by slow freezing of the samples in isopropanol and storage in liquid nitrogen. After cryopreservation, the viability of the EBs was assessed using the LIVE/DEAD assay directly on the microfluidic chip (Fig. 2B). Moreover, the cryopreserved and differentiated EBs were also positive for ovarian lineage markers, such as AMHR, expressed by granulosa cells (Fig. 4A); FSHR, expressed by gonadotropic cells in immature ovarian follicles (Fig. 4B); and inhibin β-A (Fig. 4C). The functional activity of the differentiated tissue was confirmed further with the expression of CYP19A1, showing enzymatic activity for the secretion of estradiol (Fig. 4A).

**Cryopreserved EB-Microfluidic Chips Recovered Steroidogenic Function When Thawed and Cultured**

The presence of the steroid hormones estradiol, testosterone, and progesterone within the conditioned media collected from the dynamic culture of EBs before and after cryopreservation was detected using ELISA analysis. The samples for 24-hour period were collected at days 1, 5, 11, 15, and 21 and stored frozen until analysis. The estradiol level present in the collected samples from the non-cryopreserved samples was stable at 64–79 pg/ml for the 21-day period (Fig. 5). After cryopreservation of the EB-containing microfluidic chip, the estradiol levels had decreased but not to a significant amount (54–62 pg/ml) compared with the 21-day culture period. Secretion of progesterone in the 21-day period showed...
EBs, embryoid bodies; NFs, neurofilaments.

Cryopreservation, the AMH levels were similar at 19 served channels were 17 testosterone, 54.33 pg/ml. The AMH levels for the noncryopre-

The basal levels of the steroid hormones in blank EB media were 

a similar trend for the samples with and without cryopreservation. 

A similar trend for the samples with and without cryopreservation. 

The range of progesterone for the noncryopreserved sample was 144–423 pg/ml for the 21-day period. The level of secreted testos-
terone fluctuated from 76 to 141 pg/ml for the noncryopreserved 

The AMH results support the pres-

Moreover, microfluidic cultures provide advantages such as 

the ability to mimic native-like environments and investigate bi-

ological systems [18, 28, 29]. The physical and chemical properties 

of cellular microenvironments (e.g., matrix stiffness, gradient of 

chemokines and growth factors) are known to significantly influ-

ence and direct the differentiation process of stem cells [26, 

30–33]. Microfluidic systems also enable minimization of the 

sample size, allowing costly reagents to be used in smaller quan-
ties, and provide a dynamic platform for high-throughput 

screening of chemicals or drugs [34, 35]. We used these advan-
tages of the microfluidic platforms to differentiate EBs toward 

ovarian tissue. Steroidogenic cells of the ovary are the primary en-
docrine tissue of the female reproductive tract, and they are crit-
tical to normal female development, reproductive function, and 
maintenance of health.

Differentiation of EBs Toward Ovarian Tissue in Microfluidic Systems

In the present study, by introducing dynamic microfluidic culture, 

we have extended our previous work, in which we showed that differentiation of EBs under static culture conditions can develop 

trophoblastic tissue that secretes estradiol, progesterone, and 

human chorionic gonadotropin (hCG) [21]. We have demon-

strated the presence of granulosa and gonadotropic cells within 

the EBs differentiated in the designed microfluidic system. To-

gether with the hormone secretion profiles, these findings have 

confirmed the ovarian tissue characteristics of the differentiated 

EBs. Similar findings were reported by Lipskind et al. [13], who 

found expression of ovarian antigens in the differentiating EBs 

under dynamic culture conditions. In the present study, we have vali-
dated the functional performance of differentiated EBs in the 

microfluidic system. These data show the dynamics of the gener-
ated microenvironment on the chip. Taken together, these results 
suggest that a dynamic flow system using microfluidic chips is a vi-
able option for differentiation of ESC-derived EBs that will suc-

cessfully differentiate toward functional ovarian tissue.

Cryopreservation of the EB Differentiation Platform

Traditional drug development and therapeutic approaches to 
cure diseases have been based on mimicking the synthesis of 
natural molecules or designing biologically active compounds. 
The activity of a therapeutic agent and the physiological re-
sponse can vary greatly among patient populations [10]. The

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Fig. 3. Germ layer differentiation and stemness properties of EBs. Differentiation and stemness of EBs were analyzed and compared with static conditions after 21 days of culture in a microfluidic chip. Mesoderm was stained for α-SMA, ectoderm for NFs, and endoderm for α-FP. Stem cell markers SSEA-1, Oct4, and Nanog were confirmed in EBs cultured in both static conditions and microfluidic chips. Scale bars = 100 μm (A–C) and 50 μm (D–F). Abbreviations: α-FP, α-fetoprotein; α-SMA, α-smooth muscle actin; DAPI, 4′,6-diamidino-2-phenylindole; EBs, embryoid bodies; NFs, neurofilaments.

DISCUSSION

The current clinical approaches to regenerative medicine aim to use pluripotent stem cells in cell- and gene-based therapies and tissue engineering applications. Because the capacity to form trophoblastic clusters and secrete steroidogenic hormones such as estradiol has been demonstrated, the idea has emerged to use pluripotent stem cells as in vitro agents for the secretion of endocrine hormones [14, 21]. Existing tissue culture methods face specific challenges, including elucidating specific differentiation signals, reproducing in vivo-like differentiation conditions, tissue tolerance, and the long-term viability of the differentiated tissues in culture [20, 22].

Designing a Microfluidic Device for Sustainable Long-Term Dynamic Culture of EBs

We have described an innovative culture system (Fig. 1) to grow, differentiate, and cryopreserve EBs in a microfluidic environment

that allows the development of functionally specialized cells and 
tissues, such as ovarian cells and endocrine tissue. EBs are formed from embryonic stem cells or induced pluripotent stem cells (iPSCs) and have the potential to differentiate into any desired cell type, such as cardiac cells [23], osteogenic and chondrogenic cells [24], neurons [25], insulin-secreting β-cells [26], and steroid hormone-secreting cells [21]. EBs are three dimensional; thus, their growth and duration in culture have been restricted by physical limitations such as the penetration of media nutrients to the EB’s core. In the present study, we attempted to address the considerations regarding the survival of EBs using continuous laminar flow within a microfluidic system. We have demonstrated sustained high viability of EBs for up to 21 days, with high proliferation activity within the microfluidic system, indicating favorable culture conditions. We also observed dead cells within the EBs that can be explained by the progress of cell cycle dynamics, in which cells grow and die [27].

We have described an innovative culture system (Fig. 1) to grow, differentiate, and cryopreserve EBs in a microfluidic environment
current trends in medicine are focusing on personalized approaches specific to the patient, developing customized tools for curing diseases. The microfluidic culture system presented in our study combines stem cell biology with bioengineering to formulate a platform for personalized medicine, in which a dynamic microfluidic system can reflect the natural in vivo environment. To show the potential for future clinical applications, which require “on demand use,” we tested and found that the microfluidic system can be successfully cryopreserved and thawed on demand with viable and functional differentiated EBs. These advances could be translated to useful applications such as in cell-based therapies.

Toward Personalized Medicine

An exciting application of the presented platform in regenerative medicine is the development of patient-specific microfluidic treatment modules. The potential applications of such a system are far-reaching for the treatment of other endocrine or neurohormonal disorders, such as diabetes with insulin replacement, Parkinson’s disease with dopamine replacement, or ovarian failure with estrogen and progesterone replacement. For each of these situations, as the specific signals that direct the differentiation of embryonic and pluripotent stem cells into the desired cell types are unraveled, one could use such microfluidic cassette cultures to harvest secreted bioidentical hormones and to cryopreserve the differentiated EBs within the chip for future use, as needed. Similar to the medication cartridges used today, in the foreseeable future, patients might receive autologous personalized treatment using their own iPSCs that have been differentiated into the desired secretory cell and grown in individual microfluidic chips. For the production of personalized biological agents, in addition to device simplicity and ease-of-use, reproducibility, reliability, and robustness through automation are required by regulatory agencies [12]. To allow the presented system to meet the levels of production at physiologically relevant hormone concentrations, high-throughput, multiplexed systems are needed for hormone therapies.

Previous studies have demonstrated the ability of EBs from human ESCs to produce functional trophoblastic tissue secreting estradiol, progesterone, and hCG [21] and ovarian granulosa-like cells secreting AMH and follicle-stimulating hormone [36]. With the discovery of iPSCs, excitement has been heightened in the field of regenerative medicine, because a primary obstacle to cell-based therapies has been the antigenic matching of tissue. With iPSCs, we have the option of developing autologous patient-specific treatment systems using pluripotent iPSCs autologous to the patient [37]. Regarding hormone replacement therapy, in recent years, concerns have been raised by studies such as the Women’s Health Initiative, regarding the risks associated with the use of synthetically produced hormones [4]. Combining the autologous nature of iPSCs with the potential to differentiate iPSC-derived EBs into steroidogenic cells, it will be possible to produce bioidentical hormones for patient treatment [38]. A potential future direction with the presented microfluidic platform will be to further evaluate human iPSCs for clinically relevant applications.

CONCLUSION

The present study has demonstrated several innovative advancements in regenerative medicine and microfluidic systems. We showed that microfluidic chips present a viable system for maintaining mouse EB growth and differentiation. Also, estradiol and progesterone were produced at physiologically relevant levels. Finally, functionally established microfluidic chips with EBs can be cryopreserved and thawed with restoration of function for use at a later time point. These findings strongly support the potential use of microfluidic chips for future personalized hormone therapy.

Figure 4. Embryoid bodies cryopreserved in a microfluidic channel showing differentiation to ovarian tissue markers: AMHR type II and estrogen biosynthesis enzyme aromatase Cyp19A1 (A), FSHR (B), and INHB-A (C) markers. Scale bars 200μm. Abbreviations: AMHR, anti-müllerian hormone receptor; DAPI 4′6-diamidino-2-phenylindole; FSHR, follicle-stimulating hormone receptor; INHB-A, inhibin β-A.

Figure 5. Steroid hormones secreted by mouse embryoid bodies in a microfluidic chip detected by enzyme-linked immunosorbent assay analysis after 21 days of culture. Noncryopreserved samples (black bars) and cryopreserved samples (white bars). Hormones estradiol (A), progesterone (B), and testosterone (C).
therapies. This approach could be adapted to broad clinical applications, such as the generation of patient-specific β-islet cells or a drug-screening platform for patient-derived tumorigenic cells.

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AUTHOR CONTRIBUTIONS

S.G., J.S.L., I.P., S.C., M.D.N., and B.G.-N.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; U.A.G.: conception and design, final approval of manuscript; R.M.A.: conception and design, financial support, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; U.D.: conception and design, financial support, provision of study material or patients, data analysis and interpretation, manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

U.D. is a founder of, and has an equity interest in: (a) DxNow Inc., a company that is developing microfluidic and imaging technologies for point-of-care diagnostic solutions, and (b) Koek Biotech, a company that is developing microfluidic IVF technologies for clinical solutions; his interests were reviewed and are managed by the Brigham and Women’s Hospital and Partners HealthCare in accordance with their conflict of interest policies. The other authors indicated no potential conflicts of interest.

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