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# MCF10A-Z Cell Line in Co-Culture as In vitro Model of Mammary Epithelial Drug Transport

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# MCF10A-Z: Assessment of Novel Cell Line for Use in Co-Culture

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## INTRODUCTION

- Recent studies have found that lactating women ingest an average of three different medications while lactating[1]. These drugs are transported across the mammary epithelium and into the breastmilk, then subsequently ingested by the infant. Current work aims to develop *in vitro* models representative of transport across the mammary epithelium to allow medical providers and lactating women to better understand the amount of medication reaching an infant
- The complex structure of the mammary epithelium makes it difficult to produce models accurately predicting drug movement across the epithelium and into milk. Single-cell cultures lack the distinct cell types expressing various transporters of drug molecules. A **co-culture of luminal and basal** expressing cells may have better representation of the distinct cell types and functions in the mammary epithelium.

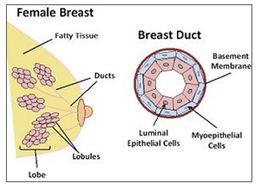



Figure 1: Model of the Human Mammary Epithelium[2]

- The novel mammary cell line, **MCF10A-Z**, shows distinct variation from the initial MCF10A line. These changes in morphology and function suggest it may be an isolated, luminal-expressing subpopulation of the traditional line, making it useful to represent luminal mammary cells in co-culture.

## OBJECTIVE

- Before use as a mammary luminal cell in a co-culture model, further examination of the traits of the novel MCF10A-Z line must be done to assess luminal expression.
- The goal was to compare the physical morphology of MCF10A-Z to the parent line, MCF10A, in terms of phenotypic appearance, growth rate, and response to trypsinization.
- Function of the cellular barriers in both cell lines were also compared in order to draw conclusions about the luminal characteristics of the MCF10A-Z line

## METHODS

- Culture of normal MCF10A (Georgia Tech, GT) and MCF10A-Z were initiated in cell dishes on the same date. Each progression was performed simultaneously; cells were counted every progression & each dish was seeded at the same density between lines. Cells were seeded in 12-well Transwell plates.
- **Growth rates** of each line were compared after notable differences were observed following seeding and the first progressions.
- The **physical characteristics** of each cell line was assessed at varying levels of confluence, the phenotypic appearance used for imaging and data analysis was as the cell lines neared 95 - 100% confluence.
- Other physical and functional aspects of the cells were assessed by monitoring and comparing the **time for complete trypsinization** of each line. The difference in trypsinization length could be indicative of different types of membrane-bound proteins present or differences in the junction proteins between cells
- Transepithelial electrical resistance (TEER) values of each line in 12-Well Transwell plate inserts (area = 1.12 $cm^2$) were found using the EVOM2 Voltmeter. The formula to calculate the values is as follows:

**TEER = Resistance value (Ω) x Insert area ($cm^2$)**

  - These values indicate the development of tight junction proteins between cells to form the cellular barrier, important to stopping passive diffusion across the membrane and forcing molecules to follow active transport routes more representative of *in vivo* processes.
  - Zonula occludens-1 (ZO-1) is a TJ some studies have found to be expressed in luminal, but not basal, cells[3]. Previous staining of MCF10A-Z by our lab has shown ZO-1 expression.

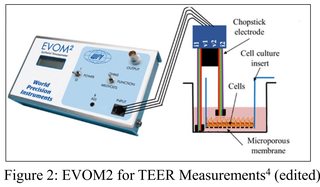



Figure 2: EVOM2 for TEER Measurements[4] (edited)

## RESULTS

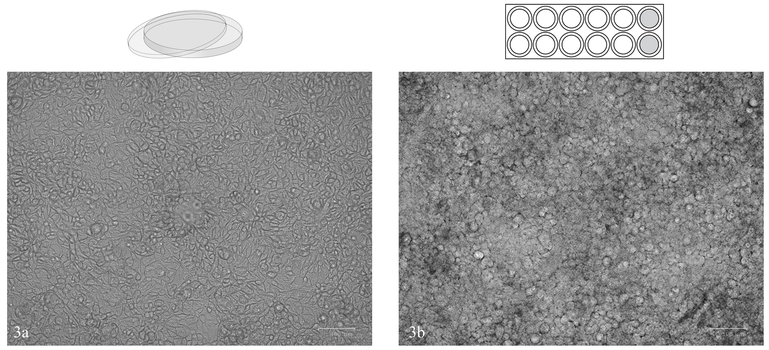



Figure 3a &3b: MCF10A-Z grown to confluence in cell dish; cells grown in Transwell inserts, respectively. Images taken with cell fluorescence imager

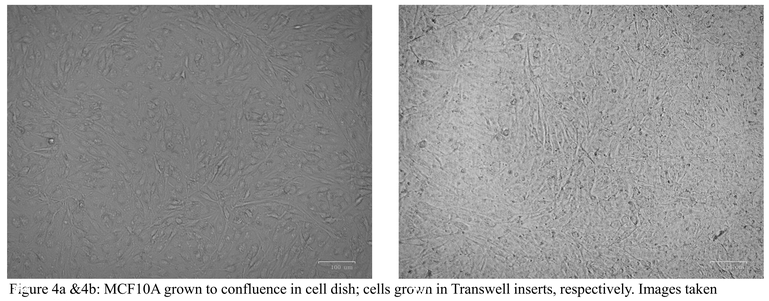
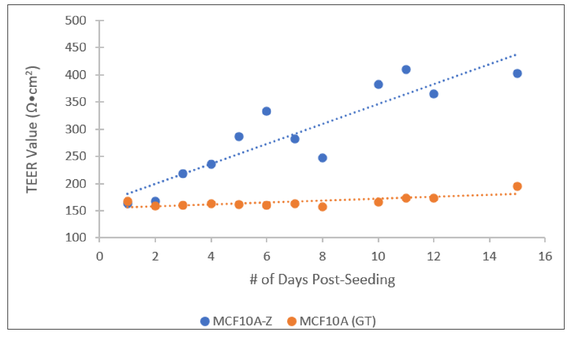



Figure 4a &4b: MCF10A grown to confluence in cell dish; cells grown in Transwell inserts, respectively. Images taken with cell fluorescence imager

Table 1: TEER Values of MCF10A and MCF10A-Z Over Time




| Comparison of Cell Physical Characteristics | |
|---|---|
| MCF10 (GT) | MCF10A-Z |
| • Elongated cells with lower confluence<br>• Slower growth rates<br>• Easier trypsinization (8-10 minutes). Normal cell pellet produced with centrifugation<br>• Lower final cell counts: 1.75 x $10^6$/mL in 2mL media | • Cobblestone cells with dense confluence<br>• Faster growth rates<br>• Increased difficulty with trypsinization (~15 minutes). Extremely large cell pellet post-centrifugation<br>• High final cell counts: 5.25 x $10^6$/mL in 2mL |

## CONCLUSIONS & FUTURE WORK

- The cobblestone appearance of the MCF10A-Z line compared to the thinner, elongated cells of the MCF10A line is a distinct physical difference. Basal/myoepithelial cells have an elongated appearance, consistent with MCF10A, while luminal cells may have a more cobblestone morphology[5].
- The observed higher growth rates and increased cell counts of MCF10A-Z also indicate significant physical differences. It is possible that media conditions induced changes in protein expression such as EGFR, which plays a role in TJ formation[6].
- The increased trypsinization time for MCF10A-Z has many explanations. First, the increased growth of the line may make the confluent monolayer more difficult to trypsinize. Second, increased mucin protein expression, such as MUC1, could make trypsinization more difficult. MUC1 forms a protective barrier around epithelial cells via a mucosal surface, protecting cells from extreme conditions (i.e. trypsinization)[7].
- Finally, the higher TEER values of MCF10A-Z suggest the presence of tight junctions between cells, forming a more coherent epithelial barrier. This could be due to expression of Crumb3, found to induce TJ formation when exogenously expressed[8].

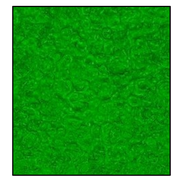


Figure 5: ZO-1 Staining of MCF10A-Z in current project, imaged via Olympus IX73 Inverted Microscope. Staining diffuse through cytoplasm

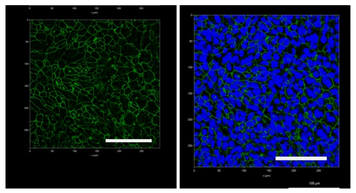


Figure 6a & 6b: ZO-1 staining of MCF10A-Z and ZO-1 staining overlaid with DAPI staining, imaged using confocal microscope in previous study by Zhang Lab

- Future work should first refine the ICC staining process for ZO-1 to better visualize and compare the tight junctions present in MCF10A-Z. Following tight junction assessment, it would be useful to examine protein expression that may be causing the phenotypic and functional differences, starting with EGFR, MUC1, and Crumb3.

## REFERENCES

1. Lutz BH, Bassani DG, Miranda VIA, Silveira MPT, Mengue SS, Dal Pizzol TDS, da Silveira MF, Bertoldi AD. Use of Medications by Breastfeeding Women in the 2015 Pelotas (Brazil) Birth Cohort Study. Int J Environ Res Public Health. 2020 Jan 16;17(2):568. doi: 10.3390/ijerph17020568. PMID: 31963112; PMCID: PMC7014443

2. Human breast cells in cancer research. Lifeline Cell Technology. June 1, 2015. https://www.lifelinecelltech.com/optimal-systems-for-breast-cancer-research/.

3. Wang J, Zhu HH, chu M, Liu Y, Zhang C, liu G, yang X, yang R, Gao WQ. Symmetrical and asymmetrical division analysis provides evidence for a hierarchy of prostate epithelial cell lineages. Nat commun. 2014 Aug 28;5:4758. Doi: 10.1038/ncomms5758.

4. Raut, Bibek & Chen, Li-Jiun & Hori, Takeshi & Kaji, Hirokazu. (2021). An Open-Source Add-On EVOM® Device for Real-Time Transepithelial/Endothelial Electrical Resistance Measurements in Multiple Transwell Samples. Micromachines. 12. 282. 10.3390/mi12030282.

5. Visvader JE, Stingl J. Mammary stem cells and the differentiation hierarchy: current status and perspectives. Genes Dev. 2014 Jun 1;28(11):1143-58. doi: 10.1101/gad.242511.114. PMID: 24888586; PMCID: PMC4052761.

6. Muranen T, Iwanicki MP, Curry NL, Hwang J, Dubois CD, Coloff JL, Hitchcock DS, Dlish CD, Brugge jJS, Kalaany NY. Starved epithelial cells uptake extracellular matrix for survival. Nat commun. 2017 Jan. 10;8:13989. Doi: 10.1038/ncomms13989. Pmid: 28071763; pmcid: pmc5234072.

7. Chen W, Zhang Z, Zhang S, Zhu P, Ko JK, Yung KK. MUC1: Structure, Function, and Clinic Application in Epithelial Cancers. Int J Mol Sci. 2021 Jun 18;22(12):6567. doi: 10.3390/ijms22126567. PMID: 34207342; PMCID: PMC8234110.

8. Fogg VC, Liu CJ, Margolis B. Multiple regions of crumbs3 are required for tight junction formation in MCF10A cells. J cell sci. 2005 jul 1;118(pt 13):2859-69. Doi: 10.1242/jcs.02412.

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