NOVEL IN VITRO MODEL TO GROW AND CULTURE OVARIES

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ABSTRACT

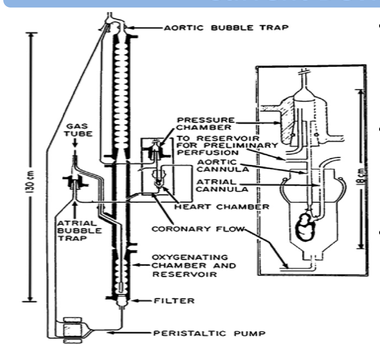
As cancer chemotherapy treatments become more sophisticated, there has been an associated increase in patient survival time. As a result, the severity of long-term side effects of chemotherapy has become apparent. One of these side effects is primary ovarian insufficiency (POI), which is seen in 40% of reproductive age breast cancer survivors. Specifically, studies have shown that doxorubicin (DXR) chemotherapy is associated with ovarian insult. The exact model of this insult has not been determined. To investigate, study, and ultimately solve this problem, a novel device for extensive study of mature ovaries *in vitro* is needed, as no systems currently exist. Through a careful design process and analysis, a bioreactor for ovarian culture that exhibits intravenous delivery of media to the ovary was fabricated. This device and method provides nutrients to the ovary in a physiologically accurate manner, while providing a controlled environment. Testing of the proposed design has yielded a simplified and successful protocol for ovarian artery isolation and cannulation, as well as a proven concept of method. Flowing fluid through the vasculature successfully saturated the ovary and excess fluid was observed exiting the ovary via diffusion. Future testing of the effectiveness of the media delivery system is needed in order to optimize a general protocol for extended ovary culture. Ultimately, our device will aid in determining the mechanism of ovarian insult, knowledge that will help reduce or even prevent primary ovarian insufficiency in surviving cancer patients.

BACKGROUND

Motivation

- Premature ovarian failure is apparent in:
  - 40% of breast cancer survivors [1]
  - 8% of childhood cancer survivors [1]
- Doxorubicin (DXR) chemotherapy causes primary ovarian insufficiency (POI)
- Cellular/tissue mechanism of DXR toxicity is not understood [2]
  - A method for experimentation is necessary
- No current culture system models are physiologically relevant

Current Devices



- Neonatal mouse ovary culture [3]
  - Ovaries can only be cultured for 1-15 days
- *In vitro* organ cultures have been successful [4]
  - None specialized for the ovary
- Tissue Bioreactors
  - Liver
  - Kidney
  - Langendorff Heart (**Figure 1**)

Figure 1. Modification of the isolated heart perfusion model (Langendorff), which allows measurement of the left ventricle work [5].

PROBLEM STATEMENT

- To develop an *in vitro* ovary culture system that:
  - Maintains tissue viability
  - Is sterile and biocompatible
  - Facilitates assessment of DXR chemotherapy toxicity
  - Enables future investigations of ovarian protection

DESIGN CRITERIA

- 90-100% cell viability
- Lasts for a minimum of 2 weeks and up to 3 months of experimentation
- Mimics in-vivo environment (37°C and pH ~7.4)
- Detect and measure fluid flow rates and pressure
- High repeatability
- High ease of use

FINAL DESIGN

Cannulation of Ovarian Artery

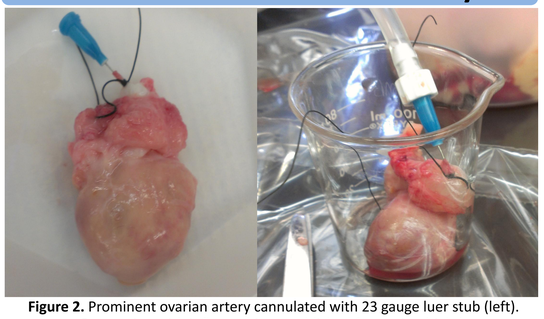


Figure 2. Prominent ovarian artery cannulated with 23 gauge luer stub (left). Connection with inflow tubing established for testing (right).

Modifications and Components

- GLS 80 Bottle (1000 mL) with modified cap for inlet/outlet ports and air filter (**Figure 3**)
- Polypropylene (PP) ovary holder funnels media to self-contained reservoir (**Figure 3**)
- Pump flows media in a closed loop from media reservoir back into ovary (**Figure 4**)

Bioreactor

Figure 3. Final assembled bioreactor with modified cap for inlet/outlet ports, air filter, and polypropylene ovary holder funnel.

TESTING AND EXPERIMENTATION

Experimental Procedure

Isolation and Cannulation

- Locate prominent ovarian artery
- Cut away extraneous tissue
- Insert 23 gauge luer stub into arterial opening
- Tie off artery with nylon thread
  - Prevents backflow
  - Maintains stable luer stub connection

Flow Testing

- Flow water dyed with trypan blue through ovary at 3.5 mL/min
- Short Term Testing
  - Two minutes between data collection for a total of 10 minutes
- Long Term Testing
  - Ten minutes between data collection for a total of 60 minutes

Experimental Setup

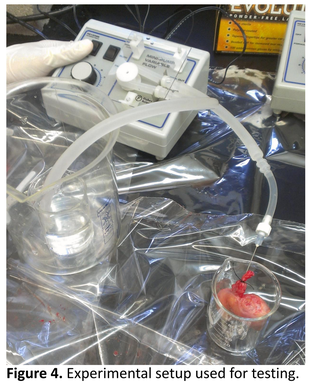


Figure 4. Experimental setup used for testing.

Materials

- Variable Flow Mini Pump II
- 1/4” Tygon tubing
- 1/4” to 1/8” reducers
- 1/8” tubing
- Luer adapter
- Luer stub
- 3-0 Nylon thread
- Trypan blue dye
- Balance

RESULTS

Table 2. Results of short term flow test.

Short Term Flow Testing (10 min)				
Ovary	Initial Mass (g)	Final Mass (g)	Total Outflow (mL)	Average Outflow Rate (mL/min)
1	15.22	20.68	18.2	1.82
2	13.49	17.52	21.3	2.13
3	18.66	26.06	11.9	1.19

Figure 5. Dissected ovaries after harvest (left), 10 minutes of testing (middle), and 60 minutes of testing (right). After extended flow, the ovarian vasculature and tissue was dyed blue as a result of the trypan blue.

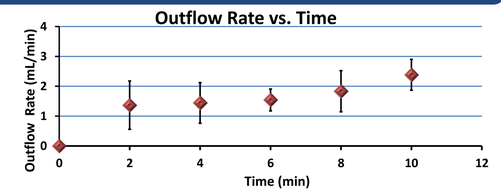


Figure 6. Representation of measured outflow vs. time during a short term test.

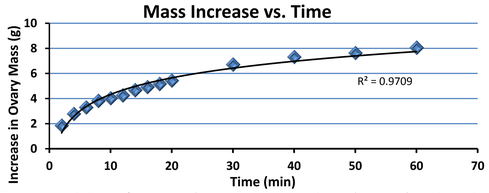


Figure 7. Representation of measured mass increase vs. time during a long term test.

MATERIALS AND EXPENSES

Table 1. Materials used for a single bioreactor and their corresponding prices.

Part/Material	Quantity	Unit Price	Price	Supplier
Polypropylene sheet (12 x 12”x1.5”)	1	$59.17	$59.17	MSC Direct
1/8"FNPT x 1/4"Hose ID Polypropylene Female Adapter	2	$1.30	$2.60	US Plastic
Natural Polypropylene Reduction Couplers 1/4" x 1/8" Tube ID	2	$0.53	$1.06	US Plastic
1/4" ID x 5/8" OD x 3/16" Wall Tygon® S³™ E-3603 Vacuum Tubing	1	$5.73	$5.73	US Plastic
1/8" ID x 1/4" OD x 1/16" Wall Tygon® E-1000 Tubing	10	$0.99	$9.90	US Plastic
Luer Adapter M 1/8 PP 25 Pack	1	$13.50	$13.50	Cole Parmer
1/4-20 Threaded Rod (.250 dia.) x 4 feet	1 ft	$4.82	$1.21	US Plastic
1/4" Polypro Threaded Adapter With 1/8 NPT Thread	2	$0.33	$0.66	US Plastic
DURAN® GLS 80 wide mouth graduated laboratory bottles with cap and pouring ring	1	$19.88	$19.88	Sigma Aldrich
Total Cost			**$113.71**	

FUTURE WORK

Materials

- Slow flow pump
- Oxygenator to expose fluid to oxygen and remove carbon dioxide

Optimization of Flow Rate

- Further testing of current experimental procedure
- Determine physical limits of artery
  - Maximum flow rate before rupture
  - Minimum flow rate for circulation

Future Experimentation

- Ensure necrosis does not occur in a short duration (less than one week)
- Incrementally increase test duration from days to months
- Fabricate multiple systems for long-term testing
- DXR and DXR protecting agent administration

Viability Testing

- Prove efficacy of device through viability testing
  - TUNEL Assay
  - Flowcytometry
  - Calcein AM

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