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Womb-on-a-chip biomimetic system for improved embryo culture and development

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ABSTRACT

The 3D biomimetic microsystem described in this research integrates various functions of the fallopian tube to serve as an in vitro platform for fertilization and embryonic development. This device mimics the in vivo embryo-endometrial stromal cell monolayer coculture to enhance the group culture of embryos and mechanically culture the embryos in a dynamic manner to enhance their development. The microdevice presented here is compared with static culture for embryonic development rate in the presence and absence of epithelial cells. The results suggest that the dynamic biomimetic microsystem enhances the rate of embryo development, providing a culture environment that closely mimics the in vivo microenvironment. This all-in-one biomimetic microsystem has the potential to replace the present embryo culture platforms used for assisting in vitro fertilization.

1. Introduction

A genuine increase in infertility has been observed due to various factors including stress, environmental pollution, increasing age, smoking, consumption of alcohol, sexually transmitted diseases, etc. In vitro fertilization (IVF), a state-of-the-art technology, increases the rate of pregnancy, offering hope to those with fertility issues. During IVF, fertilized eggs in the blastocyst stage are transferred to the woman's uterus for implantation and further development. Since the invention of IVF, research advancements have been made in areas such as chemically and physically improving the culture environment of the pre-implantation embryos and developing specialized culture surfaces [1,2]. In the past decade, various static culture platforms, i.e. microdrops [3], ultramicrodrops [4], microwells [5] and microchannels [6–9] have been developed to confine the embryos in separated wells [10] or to group culture them [11–13], considering the role of media volume [14] and growth factors [15] necessary for embryonic development. In comparison with individual culture, group culture of embryos improves rate of blastocyst development; most likely via the production of autocrine and paracrine factors [16].

Traditional static culture systems fail to mimic the dynamic fluid environment in the fallopian tube [17]. Given the need for physical stimulation in embryonic growth and development [18], dynamic culture platforms that use shaking/rotation [19,20], tilting [21], vibration [22] and controlled fluid flow [23] to mildly stimulate the embryo have been studied for use in embryonic development. Moreover, reports have indicated that the co-culturing of embryos with endometrial cells has proven beneficial in embryo culture and development [24–28].

In an attempt to overcome developmental arrest of early embryos cultured in media alone, various coculture systems have been developed including Vero cells (an epithelial cell line derived from Green monkey kidney) [29], human tubular cells [30], uterine fibroblasts [31], granulosa cells [32] and human endometrial epithelium [33]. In most cases, these studies have demonstrated the beneficial effects of various cellular monolayers on the development of mammalian embryos when compared to embryos cultured in media alone. In addition, the embryo trophic effects of these

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cellular monolayers are known to be epithelium rather than hormone dependent [34], and are neither species [35], nor organ-specific [29]. The mechanism of action by which cell monolayers enhance blastocyst formation could be attributed to removal of toxins from the culture medium, or secretion of embryo trophic substances and embryo-cell interaction [29,36,37].

Although all of the parameters affecting embryo culture have been addressed using different culture platforms, a stand-alone culture device is needed, which not only mimics the in vivo micro environment, but also considers all the parameters affecting embryonic development. The primary goal of this research is to develop an autologous endometrial co-culture chip accompanied with group culture of embryos as a necessary approach to deal with IVF and partly mimic the physiological function of the reproductive system. The advancements in micro fabrication technologies have made it possible to imagine the culture platforms wherein control of the embryonic microenvironment is feasible. Based on the micro fabrication technologies [38,39], a three-dimensional (3D) perfusion platform have been developed for the co-culture of embryos with endometrial stromal cells that simultaneously exposes the embryos to mild mechanical stimulation with the help of a specially designed culture chamber. The microfluidic device also features the automated generation of different concentrations of progesterone to investigate the role of progesterone concentration in the culture of endometrial stromal cells.

2. Materials and method

2.1. Device design and fabrication

The microfluidic device incorporates different parts that performed different functions on a chip. In the female body, during the menstruation cycle the serum level of steroid hormones varies in a phase dependent manner. To mimic this phenomena of change in hormone level in different phase of menstrual cycle and to study its effect on the embryo development rate, we fabricated the gradient channel for automated generation of different concentrations of steroid hormone. The chip as shown in Fig. 1 is comprised of an upstream concentration gradient generator (width: 250 μm, height: 230 μm), which is integrated with a diamond-shaped passive micro-mixer (width: 200 μm, height: 230 μm) that generates six different homogeneous concentrations of progesterone. The micro-mixer continuously split and merge the liquids, increasing the contact area between liquid molecules, thus increasing the mixing efficiency. The generated concentrations then enter the culture chamber, wherein the endometrial stromal cells are cultured on a porous polydimethylsiloxane (PDMS) membrane sandwiched between the culture chamber (radius: 3 mm, height: 230 μm) and the perfusion chamber. Perfusion system replenishes the used culture medium and nurtures the endometrial stromal cells. The main specifications and goals of our microfluidic channel design are (i) gradient distribution for specific concentrations of steroid hormones in six culture chambers, (ii) complete mixing for homogenous concentrations of steroid hormones in individual culture chamber, (iii) similar flow speed/rate for the six culture chambers to maintain uniform culture conditions with respect to the flow speed/rate. To simplify our microfluidic channel design, gradient generator not only generated different concentrations but it also maintained a uniform flow rate. The diamond-shaped passive micro-mixer was used to enhance the mixing in our microfluidic channel design.

The perfusion channel (width: 250 μm, height: 230 μm) on the bottom provides 10 nM of estrogen by perfusion through the porous membrane to the endometrial stromal cells cultured on the porous membrane. The estrogen is pumped into the perfusion chamber at a very slow flow rate of 1 μL/min. Progesterone and estrogen modulated the proliferation and differentiation of endometrium tissue for successful embryo implantation. In this work, we have treated the endometrial stromal cells with progesterone [40] and estrogen to mimic the in vivo situation in the womb for embryo implantation.

In vivo fertilization of the ovum usually occurs in the ampullary region of the fallopian tube. In the ampulla of the fallopian tube, the embryo is propelled by the lining cilia of tubular mucosa [41,42]. In an attempt to mimic the in vivo conditions in the uterine tube, a PDMS culture chamber was designed with semi-circular curved microstructures. The culture chamber of the chip was designed to mimic this function of the fallopian tube wherein the flow velocity in assistance with the microstructure was exploited to provide stimulation to the embryos in resemblance with the beating of cilia. The flow driven mechanical stimuli generated in the culture chamber may assist the embryo development. When the embryos were introduced in the culture chamber through the left inlet, the embryos get positioned randomly in the region of minimal velocity i.e. near the curved structures in the culture chamber, where they receive mild stimuli due to minimal flow velocity in that region similar to the beating of cilia in fallopian tube. From the bottom, a pneumatic channel (width: 250 μm, height: 230 μm) is integrated into the gradient channel to stop the flow to the culture chamber.

This 3D microfluidic device is produced by precise alignment and bonding of three layers prepared by casting PDMS against a photolithographically prepared SU-8 master. The fabrication process shown in Fig. 2(A)–(C) is as follows: the SU-8 photoresist was spun on a four-inch Si wafer, exposed, and then developed using a photolithography process to obtain an SU-8 master. The PDMS channels were created using a Sylgard 184 silicone elastomer mixture (Dow Corning Corporation, Midland, USA) at a weight ratio of 10:1 (base:curing agent). The mixture was degassed to remove the bubbles and poured over the SU-8 master. The PDMS was cured by baking in an oven at 100 °C for 15 min. After cooling, the PDMS was peeled off and cut to obtain the top and bottom PDMS channels. To prepare the microporous PDMS membrane, a PDMS mixture with n-Hexane at a weight ratio of 6:1 was spin-coated onto an array of 40 μm columnar structures at 1800 rpm for 30 s, and then baked and cured. After cooling, the microporous membrane was peeled slowly and further alligned and bonded between the two PDMS structures, as shown in Fig. 2(F) and (G).

2.2. Human endometrial stromal cell culture

Human endometrium was obtained from surgical specimens of normally cycling women undergoing hysterectomy for benign reasons, in accordance with the guidelines of the Declaration of Helsinki after informed consent and with approval by the Chang Gung Memorial Hospital Institutional Review Board. The tissue samples used for this study were histologically normal. Endometrial stromal cells were separated from the glandular epithelium after collagenase digestion and cultured using an established in-vitro model as previously described [43].

Human endometrial stromal cells were cultured and grown to confluence in 75% Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY, USA) and 25% MCDB 105 medium (Sigma, St. Louis, MO, USA) comprising antibiotics (100 U/ml penicillin and 100 U/ml streptomycin) (Sigma-Aldrich Co.), 5 μg/mL insulin (Sigma), supplemented with 10% charcoal-stripped fetal bovine serum (FBS) (Gibco, USA), 10% FBS (Invitrogen) and antibody. The cells were incubated at 37 °C under a humidified atmosphere of 5% CO_2 and 95% air. The subculture procedure was conducted under a cell culture hood. In the first step, the spent culture media was aspirated from the flask and the cells were gently washed with 10 mL Dulbecco's Phosphate-Buffered Saline