**3 Requirements and Analysis**

3.1 Methodology

For the development of the program to discover the effect age has on heart attacks, an Agent Based Model will provide the best results for the user. ABMs model each cell individually with their own parameters, allowing for a more distributed representation of the cells, such as each cell can vary in radius slightly from each other. An ABM also provides a graphical output of how the cells move, allowing us to better understand what’s happening with the emergent behaviour in a more visual way. The ABM approach is better than a continuum approach as in continuum modelling there is no individual agent representation and so approximations may be too significant to produce reliable results. Cellular automata wasn’t chosen as it would incorrectly model the endothelial cells on the environment, not allowing them to migrate into the wound and therefore not answering the research question.

3.2 Aims and Requirements

The main aim of this project is to demonstrate and help professional understand further the affect ageing, and other physiological factors, has on the ability for a wounded area of ECs to repair itself. The main observation will be time taken for the ECs to divide and move into the gap of the wound, once more forming a confluent layer.

To facilitate the main aim, we’ve seen the benefits several current software have, to form the start of the project; however, they lack the correct logic or behaviours that occurs within blood vessels. Below, I outline the functional and non-functional requirements, parameters, and rules that need to be met to produce an accurate and correct model.

3.2.1 Functional Requirements

|  |
| --- |
| **It is critical that the system:** |
| Uses an appropriate time scale for each iteration |
| Creates a wound when a confluence is made |
| Model’s Senescent cells |

Table 3.1: Critical functional requirements

|  |
| --- |
| **It is important that the system:** |
| Produces quiescent cells when proliferation is no longer possible |
| Models quiescent cells differentiating to proliferating cells |
| Models proliferating cells differentiating to senescent cells |
| Tells the user how long it took for wound healing to occur |
| Produces graphs of cell locations each iteration |

Table 3.2: Important functional requirements

|  |
| --- |
| **It is desirable that the system:** |
| Forms a confluence before being wounded |
| Models Senescent Cells as barriers |
| Stops the simulation when second confluence is formed |

Table 3.3: Desirable functional requirements

|  |
| --- |
| **It is optional that the system:** |
| Models senescent cell death |

Table 3.4: Optional functional requirements

3.2.2 Non-functional Requirements

|  |
| --- |
| **It is desirable that the system:** |
| Is simple to run from the command line |
| Is commented well for future development |

3.5: Non-functional requirements

3.2.3 Parameters

These parameters have either been gained from literature review or are an educated guess which will be refined heuristically on the final product.

|  |  |  |
| --- | --- | --- |
| **Parameter Name** | **Data** | **Source** |
| PC diameter | 10-20μm | Literature Review |
| Senescent cell diameter | < 100μm | Literature Review |
| PC speed | 1μm/min | Educated Guess |
| Senescent cell speed | 0 | Literature Review |
| PC direction | Random | Educated Guess |
| PC growth factor | 2 x during proliferation | Literature Review |
| Cell turnover | 50 times | Literature Review |
| PC turnover time | 24hrs | Literature Review |
| Senescent cell turnover time | 3 days | Literature Review |
| Time period | 6 hours | Educated Guess |

Table 3.6: Values associated with the parameters for the software.

3.2.3 Rules

|  |  |
| --- | --- |
| **Rule Name** | **Behaviour** |
| Mitosis | * Splitting enlarged EC into 2 equal sized half cells. |
| Apoptosis | * When turnover limit reached, enter * Remove cell from environment |
| Quiescence | * When no more proliferation possible, enter * When proliferation possible, exit * No mitosis |
| Senescence | * When cell turnover hit, enter * When cell has been quiescent for long enough, enter * Static * Enter cell growth * No mitosis * Cell turnover = 3 years |
| Collision Correction | * Adjust overlapping cells so they no longer are |
| Cell growth | * Double in size for ECs * Grow up to 10 times in size for senescent cells |

Table 3.7: Check-list of the behaviours each implemented rule should have.

3.2.5 Emergent Behaviours

Emergent behaviours arise through the interaction of the above rules and are not hard-coded, but observed. Some of these behaviours in action include the formation of tissues and organs and the expansion of tumours. For this project, I expect to see an emergent behaviour of wound healing when the blood vessel is damaged, by having the Quiescent cells differentiate back to Proliferating cells (PCs) due to the increased space, and these PCs migrating and proliferating to fill the space; once more forming a monolayer of cells which will differentiate back to Quiescent Cells. Another expected emergent behaviour is the obstruction of migration of PCs from the Senescent cells leading to delayed healing, increasing the chances of forming an atheroma and blood clot, leading to a heart attack.

3.3 Areas not Covered

Either due to time or computational constraints there are a few areas that this project will not be covering. Firstly, due to the lack of understanding the advanced Biology of the inner workings of ECs, I will be unable to implement all the of rules biologists have found that cause cellular senescence.

Another area I will not be covering are the multiple ways the endothelial monolayer gap can be filled during healing. I am only modelling the spreading of adjacent ECs into the gap due to the decrease in pressure caused by the lack of cells pushing back. The other ways the gap can be filled include: hyperplasia of existing endothelial cells and engraftment of circulating endothelial progenitor cells [7].

I am also assuming, that I am modelling ECs from a healthy person with a Hayflick limit of 50, 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 [18].

I will not be creating a graphical user interface (GUI) for the user to change parameters on the fly in the simulation. All parameters will be set at the beginning of the simulation and shall remain unchanged. To observe the effect of the changing parameters, several simulations must be run with varying initial conditions.

3.4 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 then provide a mitigation plan to decrease severity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **Likelihood** | | | |
| Very unlikely  1 | Unlikely  2 | Likely  3 | Very Likely  4 |
| **Impact** | Negligible 1 | 1 | 2 | 3 | 4 |
| Low 2 | 2 | 4 | 6 | 8 |
| Significant 3 | 3 | 6 | 9 | 12 |
| Catastrophic 4 | 4 | 8 | 12 | 16 |

Table 3.8: Risk Rating Matrix where Risk Rating = Likelihood x Impact

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Risk Event | Likelihood | Impact | Risk Rating | Mitigation |
| Loss of developers’ code | 1 | 4 | 4 | Backups of the developers’ machine are take daily to an external hard-drive. The code will also be tracked on GitHub. |
| External event prevents progression | 2 | 3 | 6 | Careful project planning implementation of contingency plans if developer starts to fall behind. Some weeks are designed to have less work in case developer needs to catch up. |
| Optimistic project plan | 3 | 3 | 9 | Enough time must be given to the development of the software and is something that shouldn’t be rushed. Adjustment to project plan may be required if developer start to lag. |
| Completion of code hinders completion of dissertation | 2 | 4 | 8 | Enough time will be given to produce several drafts of the final dissertation in the project plan. |
| New functions not working with current software | 2 | 3 | 6 | Ensuring there are no compatibility issues and correct design practices are followed, such as the creation of UML diagrams showing function interaction. |
| Contact resolution scalability not fixed | 3 | 4 | 12 | Review of different software for contact resolution. Decreasing experiment area is a last resort to ensuring a confluence can be modelled. |
| Lack of accurate data | 4 | 3 | 12 | Continual reviewing of papers surrounding the topic for any extra hints. Otherwise a heuristic approach with several simulations should provide accurate results. |
| System too slow for use under standard conditions | 3 | 4 | 12 | Avoid implementation of nested loops, and constantly assess performance. Possibility of running simulation on Iceberg. |
| Requirements change during development | 1 | 3 | 3 | The code will be implemented in an Object Orientated manner, providing modularity of functions with little refactoring. |

Table 3.9: Risk identification, analysis and planned mitigations.

3.5 Evaluation and Testing

Tests will mainly focus on what occurs after the wound has been created. For this to occur, a confluence must be formed. To save time, one simulation can be run at the desired environment size to determine the number of cells the simulation stabilises towards so this can be used as the starting condition of future tests, saving time as confluence formation won’t be simulated. Theoretically, if the environment is 2500μm2 and each proliferating cell can grow up to 10μm in diameter, 2500 cells can fit onto the environment. However, this doesn’t factor the size of senescent cells or the fact cells can be of different sizes.

There are several tests that could be used to measure the success of the project once everything’s completed.

Test 1 would involve the variation of age and the subsequent measurement of change in time for the wound to heal. To vary age, as shown in the literature review, the number of starting senescent cells within the model will change, with younger patients having fewer senescent cells and elderly patients more. This test is paramount as it will be the main evidence used to answer the main aim.

Test 2 involves varying the wound size and observing the time taken for the wound to heal for each age group.

Test 3 involves producing simple test cases on the simulation to show the rule behaviours in a controlled environment where no other rules are acting on the cell. This will show that each rule works on the micro scale and therefore will work when scaled up to macro size.

Test 4 involves qualitative validation of whether the emergent behaviour looks like the predicted behaviour.

# Test 5 involves local sensitivity testing: if a parameter is varied by a small amount, what is the change in the model? This result can then be used as further calibration of the model parameters as feedback [A validation methodology for agent-based simulations]. However, this type of testing has its limitations as it only varies one parameter at a time, whereas the interaction between parameters could be more important.

Test 6 would involve performing a statistical test on the time taken for the wound to close whilst varying the percentage of Senescent cells in the model. If the simulation is run enough times, Student’s T test can be used to determine whether the increase in Senescent Cells is significant enough to change the model’s behaviour. However, this test is very rigorous and requires a lot of simulated data and real world experiments to analyse the model and so may not be feasible.

The evaluation of my work will include the results I gather from the tests above and comparing them against current literature showing blood vessel wound healing in vitro.