

MimEX™

Tissue Processing Kit

Catalog Number MIM001

Reagents for the processing of tissue samples for isolation of adult epithelial stem cells.

This package insert must be read in its entirety before using this product.
For laboratory research use only. Not for diagnostic use.
The safety and efficacy of this product in diagnostic or
other clinical uses has not been established.

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INTRODUCTION

Adult stem cell populations are found in most tissues of the human body and provide a long-lived source of self-renewing cells for both tissue homeostasis under normal conditions as well as for regeneration during disease or injury. In the adult, tissue specific stem cells can be either unipotent or multipotent. Tissue-specific adult stem cells exhibit an epigenetic memory conferring tissue specificity and regional identity. Moreover, adult stem cells may hold the keys to understanding aging, disease progression, and the development and spread of cancer.

The MimEX™ Tissue Model System allow for the expansion and differentiation of ground-state, adult stem cell populations. These cells create sustainable and accessible 3-dimensional tissues *in vitro* and provide a valuable tool for biomarker discovery, disease modeling, drug screening, and developmental biology studies. The MimEX™ Tissue Processing Kit provides reagents for the dissociation of epithelial tissue and subsequent isolation of adult epithelial stem cells that are capable of maintaining regional identity, genome stability, and disease state (1-2).

PRINCIPLE OF THE ASSAY

The MimEX™ Tissue Processing Kit contains reagents and supplies for the collection and dissociation of adult epithelial tissue samples for the isolation of adult epithelial stem cells. The quantity of each component is sufficient to dissociate 1 tissue sample for stem cell isolation, up to 3 cm in size.

TECHNICAL HINTS & LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix or substitute reagents with those from other lots or sources.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

PRECAUTION

The acute and chronic effects of overexposure to reagents of this kit are unknown. When handling kit reagents and biohazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF UNOPENED MATERIAL	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
MimEX™ Tissue Collection Media	390246	30 mL of ready to use media.	Store at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer.*	Store at $2-8^{\circ}\text{C}$ for up to 2 weeks.*
MimEX™ Stem Cell Isolation Media	390245	250 mL of ready to use media.	Store at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer.*	Store at $2-8^{\circ}\text{C}$ for up to 2 weeks or aliquot and store at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer for up to 3 months.* Avoid repeated freeze-thaw cycles.
MimEX™ Dissociation Solution	390615	10 mL of a ready to use solution.		
Scalpels	608106	2 individually wrapped scalpels.	Store at room temperature until use.	Store at room temperature until use.

*Provided this is within the expiration of the kit.

OTHER SUPPLIES REQUIRED

Materials

- Tissue sample, maximum size 3 cm
- 6-well culture plates (or other, as needed)
- 10 cm petri dishes
- Pipettes and pipette tips
- 50 mL centrifuge tubes
- 15 mL centrifuge tubes
- Serological pipettes
- Dissecting scissors
- Irridectomy scissors
- Fine forceps
- Blunt forceps
- Angled dissection scissors

Reagents

- DMEM/F12
- MimEX™ Irradiated Fibroblast Kit (R&D Systems®, Catalog # MIM005)
- MimEX™ Expansion Media (R&D Systems®, Catalog # MIM003)

Equipment

- 37°C and 7.5% CO_2 incubator
- Centrifuge
- Hemocytometer
- Inverted microscope
- Dissecting microscope
- 37°C water bath
- Orbital shaker, 37°C

REAGENT & MATERIAL PREPARATION

MimEX™ Tissue Collection Media - Thaw the MimEX™ Tissue Collection Media at 2-8 °C overnight.

MimEX™ Stem Cell Isolation Media - Thaw the MimEX™ Stem Cell Isolation Media at 2-8 °C overnight.

MimEX™ Dissociation Solution Media - Thaw the MimEX™ Dissociation Solution at 2-8 °C or room temperature.

PROCEDURE FOR THE ISOLATION & EXPANSION OF HUMAN ADULT EPITHELIAL STEM CELLS FROM BIOPSY TISSUE

Plating MimEX™ Irradiated Fibroblasts for Stem Cell Expansion

The following protocol is to plate one vial of MimEX™ Irradiated Fibroblasts into 2 wells of a 6-well plate. If an alternate plate size is desired, adjust volumes and cell numbers accordingly.

1. Place the 6-well plate at 2-8 °C for 10 minutes.
2. Dilute Cultrex® Stem Cell Qualified BME 1:10 in ice cold DMEM/F12. Keep on ice. Diluted BME can be stored at 2-8 °C for up to 1 week.
3. Coat wells with diluted Cultrex® BME mixture. Pipette 1 mL into the center of the well, swirl gently to coat the entire well, and remove by pipette while tilting the plate. Ensure there are no air bubbles during the coating. Quickly add the volume to the second well and repeat for all wells needed. Return the diluted Cultrex® BME mixture to ice every 3-4 wells to keep it cold and prevent premature polymerization.
4. After all wells are coated, tilt the plate at an angle for 15 seconds. Using a pipette remove and discard any remaining Cultrex® BME that pools at the bottom of the wells.
5. Incubate the plate at 37 °C/7.5% CO₂ for 10 minutes. At the end of the incubation, the wells have a shine when held at an angle in light. Do not allow the plates to become dry.
6. While the BME-coated plates are incubating, retrieve the needed vials of MimEX™ Irradiated Fibroblasts from liquid nitrogen storage. Thaw in a 37 °C water bath.
7. Aliquot the needed volume of MimEX™ Fibroblast Media (2 mL/well) into a 50 mL centrifuge tube. Each vial of MimEX™ Irradiated Fibroblasts is enough to coat 2 wells of a 6-well plate, or the equivalent surface area.
8. Add 0.5 mL of MimEX™ Fibroblast Media to the cryovial of fibroblasts and gently pipette to re-suspend. Slowly add the cells dropwise to the media in the 50 mL centrifuge tube, with gentle swirling. Do not pipette up and down vigorously.

Note: Vigorous treatment of MimEX™ Irradiated Fibroblasts may impact viability and performance.

Preparing Plates with MimEX™ Irradiated Fibroblasts *continued*

9. Rinse the cryovial once with 0.5 mL of the resuspended fibroblasts. Slowly add the media back to the 50 mL centrifuge tube dropwise while mixing by gentle swirling.
10. Add the fibroblast suspension to the coated wells, 2 mL/well. Ensure there are no air bubbles in the wells.
11. **Critical Step:** Evenly distribute the cells in the well by the **MimEX™ Cell Plating Procedure**.
 - a. Place the plate in the incubator. Allow media to become still.
 - b. Slide the plate forward and backward evenly three times. You should observe a wave traveling forward and back. Avoid swirling the media.
 - c. Allow the media to become completely still (about 3 seconds).
 - d. Slide the plate side to side evenly three times. A wave should be observed traveling side to side. Avoid swirling the media.
 - e. Allow the media to become completely still (about 3 seconds).
 - f. Repeat steps a-e two more times (for a total of three times).
12. **Critical Step:** Incubate the plate in a **37 °C/7.5% CO₂** incubator overnight **without disturbing the plate**.
13. As early as possible the next day, check the density and distribution of the cells. Replace the media with fresh MimEX™ Fibroblast Media.

Note: *If fibroblasts are not used the next day, exchange MimEX™ Fibroblast Media the morning of the procedure. Plated fibroblasts must be used within 3 days.*
14. One to two hours prior to plating dissociated biopsy tissue onto the fibroblast-coated plate, replace MimEX™ Fibroblast Media in each well with 2 mL of MimEX™ Expansion Media.

Tissue Dissociation and Stem Cell Isolation

1. Tissue used for stem cell isolation should be harvested and stored directly in the MimEX™ Tissue Collection Media tube provided in this kit. Tissue can be stored up to 48 hours on ice prior to processing. Increased time between harvest and processing can result in reduced yield of stem cell colonies. **Do not freeze the tissue.**

Note: *It is recommended to use a 1 cm x 1 cm section of tissue or larger to maximize the number of potential colonies. Smaller sections can also be used. For tissue < 5 mm x 5 mm a 12-well plate is recommended.*
2. Remove the tissue from the MimEX™ Tissue Collection Media and place it in a 10 cm petri dish. Wash tissue 1-2 times in 5 mL of MimEX™ Stem Cell Isolation Media. This can be done by pipetting the media onto the tissue, swirling, and removing the media.

Note: *Depending on tissue procurement source, surgeon, procedure, the tissue can vary in size and appearance. Typically, for cancer resections, a large tissue specimen is provided. These are often cut longitudinally and are inverted inside-out, so the epithelial layer (the lumen) is facing outward (**Figure 1**). If mucus and debris is apparent, this can be gently scraped away from the tissue.*

Tissue Dissociation and Stem Cell Isolation *continued*

- Depending on the size of the tissue, you may need to divide the tissue into 2 to 4 pieces using broad forceps and a scalpel (or dissecting scissors).

Note: *Fragments of the tissue samples can be frozen for DNA and/or RNA extraction or submerged into 4% PFA (or formalin) for histology. This is recommended for downstream comparison of the source tissue with both in vitro-derived adult stem cell populations and differentiated tissues.*

- Under a dissecting microscope, confirm that the epithelial layer (lumen) is exposed towards the outside of the specimen. This can be identified by its yellow/orange appearance and lack of blood vessels and fat tissue. Scrape away mucous film layer covering the epithelium if necessary (**Figure 1**).

Note: *There are different strategies to isolate the epithelial tissue. Below describes two potential methods.*

- Using a blunt forceps, grasp one end of the tissue along the stroma. Take a fine forceps in the other hand and insert it between the luminal and stromal layers (**Figure 2**). Open the forceps and repeat opening and closing the forceps until a sheet begins to separate from stroma. Gently grab this layer with forceps and peel tissue away from underlying stroma until an epithelial sheet is exposed. The fine forceps or dissecting scissors may be used to liberate the epithelial tissue from underlying stroma.
 - Alternatively, blunt forceps can be used to guide/hold the tissue and dissection scissors can be lowered flat against long axis of tissue specimen and used to cut sheet of epithelium away from stroma (**Figure 3**).
- Place the liberated epithelial tissue into a new 10 cm petri dish and add a small amount of MimEX™ Stem Cell Isolation Media. The rest of this protocol is done under high magnification of a dissecting microscope.
Critical Note: *It is important that MimEX™ Stem Cell Isolation Media and the tissue be kept as cold as possible and should be kept on ice during this procedure.*
 - View liberated epithelial tissue under high magnification. Hold with forceps and examine tissue for remaining stroma and adipose tissue (**Figure 4**). Carefully remove the stroma and adipose tissue with fine forceps and/or angled dissecting scissors. At this step the tissue may begin to dissociate. Be sure to collect all epithelial tissue and keep stroma tissue separate.
 - Observe final epithelial tissue under high magnification to verify the presence of key cyto-architectural components found within the organ of dissection, such as intestinal crypts, when dissecting intestinal epithelia (**Figure 5**).
 - Collect cleaned epithelial tissue into new 15 mL centrifuge tube. Add MimEX™ Stem Cell Isolation Media to a final volume of 10 mL. Move to a biological safety cabinet and transfer tissue to a new 10 cm petri dish for the remaining procedure.

Tissue Dissociation and Stem Cell Isolation *continued*

9. Remove excess media, leaving just enough to keep tissue moist.
10. Using two scalpels, mince the tissue with even downward motions using low to moderate force. Be careful not to scrape the scalpel against the bottom of the petri dish as this will cause slivers of plastic to mix with the tissue. Move down the length of the tissue while mincing in a typewriting-like fashion. This step will typically take at least 10 minutes. Depending on the size and composition of your tissue it may take up to 30 minutes.
Note: *Do not let the tissue become dry. Add additional MimEX™ Stem Cell Isolation Media as needed to maintain moisture.*
11. Continue until tissue is a soup-like consistency.
12. Use the scalpel to scoop the tissue into a 50 mL centrifuge tube containing 10 mL of MimEX™ Dissociation Solution.
13. Using a P1000 pipette, wash the petri dish with MimEX™ Dissociation Solution and transfer solution into the 50 mL centrifuge tube. Repeat as needed to ensure the majority of tissue is transferred.
14. Rinse scalpels with MimEX™ Dissociation Solution to transfer any remaining epithelial cells to the 50 mL centrifuge.
15. Secure the 50 mL centrifuge tube at a 30° angle onto an orbital shaker at 37 °C. This can be done by taping the centrifuge tube to a styrofoam container and securing that onto an orbital shaker.
16. Set the orbital shaker at ~120 rotations/minute for 30 minutes.
17. After 30 minutes, check the biopsy solution. It should now appear cloudy with no large tissue pieces. Sometimes the tissue will aggregate, but this can be easily dissociated with a pipette.
18. Using a 5 mL pipette, triturate the dissociated biopsy up and down 10-20 times. Take 1 mL and transfer to one well of a 24-well tissue culture plate (or any sterile plate for observation).
19. Check for completed digestion. You should observe many 2-5 cell clusters (**Figure 6**), some single cells, and few large cell clusters. If there are very few small cell clusters and no single cells, continue incubation in MimEX™ Dissociation Solution for an additional 10 minutes. Check cells again and repeat as needed. Return cells to the 50 mL centrifuge tube containing dissociated epithelial cells. Do not exceed 1 hour incubation in the dissociation solution.
20. Add 20 mL of MimEX™ Stem Cell Isolation Media (final volume is 30 mL), mix gently, and centrifuge at 200 x g for 6 minutes (at 2-8 °C if possible).
Note: *If there are many large tissue clumps you can pass cell suspension through a 100 µm cell strainer, but this may reduce the number of primary colonies if you did not start with ample amounts of tissue. If a cell strainer is used, add the 10 mL of suspended cells to the cell strainer fixed atop a new 50 mL centrifuge tube. Wash 2 times with 10 mL of MimEX™ Stem Cell Isolation Media (final volume 30 mL).*

Tissue Dissociation and Stem Cell Isolation *continued*

21. Remove supernatant without disrupting cell pellet. Leave 3-5 mL of solution to ensure maximal cell recovery.
22. Re-suspend the pellet in 30 mL of cold MimEX™ Stem Cell Isolation Media (~35 mL final volume), mix gently with 10 mL pipette, and centrifuge at 200 x g for 8 minutes at 2-8 °C.
23. Repeat steps 21-22 four times (5 washes total). Mix by inverting the centrifuge tube. Avoid excessive pipetting.
Note: *These washing steps are necessary to remove dissociation buffer enzymes, contaminants, and erythrocytes from the biopsy.*
24. After the final centrifugation, remove as much supernatant as possible without disturbing the pellet. Add 20 mL of room temperature MimEX™ Expansion Media, resuspend by pipetting (minimize bubble formation), and perform final centrifugation at 200 x g for 8 minutes.
25. Remove supernatant leaving pellet undisturbed.
26. Re-suspend the pellet in 2.5 mL of warm (37 °C) MimEX™ Expansion Media. Mix well with a 5 mL pipette. Minimize bubble formation
27. Take 6-well plate with pre-plated MimEX™ Irradiated Fibroblasts prepared as described above, remove existing media, and add 2.5 mL of cell suspension to each well. Transfer to 37 °C incubator set at 7.5% CO₂.
28. **Critical Step:** Evenly distribute the cells in the well by the **MimEX™ Cell Plating Procedure**.
 - a. Place the plate in the incubator. Allow media to become still.
 - b. Slide the plate forward and backward evenly three times. You should observe a wave traveling forward and back. Avoid swirling the media.
 - c. Allow the media to become completely still (about 3 seconds).
 - d. Slide the plate side to side evenly three times. A wave should be observed traveling side to side. Avoid swirling the media.
 - e. Allow the media to become completely still (about 3 seconds).
 - f. Repeat steps a-e two more times (for a total of three times).
29. **Critical Step:** Incubate the plate in a **37 °C/7.5% CO₂** incubator overnight **without disturbing the plate**.
30. After 48 hours, exchange media with 2 mL of fresh prewarmed MimEX™ Expansion Media.
Optional: *To improve colony yield, the supernatant can be centrifuged at 200 x g for 8 minutes and re-plated on a fresh plate of MimEX™ Irradiated Fibroblasts.*
31. Exchange media every other day and monitor for colony outgrowth. Follow culturing protocol as described in MimEX™ Expansion Media (R&D Systems®, Catalog # MIM003).
32. Monitor cultures daily. Colonies should become visible after 2-3 weeks. Colonies should be passaged before they start to show signs of differentiation (**Figure C and D**).
Optional: *Isolation and expansion of individual adult stem cell clones is recommended. Colony selection should be performed during the first few passages of adult stem cells from biopsy tissue. Clones can be picked directly from individual adult stem cell colonies, similar to picking induced pluripotent stem cell colonies. Alternatively, isolate and expand a single adult stem cell by performing a serial dilution into a 96-well plate pre-coated with MimEX™ Irradiated Fibroblasts.*
Note: *It is recommended to expand the stem cell colonies for 3 or 4 passages before performing tissue differentiation in an air-liquid interface.*

TROUBLESHOOTING TIPS

- If the tissue is not well processed and some stroma is included in the cell suspension, this can lead to fibroblast contamination in the stem cell cultures.
- Fibroblast contamination is visible and can be detected after 2 or 3 passages. In contrast to tightly packed, regular shaped, round adult stem cell colonies, human fibroblasts can be identified by their irregular and elongated morphologies, ranging from thin bipolar cells to spread multipolar cells. They will be most evident as they grow over the adult stem cell colonies (**Figure 7**).
- If a fibroblast contamination is detected, fibroblasts can be removed using the MagCelect™ Plus Human EpCAM⁺ Cell Isolation Kit (R&D Systems®, Catalog # MAGH121) according to the manufacturer's instructions. The EpCAM-positive isolated cells are then plated onto a new plate of MimEX™ Irradiated Fibroblasts and cultured as described above. This process can be repeated if needed.

PROTOCOL ILLUSTRATIONS

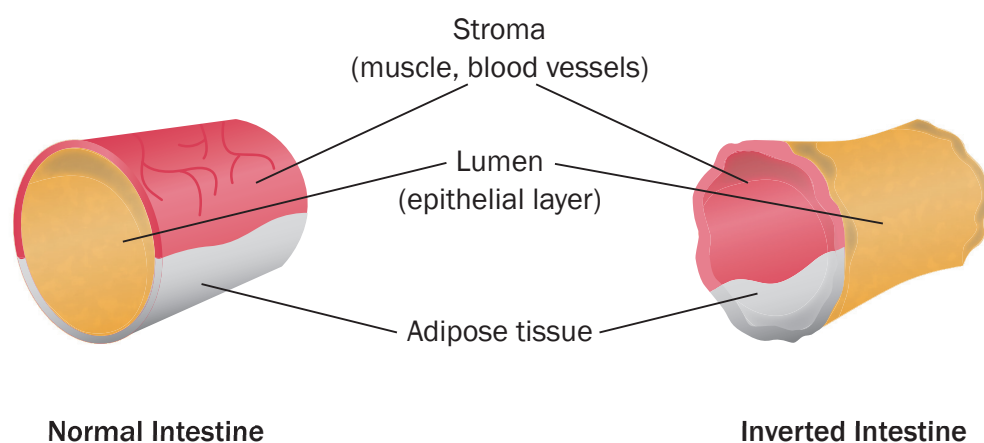


Figure 1: Identifying Epithelial Tissue in a Biopsy Sample. Using adult gastrointestinal tissue as an example, this figure illustrates the identification of the epithelial layer amongst surrounding stromal and adipose tissue. The degree of difficulty in isolating epithelial tissue from a biopsy will vary by organ and biopsy sample preparation.

PROTOCOL ILLUSTRATIONS *CONTINUED*

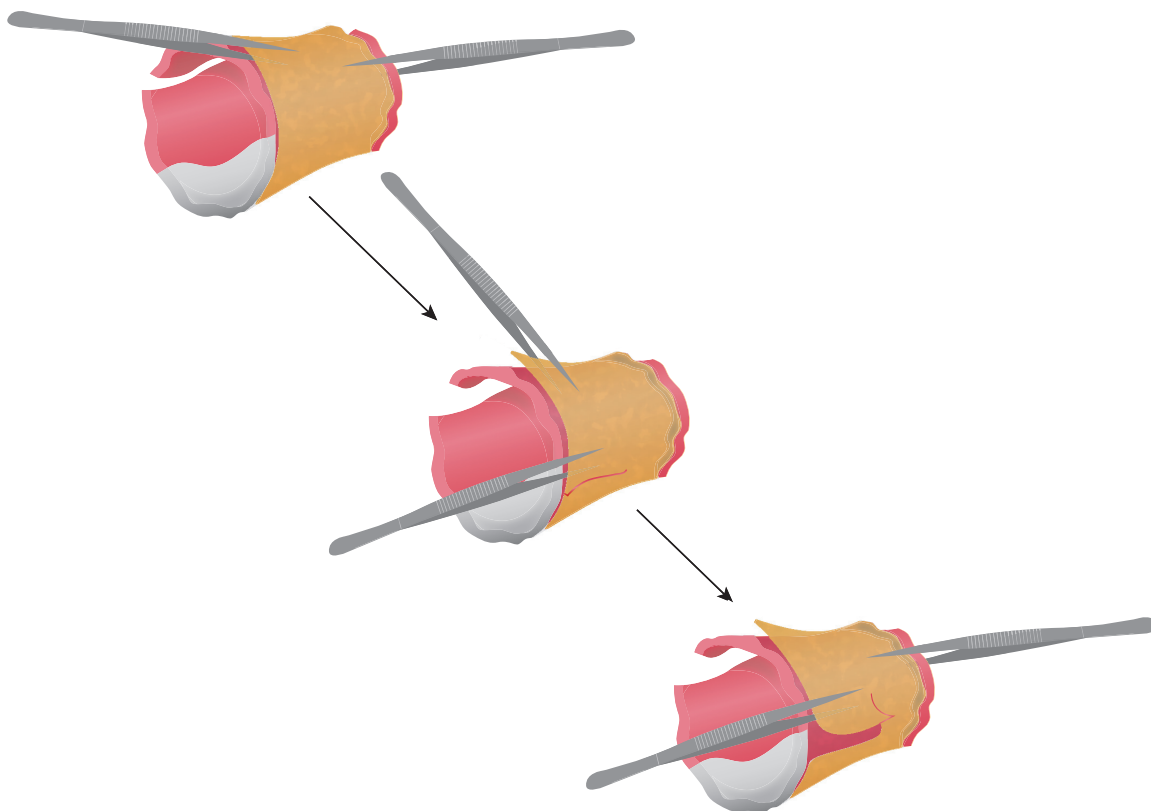


Figure 2: Isolation of Epithelial Tissue from Stromal and Adipose Tissue - Method 1. Using adult gastrointestinal tract tissue for an illustrated example, this figure demonstrates epithelial layer dissociation using forceps. The degree of difficulty in isolating epithelial tissue from a biopsy will vary by organ and biopsy sample preparation.

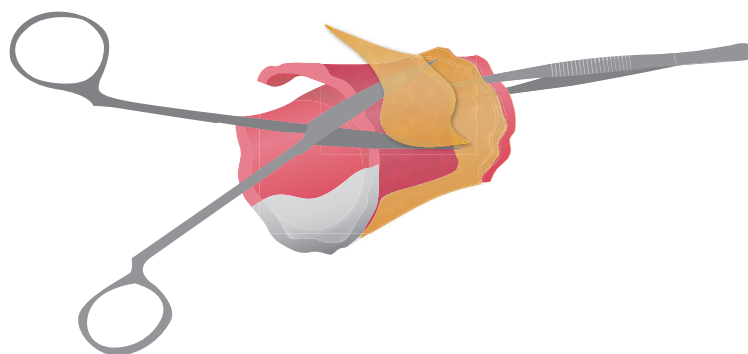


Figure 3: Isolation of Epithelial Tissue from Stromal and Adipose Tissue - Method 2. Using adult gastrointestinal tract tissue for an illustrated example, this figure demonstrates epithelial layer dissociation using blunt forceps and a scissors. The degree of difficulty in isolating epithelial tissue from a biopsy will vary by organ and biopsy sample preparation.

DATA EXAMPLES

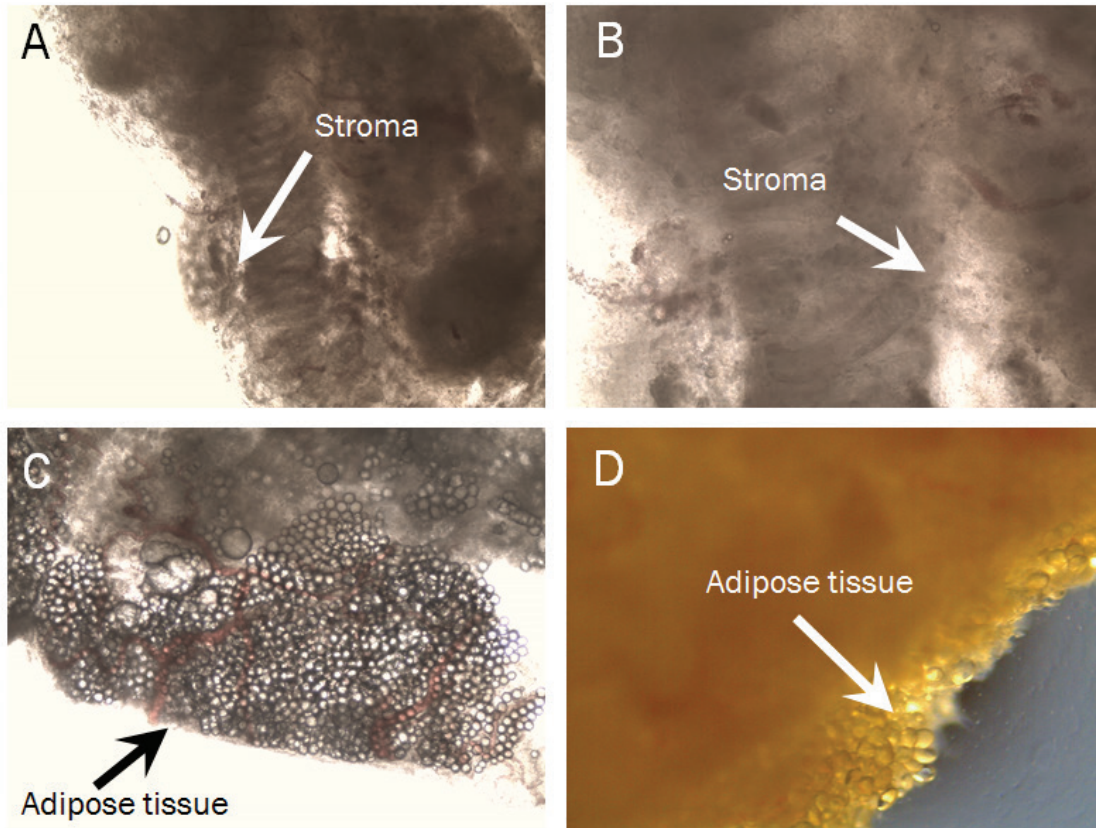


Figure 4: Identification of Stroma and Adipose Tissue in a Human Intestinal Tissue Biopsy.

Human intestinal tissue was dissected using the protocol provided in the MimEX™ Tissue Collection Kit. Following gross dissection of epithelial tissue, remaining stroma and adipose tissue should be removed under high magnification. **(A, B)** Example images of remaining stroma following dissection. **(C, D)** Example images of remaining adipose tissue following dissection.

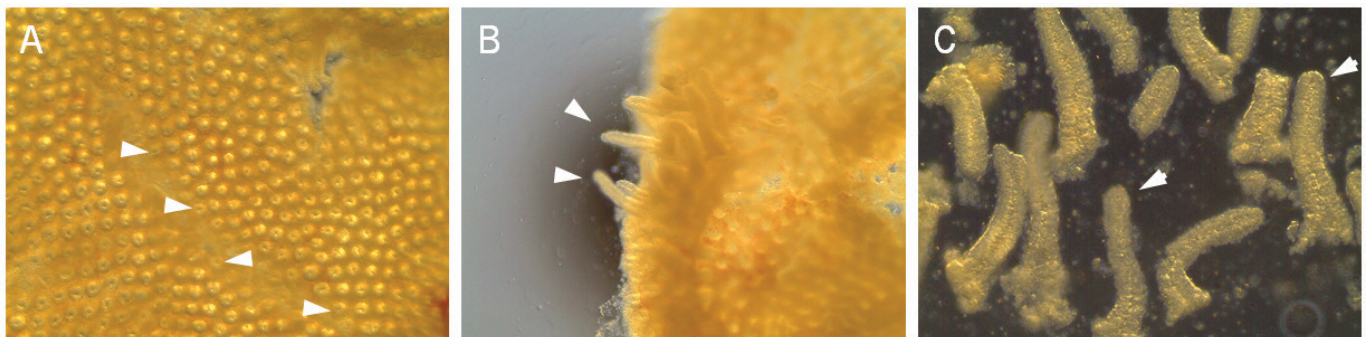


Figure 5: Intestinal Epithelium after Dissection from Surrounding Stroma and Adipose Tissue.

Human intestinal tissue was dissected using the protocol provided in the MimEX™ Tissue Collection Kit. Low **(A)**, medium **(B)**, and high magnification **(C)** images of intestinal crypts (arrowheads).

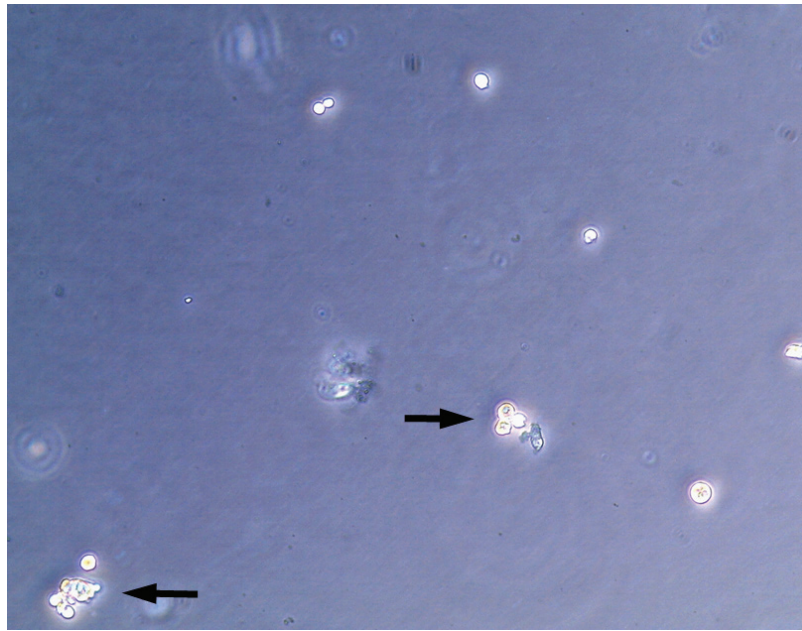


Figure 6: Optimal Density of Adult Stem Cells Following Tissue Biopsy Dissociation and Digestion. Adult human intestinal epithelial tissue was digested and dissociated using the protocol described in the MimEX™ Tissue Collection Kit. A complete dissociation should yield mostly clusters of 2-5 cells (arrows). Some single cells and few large cell clusters may also be observed. If there are very few small cell clusters and no single cells, continue incubation in dissociation solution for 10 minutes. Check cells again and repeat as needed.

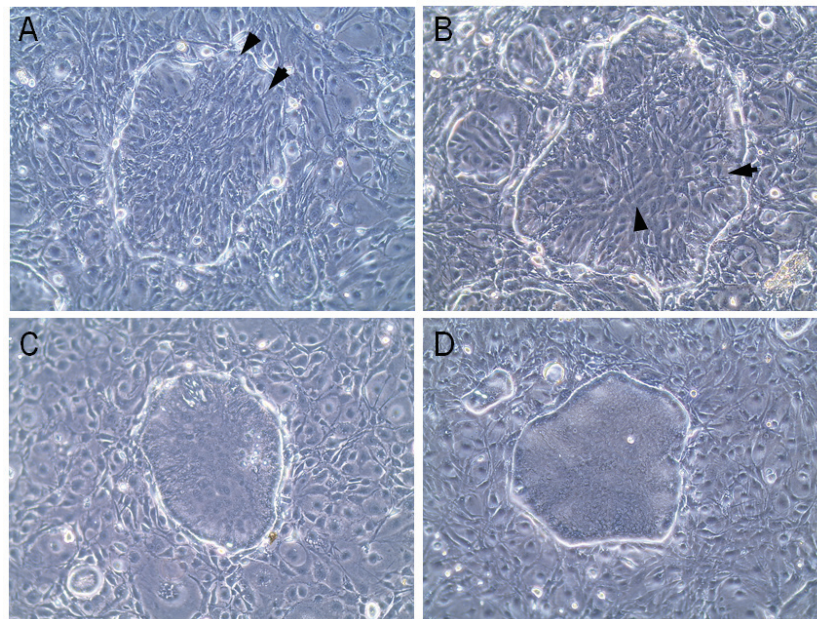


Figure 7: Removal of Human Fibroblasts from Stem Cell Cultures Following Biopsy Dissection. (A, B) Images of human fibroblast overgrowth (arrows) on the top of an adult epithelial stem cell colony after 2-3 passages in culture. (C, D) Adult stem cell colonies after human fibroblast removal using MagCollect™ Plus Human EpCAM⁺ Cell Isolation Kit (R&D Systems®, Catalog # MAGH121).

REFERENCES

1. Wang, X. *et al.* (2015) Nature **522**:173.
2. Yamamoto, Y. *et al.* (2016) Nature Comm. **7**:10380.

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