Investigating the effect of Toyocamycin on Single A549 cells using Scanning Ion Conductance Microscopy

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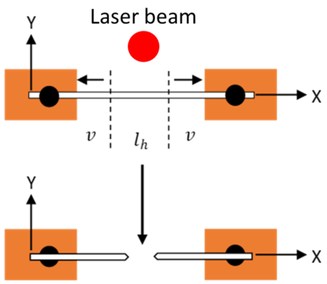
Summary

Adenocarcinoma is a common cancer of the lung, and the identification of new anticancer drugs effective against lung cancer will open new therapeutic opportunities such as combination therapy. Toyocamycin is an antibiotic that has shown effect on cancer cells such as multiple myeloma and pancreatic cancer cells. By using scanning ion conductance microscopy (SICM), toyocamycin is found effective against adenocarcinoma cells such as A549. The drug induced membrane blebbing, cell shrinkage, and apoptotic volume decrease. The technique is suitable in continuous mapping of the topography of live single cells before and after treatment with anticancer drugs, due to its label free and non-invasive nature. Herein, we have shown that toyocamycin is effective against A549 cells, and other biochemical assays could be used to further confirm the effect of the drug.

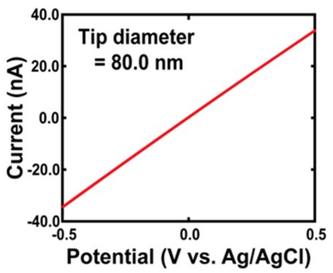
SICM as a technique for 3D live cell imaging

Fabrication of SICM imaging probe using a laser puller



Nanopipettes are fabricated from quartz capillary tube with I.D: 0.5 or 0.7mm and O.D: 1 mm

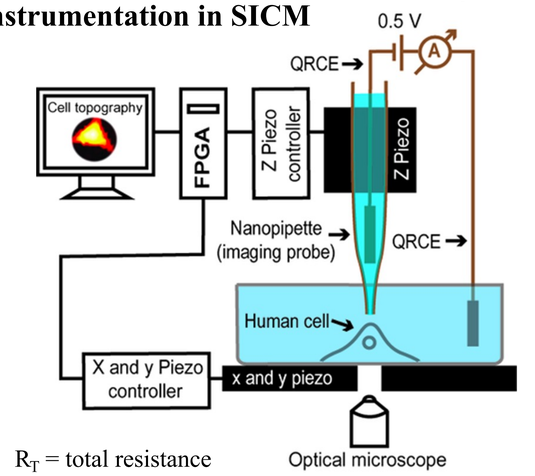
Characterization of SICM imaging probe in 1 M KCl using cyclic voltammetry



Equation used for calculating nanopipette size

$V = IR_p$ $\quad R_p = \frac{1}{\sigma \pi r_i} \frac{1}{\tan \alpha}$

Instrumentation in SICM



Advantage of SICM
- Non-invasive
- Label free
- Nanoscale resolution

Limitations
- Low throughput
- Susceptible to current drift
- Realtime studies with SICM is challenging

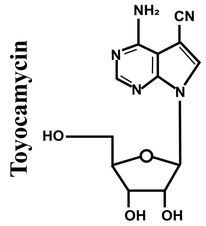
$$I(A) = \frac{V}{R_p + R_{AC} + R_s}$$

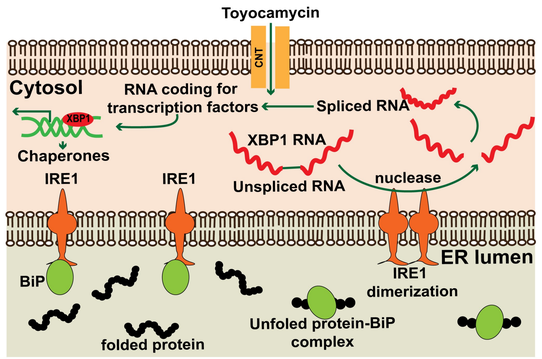
$$R_T = R_P + R_{AC} + R_S$$

R_T = total resistance
R_P = nanopipette resistance
R_S = solution resistance
R_{AC} = access resistance

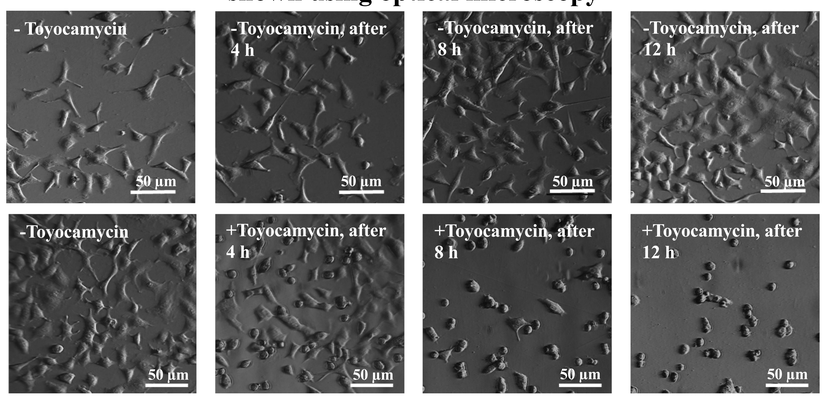
Toyocamycin induces apoptosis in A549 cells

Toyocamycin leads to an abundance of unfolded protein within a cell and high levels of caspase 3 to signal apoptosis

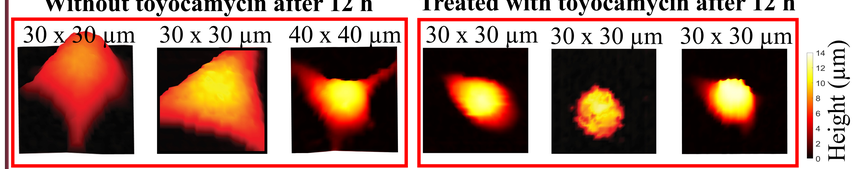




Toyocamycin alters the native morphology of A549 cells as shown using optical microscopy

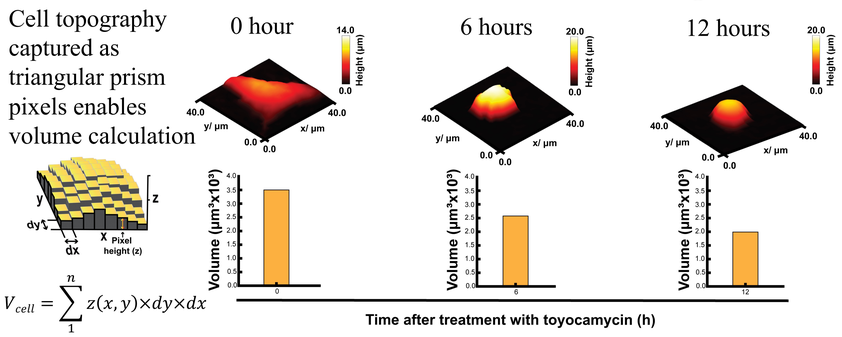


Toyocamycin alters the native morphology and volume of A549 cells observed using SICM



Continuous volume measurement of A549 cells using SICM

Cell topography captured as triangular prism pixels enables volume calculation

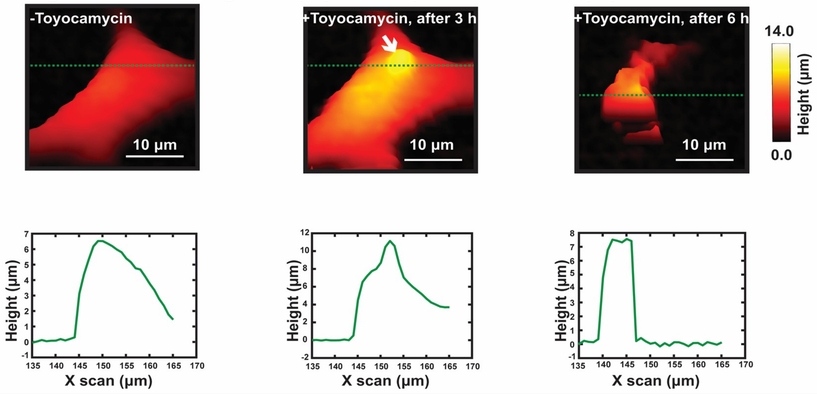


$$V_{cell} = \sum_{1}^{n} z(x,y) \times dy \times dx$$

Membrane blebbing induced by toyocamycin

Toyocamycin induces membrane blebbing in A549 cells

Membrane blebbing in this study (a hallmark of apoptosis) is a result of apoptosis induced through toyocamycin. This protrusion of the cellular membrane increases throughout the final stages of apoptosis to produce smaller apoptotic bodies.



Conclusion

We have demonstrated SICM as a powerful tool for the studies of drug-induced apoptosis. Cells were treated with toyocamycin, and it was determined that there was a drastic change in the morphology at the higher molarities (30-50 µM). However, lower concentrations (10 and 20 µM) had a minor affect in the overall morphology yet did decrease the cell growth rate. Using SICM, the continuous measurement of the topography of live single cells can be followed where information about cell morphology, apoptotic volume decrease, and membrane blebbing can be obtained.

Future direction

Future research includes fabricating pH sensors using a double nanopipette, where one of the barrels will be carbon pyrolyzed, and then functionalized with IrO_2 pH sensitive material, while the other barrel will be filled with 0.1 M KCl for topography mapping. This multifunctional nanoscale electrode can be further used to understand the morphology and pH changes due to toyocamycin.

References and Group Information

- Chiao Chicy Chen; Zhou, Y.; Baker, L. A. Scanning Ion Conductance Microscopy. **2012**, *5* (1), 207–228.
- Park, S.-G.; Kim, S.-H. et al., Toyocamycin Induces Apoptosis via the Crosstalk between Reactive Oxygen Species and P38/ERK MAPKs Signaling Pathway in Human Prostate Cancer PC-3 Cells. *Pharmacological reports: PR* **2017**, *69* (1), 90–96.
- Ri, M. et al., Identification of Toyocamycin, an Agent Cytotoxic for Multiple Myeloma Cells, as a Potent Inhibitor of ER Stress-Induced XBP1 MRNA Splicing. *Blood Cancer Journal* **2012**, *2* (7), e79–e79.

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