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Review

# Female hormone release of microencapsulated *Xenopus laevis* ovarian cells

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ABSTRACT

Cell microencapsulation has potential for the treatment of endocrine diseases. This study aims to probe the feasibility of *Xenopus laevis* as an animal model for cell microencapsulation and transplantation and to evaluate the female hormone release of microencapsulated *X. laevis* ovarian cells. The cells were harvested, cultured and microencapsulated into alginate–chitosan–alginate microcapsules with an electrostatic generator. The estradiol and progesterone releases of the microencapsulated *X. laevis* ovarian cells were investigated both *in vitro* and *in vivo*. The results showed that the microencapsulated cells kept secreting estradiol and progesterone *in vitro* for 60 days. After transplantation, serum estradiol and progesterone levels in ovariectomized *X. laevis* remained elevated for 60 days. *X. laevis* has been proved to be a suitable animal model for cell microencapsulation and transplantation. Microencapsulated ovarian cells may be considered as a promising endogenous drug delivery system for the treatment of deficiency of female hormones.



**Contents**



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## 1. Introduction

When women enter the menopause, they may suffer from menopausal symptoms such as urogenital atrophy, sexual dysfunction and intense emotional reactions. Reduced secretion of female hormones such as estradiol (E2) and progesterone (P4) plays a key role in menopausal syndrome and causes a great risk of diseases (Nelson, 2008).

Hormone replacement therapy (HRT) is a common drug therapy to suppress menopausal symptoms (Genazzani and Gambacciani, 1999). However, long-term HRT entails serious side effects such as venous thromboembolism, stroke, coronary heart disease, and cancers (Dietel et al., 2005; Rossouw et al., 2002). The problem of the side effects may be resolved if endogenous female hormones can be provided (Feng et al., 2007) because the hormone release can be regulated by hypothalamus–pituitary–gonad (HPG) axis, which is an essential regulation pathway for reproduction in vertebrates. Ovarian tissue transplantation has been reported to be able to regain ovarian function and maintain female hormone levels (Deng et al., 2009; Schmidt et al., 2005). However, immunological rejection threatens the viability of grafted tissues, thus immunosuppressants are routinely required.

Long-term transplantation of tissue cells could be achieved by cell microencapsulation in the absence of immunosuppressant treatment. Alginate-based microencapsulation of cells was introduced by Lim and Sun (1980) and it has provided a series of promising therapeutic treatments for diabetes, cancer, hypoparathyroidism, renal failure, etc. (Orive et al., 2003). Cell microencapsulation physically entraps cells from the outside environment within a semipermeable membrane (Uludag et al., 2000), permitting the entry of nutrients and oxygen as well as the exit of therapeutic products. Furthermore, the membrane isolates cells from the host immune system, preventing antibodies and other immunologic moieties from penetrating through the membrane and destroying the cells as foreign invaders (Orive et al., 2004). Alginate–chitosan–alginate (ACA) microcapsule has been attracting interest for its biocompatibility, good stability, and suitable permeability (Gaserod et al., 1999; Hernandez et al., 2010; Holme et al., 2008; Onishi and Machida, 1999). Its membrane is created by the reaction between the polyanion alginate and the polycation chitosan. It has been applied to investigations on cell microencapsulation (Baruch and Machluf, 2006; Graff et al., 2008; Haque et al., 2005; Pound et al., 2006; Zhang et al., 2008).

Electrostatic generator can be used to prepare spherical and uniform alginate gel beads (Barnett et al., 2011; Manojlovic et al., 2006), and the microbead size can be controlled by adjusting needle diameter, electrode distance and electrostatic potential (Bugarski et al., 1994; Goosen et al., 1997). The electrostatic field has been proved to be safe for microencapsulated cells and does not lead to cell dysfunction (Bugarski et al., 1993; Nedovic et al., 2001; Poncelet et al., 1994).

African clawed frog (*Xenopus laevis*) is a major amphibian model and widely employed for studies on genomics (Khokha, 2012), endocrinology (Kloas and Lutz, 2006), toxicology (Franchini et al., 2010) and developmental biology (Mereau et al., 2007). *X. laevis* as an animal model has many advantages, such as: (1) as a vertebrate, its sex hormone synthesis is regulated by HPG axis; (2) anesthesia can be accomplished conveniently by immerging in narcotic solution; (3) it is docile, harmless and easy to handle, thus accidental injury to researchers can be avoided; (4) it can be kept easily and inexpensively.

The purpose of this study is to probe the feasibility of *X. laevis* as an animal model for cell microencapsulation and evaluate the female hormone release of microencapsulated *X. laevis* ovarian cells. The cells were harvested, cultured and microencapsulated into ACA microcapsules using an electrostatic generator. The female hormone release of the microencapsulated cells was investigated both *in vitro* and *in vivo*.

## 2. Materials and methods

### 2.1. Experimental animal

Two years old female *X. laevis* were kept in fresh water tanks with a filtration system under ambient temperature (25 ± 2 °C) and fed with commercial fish food. The light:dark cycle was 12:12 h. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals set by the National Institutes of Health, and approved by the Animal Care Committee of Zhejiang University.

### 2.2. Ovariectomy (OVX)

A method described for collecting ovary tissues from *X. laevis* (Moon et al., 2006) was adopted. Briefly, *X. laevis* with average body weight of 80 g were anesthetized with 1% MS222 (Sigma–Aldrich, USA) solution in an ice bath for 15 min. The skin on the ventral side was disinfected with 10% povidone iodine and an incision was made. All the ovarian tissues were excised.

### 2.3. Ovarian cell primary culture and pregnant mare's serum gonadotropin (PMSG) treatment

The *X. laevis* ovarian tissues were placed in 60 mm culture dishes containing 10 mL of amphibian physiological saline (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, pH 7.6), chopped into small pieces and washed with amphibian physiological saline thrice. The tissue pieces were digested with collagenase I (Sigma–Aldrich, USA) at a concentration of 0.3 mg/mL at room temperature for 30 min. Then the suspension was centrifuged at 1000 rpm for 10 min, and the resultant cell pellets were washed thrice. Finally, the cells were suspended in isotonic culture medium for amphibians (60% Leibovitz's L-15 medium, Gibco, USA; 10% fetal bovine serum, FBS, Evergreen, China; 30% double distilled water), transferred to 75 mL culture flasks (Corning, USA) and placed in a humidified incubator (3111, Thermo Fisher Scientific, USA) at 25 °C. The culture medium was changed every two days.

*X. laevis* ovarian cells were seeded in a 24-well plate (Corning, USA), and then incubated with the culture medium containing 200 mM testosterone (Sigma–Aldrich, USA) and PMSG (Sigma–Aldrich, USA) at concentration of 0, 10, 20, and 40 IU/mL respectively. After 48 h incubation, the culture supernatants were collected and stored at −20 °C.

### 2.4. Immunohistochemistry

Immunohistochemistry was employed to confirm female hormone-secreting cells by detecting follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR). *X. laevis* ovarian cells were seeded in a 24-well plate, and incubated with 20 IU/mL PMSG at 25 °C for 48 h. Then the cells were rinsed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde (Boster, China). The staining was carried out according to the manufacturer's protocol of commercial strept–avidin–biotin complex (SABC) immunohistochemistry reagent kits (Boster, China). Briefly, the fixed cells were incubated in methanol containing 0.6% hydrogen peroxide at room temperature for 30 min to block endogenous peroxidase activity, and then blocked with 5% bovine serum albumin for 20 min. The cells were incubated with the primary antibody of rabbit anti-FSHR antibody (1:200; Boster, China) and rabbit anti-LHR antibody (1:100; Boster, China) respectively