

**BT307-BIOCHEMICAL ENGINEERING
CHOOSE-FOCUS-ANALYZE REPORT**

**Modeling neuronal cell death after stroke
and its treatment using stem cells**

Dhirender Beniwal,
Roll no-BT04B014,
3rd year, B-Tech,
Biotechnology,
IIT-Madras

ABSTRACT

Stroke is third largest cause of death in United States and a leading cause of long-term disability. Stroke can be classified into two major categories, namely Ischemic and Hemorrhagic. More than 80% of strokes are caused by Ischemia and hence I will discuss Ischemic stroke here. A mathematical model for neuronal cell, being the most important cells in brain, death is proposed and the validity of the model is found by comparing the results of this model with experimental evidences. Then, a model for treating stroke affected patient is discussed and theoretical time for total recovery of functionality is proposed.

INTRODUCTION

Stroke, more commonly called as “Brain Attack”, is a disorder in which the arteries to the brain become blocked or rupture; because of which brain no longer receive oxygen and nutrients from the blood and consequently results in death of brain tissue.

Stroke is a medical emergency and can cause permanent neurological damage or even death if not immediately diagnosed and treated. In the year 2003, Stroke killed 157,804 people. It is the third leading cause of death and serious, long-term disability in the United States.

There are two forms of stroke: 1-Ischemic stroke, which is due to blockage of an artery supplying blood to the brain, and 2-Hemorrhagic stroke which is caused by bleeding of an artery into or around the brain. More than 80% (nearly 88%) of the strokes are caused by ischemia and the rest by hemorrhage. I will be focusing on Ischemic stroke for further analysis.

Brain cells are of various types. Neurons are functionally most important cells as their role is to process and transmit information. Neurons communicate with one another via synapses. Glial cells provide support and nutrition and maintain homeostasis for proper functionality of neurons. Neurons, being most important brain cells, are analyzed for further analysis and comparative studies can be made for glial cells and synaptic cells.

The common carotid artery is an artery that supplies blood to the head and neck. It divides in the neck to form the external and internal carotid arteries. When this artery, leading to brain is blocked due to plaque formation, brain cells starts dying because oxygen and nutrients is limited. Brain has many sub-domains, each associated with a function like memory, speech, etc. As the lesion extends, the function associated with the region is lost. As time passes without treatment, greater functions are affected and the person can ultimately lead to paralysis of one half of the body or even death. If the patient is treated within 2-3 hours of start of stroke, he can be treated, after that there is a permanent damage observed in the person like memory loss, paralysis, problem with speech listening, thinking etc.

I will be proposing a mathematical model for neuronal cell death and check if the theoretically predicted value matches with the experimental values. Then I will propose a theoretical model for treating stroke using stem cells in sub-ventricular zone of brain and predict the approximate time taken to theoretically recover proper functionality of brain.

DEATH MODEL:

Since neuronal cell death rate due to aging is negligible compared to death rate of neurons due to stroke, brain cells are *assumed* to be growing in a stationary phase. When middle cerebral artery is occluded, whether naturally by some plaque formation or by the use of sutures, neuron cells are deprived of oxygen and nutrient supply and hence brain can be assumed as a **batch reactor** in which cells are in death phase.

A general equation for growth rate of cells is,

$$\frac{dN}{dt} = (\mu - k_d)N, \quad \text{where } \mu = \frac{\mu_m S}{k_s + S}$$

Where N = number of neurons at any instant,

μ = specific growth rate,

k_d = specific death rate,

μ_m = maximum specific growth rate,

k_s = saturation constant for neuronal cell growth when substrate is limiting,

S = substrate concentration, which may be oxygen or nutrients in this case.

During death phase, μ is very small compared to k_d because in death phase, substrate concentration is very less and hence it is neglected.

Therefore the equation becomes, $\frac{dN}{dt} = -k_d N$.

Integrating the equation, we get $\ln \frac{N_0}{N} = k_d t \implies N = N_0 \exp(-k_d t)$ equation.1

Data Used:

Data required is taken from the “Time Is Brain-Quantified”, Jeffrey L. Saver.

The total number of neurons in the average human brain is 130 billion.

Assumption used by the author in presenting the data: Given the divergent cellular architectures of the forebrain and the hindbrain and the fact that the great preponderance of ischemic strokes are supratentorial, it is most sensible to estimate neuronal circuitry in a typical ischemic stroke based on events occurring in the forebrain.

The estimated total number of neurons in the average human forebrain is 22 billion.

In patients experiencing a typical large vessel acute ischemic stroke, on an average, 120 million neurons are lost each hour.

Calculating k_d , i.e. the constant of the model:

Values are averaged over the total time span in which all neurons in forebrain die. So, first we need to find the time taken for all cell to die. Since an exponential expression (equation.1) can never give time corresponding to $N=0$, so, neuronal cells=0 essentially means $N \leq 1$. Hence, substituting $N \leq 1$ and $N_0=22$ billion in equation.1 we get,

$$T \times k_d \geq \ln(22 \times 10^9) \quad \text{equation.2}$$

Now, Average rate of loss of neurons is 120 million.

Hence, $\left\langle \frac{dN}{dt} \right\rangle = 120 \times 10^6$

Now, $\left\langle \frac{dN}{dt} \right\rangle = \frac{\int_0^T \frac{dN}{dt} dt}{\int_0^T dt}$ and $\frac{dN}{dt} = -k_d \times N \implies \frac{dN}{dt} = -k_d \times N_0 \exp(-k_d t)$

$$\therefore \left\langle \frac{dN}{dt} \right\rangle = \frac{\int_0^T -k_d \times N_0 \exp(-k_d t) dt}{\int_0^T dt} \quad \text{equation.3}$$

Integrating this we get, $\left\langle \frac{dN}{dt} \right\rangle = -\frac{N}{T}(1 - \exp(-k_d T))$

Substituting from equation.2 we get, $T \approx 183.3$ hrs.

$$\text{Equation.2} \Rightarrow k_d \geq \frac{\ln(22 \times 10^9)}{183.33} = 0.129 \approx 0.13$$

Hence, the **death model** becomes

$$\Rightarrow N = N_0 \exp(-k_d t) \quad \text{where } k_d \approx 0.13 \quad \text{equation.4}$$

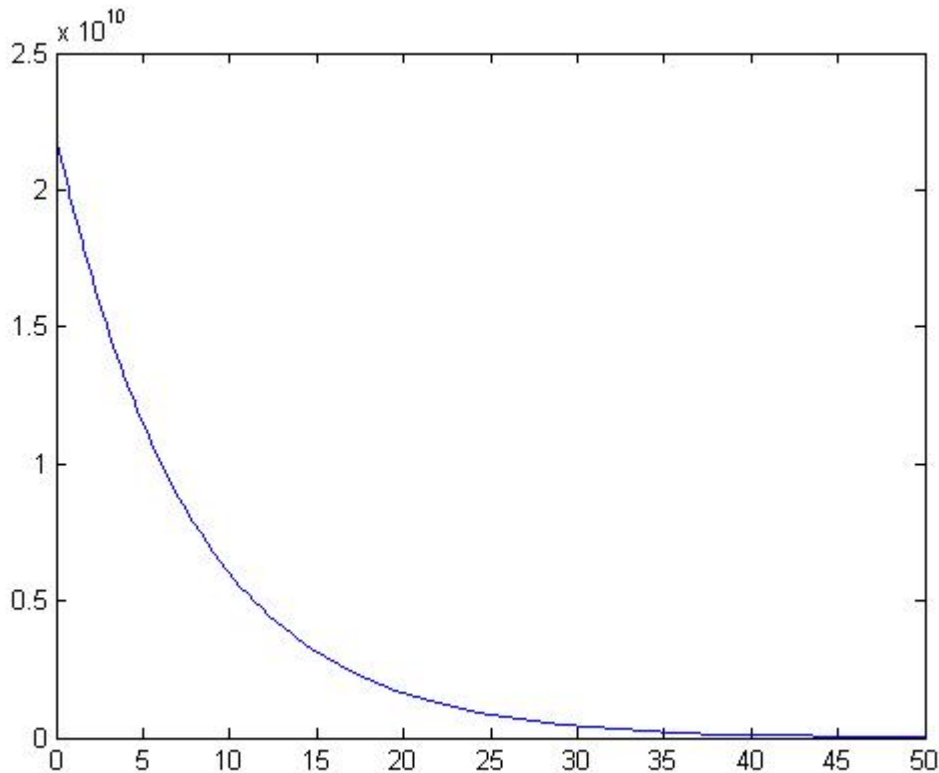


Figure1: Plot of N vs. t

This is nature of death curve of neuronal cells after stroke. Initial value of cells is taken as 22 billion. However, any value can be chosen and corresponding can be obtained.

VERIFICATION OF PROPOSED MODEL:

MRI scan of a human brain taken after 2 hours of stroke (warach, *et.al*). Also lesion volume of a rat's brain after 90 minutes of middle cerebral artery occlusion is collected from a research paper authored by Gerriets, *et.al*. Using these two experimental evidences of stroke, lesion volume will be calculated and the corresponding value using the proposed death model will be calculated. If both the values, theoretical and the experimental, of the lesion value match than validity of the theoretical death model can be assumed.

Testing validity based on data 1:

Data is taken from the paper titled “Complications and Pitfalls in Rat Stroke Models for Middle Cerebral Artery Occlusion” authored by Gerriets, *et.al*. It used using magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) techniques to calculate the lesion volume in rats after 90 minutes of their subjection to middle cerebral artery occlusion using suture technique.

Hemispheric lesion volume in rats was found to be **14.6%**.

Now, equation 4 gives,

$$\Rightarrow \frac{N_{lesion}}{N_{brain}} = [1 - \exp(-k_d t)] \times 100 = \mathbf{17.72\%}$$

(Note: Equation 4 is based on hemisphere base value).

The results almost match. Slight error is can be due to many factors, error in measurement, environmental factor etc. Hence, it can be shown that proposed death model goes with the experimental value.

Testing validity based on data 2:

First experimental data used is MRI scan of a person taken after 2 hours of attack of stroke. The MRI scan is available on the next page. From this, the volume ratios of lesion volume (stroke affected region where brain cells are dead) to brain volume can be found. This ratio can give us the approximate value of the % death of neuronal cells. Percentage of dead neuronal cells can also be estimated from the equation.

Diffusion MRI scans were enlarged to the maximum, considering the clarity. The scan available is only 2-D but brain is a 3-D entity. Hence, the image was extrapolated to be a 3-D figure. Brain was assumed to be ellipsoid and the lesion volume to be sphere. Corresponding parameters were calculated from the enlarged figure.

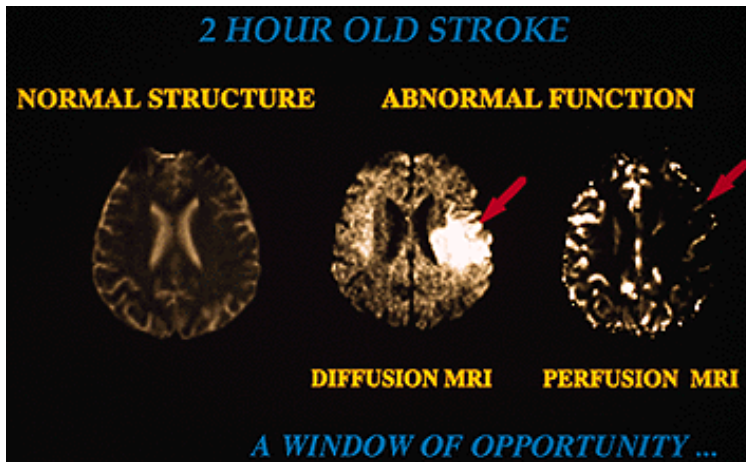


Figure 2: It shows the MRI scan of a person affected from stroke, taken after 2 hours of attack.

Diffusion MRI was considered for further studies.

Values obtained are;

Brain parameters are, $2a=20\text{cm}$, $2b=17.5$ and $2c=17.5\text{cm}$

Diameter of the lesion volume is nearly 7cm.

$$\text{Volume of brain, } V_b = \frac{4}{3}\pi \times a \times b \times c \text{ cm}^3 = 3207.04 \text{ cm}^3$$

$$\text{Volume of lesion, } V_l = \frac{4}{3}\pi \times r^3 = 179.59 \text{ cm}^3$$

$$\text{Therefore } \frac{V_l}{V_b} \times 100 = \mathbf{5.6\%}.$$

$$\text{Using equation 4, } \frac{Nl}{Nb} \times 100 = \frac{1 - \exp(-k_d t)}{2} \times 100 = \mathbf{11.44\%} \quad (\text{Note: R.H.S is divided}$$

by 2 because model is taken based on the hemisphere of brain)

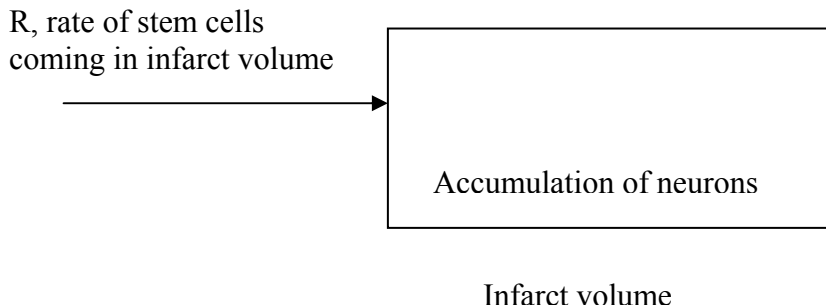
Here, there is ambiguity in the result because of many errors that I have assumed. 2-D picture was approximated to give a 3-D view. Also uniformity of lesion volume was assumed. Even there was error in assuming brain as ellipsoid but that was the best assumption that could be chosen. Accurate values can only be calculated by considering the 3-D picture and 2-D only gives an approximated result.

TREATMENT OF STROKE USING STEM CELLS:

Stem cells are present in sub-ventricular zone of brain. Some studies have shown that middle cerebral artery occlusion model (MCAO) of stroke results in enhanced proliferation in the sub-ventricular zone of brain and neuronal stem cells migrate towards the lesion site and subsequently differentiate into neuron and glial cells. Clearly, this phenomenon is insufficient to repair significant brain damage; however, a selected combination of signals (epidermal growth factor (EGF) and erythropoietin (EPO) signals) might enhance this process such that substantial and functional tissue regeneration can occur. Recently, many advances have been made in treating stroke with stem cells. Some successful stroke treatments have been done using stem cell therapy.

MODEL FOR TREATMENT:

Now, consider the Infarct volume as our system. Let us assume that the neuronal stem cells enter our system at a rate R , *be it a constant or a function of time*. ' f ' is ratio of number of neurons to number of neurons + glial cells in brain, since stem cells differentiate into neurons and glial cells.



Number **balance on stem cells** gives,

$$\dot{I} + \dot{G} - \dot{O} - \dot{C} = \frac{dA}{dt}, \text{ Here } \dot{I} = R, \dot{G} = 0, \dot{O} = 0, \dot{C} = \frac{dC}{dt}, \frac{dA}{dt} = 0$$

Therefore the equation becomes, $R = \frac{dC}{dt}$ equation.5

Assuming that the number of neurons at any instant t (after the treatment has begun i.e. the signal to amplify stem cells proliferation is applied) is 'y'. Also, it is assumed that one neuronal stem cell gives one cell (neuron or glial cell). Hence, the rate of consumption of stem cells, multiplied by the factor 'f' gives a part of generation rate of neurons.

Now, **balance on neuronal cells** gives,

$$\dot{I} + \dot{G} - \dot{O} - \dot{C} = \frac{dA}{dt}, \text{ where } \dot{I} = 0, \dot{G} = fR + \mu_N y, \dot{O} = 0, \dot{C} = 0, \frac{dA}{dt} = \frac{dy}{dt}$$

$$\text{Substituting we get, } fR + \mu_N y = \frac{dy}{dt}$$

$$\Rightarrow \frac{dy}{dt} - \mu_N y = R.$$

equation.6

Now, R is the rate at which stem cells are migrating to our system. Taking basal level of stem cells in SVZ to be N_0 , and assuming that stem cells do not accumulate here after the signal is given, i.e. they move to our system as produced. Therefore, rate of stem cell transfer to infarct volume, R, is $N - N_0$ since N_0 stem cells remain in SVZ at any instant.

Assuming that normal growth curve applies to stem cell growth we get,

$$\frac{dN}{dt} = \mu_S N \Rightarrow N = N_0 \exp(\mu_S t)$$

$$\text{Hence, } R = N - N_0 = N_0 [\exp(\mu_S t) - 1]$$

equation.7

Multiplying both sides of equation 5 with $\exp(-\mu_N t)$ and integrating we get,

$$\Rightarrow \int d(\exp(-\mu_N t) y) = \int \exp(-\mu_N t) R dt$$

Substituting R from equation 6, we get,

$$\Rightarrow \int d(\exp(-\mu_N t) y) = N_0 \int [\exp\{-(\mu_N - \mu_S)t\} - \exp(-\mu_N t)] dt$$

Integrating from $t=0$ to $t=t$ and $y=0$ to $y=y$ it gives,

$$\Rightarrow y = N_0 \left[\left\{ \frac{\exp(\mu_N t) - \exp(\mu_S t)}{\mu_N - \mu_S} \right\} - \left\{ \frac{\exp(\mu_N t) - 1}{\mu_N} \right\} \right]$$

equation.8

Here, μ_N = specific growth rate of neuronal cells,

μ_S = specific growth rate of neuronal stem cells,

R=rate of input of stem cells to the infarct volume.

y=no. of cells in the infarct volume at any instant.

Equation 7 can be used to calculate the time required to regenerate total functionality of brain after the signals are applied. This will be the theoretical time that will be required to produce the number of cells that are lost due to death after stroke. IT is clear from the equation that this time will be dependent on size of damage, greater time will be taken for a greater size of damage. Practically 100% functionality cannot be obtained. Practically, only 40-60% of regeneration of function is observed in the experiments done.

Data Used:

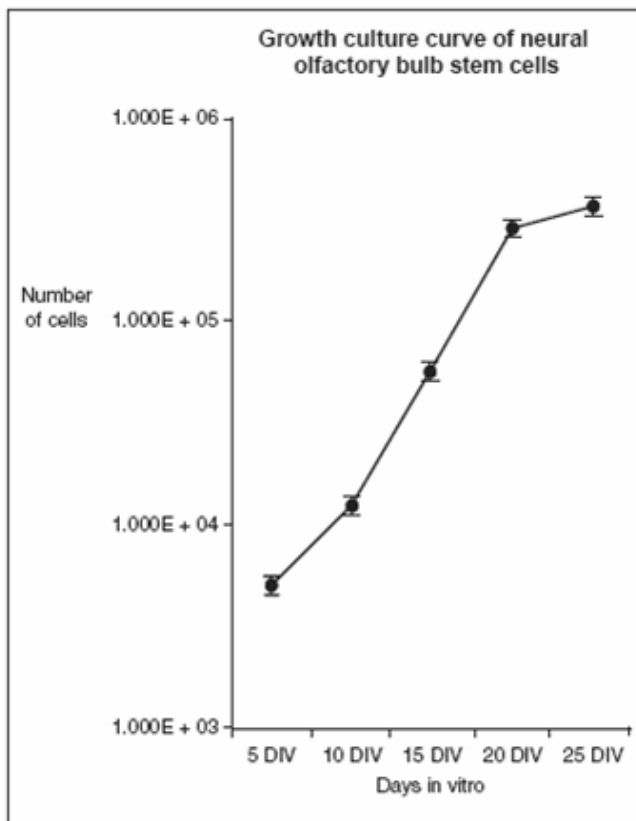


Figure 3 : As quoted in the paper “After a period of relatively slow growth (time of doubling between 5-10 DIV is 5 days), the cells quickly expand (time of doubling 15-20 DIV is 2.5 days). The cells were cultured in a growth medium containing mitotic factors (20 ng/ml of EGF and 10 ng/ml of bFGF)”.

Hence, $\Rightarrow \mu = \frac{\ln 2}{T}$

Here μ =specific growth rate,
T=doubling time in the exponential phase.

Therefore, $\mu_s = 0.27725$ per day.

Source: This image was taken from the paper titled” Isolation and Characterization of Neural Stem Cells from the Adult Human Olfactory Bulb” authored by Pagano, *et.al*.

Initial concentration of stem cells in SVZ is approximately 10^6 cells/ml (Rowley SD, et.al). Average volume of a typical human Brain is nearly 1450ml. Volume of SVZ is nearly $1/6^{\text{th}}$ that of brain. Therefore, N_0 is assumed to be 2.42×10^8 .

μ_N , i.e. specific growth rate of neuronal cells is interpreted from World Wide Web to be approximately 0.2 / hour.

Using this data and plotting the graph of y vs. t using equation 7, value of time can be found out from the graph for the recovery of a given number of cells (that is, value of t for a given y can be found.)

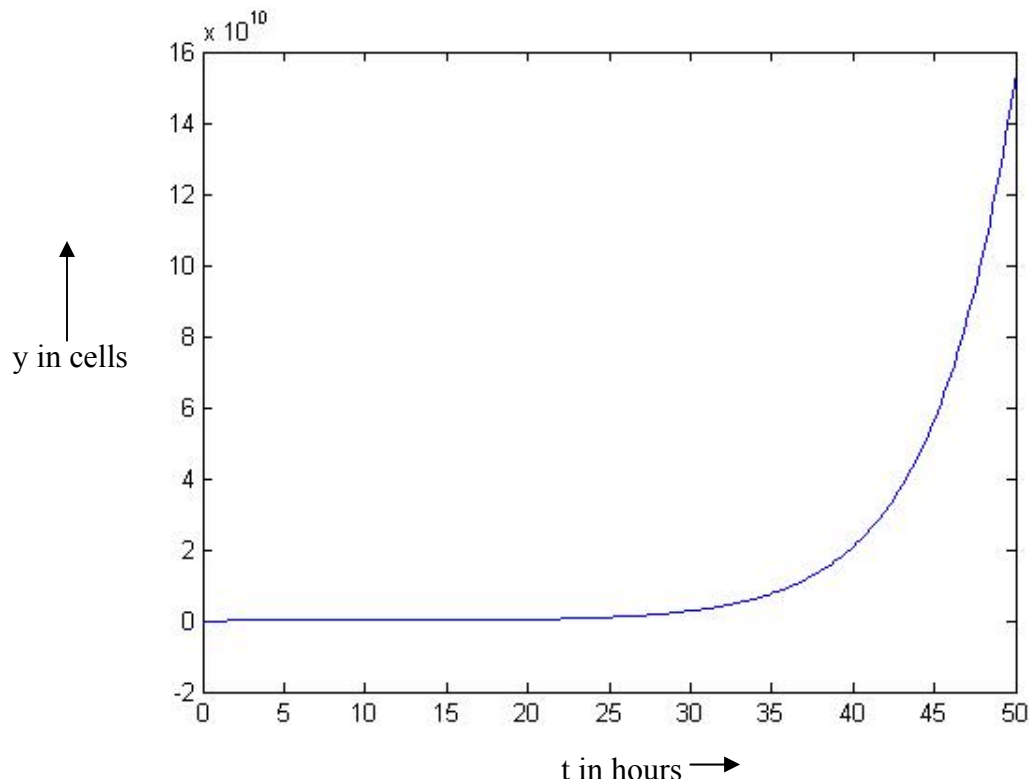


Figure 4: plot y vs. t generated using matlab.7.

Using this graph, theoretical time required to regenerate any given number of cells (y) can be calculated. Ideally, this graph will keep on increasing with time forever resulting in excess amount of cells produced which is not required. But, this will not happen because with cells growth, substrate will be used and when the required number of cells is reached, the cells will go to stationary phase and will remain constant thereafter.

RESULTS AND DISCUSSIONS:

1) Death model of neurons in stroke:

The proposed model was verified using two experimental data; 1- lesion volume of a rat's brain after 90 min of MCAO, 2- Diffusion MRI scan of a patients brain after two hours of attack of stroke.

Proposed model gave reasonably good match for the 1st data. First data is collected from a research paper, in which lesion volume from MRI scan was found using sophisticated techniques. Hence it can be assumed that the lesion volume given by experiments is more accurate and the proposed model rightly predicts it. Hence model predicts the percentage of lesion volume with reasonable accuracy, at any given time after stroke has occurred.

However, in 2nd case, there was a poor match of lesion volume between the one calculated using the model and that approximated from the MRI scan pictures. This may be explained because of approximation used in the calculation of lesion volume from diffusion MRI scans. Lack of good techniques in calculating the lesion volume using MRI scan, lead to making approximations, which could be one of the reasons for the mismatch. Hence, due to the unreliability of the data, the accuracy of the proposed model cannot be verified extensively.

Hence more experimental comparisons are to be done to establish that the death model of neurons after stroke holds good and if the model fails, a better model has to be proposed.

2) Treatment of stroke using stem cells:

The lesion volume was assumed as a reactor. When some external signals are applied, stem cells present in SVZ proliferates and migrates to the infarct volume and differentiates in neuronal and glial cells to recover the damage due to stroke. This process was modeled and a curve was generated which predicts the theoretical time taken to repair total damage, i.e. all dead cells in the lesion volume are theoretically replaced by stem cells. Practically, only 40-60% regeneration of damage can be done and hence the predicted time will be greater than the actual time taken for regeneration.

CONCLUSION:

In this exercise, I have tried to establish a quantitative approach to stroke. The model predicted for death of neurons after stroke gave reasonable results when compared to experimental values. However, more analysis is to be done to establish that this model predicts correctly in most of the cases. If the model does not hold for most of the cases, than a more general equation is to be predicted.

Treatment of stroke using stem cells is modeled and it gives the required theoretical time required to recover the damage due to stroke. This model has only been analyzed only for time and some improvement in the model can let us do more detailed analysis. Further studies have to be done to make models for other types of treatments.

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