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REGULAR ARTICLE

Differentiation of human endometrial stem cells into germ cell – Like cell in fibrin scaffold

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Germ cells

Abstract Recent studies on stem cells differentiation into germ cells have changed scientists' attitude to reproductive problems as well as infertility topics. It is supposed there are promising and new approaches in treatment of infertile couples and numerous advances will be made in reproductive medicine in near future. Application of embryonic stem cells for clinical trials is limited due to high potent of tumorogenicity and ethical issues. Therefore, pluripotent cells taken from adult tissues or organs, could be a good alternative for gamete production. Herein, we hypothesize to stimulate human endometrial stem cells (hEnSCs) differentiation into germ cell-like cells by culturing in retinoic acid (RA) as 2D medium and then in fibrin as 3D scaffold. Germ cell markers such as DAZL, DDX4 and Dppa3, will be assessed by immunofluorescence and real-time PCR. Fibrin mechanical properties will be examined by rheology analysis and cell viability will be determined by MTT assay. Specific markers expression and the cells' integrity will be detected by immunofluorescence staining and SEM analysis respectively. We suggest differentiation of hEnSCs into germ cell-like cells in a medium containing 10^{-5} M RA in which the specific markers were expressed properly in both 2D and 3D medium cultures. Additionally, fibrin scaffold will offer a proper 3D scaffold for hEnSCs-derived germ cell-like cells.

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Introduction

According to World Health Organization definition Infertility is "a disease of the reproductive system defined by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual activities". Infertility in a couple who have never had a child is known as primary infertility. Failure to conceive following a previous pregnancy is known as secondary infertility. Infertility may be caused by infection in the man or woman. Assisted Reproductive Technology (ART) has been developed as new methods of infertility treatments [1]. The latest method is stem cell applications for the purpose of infertility treatment and in vitro production of gametes [2,3].

Recent studies showed that a good alternative for the production of germ cells in the laboratory is adult stem cell obtained from human endometrial tissue with its pluripotent characteristics. To date, hEnSCs differentiation to germ cell – like cells has not offered and this study presents a novel hypothesis of germ cell – like cells differentiation with its markers expression both in ICC and real-time PCR for the first time.

The fibrin gel would preserve a suitable 3D hydrogel structure as a viscoelastic material. Viability of differentiated cells will be examined through MTT test, in order to prove no evidence of toxicity. To have the microstructure of fibrin as a 3D mesh, SEM images will be taken in which nano-fibers cross and entangle with high porosity for nutrient transfer and cell penetration. Finally immunohistochemistry will show that differentiated hEnSCs to germ cell – like cells reserve their nature as positive expression of DAZL and DDX4 markers were evident in suspended cells after 7 days in culture.

Hypothesis

In recent years, stem cell therapy has offered novel therapeutic strategy to managing infertility and reproductive disorders and scientists have concentrated on them to produce gametes in vitro [4]. They demonstrated that mouse embryonic and bone marrow stem cells can be differentiated into oocyte-like cells and male germ cell-like cells respectively, which express germ cell specific markers [5,6]. Other studies showed derived human male germ cell-like cells from bone marrow stem cells in RA culture medium [7,8]. Human EnSCs are readily available sources of adult stem cells in the uterus which are multipotent and express CD146 marker [9]. We postulate the suitable conditions which promoted hEnSCs differentiation into germ cell-like cells by adjusting the best concentration of RA in 2D medium. Afterwards, the differentiated cells will be cultured in a 3D fibrin scaffold and their viability and properties will be evaluated (Fig. 1).

Discussion

Evaluation of the hypothesis

The hypothesis can be evaluated by using flow cytometry for detection CD146, CD90 as stem cell markers in the isolated endometrial stem cells. The next step is to investigate the ability of human endometrial adult stem cells to differentiate into the germ cell-like cells .For this purpose the endometrial stem cells will be induced by RA by optimum concentration of 10^{-5} M after 7 days. Then, characteristic cell markers such as Dazl, DDX4 and Dppa3 will be determined by immunofluorescence and real- time PCR assays. After encapsulating the hEnSCs-derived germ cells in fibrin gel, cell differentiation and viability will be assessed by culturing for 7 days subsequently. Structural and mechanical properties of the fibrin scaffold will be examined by rheological analysis and the porosity will be examined by SEM. Also viability of cells will be analyzed using MTT assay.

Endometrial stem cells versus other sources of stem cells

Human endometrium undergoes cycles of growth and regression, extensive restructuring and remodeling during the female reproduction life [10]. Human EnSCs are readily available sources of adult stem cells in the uterus and can be highlighted for their multipotent and differentiation properties [9]. Their

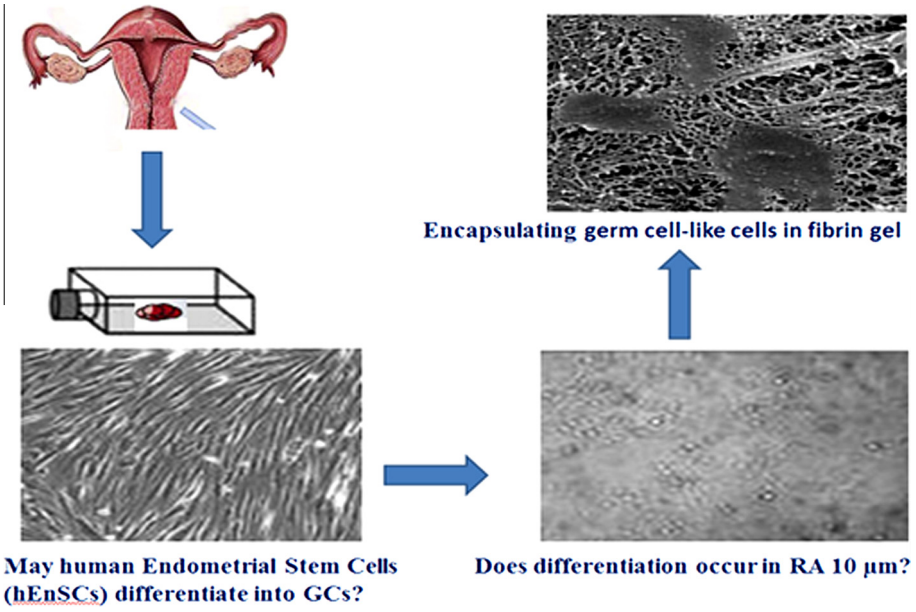

Fig. 1 Schematic presentation of our hypothesis.