**Dissertation:**

**Abstract (Aim 1/100 words)**

**Introduction (Aim: 572/600 words)**

Background Information:

The cells which line our blood vessels are called Endothelial cells (EC), which form a layer known as the Endothelium. This layer of cells is able to repair itself after injury, which is essential to good health, however, the repair process becomes slower with age due to the cells becoming senescent.

These cells are generally in a confluent layer, therefore a larger number of cells are no longer dividing, however, when they’re wounded, such as an atheroma, the confluence is broken and the cells leave this phase to continue dividing, repairing the damaged tissue. This process is slower in elderly patients due to many senescent cells, or if the same area is damaged a second time after repair. This is due to scar tissue being less capable of mitosis and repair.

Aims and Objectives:

The main aim of this project is to estimate the affect ageing has on the ability for blood vessels to heal after being scratched. The implications of this project will help professionals further understand the process of wound healing and to provide further insights into the conditions affecting the deadly disease atherosclerosis, which can lead to strokes and heart attacks.

The way the main aim will be implemented requires the development of an agent based model to encapsulate the key behaviours associated with endothelial cells, including: cell proliferation, apoptosis, and senescence. This model will record the time taken for the wound to repair itself, and observe any emergent behaviour that takes place through the mitosis and movement of the cells. For the basis of producing this agent based model, I’ll be developing on top of already developed code by Marziah Tehranis’ code, giving the agents and environment differing behaviours.

I’ll be observing the difference between elderly and younger cells to see how much, if any, age affects repair time.

This project has ample room for expansion; some of these aims include: modelling the problems associated when the endothelium layer doesn’t sufficiently repair in time, and the effect on endothelium repair after successive tears (allowing significant scar tissue to build up) showing the differences in speed and process of the repair. It would also be beneficial to model a more realistic vessel shape as the blood flow turbulence has a dramatic effect on healing ability.

Constraints:

Unfortunately, this aims of this project are currently hindered by several constraints that agent based models inherently possess.

So far in my testing of Marzihas code, it has become obvious that the level of computing power on my personal computer is only capable of modelling areas of around 0.1mm2 within a reasonable amount of time, without many agents. However, when I increase the area to the size desirable for the project, 1mm2, the number of interactions between all the agents is too large and therefore not feasible.

Another constraint on this project is the lack of specific and accurate data I can use as parameters within the program, which can end up leaving the simulation less useful.

 Summary of Report:

Over the next few pages, I’ll summarise the literature I’ve read to date, picking out any data that could be used as parameters, go through the current state of Marzihas code and how I’ll adjust it to this project. Next, we’ll discuss in detail the aims and objectives, what will not be covered and why, and any experiments or tests I’ll be carrying out at the end of the project. Finally, I end on a conclusion on what’s been found so far, my achievements to date and a project plan to take through into semester 2.

**Literature Review (Aim: 1439/1500)**

Within our bodies, there are several systemic factors, such as: obesity, heredity factors and age that contribute to the ill-health of a person[8 – Warboys]. The areas that seem to cause the greatest deal of harm tend to be at branches within the blood vessel, where there is a turbulent flow of blood [8 – Warboys]. At these sites, we tend to see an increase in senescent cells which can increase the time taken for a wound to heal. Evidence shows that with an increase in age, there tends to be an increase in the number of senescent cells, leading to long term health problems such as atherosclerosis or plaque formation, potentially breaking off a capillary, causing heart attacks or strokes

The Endothelial Cell Cycle:

Firstly, it’s important to fully understand the mechanisms by which our ECs divide and any biological factors that can change its behaviour. ECs are a specific type of Eukaryotic Cell that line our blood vessels. When these cells are healthy, they secrete molecules, such as hormones, into the blood stream to maintain homeostasis [7 - JD Pearson]. This is vital as it helps fend off disease progression, keeping the individual healthy.

EC’s, like other Eukaryotic Cells undergo several distinct phases during replication as shown in the diagram below, however have another stage between G1 and S Phase.

source: Figure 14.1 Phases of cell cycle <https://www.ncbi.nlm.nih.gov/books/NBK9876/figure/A2435/?report=objectonly>

Stages G1, S and G2 are called Interphase; this is the time when the cell is increasing in size, and the lengths of time in each stage are proportional to their relative lengths. As shown in the figure, during S phase, the DNA is replicates an identical copy of itself which moves onto M phase (mitosis), when the enlarged cell splits into 2 identical daughter cells [2- The Cell]. The length of time for a normal Eukaryotic Cell to undergo proliferation is around 24 hours, with 1 hour of that being the M phase, therefore 23 hours (96%) of the time is during cell growth and DNA replication, during which time the cell grows to be about twice its size [2- The Cell].

However, for ECs there is another cycle between the G1 and S phase. This is called the G0 phase and generally known as the quiescence state. This is a state of inactivity, usually induced when EC proliferation is no longer required. If there is a stressor, such as a decrease in external pressure due to the ECs spreading out or moving, the quiescent cell can move out of G0 back into the normal eukaryotic cell cycle [Find concrete source]. However, if the EC stays in the quiescent state for too long, it’s possible for it to develop into a senescent cell over time where it will never return to the normal cycle [Find concrete source].

In general, ECs are long, flat cells around 5-10μm in radius and 1-2μm wide [6 - Blue Histology].

Ageing:

* Important behaviours
  + Telomere length -> short go quiescent or senescent
  + Quiescent when confluence
    - No more room to divide
  + 50 CRDs (proliferation) until cellular arrest

Senescent Cells:

It has been noted the senescent ECs have several characteristics which differ them from normal ECs. First of all, they are unable to undergo mitosis and therefore never divide, they become enlarged after entering this state [8 - Warboys] and slow down surrounding ECs. Warboys suggests that senescent ECs could be the main contributor and initiator of atherosclerosis. In vitro, it has been seen that senescence in the ECs increases during a turbulent, disturbed flow, from 1% of EC being senescent using a 13 dynes/cm2 uniform flow compared to just over 2% senescent EC when exposed to a flow fluctuating between +/- 5 dynes/cm2 at 1Hz. It’s also noted that for these two categories, the number of multinucleate cells with a diameter > 100µm increased from 0.5% to 1.5%. This provides very useful starting data for the cells in my model. This increase in number of senescent EC is believed to be due to an increase turnover rate of ECs at these turbulent atheroprone sites. Meaning that this increased level of proliferation should be considered when developing my senescent cell model. It can also be hypothesised here that in general, over time, more cell proliferation will occur and thus there will be an increase in the total number of senescent cells within the environment.

Another important fact Warboys reveals is that due to the size of the senescent ECs, this has a detrimental effect to the speeds of its neighbouring cells, acting as a blockage, and slowing them down. This can hinder wound healing as it will take longer for healthy mitotic ECs to fill the gap. As mentioned above, there’s is also an increase in the number of senescent cells over time, therefore I expect my model to show that with age, it takes longer for any wounds to heal.

* Slow down surrounding cells
  + How?
* Undergo apoptosis?
* What size do they grow to?
  + How long does this take?

Atheroprone Sites:

Not all ECs within our blood vessel have the same physiological behaviours; this is due to the differing environmental factors within the vessels, discussed above. This leads to parts of our blood vessels under going higher levels of injury than others. In fact, the main disease this project is aimed at further understanding, atherosclerosis, is rather specific, and can be most commonly be found at the bends or branches of arterial trees [1 – Chaudhury]. These bends and branches are known as atherosusceptible sites, which have enhanced proinflamitory activation, due to the constant activation of c-Jun N-terminal kinase (JNK) [1 – Chaudhury]. These atherosusceptible sites therefore have a higher rate of injury and cell turnover compared to EC at atherprotected sites [3 Gerrity, 4 Hansson, 5 Hu]. Analysis by Chaudhury et al showed that the ECs at Atheroprone sites express proteins that respond to lipopolysaccharides by priming for apoptosis and proliferation [1 – Chaudhury]. They also state that wherever JNK1 is active is where apoptosis and EC turnover occur in arteries.

I will therefore be looking at branches and bends within my model as they are the areas where there is the highest level of turbulence and concentration of JNK; leading to the greatest injury of the endothelium wall. Which, in turn has the greatest concentration of EC apoptosis and proliferation.

Methods of Modelling:

There are two clear options for modelling the interactions between ECs and senescent cells. Cellular automata (CA) is an orthogonal grid of similar cells that interact with their neighbouring cells. Its advantages are that runtime is extremely quick and it can produce complex macro-scale emergent behaviour of the interacting cells [[eBook](https://link.springer.com/book/10.1007/3-540-47849-3)]. However, the disadvantages are that due to the orthogonal grid, cells are fixed in place, unable to move; this is very much a simplification of the project as ECs move around on the endothelium to fill gaps and is an important factor for wound healing. Another disadvantage of CA is that it can only model local interaction between neighbouring cells, therefore any change further away from the cell won’t be noticed until it cascades down the subsequent neighbouring cells over several iterations

Whereas an Agent Based Model is <>. For these reasons, I believe it’s best to complete this project using an Agent Based Model.

* Talk about Dawns papers and how she’s had success with ABM

Review of Agent Based Software:

So far, I’ve tested two computer programs that use agent based modelling to allow for the type of emergent biological behaviours I’m looking for. The first program is SPARK which is a lightweight and efficient tool for ABM. Its programs are written in SPARK-PL which is translated into Java source code.

* + Looking at Innate Immune Response sample, Endothelial cells are embedded into the endothelial matrix, meaning there’s no movement of cells.
  + Much faster than Marzihas
    - Simulates 10201 ECs (grid size 101 x 101) in real time.
    - CellABM contact resolution is O(n2).
      * Are other methods, such as K-based trees<?>
      * Endotheliome runs the contact resolution as embedded C code.

The other program, is a python based ABM by Marziha Tehrani used to model the interactions between cancer cells and stem cells. It has several classes which allows the user to easily change the rules of each phase of the cell cycle along with the initial cell parameters, such as size, direction and speed.



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| --- | --- | --- | --- | --- | --- |
|  | **Comparison of Software** | | | | |
| **Spark** | **CellABM** | **Net Logo** | **Mason** | **Repost** |
| **Type** | Cellular Automata | Agent Based Model | Agent Based Model | Agent Based Model | Agent Based Model |
| **Contact Resolution** | None | Advanced implemented contact resolution, but Ο(n2) |  |  |  |
| **Language** |  | Python |  |  |  |

**Requirements and Analysis (Aim: 406/1000)**

Aims and Objectives

* Code:
  + Implement basic EC class which instantiates each cell with a random size (within a range), a direction and speed
  + Implement a Quiescent and Senescent state for the EC class
    - Moore neighbourhood for senescent slow down <ABM version?>
  + Implement logic to stop simulation when confluence has occurred
    - Once achieved, logic to strip a set segment of the cells out
      * Cell states stored in numpy array, set all to 0?
* Parameters:
  + Size of ECs
  + Size of senescent cells
    - From lit review above
  + Speed of ECs
  + Direction of ECs
  + EC growth factor
  + Cell turnover
  + Flow Turbulence
    - Increases cell turnover
* Rules:
  + Mitosis
    - Doubling size over S and G2, then splits into 2, half sized daughter cells
  + Apoptosis
    - When cell radius < threshold
    - What are the rules for senescent apoptosis?
    - What are other general apoptosis rules?
  + Into quiescent
  + Into senescent
    - Telomere shortening
    - CRD limit hit
  + Collision correction
  + Senescence slow down %
  + Senescence growth

Areas not Covered

* All rules included for becoming senescent. Warboys points out they used immunostaining and gene silencing to show other factors such as hypercholesterolemia
* Multiple ways the endothelial monolayer gap can be filled. We’re only looking at spreading of adjacent endothelial cells.
  + Ignore hyperplasia of existing endothelial cells [[paper](https://academic.oup.com/cardiovascres/article/66/2/286/270307)]
  + Ignore engraftment of circulating endothelial progenitor cells [[paper](https://academic.oup.com/cardiovascres/article/66/2/286/270307)]
* Assuming a normal human with Hayflick limit of 50 [[website](http://www.senescence.info/cell_aging.html)], ignoring deficiencies such as Werner syndrome which causes individuals to have a population growth of 53% and total replicative life span of 27% compared to normal cells [[paper](https://www.ncbi.nlm.nih.gov/pubmed/7327553)].
* No GUI to change parameters on the fly, all from command line

Risk Analysis:

I’ve included all the risks I believe are associated with my project below. I outline the nature of the risk, then give it a likelihood and impact score from 1 – 4, 1 being unlikely / negligible and 4 being very likely / project threatening.

* Generic Risks
  + Lost work
  + Personal event prevents progression
  + Optimistic schedule
  + Completion of dissertation inhibits project implementation
    - Plan dedicated time to complete dissertation after implementation
* Specific Risks
  + Classes not working together
    - Design UML diagram of full system before coding.

Evaluation of Current Work

* Test1 - vary age:
  + Increase the number of starting senescent cells and observe behaviour
* Test2 – vary wound size:

**Conclusion, Progress, and Project Plan (Aim: 347/600)**

Conclusion

Overall, I’ve decided to use Marzihas programme as it already has solved the mathematics behind the complex and possible recursive overlapping interactions between cells once they divide by mitosis or move around.

Progress

* Achieved to date

So far to date, I’ve managed to convert Marzieh’s code from python 2 in to python 3, refactored it to the industry standard of PEP8 (for standardisation and easy of reading) and added basic comments to functions with the expectation of full documentation once I’ve implemented all parts. I’ve run several simulations of Marzihas’ code as shown below. For the sake of time, I’ve limited it to 0.1mm2 with

This program is useful as it automatically outputs a graph showing the growth of each cell type over time, shown below. This can be used in my application to determine the rate of time required for the wound to heal with different starting parameters.

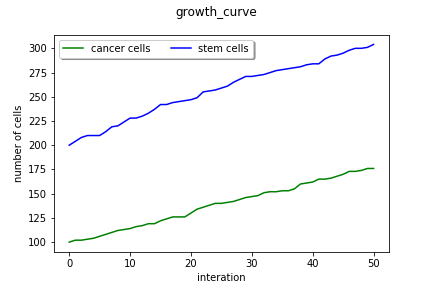
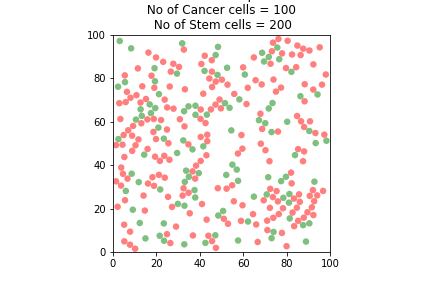
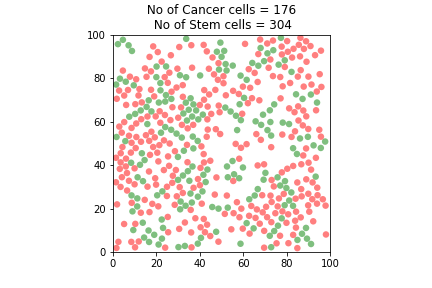


Figure taken from running Marziahs program with 0.1mm2 area, 100 cancer cells and 200 stem cells.

Marzihas code also outputs a 2D and 3D image of environment each iteration, this shows the movement of the cells over time. Which will be useful to demonstrate the emergent behaviours of wound healing with age.

A possible drawback to Marzihas code currently is the computational power required. Running the above simulation on my machine (Mac Book Pro 2.8Ghz i7) took 22 minutes and 44 seconds to compute 50 iterations. Scaling this up to 1mm2 would therefore take a significant amount of time longer. This is down to the runtime analysis of the overlap.py class. The nested for loops mean time taken is Ο(n2), therefore introducing a scalability issue.

Another downside is that Marzihas code doesn’t implement any cell growth, and each cell is the same diameter as every other cell for the whole simulation. This is a simplification which I’ll endeavour to update with my implementation.

Anticipate changes to the code.

* + Random number of proliferating cells
  + In confluence
  + Then remove a strip
  + Plot number of cells / time?

Project Plan