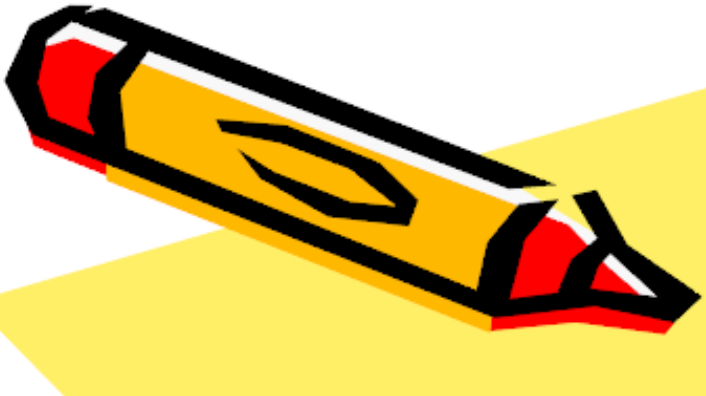


Lab 7
Primary culture

What is a primary culture?

- A primary culture is the stage of the culture after the cell isolation and before the first subculture

Stages to consider for primary culture

1. Acquisition of sample
2. Isolation of the tissue
3. Dissection and /or disaggregation
4. Culture after seeding into culture dish

Ways of obtaining primary cultures from isolated tissue

- Allow cells to migrate from the tissue fragments
- Disaggregate the tissue by the mechanical or enzymatic methods

Steps to be taken after tissue isolation

- Fat and necrotic tissue should be removed
- A sharp instrument should be used to shop the tissue to very small pieces in order not to damage the cells
- If enzymes were used to disaggregate the tissue they should be removed by gentle centrifugation
- Cell concentration in primary cultures should be more than its concentration in subcultures
- It is better to use a rich medium as Ham's F12 and if serum is needed FBS is the best one to use as it gives better survival than fetal and horse serums
- Embryonic tissues are much easier to disaggregate and proliferate more rapidly than adult cells in primary cultures

Important note

- Before working on human or animal embryos or tissues the researcher has to make sure that his work fits within the religious, medical and governmental rules.
- Animals should not be dissected in the tissue culture laboratory as animals carry microbial contamination

Common tissue sources for primary culture

- Mouse embryo
- Chick embryo
- Human biopsy material

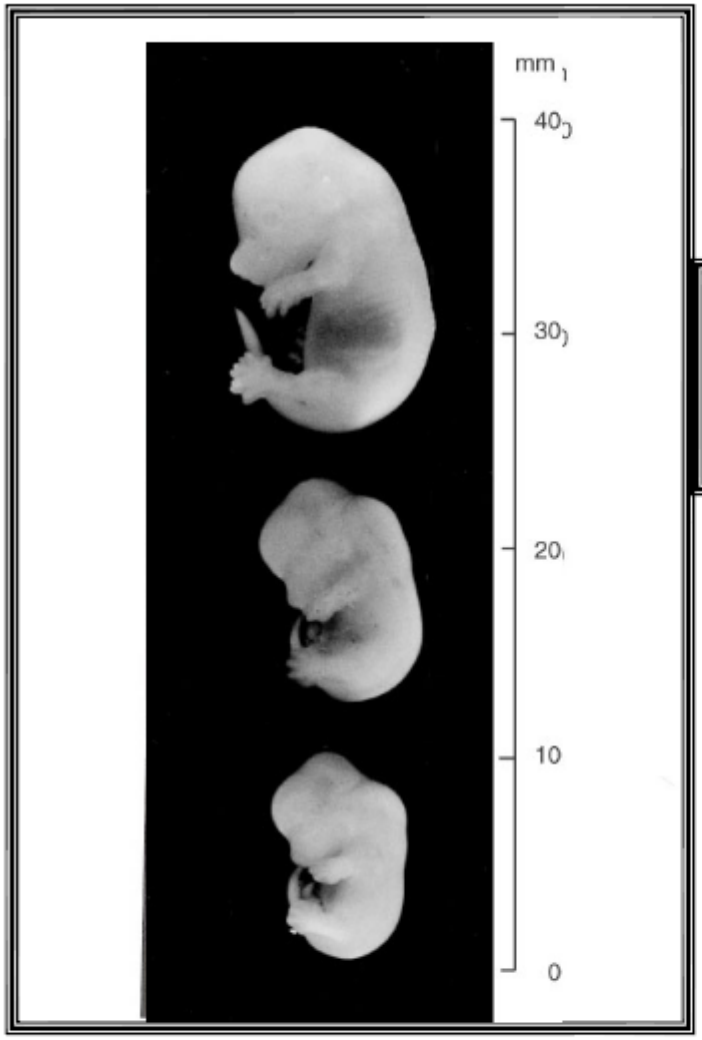
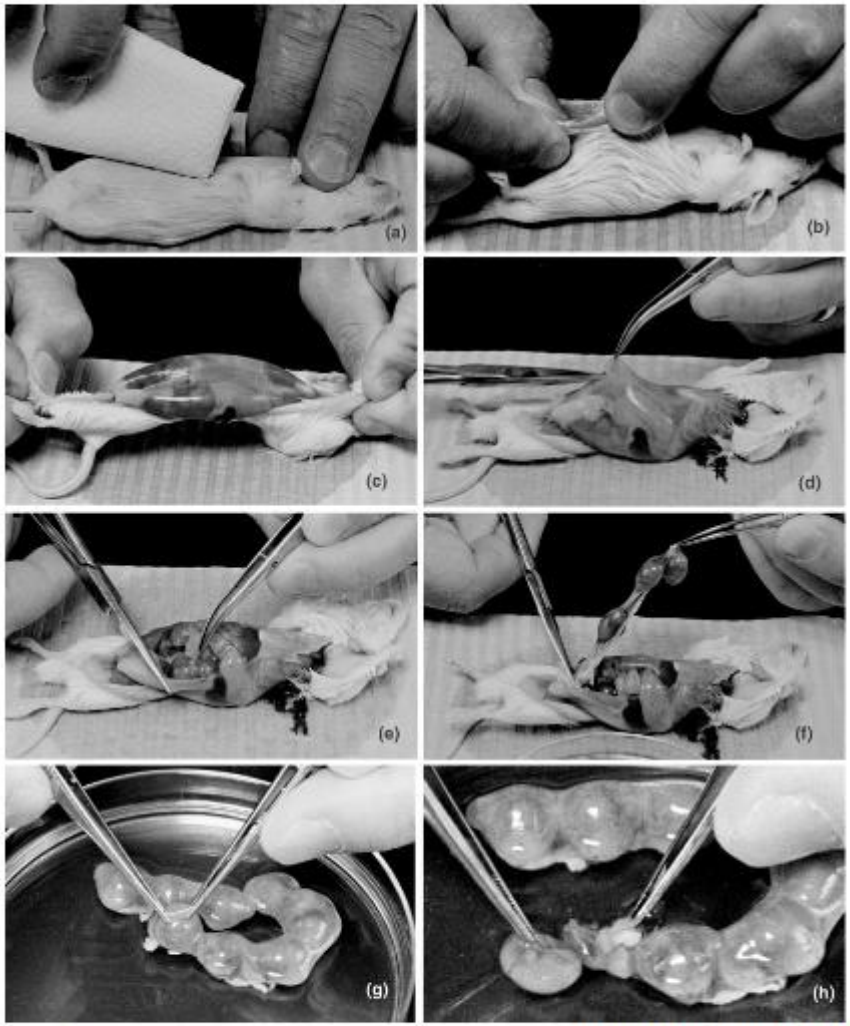


Fig. 12.2. Mouse Embryos. Embryos from the 12th, 13th, and 14th days of gestation. The 12-day embryo (bottom) came from a small litter (three) and is larger than would normally be found at this stage.

- *Freshney (2005), Culture of Animal Cells: A Manual of Basic Technique Published by John Wiley & Sons, Inc.)*



Stages in dissection of a pregnant mouse for the collection of embryos

A. Swabbing the abdomen

B. And C Tearing the skin to expose the abdominal wall

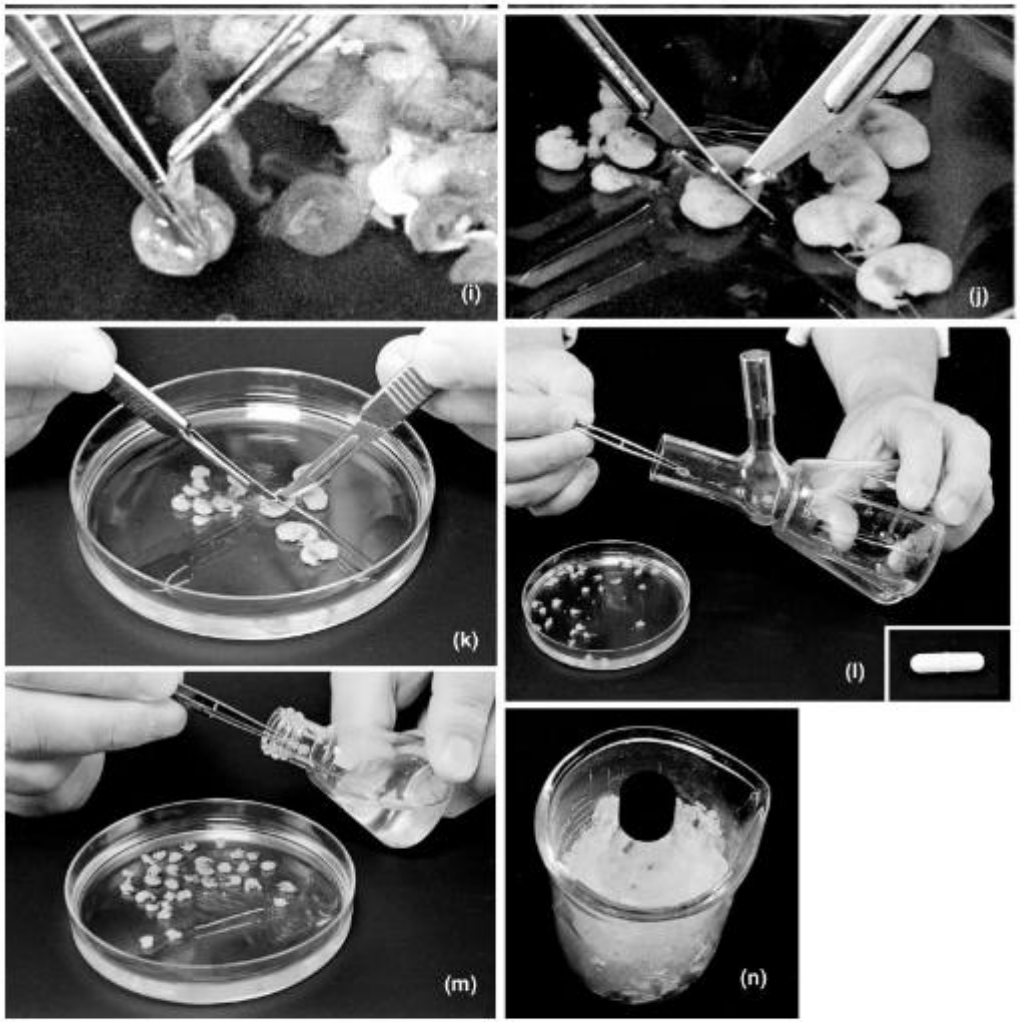
D. Opening the abdomen

E. Revealing the uterus

F. Removing the uterus

G. And h Dissecting the embryos from the uterus

- *Freshney (2005), Culture of Animal Cells: A Manual of Basic Technique Published by John Wiley & Sons, Inc.)*



I. Removing the membranes

J. Removing the head

K. Chopping the embryos

L. Transferring the pieces to trypsinization flask (for warm trypsinization)

M. Transferring the pieces to small Erlenmeyer flask (for cold trypsinization)

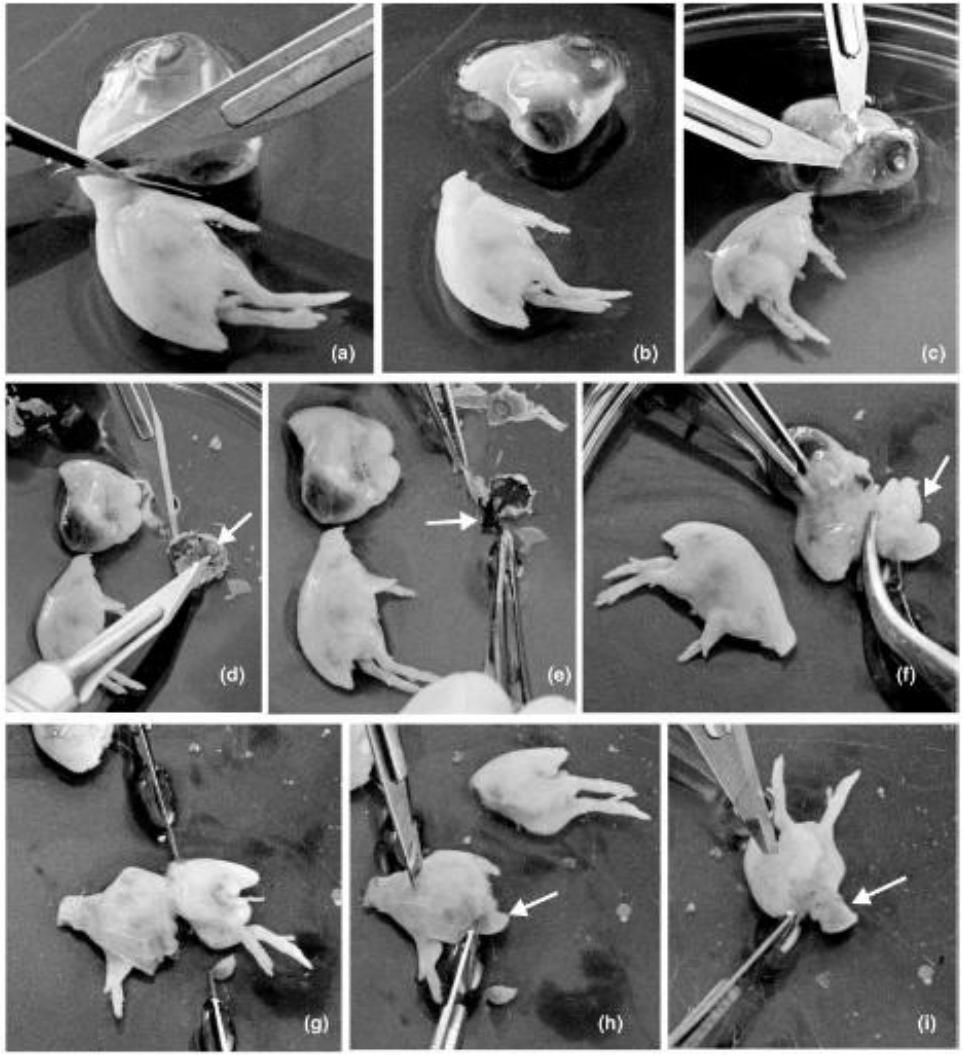
N. Flask on ice

•Freshney (2005), Culture of Animal Cells• A Manual of Basic Technique Published by John Wiley & Sons, Inc.)

Removing a chick embryo from an egg

A. Swabbing the egg with alcohol
B. Cracking the shell
C. Peeling off the shell
D. Peeling off the shell membrane
E. Chorioallantioc membrane (CAM) and vasculature revealed
F. Removing (CAM) with forceps
G. Grasping the embryo around the neck
H. Withdrawing the embryo from the egg
I. Isolated 10-day embryo in Petri dish

*Freshney (2005). Culture of Animal Cells: A Manual of Basic Technique Published by John Wiley & Sons, Inc.)



Dissection of a chick embryo

A and B. Removing of the head

C. Removing the eye

D. Dissecting out the lens

E. Peeling off the retina

F. Scooping out the brain

G. Halving the trunk

H. teasing out the heart and lungs from the anterior half

I. teasing out the liver and gut from the posterior half

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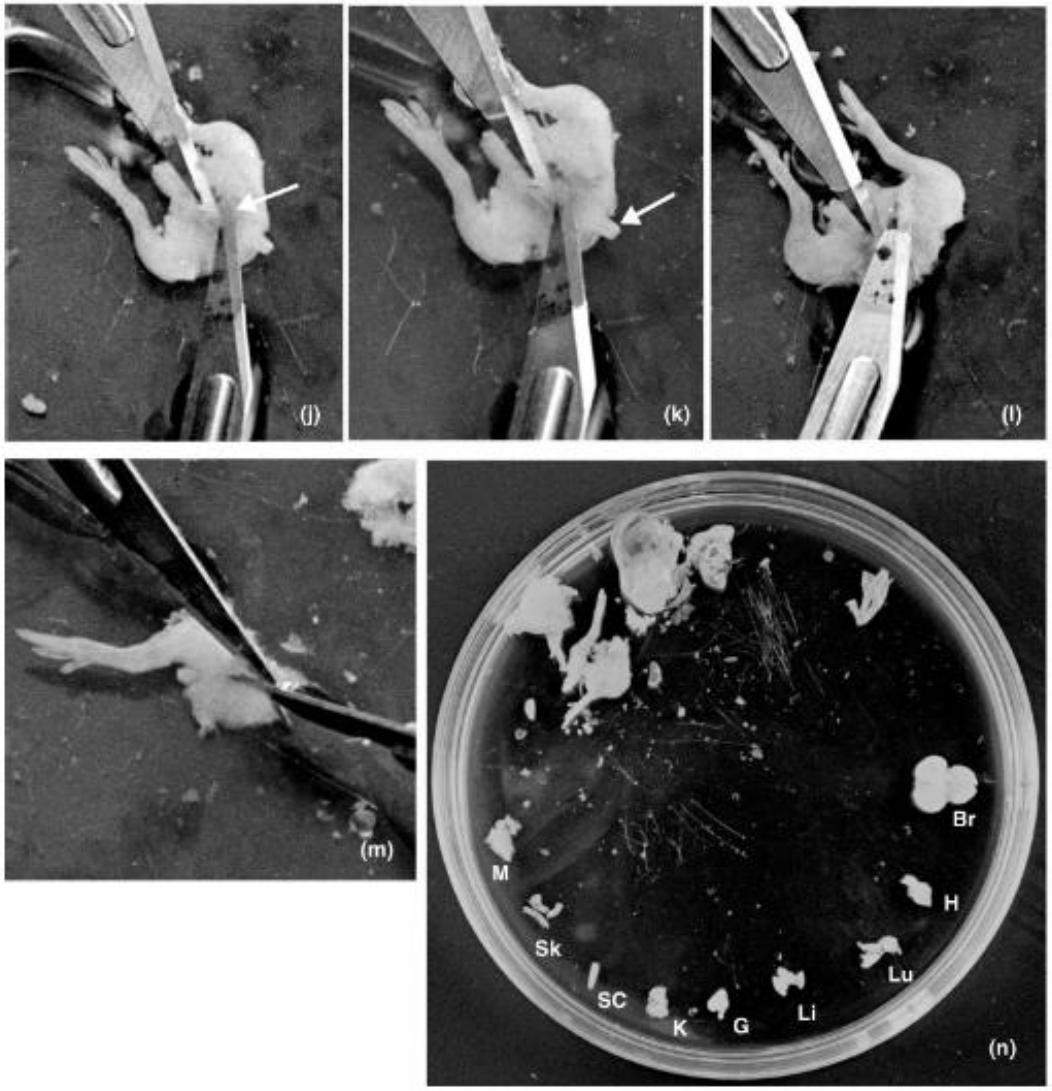

Dissection of a chick embryo

J. Inserting the tip of the scalpel between the left kidney and the dorsal body wall

K. Squeezing out the spinal cord

L. Peeling the skin of the back of the trunk and the hind leg

M. Slicing muscle from the thigh

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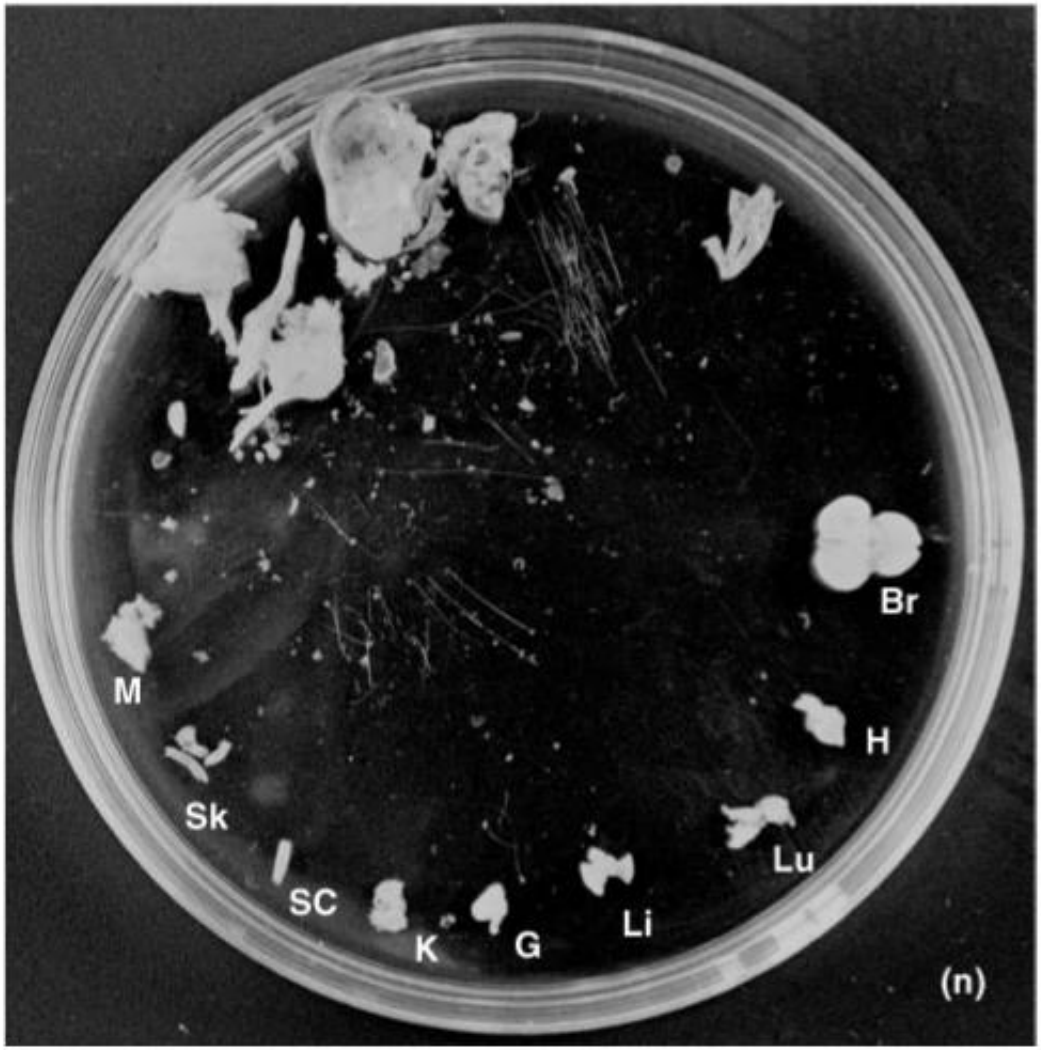

Organ rudiments arranged around the periphery of the dish

From the right clockwise, we have the following organs: *brain, heart, lungs, liver, gizzard, kidneys, spinal cord, skin, and muscle*

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Techniques used for tissue isolation and disaggregation

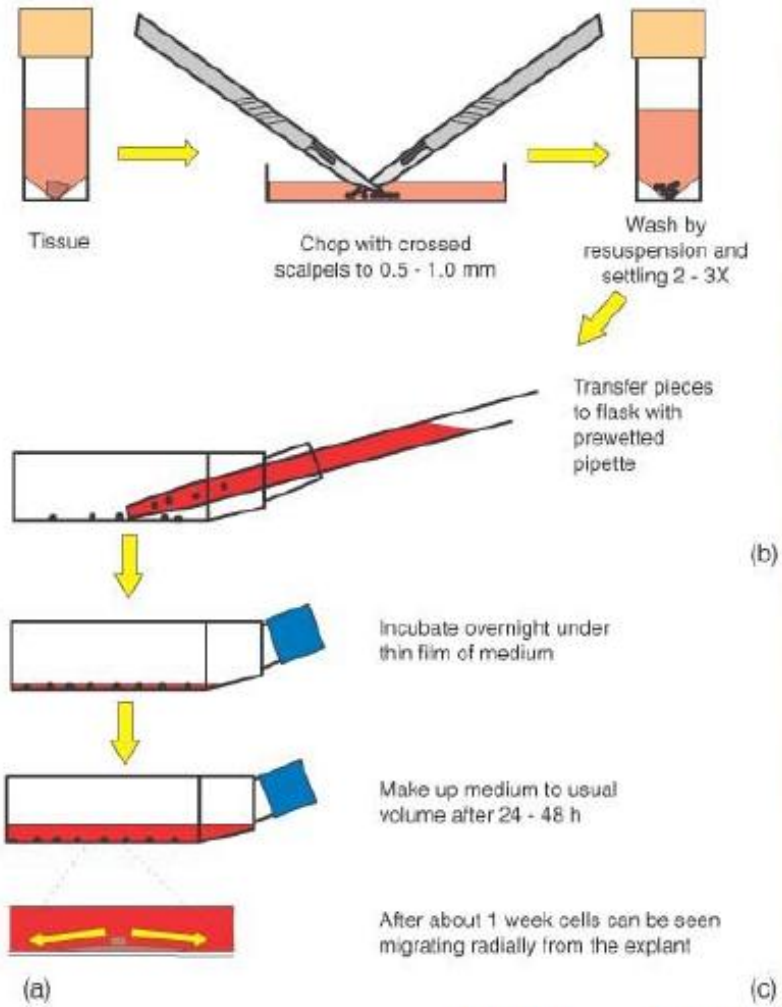
Techniques used for disaggregation of isolated tissue for primary culture

- Mechanical techniques involving dissection and maceration this method is useful for soft tissues.
- Enzymatical techniques involving the use of enzymes and is useful when a large number of cells is needed.

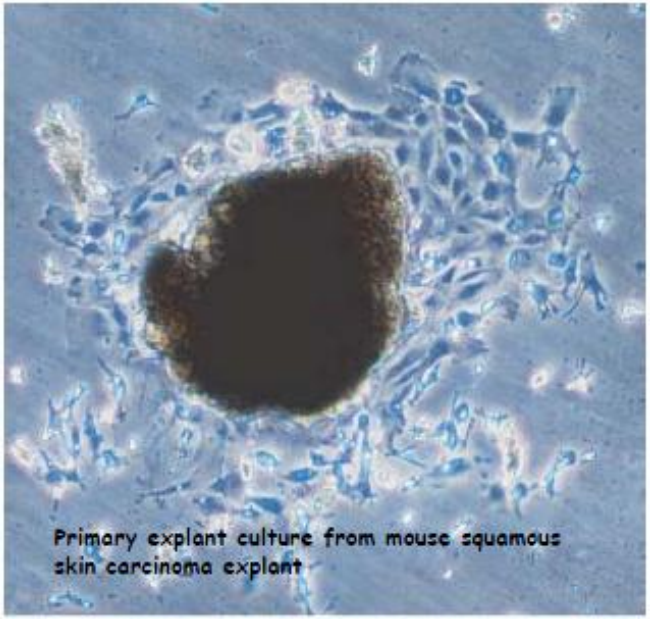
Primary explant

- This technique was the original method developed by Harrison, Carrel and others
- The purpose of primary explant is to initiate a tissue culture
- This technique is useful for small amounts of tissue example skin biopsies
- The disadvantage of this technique is the poor adhesiveness of some tissues and the selection of cells in the outgrowth
- The tissue should be shopped finely and rinsed
- The tissue pieces are seeded onto the surface of the culture flask with the medium that has high serum concentration

Primary explant culture



(a)



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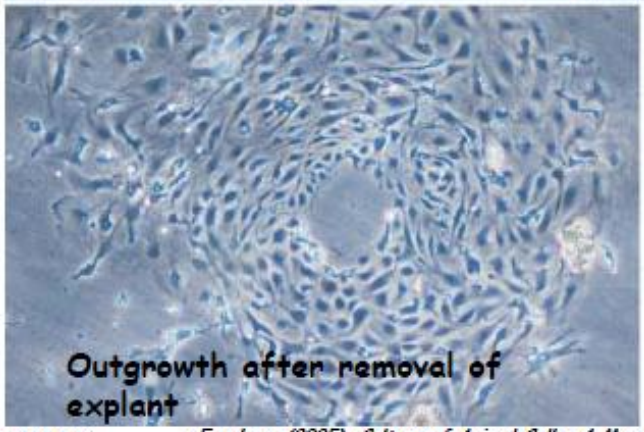

(c)

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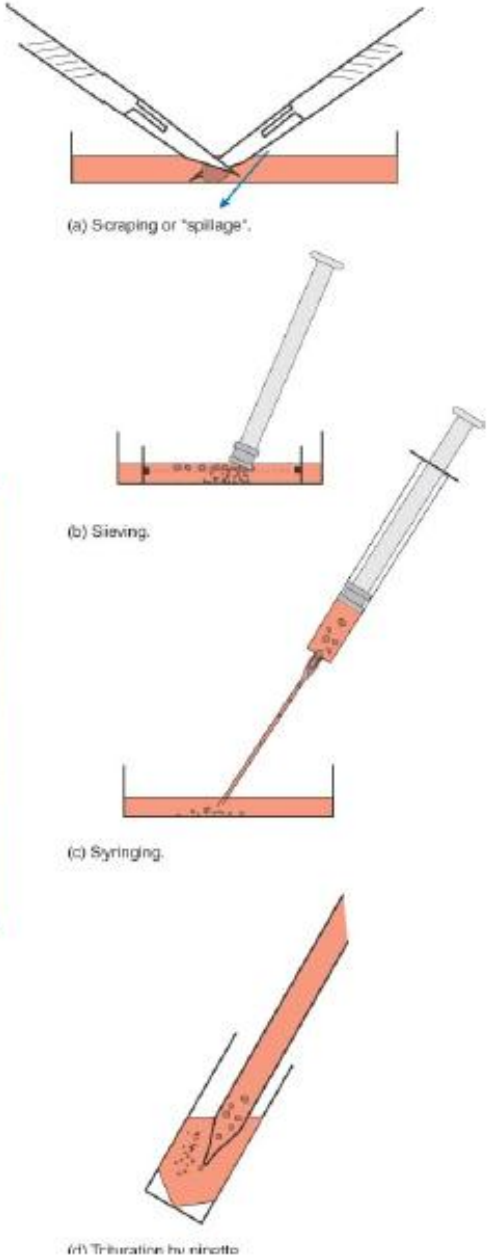
Mechanical disaggregation

- In this method the tissue is forced through a series of sieves where the mesh is gradually reduced in size until a reasonable suspention of single cells is obtained. Then the suspention is deluted and cultured
- Syringing and pipetting used in this method might cause mechanical damage to the cells

Fig. 12.13. Mechanical Disaggregation. (a) Scraping or "spillage" Cutting action, or abrasion of cut surface, releases cells. (b) Sieving Forcing tissue through sieve with syringe piston. (Falcon Cell Strainer can be used; *see* Fig. 12.8.) (c) Syringing. Drawing tissue into syringe through wide bore needle or canula and expressing (d) Trituration by pipette. Pippetting tissue fragments up and down through wide bore pipette.

Fig. 12.8. Cell Strainer. Disposable polypropylene filter and tube for straining aggregates from primary suspensions (BD Biosciences). Can also be used for disaggregating soft tissues (*see also* Fig. 12.13).



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Enzymatic disaggregation

- Warm trypsin
- Trypsinization with cold preexposure
- Other enzymatic procedures
- collagenase

Warm trypsin

- This technique is useful for the disaggregation of large amounts of tissue in short time
- It is not suitable for adult tissue that has lots of fibrous connective tissue

Trypsinization with cold preexposure

- This method is used to minimize the harm that trypsin can cause to the cells
- This method gives higher yield of viable cells with improved survival after 24 h culture, and preserves more different cell types than the warm trypsin method
- In this method no stirring or centrifugation is required

Thank you