Microencapsulation of Leydig Cells

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Problem Statement

Develop method of microencapsulating cells to allow sustained hormone release while providing a physical barrier to the host's immune system.

Motivation

Current therapies that treat hormonal imbalances include transplantation, cellular grafts, and hormone supplementation. The use of immunosuppressants associated with transplants leads to significant side effects. Hormone therapy is often impractical due to the short half-life of physiological hormones, often requiring repeated treatments. Microencapsulation of cells offers the advantages of long-term hormone release, immunoisolation, and *minimal diffusion distance*.

Sustained release of reproductive hormones is required to restore balance of the hypothalamic pituitary gonadal (HPG) axis hormones during aging to prevent degenerative processes.

Background

Client Research

Dysregulation of the hypothalamic pituitary gonadal (HPG) axis (Figure 1) as a result of decreased sex steroid secretion may be a causative factor in a wide range of diseases associated with aging (Bowen and Atwood, 2004).

An *in vivo* hormone-releasing system may restore levels of FSH and LH to physiological levels via negative feedback on the hypothalamus and pituitary gland. Use of microcapsules may enable transplanted Leydig cells to exist within the patient by providing a physical barrier to the immune system.

Hydrogels for Microencapsulation

Network of hydrophilic, crosslinked polymers. Currently used in a variety of tissue engineering applications.

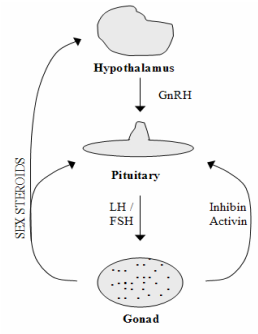

Figure 1. Hypothalamic-anterior pituitary gonadal axis, showing negative feedback loops from the gonads (Adapted from Morohashi, 1997).

Design Criteria

- Allow diffusion of relevant hormones
  - Testosterone ~300 Da, inhibin ~32 kDa, activin, FSH ~36 kDa, and LH ~30 kDa
  - Testosterone supplementation: 1.7 ng/mL serum conc. (rat model)
  - Controlled mesh size
- Provide a physical barrier to immune system (immunoglobulins ~150 kDa)
  - Molecular weight cutoff (MWCO) approximately 75 kDa
- Controlled degradation
  - Resists biological and mechanical degradation (6 mo – 1 yr)
- Encapsulated cell viability
  - 75% viability *in vitro* acceptable (1 d post-encapsulation)
  - Avoids hypoxia: <200 µm capsule diameter
  - Cell-compatible crosslinking method
- Material biocompatibility
  - Nontoxic
  - Noninflammatory – resists fibroblast overgrowth
  - Repeatability of results

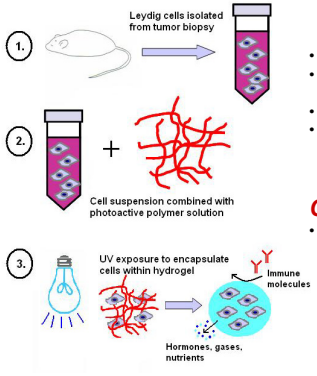

Figure 2. Basic steps in cell isolation and encapsulation within hydrogel process. Note that an established cancer cell line (MA-10) was used in the current design (Adapted from Masters, 2005).

Abstract

A method of microencapsulating cells for the long-term time release of male reproductive hormones in vivo *is desired as a treatment for a wide range of diseases associated with aging. The final design must overcome issues of biocompatibility, immune response, and hypoxia which are commonly associated with microcapsule implantation. Currently, the encapsulation of mouse Leydig cells with diacrylated polyethylene glycol (PEGDA) using a constructed microfluidic device is being pursued. Crosslinking is achieved using a photoinitiator and long-wave ultraviolet light. The viability of encapsulated cells is being examined; future work entails further capsule characterization and diffusion studies to assess capsule function* in vitro.

Final Design

Capsule Design

- Diacrylated polyethylene glycol (PEGDA) (Figure 3)
  - 8 kDa MW, 10% (w/v) solution
  - Bioinert, minimizes protein adsorption
- Crosslinking (Bryant *et al.*, 2000)
  - Photoinitiator – Irgacure 651® (Ciba Specialties)
  - UV light (365 nm, 3.9 mW/cm^2)
  - Shortest UV exposure time

Cell Culture

- MA-10 (mouse Leydig tumor cell line)
  - Produce testosterone
- Culture conditions
  - Waymouth's media, 15% horse serum, 1% penicillin/streptomycin (Invitrogen)
  - 37°C, 5% CO_2
- All cells at passage 4-5

Capsule Production

- Microfluidic device
  - Genie® flow controllers (New Era Pump Systems)
  - Mineral oil sheath flow (250 µL/min)
  - PEGDA/cell suspension flow (5 µL/min)
    - 4 x 10^6 cells/mL
  - Aqueous-in-oil emulsification
- Hydrophilic droplets crosslinked
- Microcapsules collected via oil extraction and PBS dilution
- Microcapsules immersed in culture media
- Capsule diameter: 100 - 400 µm
  - Controlled by relative flow rates and pipette tip diameter

Capsule Assessment

- Live/Dead® Viability/Cytotoxicity assay (Invitrogen)
  - Metabolism (green → live)
  - Membrane integrity (red → dead)
  - Performed on 1 µL droplets
  - Performed on PEGDA/cell suspension (prior to crosslinking) to confirm encapsulated cell presence
    - 15-30 cells/capsule

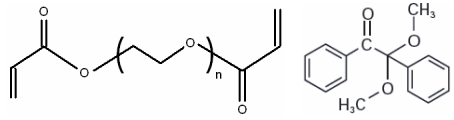

Figure 3. (Left) Repeat unit of PEGDA. Photocrosslinking reaction involves the formation of a methyl radical from a photoinitiator, which attacks the double bonds of PEGDA. A chain reaction results in a long polymer. **(Right)** Chemical structure of Irgacure 651 (benzyldimethyl-ketal) photoinitiator.

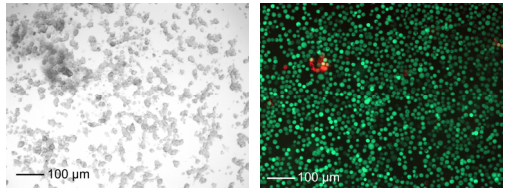

Figure 4. (Left) MA-10 cells after 1 d in culture. **(Right)** Live/Dead of MA-10 cells 10 min after plating. Both viewed at 100x.

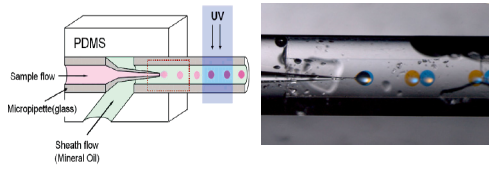

Figure 5. (Left) Schematic of microfluidic device (Jeong *et al.*, 2005). **(Right)** Droplet formation in actual device viewed using stereoscope at 50x. Outer diameter of capillary tube 1mm.

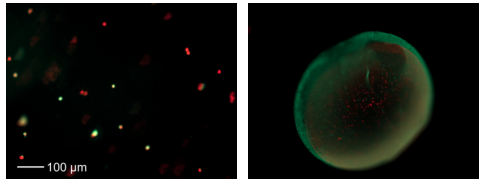

Figure 6. (Left) Live/Dead assay on MA-10 cells in PEGDA 1 h post-encapsulation. Note that these cells are not in microcapsules, but rather in small hydrogel cylinders (diameter 1 mm x length 3 mm) (100x). **(Right)** Live/Dead assay on MA-10 cells 2 d post-encapsulation in a 1 µL hydrogel droplet formed using a pipette on Parafilm (40x). Note autofluorescence of PEGDA.

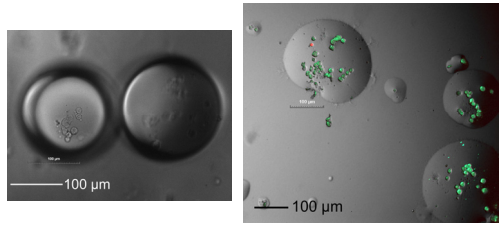

Figure 7. (Left) PEGDA microcapsules possibly including MA-10 cells. Approximate diameter is 150-200 µm (200x). **(Right)** Overlay image of visible light and Live/Dead images, used to identify cells encapsulated in PEGDA (100x). Due to the flat appearance, these PEGDA droplets appear to have fully cross-linked after settling on a polystyrene 96-well plate surface. Note that Live/Dead solution was added before encapsulation process, such that green cells do not necessarily indicate live cells post-encapsulation.

Future Work

- Further characterize microcapsules
  - Measure hydrogel swelling and degradation to determine degree of crosslinking
  - Determine appropriate crosslinking time (UV exposure)
  - Diffusion studies to determine mesh size
    - Altered by PEG molecular weight and concentration
- Continue to run cell suspensions through microfluidic device
  - Eliminate glass-tubing junction of device (aggregation)
  - Optimize collection of microcapsules from oil suspension
  - Measure hormone release, cytotoxicity of encapsulation procedure
- Implantation *in vivo* (mouse model)
  - Evaluate biocompatibility, immunoisolation, hormone release

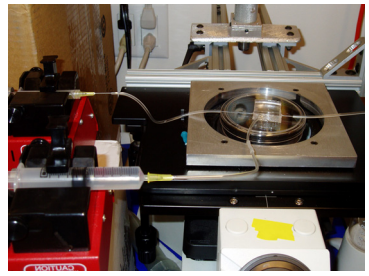

Figure 8. Microfluidic apparatus setup. Device is situated on platform, while flow controllers provide sample (PEGDA/cell suspension at 5 µL/min) and sheath (mineral oil at 250 µL/min) flows.

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