

Supplementary Information:

Tension-Induced Neurite Growth in Microfluidic Channels

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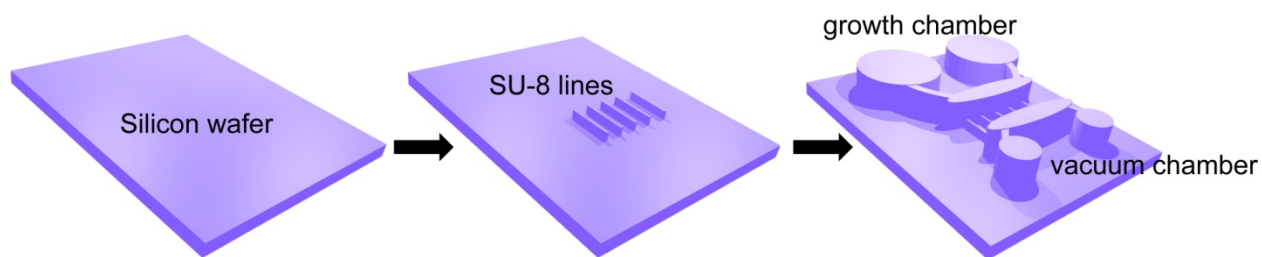


Figure S1. Schematic of the fabrication process to create the microfluidic device mold.

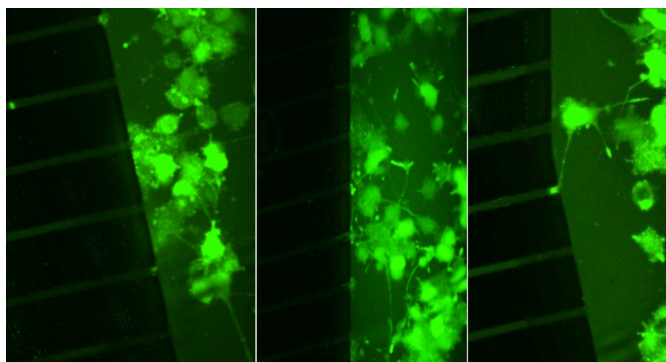


Figure S2. PC12 cells not under vacuum in the control experiment of Fig. 2b in the main text.

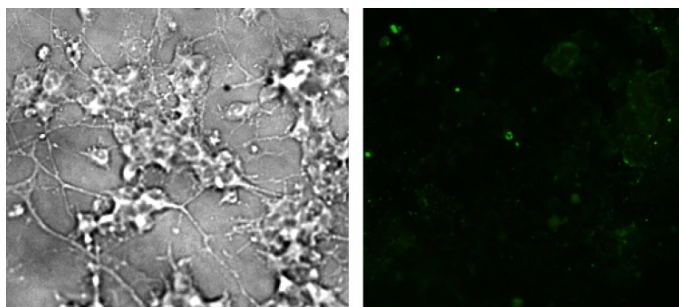


Figure S3. Control experiment for immunofluorescence (IF) staining. Transmission image (left) shows PC12 cells with neurites while the corresponding fluorescence image (right) shows no neurites or cell bodies detected from the IF assay without using the primary antibody, Tau.

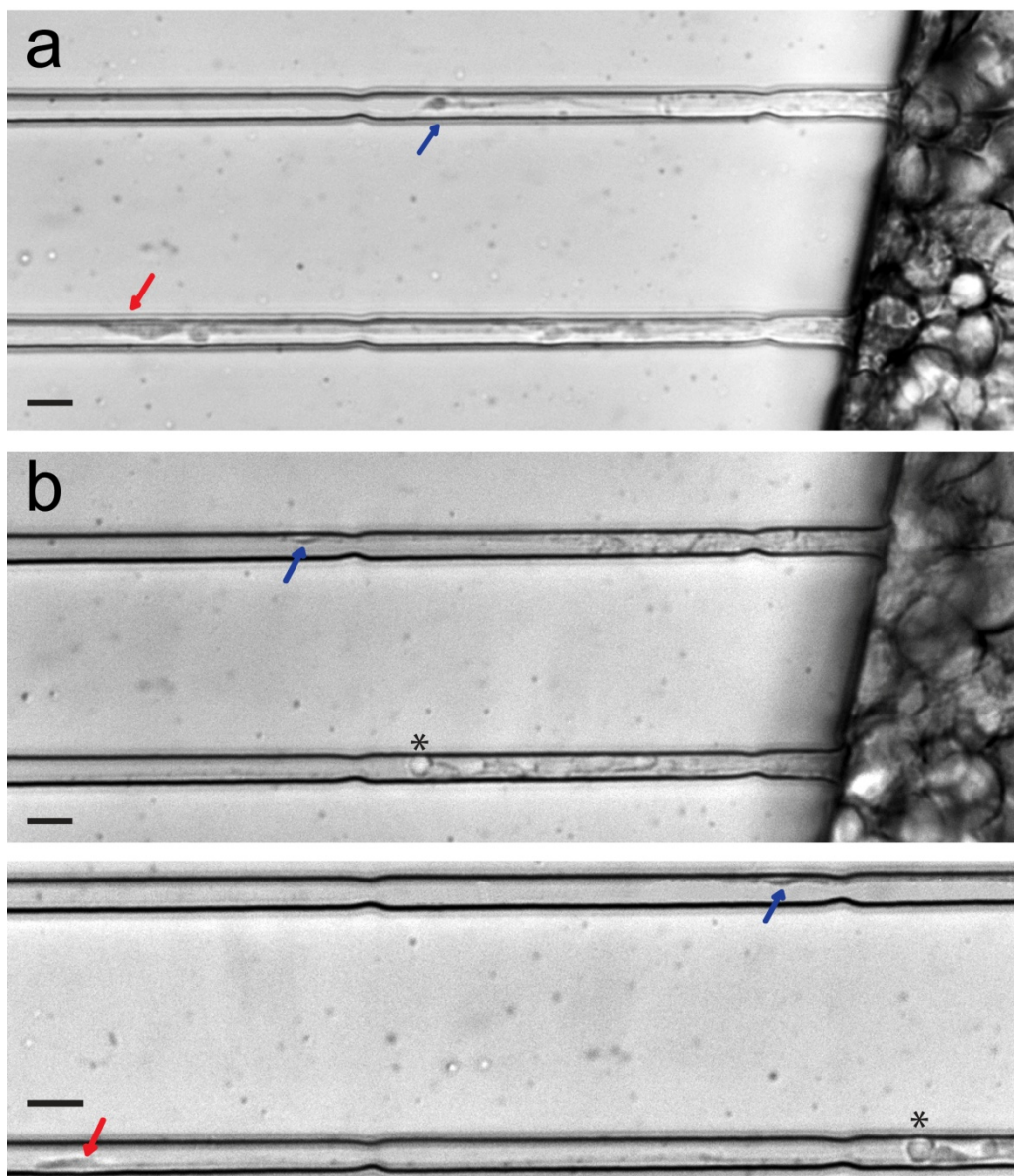


Figure S4. Stability of neurites. (a) PC12 cells, exposed to a vacuum of -600 Pa for 10 hours, were imaged by transmitted light microscopy immediately after stopping the vacuum. Arrows indicate the tips of neurites inside the microchannels. (b) Transmitted light images show neurites at 20 hours after halting the vacuum. The bottom image is a leftward extension of the top image. Asterisks (*) indicate the same spot in both images. The arrows indicate the new positions of neurite tips and clearly show that the neurites have extended following removal of the vacuum. The same color arrows show the same neurites in (a) and (b). All scale bars are 20 μm .

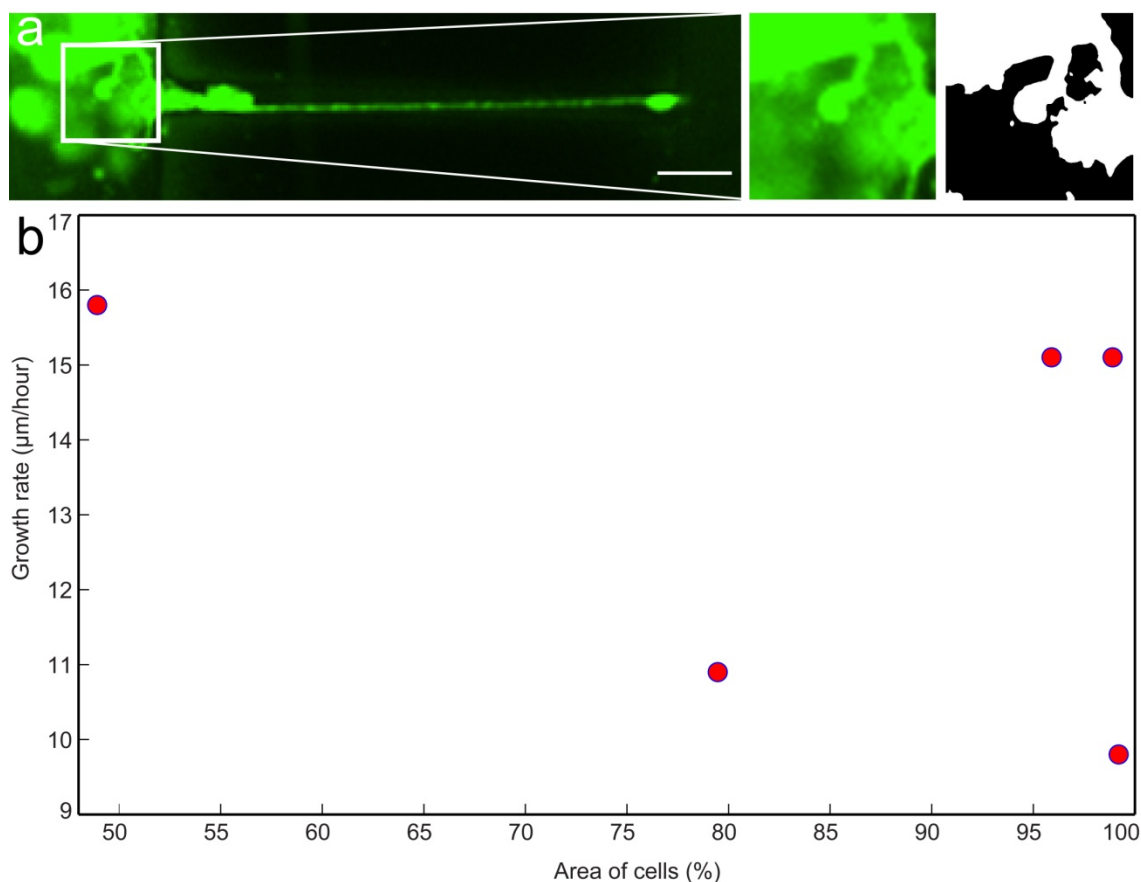


Figure S5. Growth rate of neurites versus sizes of cell body clumps in front of the microchannels. (a) The leftmost fluorescence image shows a typical neurite extending from a clump of cell bodies in front of the microchannel (exposed to -400 Pa for 12 hours). Scale bar is $30\ \mu\text{m}$. A fixed area (white box, $40\ \mu\text{m} \times 40\ \mu\text{m}$) was chosen in front of the microchannels to estimate the area occupied by cell bodies. The middle image shows a zoom-in of the white box area, and the rightmost image shows a thresholded binary image (converted in Matlab), in which the white color indicates the cell-occupied area and the black color indicates the area not occupied by cells. The area occupied by cell clumps was measured as the white area as a percentage of the total area. (b) Cell clumps in front of individual microchannels, measured as described above, were plotted against measured the neurite growth rate. Neurite growth rate does not appear to correlate in any well-defined way with cell clump size (correlation = -0.26).

Equations for the growth of neurons

The growth of neurons occurs by the polymerization of micro-tubules at the tip of the neurite. The transport of tubulin monomers occurs by diffusion. Lipid vesicles and other components are also required for the growth of the neurite. These are transported actively. In the first model we write we will account only for the diffusive transport. We will work with the model of van Veen and van Pelt [1].

1 Van Veen and Van Pelt model

Van Veen and Van Pelt approximate the flux $J = -D \frac{dQ}{dx}$ of tubulin into a neurite of length L by

$$J = -D \frac{Q_r - Q_t}{L}, \quad (1)$$

where Q_r is the concentration at the root or soma, Q_t is the concentration at the growing tip and D is a diffusion coefficient. If e is the length of a tubulin dimer then van Veen and van Pelt say that

$$\frac{1}{eV} \frac{dL}{dt} = k_{on}Q_t - k_{off}, \quad (2)$$

where V is a small volume near the tip of the neurite where the assembly takes place. van Veen and van Pelt also account for the change in length of the microtubules due to applied forces. Many measurements have shown that the microtubules are under compression and the membrane under tension in a growing neurite [3]. For example, Dennerll *et al.* found that the neurite tension varies over a broad range from 0–10,000pN. Due to the force the length L could change to kL where $k > 1$ if the microtubule is under tension and $k < 1$ if it is under compression. We will neglect this effect here and take $k \approx 1$. We also remember that force can modify the on-rate of the microtubule assembly process [4]. We say that

$$k_{on}(F) = k_0 \exp\left(-\frac{F\delta}{k_B T}\right). \quad (3)$$

where $F > 0$ is the force applied by the microtubule on the membrane. The physics behind this equation is that the probability distribution function for the gap size x between the tip of the growing microtubule and the fluctuating membrane is $p(x) = \frac{F}{k_B T} \exp(-\frac{Fx}{k_B T})$ when the fluctuations of the tip are much faster than the reaction [4]. The membrane, of course, is in tension. In fact, a rudimentary force balance on the hemispherical tip of the neurite of radius R_p gives

$$2\pi R_p \tau = \pi R_p^2 p + nF, \quad (4)$$

where τ is the membrane tension (in units of force/length), p is the pressure difference between the inside and outside of the cell, F is force exerted by a single microtubule on the tip and n is the number of microtubules impinging on the growing tip. If τ is held constant then increasing p would reduce F and enhance $k_{on}(F)$. Note that δ is about the size of one monomer and according to Howards book $\frac{k_B T}{\delta} \approx 2\text{pN} - 7\text{pN}$. Summarizing the equations

$$\frac{1}{eV} \frac{dL}{dt} = k_{on}Q_t - k_{off}, \quad (5)$$

$$\frac{dQ_t}{dt} = k_{off} - k_{on}Q_t + \frac{DA}{VL}(Q_r - Q_t), \quad (6)$$

$$\frac{dQ_r}{dt} = I - \frac{DA}{VL}(Q_r - Q_t), \quad (7)$$

where I is the rate (in units of concentration per unit time) of production of tubulin monomers in the soma and A is the area of cross-section of the neurite. The differential equations above need to be solved numerically. Typically, the initial conditions used are that $L(0) = \epsilon$, $Q_r(0) = Q_0$ and $Q_t(0) = 0$ where ϵ is a small number and Q_0 is a constant. Van Veen and Van Pelt did so and found that after a fast initial phase Q_r and L increase linearly with time and Q_t converges to a constant value. Motivated by the observations of the numerical experiments let us plug in the following into the equations of Van Veen and Van Pelt:

$$L(t) = C_1 t + C_2, \quad Q_r = C_4 t + C_5, \quad Q_t = \text{const.} \quad (8)$$

There are five unknowns above – C_1, C_2, C_4, C_5 and Q_t . We get five equations by plugging these into Van Veen and Van Pelt's equations and comparing coefficients:

$$C_1(k_{off} - k_{on}Q_t) + \frac{DA}{V}C_4 = 0, \quad (9)$$

$$C_2(k_{off} - k_{on}Q_t) + \frac{DA}{V}(C_5 - Q_t) = 0, \quad (10)$$

$$C_4C_1 - IC_1 + \frac{DA}{V}C_4 = 0, \quad (11)$$

$$C_4C_2 - IC_2 + \frac{DA}{V}(C_5 - Q_t) = 0, \quad (12)$$

$$\frac{C_1}{eV} - k_{on}Q_t + k_{off} = 0. \quad (13)$$

By solving these equations we get

$$C_1 = \frac{DA}{2V} \left[-1 + \sqrt{1 + \frac{4eV^2I}{DA}} \right], \quad (14)$$

$$C_4 = I - \frac{C_1}{eV}, \quad (15)$$

$$Q_t = \frac{k_{off} + \frac{C_1}{eV}}{k_{on}}, \quad (16)$$

$$\frac{C_5 - Q_t}{C_2} = \frac{C_1}{eDA}. \quad (17)$$

If $\frac{eV^2I}{DA}$ is small (or diffusion is very fast) then it is easy to see that $C_1 = eVI$, $C_4 = 0$. We see that k_{on} does not enter the equation for C_1 . So, even if k_{on} is increased due to an applied pressure difference p the growth rate will remain unaffected unless I is somehow changed.

2 Samuels *et al.* model

We consider now a more sophisticated model of Samuels *et al.* who add a growth dependent active transport term linear in $BQ_r \frac{dL}{dt}$ into the equations of Van Veen and Van Pelt where B is a constant. They also consider the possibility of a growth independent active transport term of the form MQ_r but assume $M = 0$ in their analysis. We would expect I , B and M to depend on external chemical factors (growth factors, nutrition, energy providing molecules) that are added to the system. The equations given by Samuels *et al.* are as follows:

$$\frac{dL}{dt} = \alpha Q_r, \quad (18)$$

$$\frac{dQ_t}{dt} = \frac{DA}{V_{tip}L}(Q_r - Q_t) + \frac{BQ_r}{V_{tip}} \frac{dL}{dt} - \frac{G}{V_{tip}} \frac{dL}{dt} + \frac{M}{V_{tip}} Q_r \quad (19)$$

$$\frac{dQ_r}{dt} = \frac{S}{V_{soma}} - \frac{DA}{V_{soma}L}(Q_r - Q_t) - \frac{BQ_r}{V_{soma}} \frac{dL}{dt} - \frac{M}{V_{soma}} Q_r. \quad (20)$$

Even if we set $B = M = 0$ these equations differ slightly from those of Van Veen and Van Pelt. First, there is no k_{off} , and second the ratio of volumes V_{soma}/V_{tip} enters the equations. By comparing the equations above with those of Van Veen and Van Pelt we expect that α should be proportional to the on-rate k_{on} , the rate of production of tubulin $I = \frac{S}{V_{soma}}$, and $G \approx 1/e$ where e is the length of a tubulin dimer. As for numbers, Samuels *et al.* say that $\frac{dL}{dt}$ is on the order of $5\mu\text{m/hr}$. Similar numbers are reported by Wissner-Gross *et al.* who did experiments on neurite growth and fitted the equations of Samuels *et al.* to their data [6]. They give the following parameters:

$$\chi_1 = \frac{DAG}{SV_{soma}} = 5.7 \pm 0.9, \quad \chi_2 = \frac{BS}{\alpha G^2} = 36.8 \pm 3.8 \quad \chi_3 = \frac{V_{soma}}{V_{tip}} = 5.2 \pm 1.3, \quad (21)$$

$$t_{sc} = \frac{V_{soma}}{\alpha G} = 6.4 \pm 0.5\text{hr}, \quad L_{sc} = \frac{V_{soma}S}{\alpha G^2} = 51.3 \pm 4.5\mu\text{m}. \quad (22)$$

Samuels *et al* integrated the ODEs numerically (for the case $M = 0$) and found that after an initial transient (that lasts about t_{sc}) the growth rate $\frac{dL}{dt}$ and the concentrations Q_r and Q_t tend to constants. Our own numerical integrations using MATLAB confirmed this result. How can we understand this? If L becomes larger and larger with Q_r and Q_t constant then the term $\frac{DA}{L}(Q_r - Q_t) \rightarrow 0$. So, we expect that for long times

$$Q_t = \frac{S}{\alpha G}, \quad Q_r = \frac{G}{B}, \quad \frac{dL}{dt} = \frac{S}{G}, \quad \text{if } M = 0, \quad (23)$$

$$Q_t = \frac{S}{\alpha G}, \quad Q_r = \frac{S}{M + \frac{BS}{G}}, \quad \frac{dL}{dt} = \frac{S}{G}, \quad \text{if } M \neq 0. \quad (24)$$

In other words, all the tubulin produced in the soma is consumed by the growing neurite. Note that the growth rate $\frac{dL}{dt}$ is independent of α (which is proportional to k_{on}) in this situation. So, even if an applied pressure changes k_{on} the growth rate will remain unaffected even in this model.

3 Diffusion limited growth

In the previous two models it was assumed that microtubule polymerization is reaction limited. In other words, a monomer falls into the gap between the tip and the growing microtubule only occasionally. In the diffusion limited case we assume that the monomer addition reaction is so fast that as soon as a gap between the tip and a growing microtubule opens up, a monomer will drop in [4]. In this case

$$\frac{dL}{dt} \approx \frac{D_1}{\delta} \frac{(F\delta/k_B T)^2}{\exp(\frac{F\delta}{k_B T}) - 1 - \frac{F\delta}{k_B T}} \quad (25)$$

Here D_1 is a diffusion coefficient (different from D) and nF is the force being exerted on the tip given by (4). When a large force is opposing the polymerization (in the absence of an externally applied pressure) then polymerization is necessarily reaction limited. If $\frac{F\delta}{k_B T} \ll 1$ then we can get

$$\frac{dL}{dt} = \frac{D_1}{2\delta} \left[1 - \frac{F\delta}{3k_B T} \right] \quad (26)$$

Since $nF = 2\pi R_p \tau - \pi R_p^2 p = T_0 - T_{app}$ we expect that the plot of growth rate against p will have a positive slope. Writing $\frac{dL}{dt}$ in terms of the rest tension $T_0 = 2\pi R_p \tau$ and applied force $T_{app} = \pi R_p^2 p$ we get

$$\frac{dL}{dt} = \frac{D_1}{2\delta} \left[1 + \frac{(T_{app} - T_0)\delta}{3nk_B T} \right]. \quad (27)$$

This equation can be compared with the data of Lamoureux *et al.* who applied known forces on growing neurites and measured their growth rates [7]. If we plot T_{app} on the x -axis and $\frac{dL}{dt}$ on the y -axis then (27) plots as a straight line with x -intercept at $T_{app} = T_0 - \frac{3nk_B T}{\delta}$. Lamoureux *et al.* find that the intercept varied between 1500pN – 4000pN. If we take $n \approx 50$ then we find that $1800\text{pN} \leq T_0 \leq 4300\text{pN}$. This is consistent with Dennerll *et al.* who report that neurite rest tensions vary over a broad range from 0 - 10,000pN [3] with a mean value of 350pN. Let us now consider the slope of the line which is $\frac{D_1}{6nk_B T}$. According to Lamoureux *et al.* the slope varies between $0.08 - 2.8\mu\text{m/hr}/\mu\text{dyne}$. Taking an average value for the slope as $1.4\mu\text{m/hr}/\mu\text{dyne}$ we get

$$\frac{D_1}{6nk_B T} = \frac{1.4 \times 10^{-6}}{3600 \times 10^{-11}} = 38.888 \text{ m/Ns}. \quad (28)$$

We tacitly assumed in the analysis above that the membrane tension remains constant even when external force is applied on the neurite. This may not be reasonable. In fact, Dennerll *et al.* have shown that the force-extension relation of PC12 neurites is linear over the range $0 \leq T \leq 5000\text{pN}$ where the extension ΔL is given by:

$$\Delta L = \frac{T - T_0}{k_{mem}}. \quad (29)$$

If we assume that the radius of the neurite remains constant for different applied tensions then we can infer that k_{mem} is related to the 2D shear modulus μ of the membrane. To infer this relation we simply set $T - T_0 = \pi R_p^2 \Delta P$ in the following formula used in micro-pipette aspiration experiments to determine the shear modulus of cell membranes [5].

$$\Delta L = \frac{\Delta P R_p^2}{2.45\mu}. \quad (30)$$

According to Dennerll *et al.* the spring constant k_{mem} varied over a range $0 \leq k_{mem} \leq 1200\text{pN}/\mu\text{m}$ with a mean value around $244\text{pN}/\mu\text{m}$. Similarly, microtubules are also elastic objects, as assumed by Van Veen and Van Pelt [1], and we can assume that the stiffness of n of them is k_{mic} . For example, if the cross-sectional area of a hollow microtubule is A_{mic} and its Young's modulus is E then $k_{mic} = nEA_{mic}$. The microtubules and the membrane together support the force T_{app} due to external pressure. An elementary calculation (assuming the springs k_{mic} and k_{mem} are in parallel) gives

$$nF = T_0 - \frac{T_{app}}{1 + \frac{k_{mem}}{k_{mic}}}. \quad (31)$$

This can be plugged into (25) to get the growth rate as a function of the applied tension. The linearized version of this equation is:

$$\frac{dL}{dt} = \frac{D_1}{2\delta} \left[1 - \frac{T_0\delta}{3nk_B T} + \frac{T_{app}\delta}{3nk_B T(1 + \frac{k_{mem}}{k_{mic}})} \right]. \quad (32)$$

We expect that $n \propto \pi R_p^2$ where R_p is the radius of cross-section of the neurite. This means that $F = C_1 - C_2 p$ where p is the pressure difference while C_1 and C_2 are two constants that depend on

the membrane rest tension and material properties respectively. Thus the growth rate is a function of p and material properties which is consistent with the analysis of Goriely and Tabor who study the biomechanics of growth in tubular fungi [11]. Let us try to estimate k_{mic} . The outer diameter of a microtubule is about 25nm and inner diameter about 14nm [8]. Its flexural rigidity is about $2.2 \times 10^{-23} \text{Nm}^2$ [9]. From this we can estimate that $EA_{mic} \approx 0.82 \times 10^{-6} \text{N/m}$. If we assume that $n \approx 50$ [10] then $1 + \frac{k_{mem}}{k_{mic}} = 1 + \frac{244}{50 \times 0.82} \approx 7$. This will reduce the x -intercept in the $\frac{dL}{dt}$ vs. F straight line by a few hundred pN, but even so our estimate for the rest tension T_0 remains consistent with Dennerll *et al.*

It will be interesting to check what happens in the equations of Samuels *et al.* when we substitute $\frac{dL}{dt} = \beta$ where β is a constant determined from the outside tension.

$$\frac{dL}{dt} = \beta, \quad (33)$$

$$\frac{dQ_t}{dt} = \frac{DA}{V_{tip}L}(Q_r - Q_t) + \beta \frac{BQ_r}{V_{tip}} - \beta \frac{G}{V_{tip}} + \frac{M}{V_{tip}}Q_r \quad (34)$$

$$\frac{dQ_r}{dt} = \frac{S}{V_{soma}} - \frac{DA}{V_{soma}L}(Q_r - Q_t) - \beta \frac{BQ_r}{V_{soma}} - \frac{M}{V_{soma}}Q_r. \quad (35)$$

Surprisingly, we note that a solution in which Q_r and Q_t are both constants is possible only if $S = \beta G$. Instead, if we assume

$$Q_t(t) = C_1 t + C_2, \quad Q_r = C_2, \quad (36)$$

and plug these into the differential equations above then we find:

$$\frac{dQ_t}{dt} = C_1 = \frac{S - \beta G}{V_{tip} - \frac{2DA}{\beta}}, \quad C_2 = \frac{\beta G - C_1(\frac{DA}{\beta} - V_{tip})}{\beta B + M}. \quad (37)$$

This type of solution is not predicted by the Samuels *et al* model or the Van Veen and Van Pelt model. Also, as expected, when $S = \beta G$, $Q_t = Q_r = C_2$. Eqn.(37) is a testable prediction of our model which assumes diffusion limited growth. This is in contrast to the reaction limited growth of Samuels *et al.* [2].

References

- [1] Van Veen, M. P. and Van Pelt, J., "Neuritic growth rate described by modeling microtubule dynamics", *Bull. Math Biol.* **56**(2), 249-273, (1994).
- [2] Samuels, D. C., Hentschel, H. G. E. and Fine, A., "The origin of neuronal polarization: a model of axon formation", *Phil. Trans. Roy. Soc. Series B* **351**, 1147-1156, (1996).
- [3] Dennerll, T. J., Joshi, H. C., Steel, V. L., Buxbaum, R. E., and Heidemann, S. R., "Tension and compression in the cytoskeleton of PC-12 neurites II: Quantitative measurements", *J. Cell Biol.* **107**, 665-674, (1988).
- [4] Howard, J., *Mechanics of motor proteins and the cytoskeleton*, Sinauer Associates, Inc., Sunderland, Massachusetts, (2001).
- [5] Hochmuth, R. M., "Micropipette aspiration of living cells", *Journal of Biomechanics* **33**, 15-22, (2000).

- [6] Wissner-Gross, Z. D., Scott, M. A., Ku, D., Ramaswamy, P. and Yanik, M. F., “Large scale analysis of neurite growth dynamics on micropatterned substrates”, *Integrative Biology*, (2010).
- [7] Lamoureux, P., Altun-Gultekin, Z. F., Lin, C., Wagner, J. A. and Heidemann, S. R., “Rac is required for growth cone function but not neurite assembly”, *J. Cell Science* **110**, 635-641, (1997).
- [8] Boal, D., *Mechanics of the cell*, Cambridge University Press, (2002).
- [9] Gittes, F., Mickey, B., Nettleton, J. and Howard, J., “Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape”, *J. of Cell Biology* **120**, 923-934, (1993).
- [10] Yu, W. and Baas, P. W., “Changes in microtubule number and length during axon differentiation”, *J. of Neuroscience* **14**(5), 2818-2829, (1994).
- [11] Goriely, A. and Tabor, M., “Biomechanical models of hyphal growth in actinomycetes”, *Journal of Theoretical Biology* **222**, 211-218, (2003).