

# Na<sup>+</sup>/K<sup>+</sup> ATPase Regulation of Breast Cancer Motility

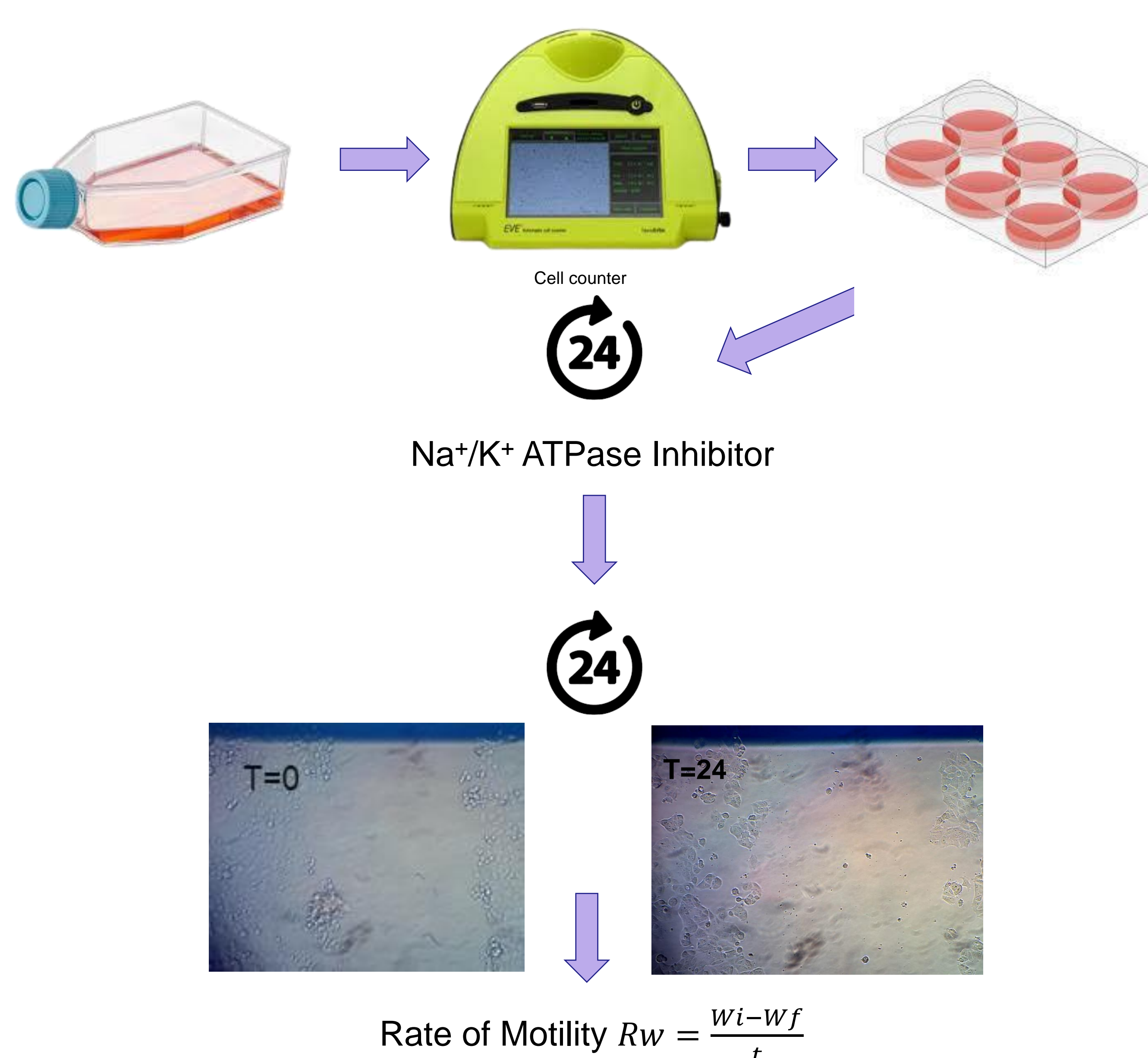
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## Introduction

Na<sup>+</sup>/K<sup>+</sup> ATPase pump is a ubiquitously expressed, energy consuming protein in cell membranes. The Na<sup>+</sup>/K<sup>+</sup> ATPase helps maintain osmotic equilibrium and membrane potential in cells. It has been found to be overexpressed in many cancers, but its impact on the development of cancer is unclear. The role that the energy consumption, ion transport and signal transduction mechanisms of Na<sup>+</sup>/K<sup>+</sup> ATPase play on impacting the behavior of cancer cells is a major field of study in our lab. The present study examines the effect of blocking Na<sup>+</sup>/K<sup>+</sup> ATPase function with different drugs on the motility of both highly and weakly metastatic breast cancer cells. We hypothesize blocking Na<sup>+</sup>/K<sup>+</sup> ATPase pump with an inhibitor will decrease the rate of breast cancer cells motility in vitro. We intend to measure the rate of motility with a in vitro wound healing assay using MDA-MB-231 a highly metastatic breast cancer cell line and MCF-7 a weakly metastatic cell line. In these experiments, we use a wound healing assay to measure cell motility in breast cancer cell lines, MDA-MB-231 and MCF-7 cell lines, a highly metastatic and moderate metastatic cell line, respectively. Cells were seeded in a six-well plate at one million per well. After 24 hours, the cells were treated with an Na<sup>+</sup>/K<sup>+</sup> ATPase inhibitor, ouabain at a concentration of 100 nM or digoxin at 500 nM concentration. Measurements of wound mark were taken at 0-hour, T=0, and 24-hour, T=24. The rate of motility of treated verses non-treated cells were compared.

## Experimental Design



## Results

**MDA-MB-231 Motility Rate Digoxin 500 nM**

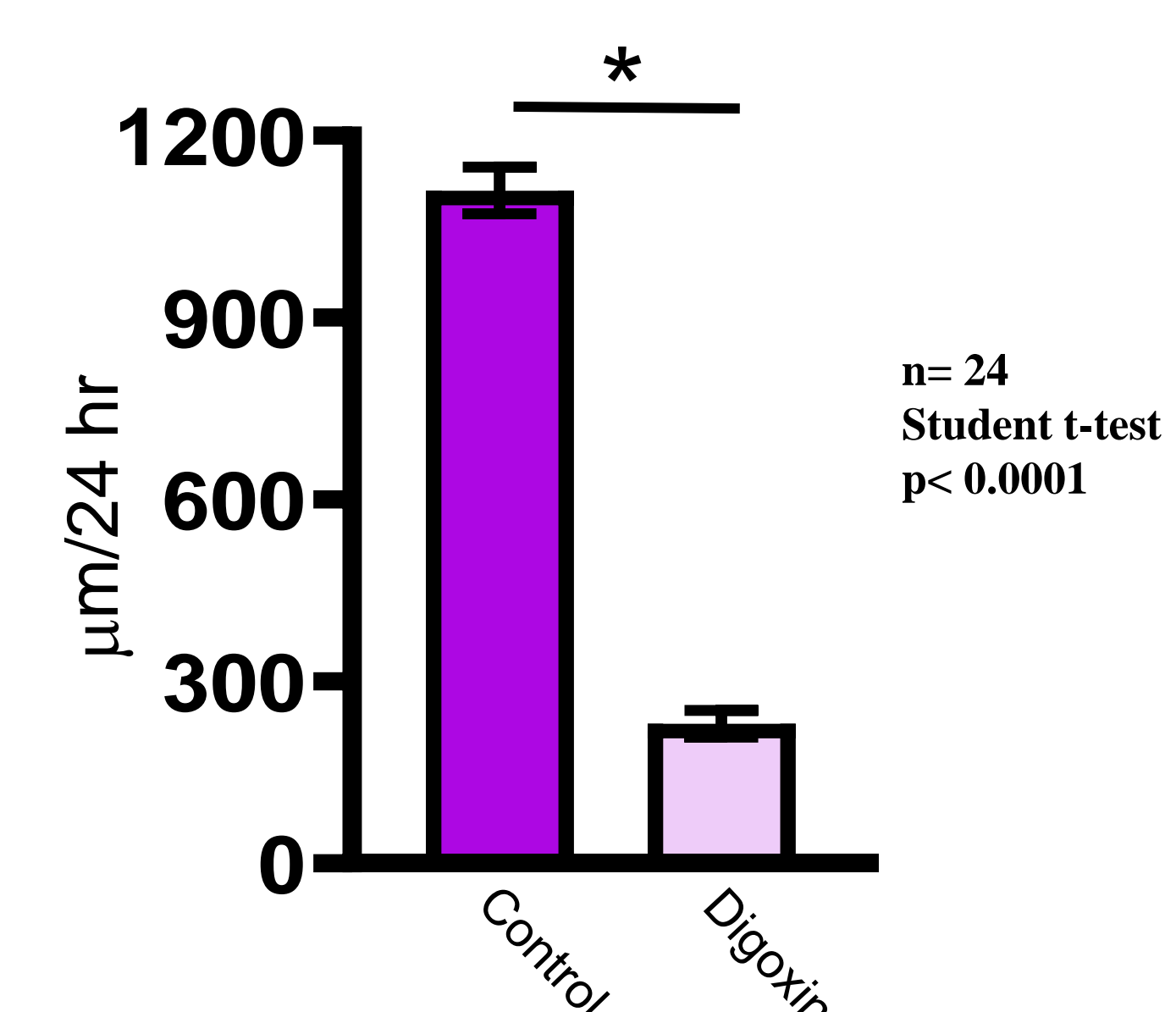


Figure 1. MDA-MB-231 motility rate of digoxin 500 nM concentration treated vs non-treated. n=24

**MDA-MB-231 Motility Rate Ouabain 100 nM**

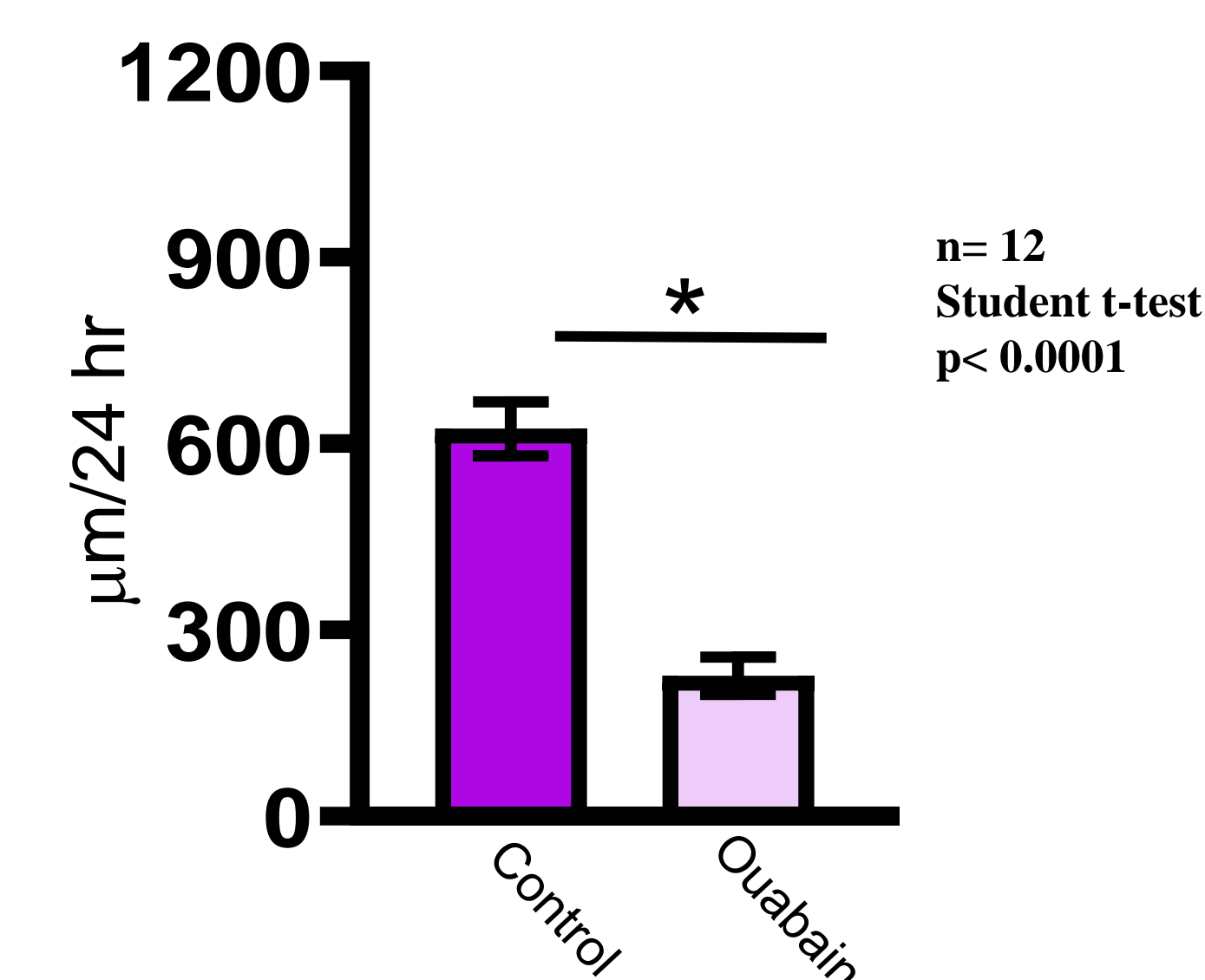
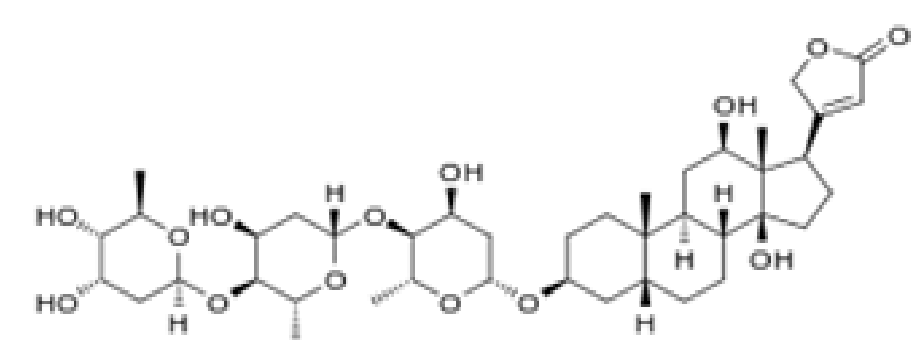


Figure 2. MDA-MB-231 motility rate of ouabain 100 nM concentration treated vs non-treated. n=12

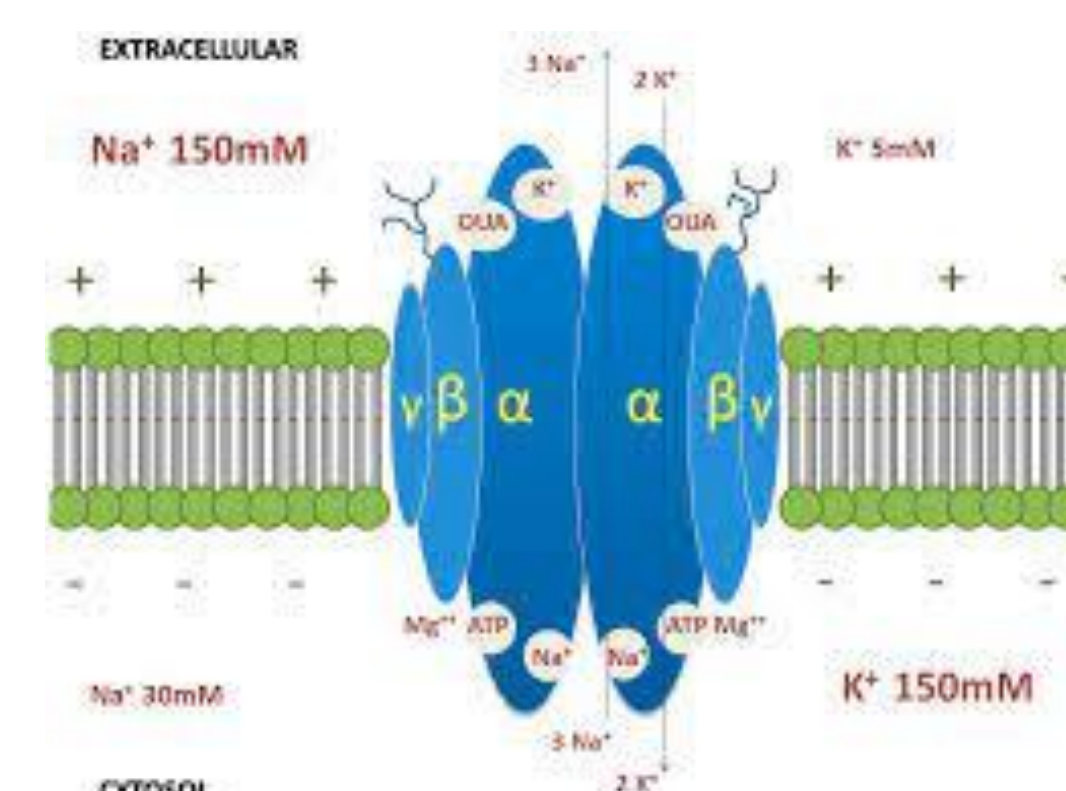
## Methods and Materials

### Digoxin



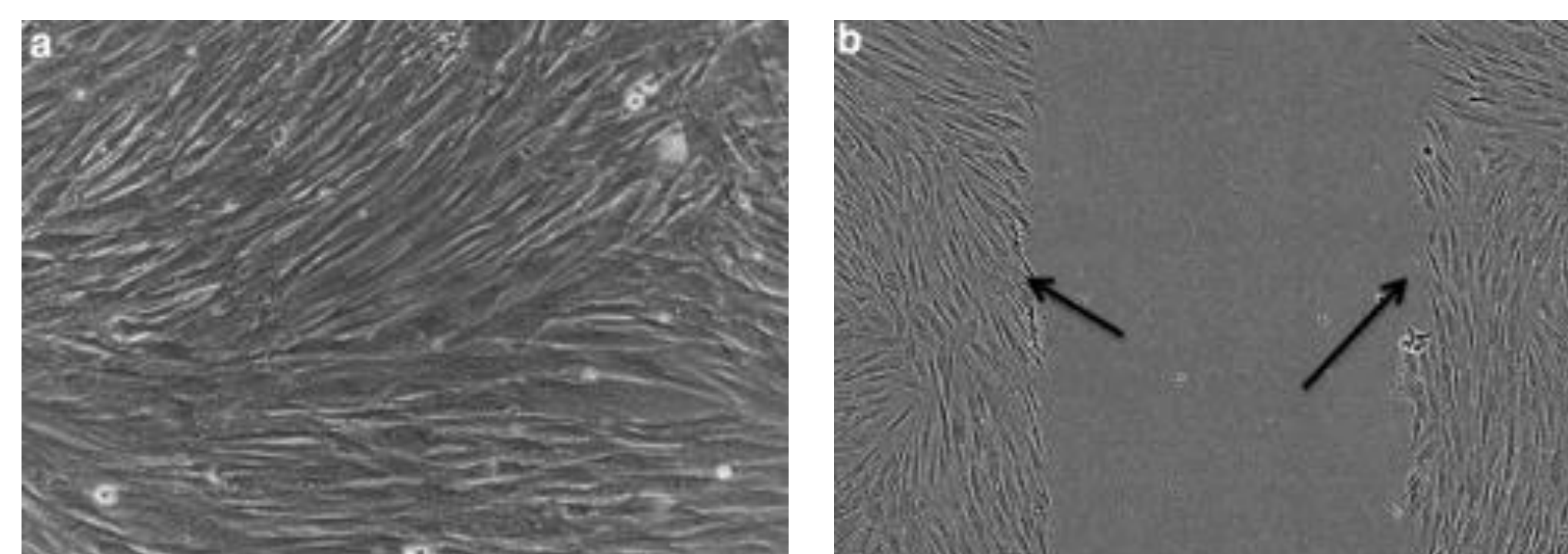
- Medication used to manage and treat heart failure and abortion
- Inhibiting the activity of the Na<sup>+</sup>/K<sup>+</sup> ATPase pump, (an enzyme that controls the movement of ions into the heart.)
- Also known as Digitalis is native to Europe, western Asia, and northwestern Africa
- Bounds to the beta subunit ( $\beta$ ) of the sodium potassium ATPase pump

### Sodium Pumps



- The sodium potassium pump (Na<sup>+</sup>/K<sup>+</sup> ATPase) is found in many cell membranes
- Powered by ATP, the pump moves sodium and potassium ions in opposite directions, each against its concentration slope
- In a single cycle of the pump, three sodium ions (3 Na<sup>+</sup>) are taken from, and two potassium ions (2 K<sup>+</sup>) are imported into the cell

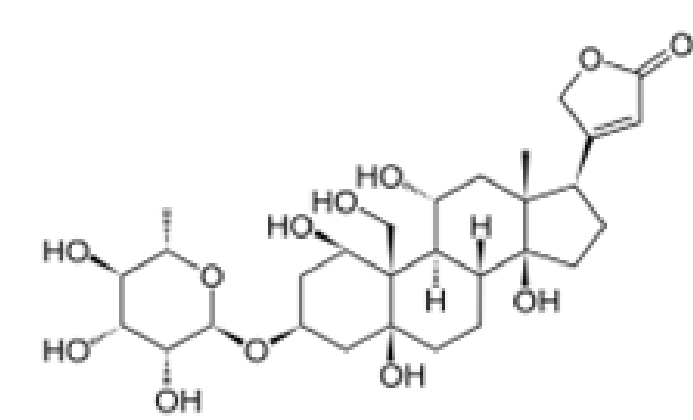
### Wound Healing Assay



The pictures came from "Research Techniques Made Simple: Analysis of Collective Cell Migration Using the Wound Healing Assay"

Wound healing is a complicated biochemical and cellular process that is needed to fix broken tissue. It includes dynamic interactions and crosstalk between different types of cells, interactions with molecules in the extracellular matrix, and the controlled release of soluble mediators and cytokines

### Ouabain



- Ouabain is used to treat hypotension and some arrhythmias
- Inhibits the Na<sup>+</sup>/K<sup>+</sup> ATPase
- Ouabain known as strophanthin, is a plant derived toxic substance that was traditionally used as an arrow poison in eastern Africa for both hunting and warfare
- Bounds to the alpha subunit ( $\alpha$ ) of the sodium potassium ATPase pump

## Conclusion

Our results suggest that blocking sodium pumps decreases cell motility of MDA-MB-231 breast cancer cells in vitro and potentially shuts energy consumption into another signaling pathway that does not play a role in cell motility. Results not shown MCF-7 treated cells were more sensitive to the 100nM concentration of ouabain Future studies will probe other pathways that take priority in the cell's homeostasis. Future studies would also include a drug concentration effect curve with MCF-7 cell lines.