Regulation of Macropinocytosis in Mammalian Cell Lines

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Macropinocytosis

- Mediates non-selective uptake of molecules
- Regulated by receptor tyrosine kinases: EGF and PDGF
- Activation of factors lead to actin polymerization → macropinosome formation
- Actin polymerization initiated by activation of GTPases working with phosphoinositide 4,5 – biphosphate
- Above factors activate proteins that bind PI(4,5)P2, actin and the Arp2/3 complex
- Binding of these factors coordinate the formation of Arp2/3 complex with an actin monomer on a pre-existing actin filament

Our Experiment

- Amiloride found to inhibit macropinocytosis
  - Prevents Cdc42 and Rac1 signaling
- EGF found to promote macropinocytosis

- Why are we Interested?
- Actin dependent ruffling – involved in cell motility
  - Tumor progression
- Immunity
  - Way in which antigen presenting cells sample surroundings for antigens
- Pathogens take advantage of macropinocytosis to infect cells

HYPOTHESIS:

The cells receiving amiloride treatment will have a significantly lower uptake of dye that the cells not receiving amiloride and the cells receiving EGF treatment will have a significantly higher uptake of dye that cells not receiving treatment.

3 mL of cells into 4 eppendorf tubes

Vortex, remove supernatant, careful not to touch cells

↓ add drug 33uL (amiloride Hek)
100uL (Epidermal Growth factor HeLa)

incubate 30min

Top up to 1mL using DMEM/10% FBS

Drug into E & C3 only

↓ vortex remove supernatant

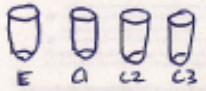
This becomes stock, add drug to E & C3 and add 100uL of dye to E & C1

Top up to one ~~ml~~ mL total using DMEM

FROM this take 100uL immediately after solutions mixed and place in new eppendorf tube. This is 0min

E = dye + drug
C1 = dye - drug
C2 = -dye - drug
C3 = -dye + drug

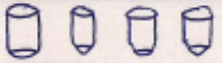
0min



↓ to this you require to "wash" cells DONE BY

Wash process add 1mL PBS, resuspend cells
- Vortex
- repeat this 3x

↓ to supernatant



+ Add 300uL of 4% PFA/PBS (Fixation) leave to incubate for 15min

Meanwhile other ninjas have take out 100uL after 10min, from first 100uL, Repeat wash, fix process.

Do this until 40min, at 10min interval

Placing in wells

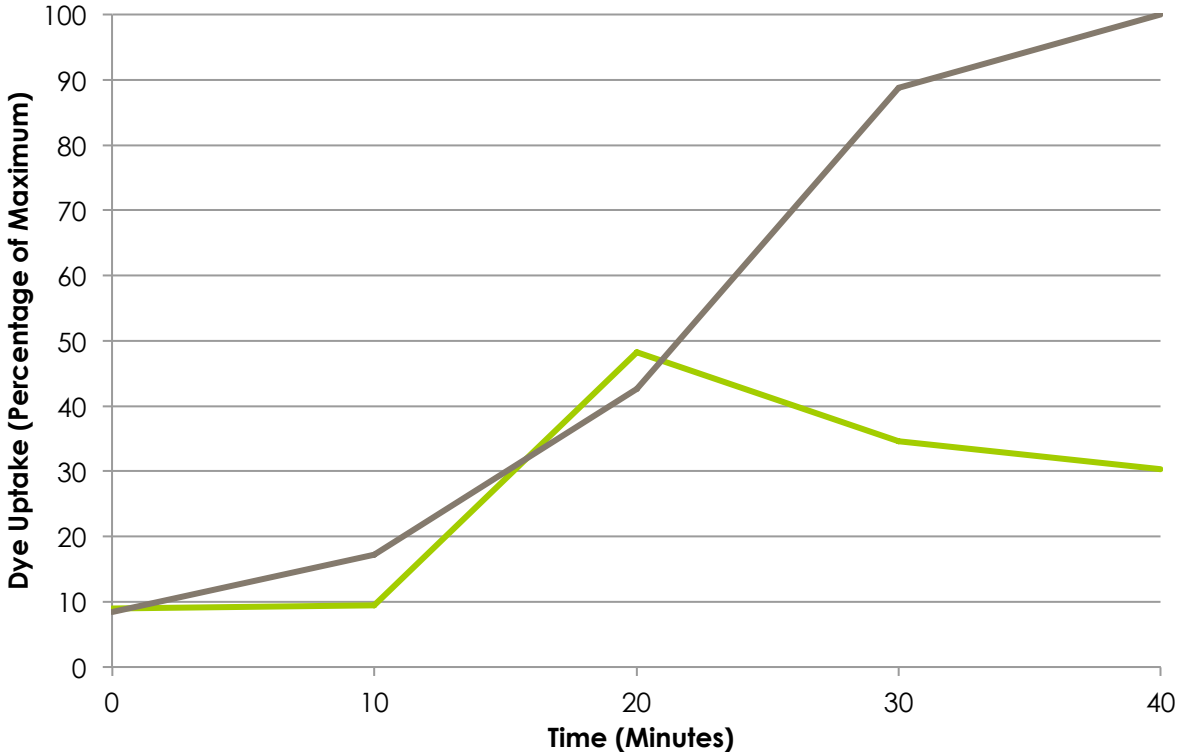
↳ From each we place 100uL in each respective well

0min	000	000	000	000
10min	000	000	000	000
20min	000	000	000	000
30min	000	000	000	000
40min	000	000	000	000

once all full
Give to tutor to analyse.

* Drug conc Amiloride 1mM
* Inhibitg conc EGF 100ng/mL

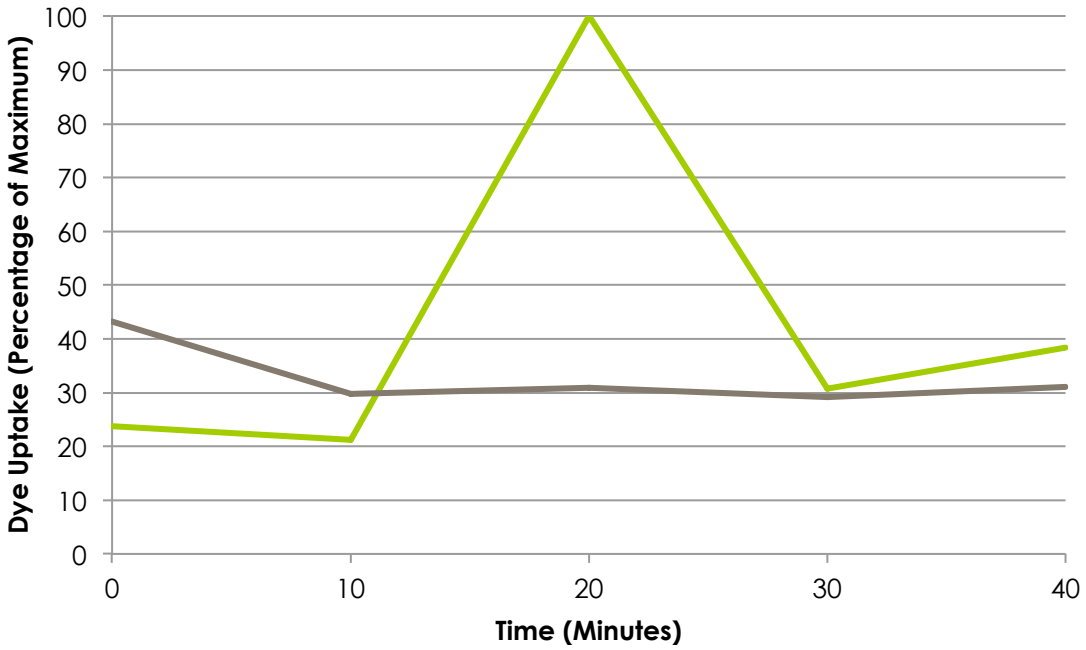
Dye Uptake in Amiloride Macropinocytosis Inhibited and Uninhibited HeLa Cells



P-value = 1.9x10↑−2

	Exp			Control 1			Control 2			Control 3		
Time (m)	Trial 1	Trial 2	Trail 3	Trial 1	Trial 2	Trail 3	Trial 1	Trial 2	Trail 3	Trial 1	Trial 2	Trail 3
0	17931	16708.6	14449.9	16990.7	16816.5	12863.6	13831.8	12813.6	6806.87	12659.2	12874.5	9500
10	14650	13789.9	17265	16378	19395.7	16429.5	10357.7	8512.42	6271.15	10095.1	8206.69	12535.7
20	44694.7	32972.8	34255.8	34094	29392.9	27685.5	6873.1	9617.98	7814.59	11254.9	11735.5	13284.5
30	35071.6	26201.3	25160.6	85142.2	41612.9	37774.1	6717.17	8982.33	9715.91	9938.85	10186.8	12098
40	27080.7	25022.4	27264.4	71501	51521.1	58918.3	8161.19	8947.54	8208.72	9846.42	9365.59	12571.4

Dye Uptake in EGF Macropinocytosis Enhanced and Unenhanced Hek293 Cells



P-value = 1.1×10^{-3}

	Exp			Control 1			Control 2			Control 3		
Time (m)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
0	27991.4	19673.3	15158.5	46904.1	28649.3	26470.5	4865.96	5493.2	4378.43	5650.41	4712.18	4503.65
10	29238.2	13025.9	13441	27755.5	28414.2	18340.9	4844.49	5006.8	4421.63	4418.89	4158.76	4261.73
20	87823.1	66621.6	63285.2	26118.1	27237.1	25507.9	4498.3	7985.84	4071.19	5706.02	5977.44	4016.72
30	29306.5	24406	24933.9	23328.8	24365.7	23494.4	4712.77	4549.65	2997.43	5532.53	5325.36	5791.25
40	37934.7	32834.6	23162.2	27468.5	26239.6	26072.1	5339.45	5644.71	6042.86	5979.94	5985.99	4427.05

Conclusions

Drug Treatments can be used to regulate macropinocytosis in mammalian cell lines.

- Amiloride Inhibits Macropinocytosis
- EGF enhances Macropinocytosis

Limitations

- Inconsistent dye uptake per endosome
- pH sensitive dye deactivated by acidic environment of the endosome
- Mechanism of inhibition/enhancement not fully understood

Future Directions

- Same drugs in other endocytotic conditions
- Behaviour of other molecules and uptake via macropinocytosis