

## Alginate Laboratory ONPRC Module 5

### Guiding Question:

How can we preserve the fertility of cancer patients?

<u>Module Question</u>	<u>Laboratory Questions</u>
How do we test fertility preservation methods?	<ul style="list-style-type: none"> <li>• How does a scientist obtain ovarian follicles for a study?</li> <li>• What are biomaterials?</li> <li>• How are biomaterials used for <i>in vitro</i> follicle cultures?</li> </ul>

### Learning Outcomes:

Define oncofertility and how the field emerged.

Describe how novel strategies (ovarian protection, follicle culture, cryopreservation, vitrification, ovarian autografts) can preserve fertility.



Brown Algae – Giant Kelp

[http://en.wikipedia.org/wiki/Alginic\\_acid#mediaviewer/File:Giantkelp2\\_300.jpg](http://en.wikipedia.org/wiki/Alginic_acid#mediaviewer/File:Giantkelp2_300.jpg) Public Domain

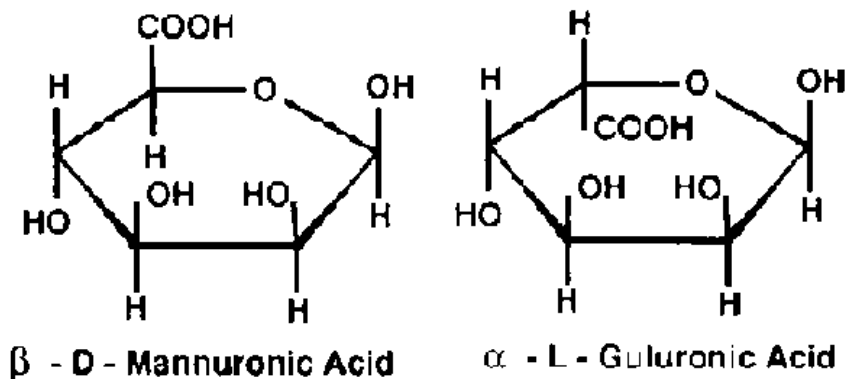


This work is licensed under a [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License](https://creativecommons.org/licenses/by-nc-sa/4.0/).

## Biomaterials Vocabulary

**Alginate:** a linear co-polymer that appears as a viscous gum and is abundant in the cell walls of brown algae, such as kelp and laminaria.

Alginate  
components



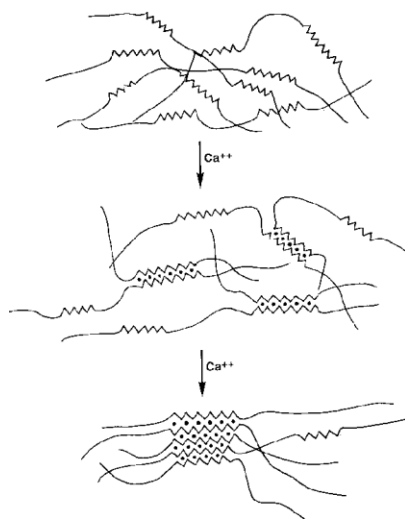
<http://www.fao.org/docrep/x5822e/x5822e04.htm>

[© FAO] [Chapter 2 – Production, Properties and Uses of Alginates by Dennis J. McHugh], [Page 3]  
[Downloaded 10/7/201] (with permission)

**Bioengineering:** biological or medical application of engineering principles or engineering equipment – called also *biomedical engineering*

**Biomaterial:** a natural or synthetic material (as a metal or polymer) that is suitable for introduction into living tissue especially as part of a medical device (as an artificial joint)

**Crosslinking:** the process of chemically bonding two or more molecules by a covalent bond, see below.



**Hydrogel:** a gel composed of one or more polymers suspended in water; alginate is a hydrogel

**Polymerization:** a chemical reaction in which two or more molecules which combine to form larger molecules that contain repeating structural units. Alginate becomes a gel when it polymerizes in a solution of calcium chloride salt.

<http://www.fao.org/docrep/x5822e/x5822e04.htm>

[© FAO] [Chapter 2 – Production, Properties and Uses of Alginates by Dennis J. McHugh], [Page 24] [Downloaded 10/7/201] (with permission)

## Follicle Culture Vocabulary

**Culture:** the act or process of cultivating living material in prepared nutrient media

**In vitro:** outside the body and in an artificial environment

**Tissue Culture:** the process or technique of making tissue grow in a culture medium outside the organism



# What are biomaterials?

## Alginate Procedure:

- Partially fill plastic dropper or 1 mL syringe (up to 0.1 mL) with alginate
- Pipette 5 mL calcium solution into a small Petri dish
- Holding syringe 4-6 inches above the calcium solution, slowly allow drops of alginate to fall into the solution; try different heights
- Allow alginate droplets to cross-link for 2 minutes
- Fill the small plastic vial with 1 ml calcium chloride solution; cap off
- Locate an alginate bead and **carefully** remove it from the solution using forceps; the beads are fragile
- Place bead in micro-centrifuge tube containing 1 mL calcium solution to take home



[http://blog.oncofertility.northwestern.edu/wp-content/uploads/2010/11/44061\\_1195.jpg](http://blog.oncofertility.northwestern.edu/wp-content/uploads/2010/11/44061_1195.jpg)



<http://www.cascadehealthcaresolutions.com/maxorb-extra-ag-silver-alginate-rope-dressing-p/msc9412z.htm>

[http://en.wikipedia.org/wiki/Dental\\_impression#mediaviewer/File:Alginate\\_impression\\_body.jpg](http://en.wikipedia.org/wiki/Dental_impression#mediaviewer/File:Alginate_impression_body.jpg)

<http://foodingredients.wikispaces.com/Huan+Jia's%C2%A0Weekly+Lecture+Summary>  
<http://creativecommons.org/licenses/by-nd/2.5/> No changes have been made.

### Questions to Think About:

- What biomaterials can you name?
- Where does alginate come from?
- Where might you find alginate in your home?
- Why is alginate a good biomaterial for ovarian follicle culture?

### Students Notes or Questions:

---



---



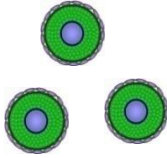
---



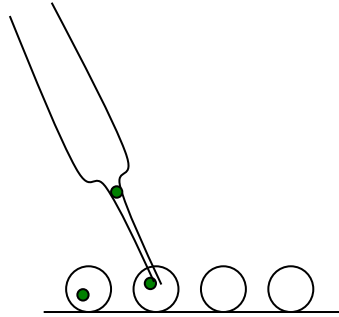
---

# How are biomaterials used for *in vitro* follicle cultures?

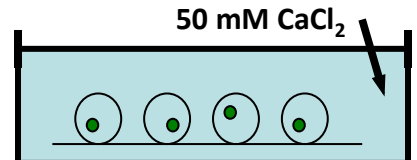
## Follicle Encapsulation:



1. Mechanically isolate individual follicles



2. Pipette individual follicles into ~2-3 $\mu$ L droplets of alginate on a mesh

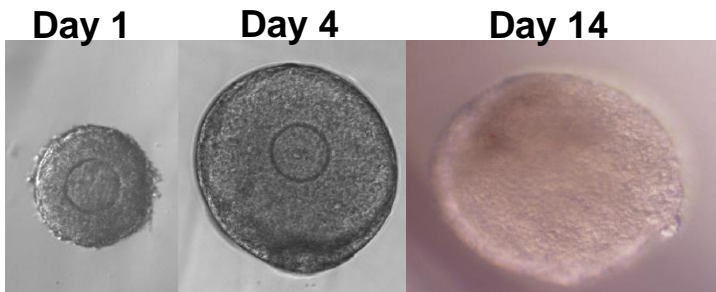


3. Invert mesh with forceps and hit side of Petri dish containing 50mM  $\text{CaCl}_2$ ; let beads sit for 2 min to gel before transferring to plates for culture

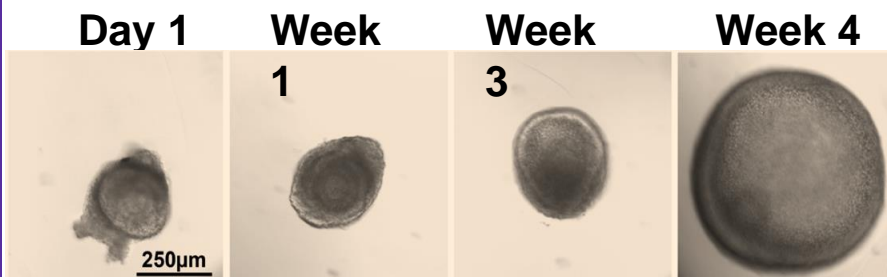
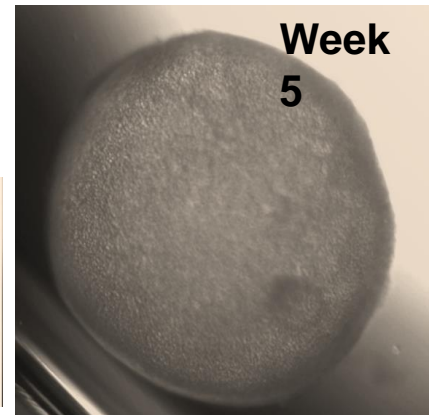
Drawings: Oncofertility Consortium



**Mouse**



**Rhesus Monkey**



Photos: Dr. Mary Zelinski, PhD, ONPRC

### Questions to Think About:

What happens when a follicle is cultured in just a petri dish?

What does the alginate provide that the follicle needs to grow?

### Students Notes or Questions:

---



---



---



---