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Engineered multilayer ovarian tissue that secretes sex steroids and peptide hormones in response to gonadotropins

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ABSTRACT

Although hormone replacement therapy is an option for the loss of ovarian function, hormone delivery through pharmacological means results in various clinical complications. The present study was designed to deliver sex steroids by a functional construct fabricated using encapsulation techniques. Theca and granulosa cells isolated from ovaries of 21-day old rats were encapsulated in multilayer alginate microcapsules to recapitulate the native follicular structure. Cells encapsulated in two other schemes were used as controls to assess the importance of the multilayer structure. The endocrine functions of the encapsulated cells were assessed *in vitro* for a period of 30 days. Encapsulated cells showed sustained viability during long-term *in vitro* culture with those encapsulated in multilayer capsules secreting significantly higher and sustained concentrations of 17 β-estradiol (E_2) than the two other encapsulation schemes ($p < 0.05$, $n = 6$) in response to follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In addition, cells in the multilayer microcapsules also secreted activin and inhibin *in vitro*. In contrast, when granulosa and theca cells were cultured in 2D culture, progesterone (P_4) secretion increased while E_2 secretion decreased over a 30-day period. In summary, we have designed a multilayer engineered ovarian tissue that secretes sex steroids and peptide hormones and responds to gonadotropins, thus demonstrating the ability to recapitulate native ovarian structure *ex vivo*.

1. Introduction

Ovaries serve as gonads as well as endocrine glands, and are the primary physiological source for sex steroids. Ovarian follicles are the fundamental units of ovaries and each produces a single oocyte (germ cell), steroids, and protein hormones to regulate the reproductive cycles in females [1,2]. The ovarian hormones produced by the follicles play a crucial role in maintaining the ovarian cycle, determining secondary sexual characteristics and preparing the endometrium for implantation. The follicles become corpora lutea after ovulation and produce hormones in order to maintain the pregnancy if the oocyte is successfully fertilized [3,4]. During the follicular phase (pre-ovulatory phase) estrogens are the predominant steroids produced by the functional follicle and in the luteal phase (post-ovulatory phase) progesterone is the major steroid produced by the corpus luteum. As far as estrogen biosynthesis is concerned there exists a two-cell two-gonadotropin concept [5–9], which involves theca cells, granulosa cells, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Theca cells in the periphery of the follicle possess the key enzyme CYP17A1 (17, 20 lyase) to synthesize the aromatizable androgens (androstenedione and testosterone) under the influence of LH. These aromatizable androgens are then converted by CYP19 (aromatase) in the granulosa cells into estrogens, a process regulated by FSH [10,11]. This cyclic pattern of sex steroid synthesis and secretion occurs during every ovarian cycle until all the embryonically formed primordial follicles for the production of functional follicles are exhausted.

Loss of ovarian function caused by surgical resection, ablative therapy, or menopause not only affects the reproductive ability but also leads to cessation of sex steroid production by the ovary leading to various physiological consequences in women [12,13]. Ovarian hormone-deprived conditions results in various pathological conditions ranging from urogenital complications to osteoporosis [13,14]. Although hormone replacement therapy (HRT) is able to compensate for the loss of ovarian hormone production, hormone delivery through pharmacological means results in consistently

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higher serum concentrations and clinical complications including increased incidence of heart disease and cancer depending on the HRT regimen used [15–19]. Therefore, cell/tissue-based hormone therapy that provides more physiological serum levels of hormones is an appealing alternative for treatment of ovarian hormone-deprived conditions. We hypothesize that the endocrine cells would respond to the endogenous levels of gonadotropins and secrete sex steroids and, in turn, the gonadotropins would be

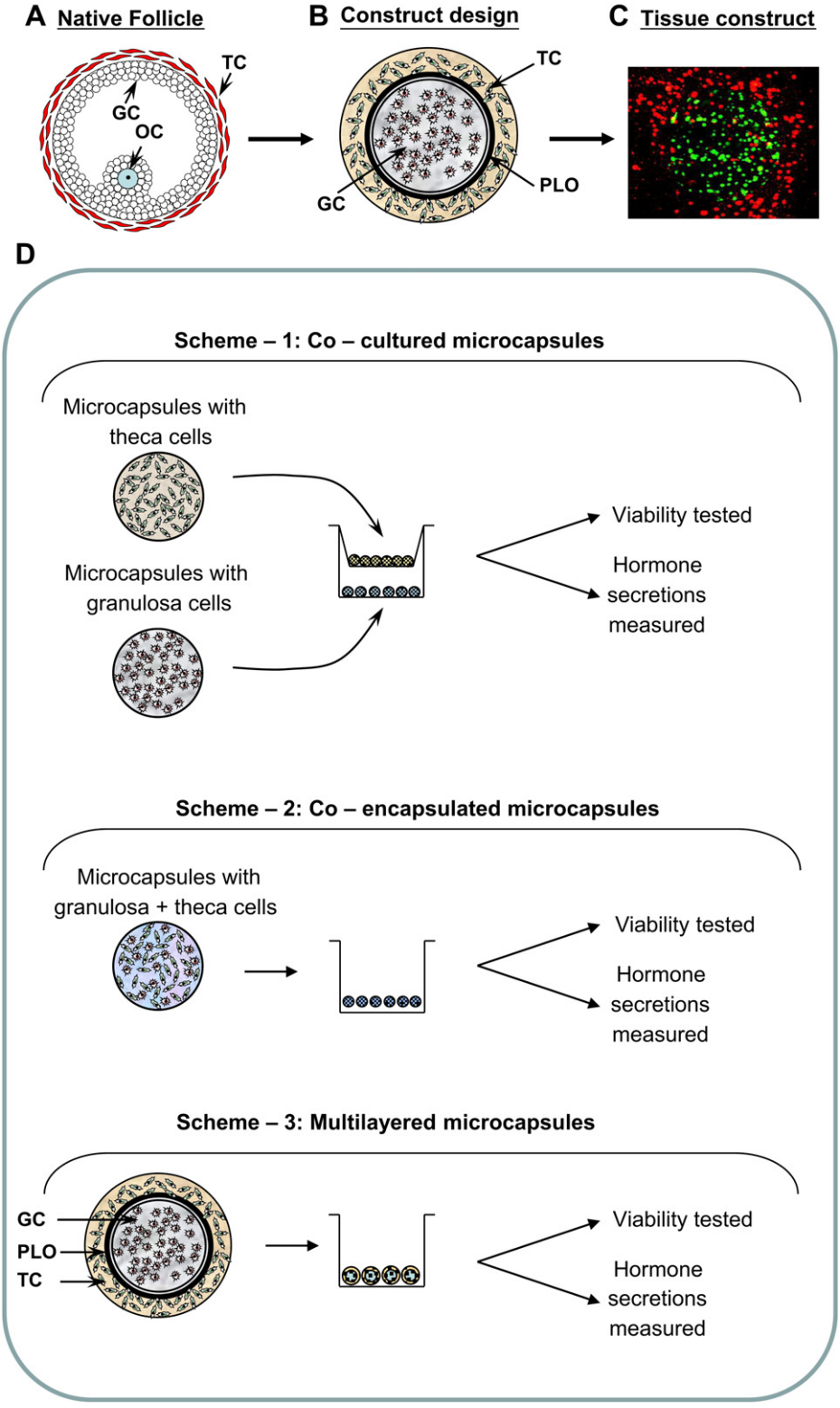

Fig. 1. (A) Schematic diagram of an ovarian follicle. (B) Approach of using multilayered alginate microcapsule to mimic native follicular structure. (C) 3D - confocal image of microcapsules demonstrating compartmentalization of different cells - distribution of CellTracker green-labeled cells (granulosa) in the inner layer and CellTracker orange-labeled cells (theca) in the outer layer. (D) Outline of the study demonstrating the co-cultured/co-encapsulated/multilayer microcapsules as the three approaches used. GC – granulosa cells; OC – oocyte; TC – theca cells; PLO – poly-L-ornithine.