Endometrial regeneration using cell sheet transplantation techniques in rats facilitates successful fertilization and pregnancy

Goro Kuramoto, M.D., Ph.D.,[a,b] Tatsuya Shimizu, M.D., Ph.D.,[b] Soichi Takagi, Ph.D.,[b] Ken Ishitani, M.D., Ph.D.,[a,c] Hideo Matsui, M.D., Ph.D.,[a] and Teruo Okano, Ph.D.[b]

[a] Department of Obstetrics and Gynecology and [b] Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University; and [c] Department of Obstetrics and Gynecology, Kitasato University Kitasato Institute Hospital, Tokyo, Japan

Objective: To regenerate functional endometrium tissue using "cell sheet" techniques as a regenerative medicine approach to address endometrial disorders causing female factor infertility.
Design: In vivo experimental study.
Setting: Preclinical surgical and biomedical research laboratories.
Animal(s): Green fluorescent protein (GFP) transgenic rats [SD-Tg (CAG-EGFP) rats] and nude rats (F344/NJcl-*rnu*/*rnu*).
Intervention(s): GFP-positive rat uterine-derived cells as cell sheets were transplanted into resected rat uterine endometrial sites. Transplanted cell sheet areas were then analyzed using macroscopic observations and histological analysis including immunohistochemistry. Subsequently, crossbreeding was performed to establish fertility and confirm pregnancy in the rat-regenerated uterus.
Main Outcome Measure(s): Morphologic and biochemical markers of regenerated endometrium and establishment of pregnancy in otherwise sterile animals.
Result(s): After cell sheet transplantation, regenerated endometrium was confirmed as GFP-positive tissue engraftment both visually and under histological analysis. After crossbreeding, GFP-positive tissue areas and living fetuses were observed in the transplantation group.
Conclusion(s): Cell sheet transplantation can regenerate endometrial tissue with histological structure and physiological function supporting pregnancy similar to normal endometrial tissue. Translation of this endometrial cell sheet transplantation method to human patients with endometrial disorders could yield a novel therapy for uterine infertility. (Fertil Steril® 2018;110:172–81. ©2018 by American Society for Reproductive Medicine.)
This abstract is available in Spanish at the end of the article.

Key Words: Endometrial regeneration, uterine infertility, tissue engineering, regenerative medicine, cell sheet

Pregnancy occurs when a fertilized egg successfully implants into the uterine endometrium. Endometrial disorders due to dilation and curettage (1–4), posthysteroscopic surgery (5, 6), or retained uterine contents (7) can result in uterine infertility. Schenker and Margalioth (8) reported that 43% women with intrauterine adhesions are infertile. Pathologically, diseased endometrial tissue is characterized by fibrosis and adhesions, and adhesions with connective tissue lack any endometrial lining in disease (9, 10). The prognosis for such disease (i.e., normal menses and fertility) depends very much on the severity and extent of the adhesions (10). Some researchers report that surgical synechiotomy (11) and hormonal drugs (11) are effective against intrauterine adhesions, and

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Reprint requests: Tatsuya Shimizu, M.D., Ph.D., Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo, 162-8666, Japan (E-mail: shimizu.tatsuya@twmu.ac.jp).

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intrauterine device placement (12) is reported as generally therapeutic against intrauterine adhesions. However, for some severe endometrial disorder patients, these interventions are ineffective, and these cases are found to be irreparable (10, 13). Therefore, new therapeutic approaches that regenerate normal endometrial tissue are needed for severe cases.

Regenerative therapies based on cellular approaches have the potential to provide new treatment methods. Tissue engineering is commonly used experimentally to transfer cell scaffold combinations for tissue regeneration and for forming surrogate organs in vitro (14). Many bioabsorbable polymer scaffolds have been developed for three-dimensional tissue production, using seeded cells on scaffolds (15, 16). However, scaffold-based tissue engineering structures such as collagen often produce lower cell densities. Moreover, polymer scaffolds can also induce host inflammatory reactions during the absorptive process in vivo (17). Few cell scaffold strategies are translated clinically. As an alternative, our laboratory has described a new technology termed "cell sheet engineering" to fabricate contiguous cell sheets and their multilayers without scaffolds, using thermoresponsive cultureware to harvest and manipulate these constructs from diverse cell sources (18). Because these sheets retain native adhesive extracellular matrix, including fibronectin (19) and laminin (20), on their basal surfaces, these cell sheets readily transplant without suturing (21). This technology has been applied in various clinical studies (22–26) and in basic research activities (27–30).

Previously, endometrial regeneration has been reported using a decellularization technique (31, 32) and collagen scaffolds (33). However, only partial endometrial regeneration is reported, and it is unclear whether fertilized eggs could implant successfully into such partially regenerated endometrium.

This study aimed to regenerate endometrial tissue in rats to yield native, functional endometrium using endometrial cell sheet transplantation. Results describe a method to produce cell sheets from rat primary endometrial cells, regeneration of endometrium, and establishment of pregnancy on regenerated endometrium in a rat model.

MATERIALS AND METHODS

Animals

Female green fluorescent protein (GFP) transgenic rats (SD-Tg [CAG-EGFP] rats) age 3 weeks (n = 13) were used to collect endometrial cells for fabricating three-layer cell sheets, and female adult nude rats (F344/NJcl-*rnu*/*rnu*) age 9 weeks (weight approximately 150 g) were used for all transplantation experiments; animals were purchased from Japan SLC and CLEA Japan, respectively. The cell sheet transplantation group used 38 rats total, and the nontransplantation group used 25 rats; further details are provided in Supplemental Table 1. Male adult rats (F344/NJcl-*rnu*/*rnu*) used for mating were purchased from CLEA Japan. All animals were housed in a temperature-controlled room at approximately 22°C with a 12-hour light/dark cycle. Food and water were available ad libitum. All animal use was approved by the Animal Welfare Committee of Tokyo Women's Medical University (approval no. 14-84, 2014). This preclinical study is based solely on small animal studies, and therefore Institutional Review Board approval was not necessary.

Preparation of Endometrial Cell Sheets

Uteri were resected from 10 female 3-week-old GFP rats. After removing the myometrium physically with tweezers, the remaining endometrial tissue was treated with 0.5% trypsin solution containing 0.2% ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich) at 37°C for 20 minutes with continuous shaking. Suspended endometrial cells were isolated by 100- and 40-μm pore size cell strainers (BD Falcon 352360 and 352340, respectively; BD Biosciences). Two hours after seeding on normal 100-mm dishes, supernatant (epithelial) cells were collected and reseeded on temperature-responsive poly(*N*-isopropylacrylamide)-grafted cell culture inserts (UpCell insert, CellSeed) at a density of 5.0×10^6 cells per insert with phenol red-free Dulbecco's modified Eagle medium and Ham's F-12 at a volume ratio of 1:1 (DMEM/F12; Life Technologies) supplemented with 10 μg/mL insulin, 10 μg/mL transferrin, 0.038 μM selenite, 100 μg/mL hydrocortisone, 2.5 nM retinoic acid, 100 μm L-ascorbic acid, 10 ng/mL epidermal growth factor, 100 U/mL penicillin, and 100 mg/mL streptomycin. Adherent (stromal) cells were collected with 0.5% trypsin solution containing 0.2% EDTA treatment for 5 minutes and were reseeded on temperature-responsive poly(*N*-isopropylacrylamide)-grafted cell culture dishes (UpCell CS3007, CellSeed) precoated with fetal bovine serum for 3 hours at a density of 2.5×10^6 cells per dish with DMEM/F12 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. After 4 days of incubation, confluent cells from each approach were harvested as cell sheets by reducing culture temperature from 37°C to 20°C for 1 hour after scraping the dish edge basal surface with a pipette tip. Layered cell sheets were assembled from layering one supernatant-derived cell sheet (endometrial-epithelial cell sheet) and two adherent cell sheets (endometrial-stromal cell sheet). GFP fluorescence activity of the resulting three-layer GFP cell sheet was confirmed with fluorescent microscopy.

Cell Sheet Transplantation

The left uterus of a 10-week-old nude rat was cut lengthwise and opened, and the endometrial layer was physically resected using tweezers (Fig. 1A). The defect area was circumferentially 10 mm in length such that it matched the size of the cell sheet. The remaining myometrium was pulled to the left and right using 7-0 nylon sutures at 10 points (Fig. 1B). In the transplantation group, the three-layer endometrial cell sheet was transplanted to the resected area using a plastic support device. Fluorescent microscopy was used to confirm the location of the GFP-active cell sheet after transplantation (Fig. 1C) and after wound closing (Fig. 1D). In the nontransplantation group, only the endometrium resection was performed. After implantation, the exposed uterine tissue was kept open for 1.5 hours in both groups, ostensibly to ensure