Induced Pressure Promotes Extrusion and Transient Polyp Formation in MDCK Monolayers to Maintain Homeostasis

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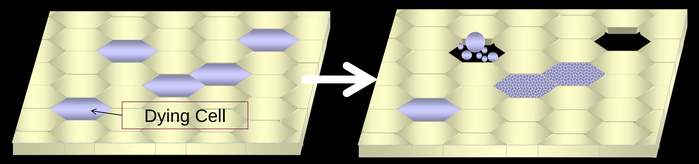
BACKGROUND

"In the human body about 100,000 cells are produced every second by mitosis and a similar number die by apoptosis"

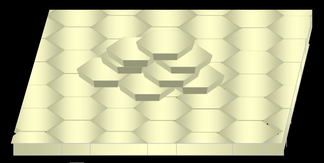
….Vaux & Korsmeyer (1999) Cell.

WHAT HAPPENS IF TOO MUCH DEATH OCCURS?



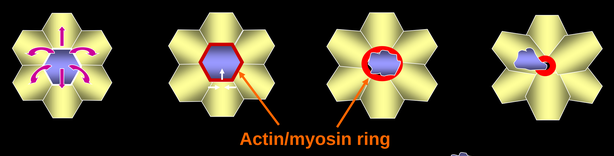
EPITHELIUM LOSES BARRIER FUNCTION

WHAT HAPPENS IF TOO LITTLE DEATH OCCURS?



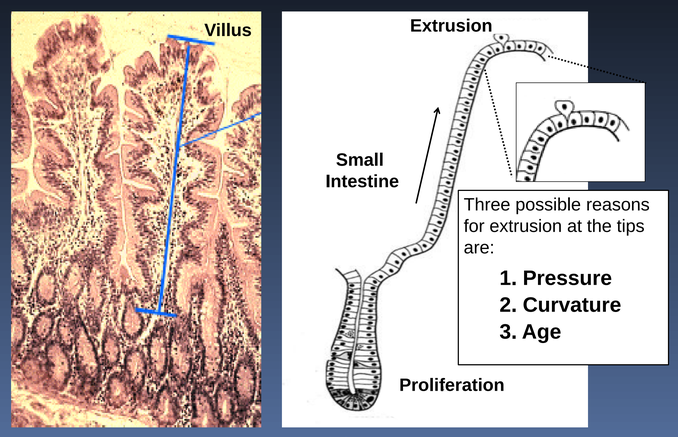
TUMORS FORM

HOW ARE DYING EPITHELIAL CELLS REMOVED?



Each dying cell sends signals to its live neighbors, which form an actin and myosin ring that squeezes the dying cell out in a process called extrusion.

THE VILLI IN THE GUT ARE A PHYSIOLOGICAL EXAMPLE OF CELL TURNOVER

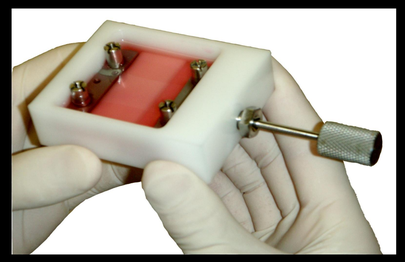


Potten (1997) Am J Physiol 273:G253-7

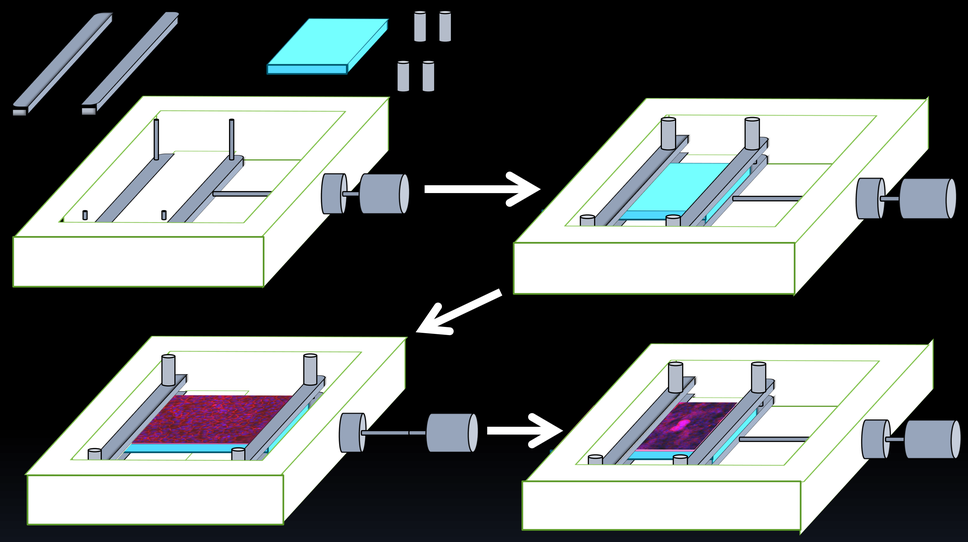
HYPOTHESIS

From the model of extrusion in the villi in the gut, we hypothesize that overcrowded cells under pressure in a monolayer will get extruded and die. Thus, we are testing if artificially producing pressure on a monolayer can induce extrusion and cell death.

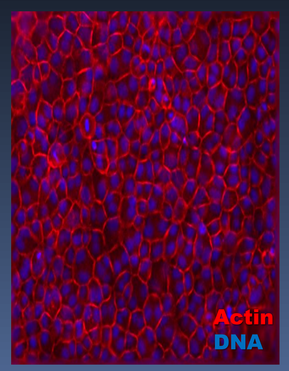
MATERIALS AND METHODS



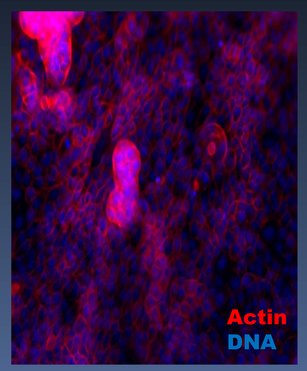
I have grown epithelial monolayers on a stretched silicone matrix, released the matrix from its stretched state, thereby increasing cell strain, and immunostained the monolayers with markers of extrusion and apoptosis. The device used to induce pressure was developed by Masaaki Yoshigi, Department of Bioengineering, University of Utah and is shown on the left. The following is an illustration of the methods:



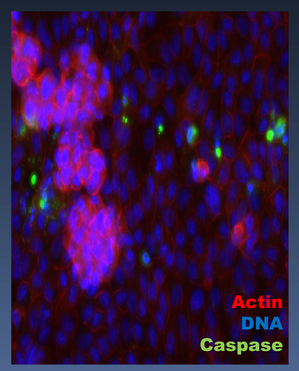
A silicone membrane was inserted into the device and stretched. Once stretched, it was seeded with epithelial cells. The cells were allowed to grow to confluency and then the membrane was released. The increased strain on the cells caused polyps to form as shown :



NORMAL MONOLAYER



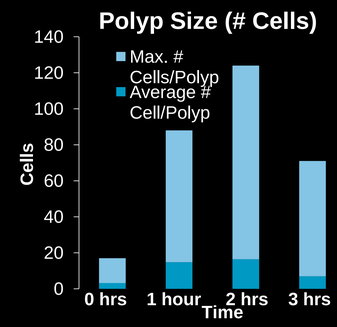
PRESSURE INDUCED MONOLAYER

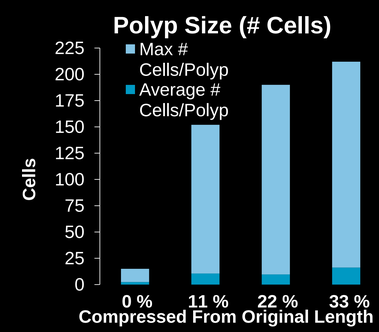


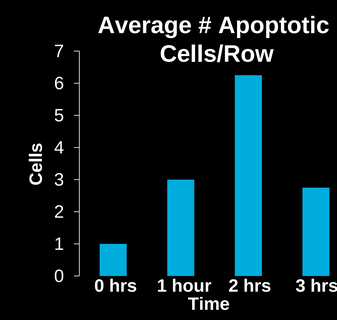
MINIMAL CELL DEATH OCCURS IN POLYPS

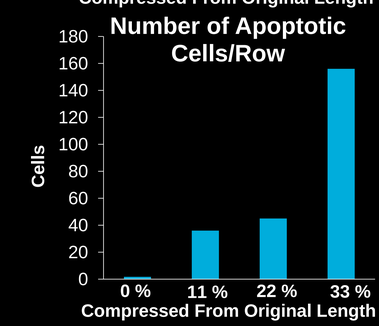
RESULTS

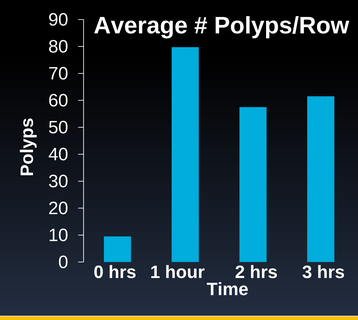
After one hour, aggregates of 2-215 cells had begun extruding and forming polyps, with less than 1% of the cells in these polyps being apoptotic. Polyps were apparent up to four hours, but were absent by 24 hours. Overall, pressure increased polyp formation in a time dependent manner. Polyp formation increased as the percentage of pressure increased.

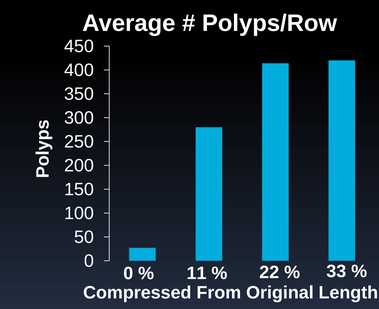












CONCLUSIONS

By using this pressure-induced cell extrusion assay with different drug agonists and antagonists, we can further delineate the signals used to initiate extrusion. As we further understand extrusion signals we will gain a broad wealth of knowledge on why tumors do or do not metastasize, leading to better treatment and healthcare for those suffering from cancer.

ACKNOWLEDGEMENTS

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