AMSI SUMMER RESEARCH SCHOLARSHIPS 2025–26

Get a taste for Research this Summer

The Mathematical Modelling of T cells as a Treatment For Cancer

Brynn Mackie

Supervised by Mason Lacy, Dr Adrianne Jenner, Associate Professor Pascal Buenzli

Queensland University of Technology

Jane Street®

AMSI AUSTRALIAN MATHEMATICAL SCIENCES INSTITUTE

Abstract

Adoptive cell transfer is a form of immunotherapy that aims to treat cancer by expanding patients' tumour-specific activated T cell populations *ex vivo*. Current processes for this treatment, such as a protocol by Dynabeads, do not re-create the natural environment of T cell activation effectively and as such expansion efficiency is limited. A new study proposes the use of mesoporous silica micro-rods that slowly diffuse interleukin-2 and are covered in T cell activating signals. Initial experiments show this method of expansion is very efficient. However, it is currently unclear how this therapy can be optimised. By mathematically modelling this process, we can understand T cell behaviour under different conditions and optimise expansion. Previous stochastic agent-based modelling showed expansion was driven by T cells concentrated around immovable micro-rods and rapid expansion could be further supported by the movement of micro-rods into heterogeneous clusters. This project aims to develop movable micro-rods to investigate this claim and explore what affects T cell behaviour and growth. Using PhysiCell, we model the expansion of T cells via micro-rods with biomechanical forces. Numerical simulations show the formation of heterogeneous micro-rod structures as the T cells push them around. The maximum rate of proliferation has been shown to have a significant impact on the rate of T cell expansion. The concentration of interleukin-2 loaded into micro-rods also directly impacted the number of cells grown over one day of simulation. The model developed has potential to be further extended in future studies to more accurately represent what physically occurs in adoptive cell transfer with micro-rods as to most accurately suggest ways to optimise the therapy.

1 Introduction

Cancer is a difficult disease to treat due to its many genetic abnormalities causing a multitude of treatment-evading properties. This includes cancer stem cells' drug resistance, metastasis, and its ability to evade the immune system [1,2]. Immunotherapy is a class of treatments for cancer that aim to boost or augment the body's natural immune response against it [3]. This project focuses on one form of immunotherapy referred to as Adoptive Cell Transfer (ACT). It involves sampling activated, tumour-specific T cells from a patient's blood or tissue sample, and expanding them *ex vivo* (outside the body) (see Figure 1). Once sufficient numbers have been reached, the activated, tumour-specific T cells are re-infused into the patient [4]. It has been shown to successfully treat melanoma, but questions remain around the most efficient way to expand T cell populations *ex vivo* [5].

The cell expansion process in ACT works by recreating the natural process in the immune system. T cell activation requires three signals - T cell receptor stimulation, co-stimulation and pro-survival cytokines [7]. These signals are provided by antigen presenting cells (APCs). APCs encounter a cancerous cell and digest it. They break down its antigens, a type of protein that the immune system recognises, into peptides. The APC then displays peptides on its cell surface which can stimulate the T cell receptor [8]. Other proteins attached to APC surface co-stimulate T cell by binding to it's co-receptors. Co-signalling regulates T cell activation,

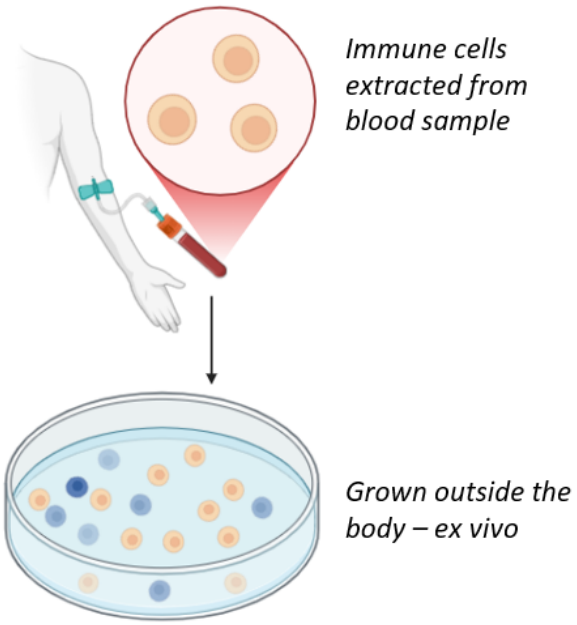

Figure 1: T cell expansion *ex vivo* in adoptive cell transfer (ACT). T cells are extracted from patient blood or tissue samples and expanded *ex vivo* before being re-infused. Created with BioRender.com [6].

differentiation and their overall function, making this a crucial step in T cell activation [9]. T cells activate when both signals are received, becoming tumour-specific activated T cells that can combat cancer growth. When T cells are exposed to the pro-survival cytokine interleukin 2 (IL-2) they are signalled to proliferate rapidly. IL-2 is a T cell growth factor released by activated T cells [10] (See Figure 2).

ACT expands T cell populations by recreating these three signals artificially by using similar stimulating and co-stimulating signals. A current method for T cell expansion being used therapeutically are micro-beads designed by Dynabeads. Magnetic beads act as artificial APCs (aAPCs) that have stimulatory signals attached. Cultures are treated with IL-2 to stimulate T cell growth [11]. While this process does recreate the three signals, its different to how they are naturally presented by APCs. This leads to less efficient expansion rates and dysfunctional T cells [7]

A novel method for expanding T cells *ex vivo* has been described by Cheung *et al.* (2018) which utilises mesoporous silica micro-rods. Micro-rods are coated in a fluid lipid bi-layer that presents membrane bound cues for T cell stimulation and co-stimulation. In cell cultures the micro-rods form a 3-dimensional scaffold which are referred to as APC-mimetic scaffolds (APC-ms). The micro-rods are loaded with IL-2 which is slowly diffused over several days. Initial experiments suggest APC-ms promote greater T cell expansion than pre-existing expansion system, like Dynabeads, as they more accurately mimic the natural presentation of cues to T cells. The system is also highly flexible and tunable, suggesting expansion efficiency can be improved [4,7]. The factors that impact expansion efficiency with APC-ms are relatively unknown and can be further explored.

Mathematical modelling allows us to explore T cell behaviour and expansion efficiency under different

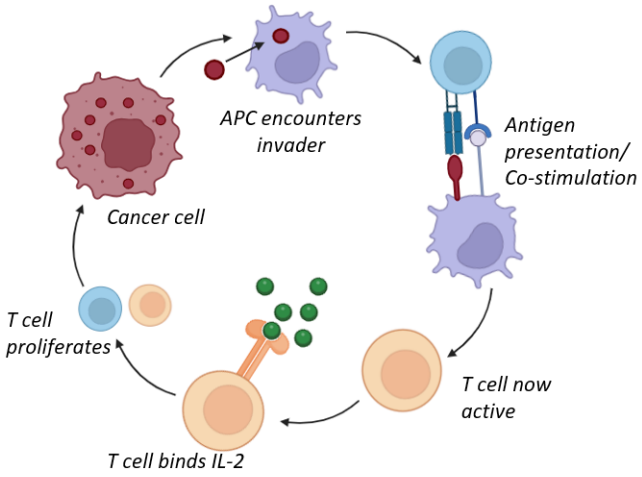

Figure 2: Process of antigen presentation and co-stimulation by antigen presenting cell (APC). APC digests cancer cell and breaks down its proteins into an antigen, which it display on its surface. After presentation, T cell activated and proliferates due to interleukin-2 (IL-2) interaction. T cells can now recognise and attack cancer. Created with BioRender.com [6].

conditions quicker and cheaper compared to laboratory experiments. Lacy *et al.* (2025) developed a novel spatio-temporal mathematical model of T cell expansion with APC-ms. Here T cells were described using a stochastic agent-based model (ABM) where individual T cells interacted with micro-rods on a 2-dimensional lattice. IL-2 was modelled deterministically. Simulations of the ABM revealed the distribution of the micro-rods correlated to the T cell expansion. Activation was shown to be initially maximised in homogeneous scaffolds, but future T cell generations activate quicker when micro-rods are clustered [12]. This model assumed micro-rods were immovable, but laboratory experiments show that micro-rods are moved by T cells [7,12].

This project develops an agent-based model that models movable micro-rods that interact with individual T cells. By utilising PhysiCell, a multicellular systems simulator, T cells and micro-rods are modelled as agents, which movements and behaviours are described with a system of ODEs and PDEs based on biomechanical forces. IL-2 is modelled with a reaction-diffusion equation which is solved numerically [13]. We use the ABM to explore how experimental set-up and model parameters affect T cell expansion. Longer simulations are used to see if micro-rod clustering occurs in this ABM to see if the model can reproduce experimental observations.

2 Statement of Authorship

The research presented in this report was the work of Brynn Mackie with contributions from Divya Joy regarding the modelling of micro-rods. This work was completed under the direct supervisor of Mason Lacy, Dr Adrianne Jenner, and Associate Professor Pascal Buenzli. This study builds on the work of Mason Lacy and has been appropriately referenced.

3 Methods

To simulate the system of T cells interaction with micro-rods, we use an agent-based model. We use an agent-based model as cell behaviour is intrinsically stochastic which this model captures. Modelling discrete agents allows us to define characteristics for and track behaviours of individual cells. This is a computational approach to recreate the behaviour of individual agents with the goal of understanding the overall result of the system. We consider the T cells and micro-rods to each be autonomous agents governed by a number of equations that describe movement, interactions, proliferation, and IL-2 secretion and uptake.

To perform agent-based modelling, we utilised a pre-existing open source framework called PhysiCell [13]. It is a multicellular systems simulator. The cells are represented off lattice. Substrate availability within the environment is determined in PhysiCell using an open-source biological diffusion solver, BioFVM [13,14]. In the below sections, key equations are highlighted that describe the most important aspects within PhysiCell for this model to be understood. It has also been highlighted where new equations not written by PhysiCell have been introduced to customise the framework to best simulate T cell expansion. Parameters listed in the below equations have either been reproduced from Lacy *et al.* (2025), Cheung *et al* (2018), or sourced from relevant documentation. They have been referenced where appropriate. Parameters that are not references have been estimated for this project. Values and their descriptions have been listed in Table 1.

Table 1: List of Parameters

Parameter	Value (units)	Physical Meaning
b	0.25 (unitless)	Strength of biased movement
r_p^{max}	4.78×10^{-4} (hr^{-1})	Maximum rate of proliferation of T cells [12]
I_p	$3.47 \times 10^{-12}(\text{ng}/\mu\text{m}^2)$	Concentration of IL-2 for which rate of proliferation is half its maximum [12]
v	$2.29 \times 10^{-4}(/\text{min})$	Probability of cell division occurring [12]
q	$6.2(\text{ng}/\mu\text{g})$	Ratio of loaded IL-2 mass to rod mass [7]
ρ_{rc}	$1.421 \times 10^{-9}(\mu\text{g}/\mu\text{m}^3)$	Mass concentration of rod cell [4]
W_i	$69.5(\mu\text{m}^3)$	Volume of T cell
W_j	$8.7(\mu\text{m}^3)$	Volume of rod cell [4]
λ_i	0.01 min^{-1}	Rate of uptake of IL-2 by T cell
λ_{ic}	0.001 min^{-1}	Rate of uptake of chemokine by T cell
S_{rc}	0.0001 min^{-1}	Rate of secretion of chemokine by rod cells
D_I	$20(\mu\text{m}^2/\text{min})$	Rate of diffusion of IL-2
D_c	$0.15(\mu\text{m}^2/\text{min})$	Rate of diffusion of chemokine

3.1 Modelling T cells

The three main components of T cell behaviour to model are their movement, activation, and proliferation. PhysiCell describes the movement of T cells with Equation 1. It states the velocity, $\mathbf{v}_i$, of the i^{th} cell is equal to the sum of forces acting upon it divided by the drag coefficient, v_i. These forces include cell-to-cell adhesion ($\mathbf{F}^{ij}_{cca}$) and repulsion ($\mathbf{F}^{ij}_{ccr}$) from some other j^{th} cell, cell-to-basement membrane adhesion ($\mathbf{F}^{i}_{cba}$) and repulsion ($\mathbf{F}^{i}_{cbr}$), and net locomotive ($\mathbf{F}^{i}_{loc}$) [13].

$$\mathbf{v}_i = \frac{1}{v_i}\left(\sum_{j\in\mathcal{N}(i)}(\mathbf{F}^{ij}_{cca}+\mathbf{F}^{ij}_{ccr})+\mathbf{F}^{i}_{cba}+\mathbf{F}^{i}_{cbr}+\mathbf{F}^{i}_{loc}\right), \tag{1}$$

where $\mathcal{N}(i)$ is the set of all cells near cell i. Net locomotive force represents the amount of force required for the cell to move towards something, for example something it is attracted to. In this model, we simulate biased movement of T cells towards the micro-rods. This was a behaviour experimentally observed by Cheung *et al.* (2018). To simulate this, we implemented a very slow diffusing substrate called a chemokine which was secreted from the micro-rods. This attracts the T cell towards the micro-rods in a process called chemo-taxis [10]. The diffusion of the chemokine can be described with the a similar equation to that shown in Section 3.2. Hence, the T cells exhibit biased movement towards this chemokine, and therefore towards the micro-rods. This movement is described by Equation 2. Here, s_{loc} is the current speed of motility of the cell, $\mathbf{d}_{\text{bias}}$ is the direction of the chemokine concentration gradient, b is the strength of the attraction, ranging from 0, no attraction, to 1, completely deterministic movement, and ξ is a random unit vector [13].

$$\mathbf{F}_{loc} = s_{loc}\left(\frac{b\mathbf{d}_{\text{bias}}+(1-b)\xi}{||b\mathbf{d}_{\text{bias}}+(1-b)\xi||}\right) \tag{2}$$

Cell movement is updated on 0.02 minute time-steps by PhysiCell.

T cells were estimated to be activated when 30 minutes of contact with micro-rods was accumulated, as demonstrated in Figure 3. This approximately represents accumulating a large enough signal from the stimulating and co-stimulating signal as described in the Introduction. The phenotype of the cell (i.e., naive or activated), is updated on 30 minute time-steps by PhysiCell.

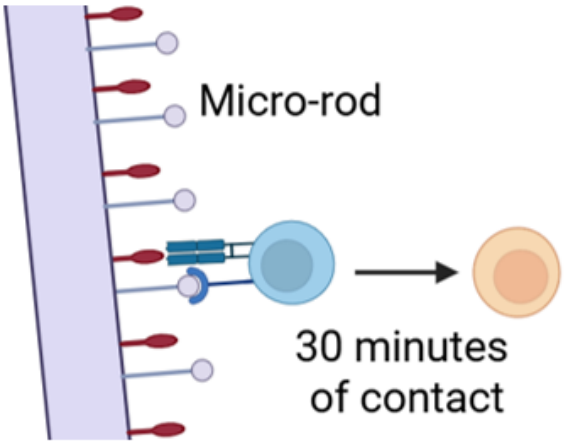

Figure 3: Process of T cell activation when interacting with stimulating and co-stimulating signals attached to lipid bi-layer on micro-rods

Once the T cell is activated, it can now proliferate. Cell division has the probability of occurring in the current time-step, described by the PhysiCell equation 3. Here, r_p is the rate of proliferation, t is the current time and Δt is the time-step [13].

$$\text{Prob(division during } [t, t+\Delta t]) = 1 - e^{-r_p \Delta t} \approx r_p \Delta t \tag{3}$$

As stated in the Introduction, rate of proliferation is dependent on the amount of IL-2 in the environment. We introduce Equation 4 to make r_p dependent on IL-2 concentration. This is described by Michaelis-Menten reaction kinetics. Here, r_p^{max} is the limiting rate of proliferation, the maximum the cell can reach, I is the concentration of IL-2 where the cell is located, and I_p is the concentration of IL-2 at which r_p is half its maximum [12].

$$r_p(I) = \frac{r_p^{max} I}{I_p + I} \tag{4}$$

The rate of proliferation is determined at each time step and supplemented into Equation 3 to update the probability of division for each time step.

3.2 Modelling IL-2 Concentration

The mesoporous silica micro-rods are loaded with IL-2 that secretes through small pores. IL-2 molecules are much smaller and more densely populated than T cells, and as such are considered as a continuous, time dependent concentration $I(x, y, t)$ (ng/μm^2) of IL-2.

PhysiCell describes the change in IL-2 concentration with a reaction diffusion equation that splits the reaction term into two sums giving Equation 5. The first term describes the rate uptake of IL-2 by T cells, while the second term describes the secretion of IL-2 by micro-rods. Micro-rods are comprised of rod cells, further discussed in Section 3.3. Here, $\mathbf{x}$ is the current location, $\mathbf{x}_i$ and $\mathbf{x}_j$ are the location of the i^{th} T cell and j^{th} rod cell respectively, W_i and W_j are the volume of the i^{th} T cell and j^{th} rod cell respectively, I_i^* and I_j^* are

the limiting terms of IL-2 secretion for T cells and rod cells respectively, and S_T is the secretion rate of IL-2 from T cells. PhysiCell breaks down this equation further, splitting the reaction term into two sums giving Equation 5. The first summation in Equation 5 describes the rate uptake of IL-2 by T cells, while the second summation describes the secretion of IL-2 by micro-rods. Micro-rods are comprised of rod cells, further discussed in Section 3.3. Further, $\mathbf{x}$ is the current location, $\mathbf{x}_i$ and $\mathbf{x}_j$ are the location of the i^{th} T cell and j^{th} rod cell respectively, W_i and W_j are the volume of the i^{th} T cell and j^{th} rod cell respectively, I_i^* and I_j^* are the limiting terms of IL-2 secretion for T cells and rod cells respectively, and S_T is the secretion rate of IL-2 from T cells [14].

$$\frac{\partial I}{\partial t} = D_I \nabla^2 I + \sum_{\text{T cells i}} \delta(\mathbf{x} - \mathbf{x}_i) W_i [(I_i^* - I) S_T - \lambda_i I] + \sum_{\text{rod cells j}} \delta(\mathbf{x} - \mathbf{x}_j) W_j [(I_j^* - I) S_r(t)] \tag{5}$$

It is noted that the dirac-delta function returns 1 when a T cell or rod cell is at location x_i or x_j respectively. While T cells do secrete IL-2 in biological systems, our model assume T cell secretion, S_T, is 0. The secretion rate from rod cells, $S_r(t)$ is notably time dependent. Secretion of IL-2 from micro-rods will decrease over the course of the experiment as the amount of IL-2 is finite. This can be represented by a negative exponential relationship, seen in Equation 6. It is defined by the parameters v, the probability of secretion occurring (/min), q, the ratio of IL-2 mass loaded in the micro-rods to the mass of the micro-rods (ng/μg), and ρ_{rc}, the mass concentration of rod cells [12].

$$S_r(t) = vq\rho_{rc}e^{-vt} \tag{6}$$

The secretion rate is calculated for each rod cell at each time step, and supplemented into Equation 5 to be numerically solved by BioFVM framework. Substrate diffusion is solved with a 0.01 minute time-step giving a result that is stable, 1st order accurate in time and 2nd order accurate in space [14].

As described above, a chemokine is used in our model. It is modelled with the same equations as detailed by Equation 5 above, but is assumed to have infinite source as it is used as a mechanism to attract T cells towards rod cells in the simulation. This chemokine is not present in experiments, but attraction is a behaviour that is seen. The uptake rate of chemokine by the T cell, λ_{ic}, secretion rate of chemokine by the rod cells, S_{rc}, and diffusion constant, D_c have different values than that of the IL-2, as listed in Table 1.

3.3 Modelling Micro-Rods

When creating the micro-rods we were limited to using pre-made agents in the PhysiCell framework. These agents are all circular, cell-like shapes. To achieve the micro-rod shape, several "rod-cells" were connected to each other utilising the cell-to-cell attraction framework. Experimental micro-rods were 70μm in length and 4.5μm in diameter, seen in Figure 4. To replicate this, 54 rod cells comprise one rod, arranged in two 27 rod-cell rows with one staggered below the other and have a volume of 8.7μm^3 per cell.

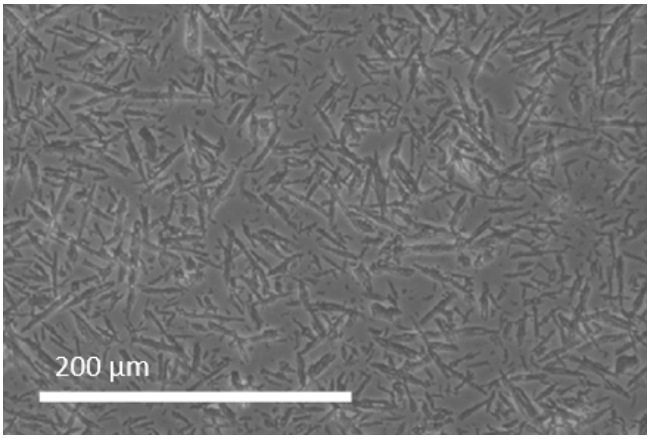

Figure 4: Representative microscopy of homogeneous mesoporous silica micro-rods reproduced from [7]

For the micro-rods to retain their shape, they had to be connected to surrounding cells in a specific manner. To ensure both rows remained connected, rod cells were connected in a triangular formation. To ensure the force of the T cells knocking into the rods didn't overcome the force of the rod cell-to-rod cell attraction, they were connected to adjacent cells up to 5 cells away. Figure 5 demonstrates the connection network. This resulted in micro-rods that didn't break when knocked but did bend slightly.

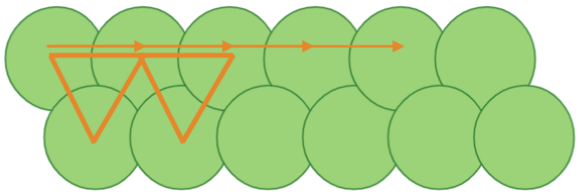

Figure 5: Diagram of rod cell connection to construct a micro-rod using PhysiCell

3.4 Initial and Boundary Conditions for Simulations

Experimentally, T cells are expanded in quasi-two-dimensional wells or dishes with more horizontal distribution then vertical. Hence, we simulate in a two-dimensional model. For computational efficiency we have a simulation domain of $\Omega : [-L, L]^2$ where $L = 200\mu$m which represents 0.1% of the surface area of a well in a 24-well culture plate.

In all simulations, there are initially 30 naive T cells. The rod density remains consistent throughout all simulations, with $1 \times 10^{-12} \mu\text{g/m}^3$. There is assumed to be no IL-2 in the environment at $t = 0$. These initial conditions are based upon the experimental values [7].
For the concentration of IL-2 and chemokine have a zero Dirichlet boundary conditions as there is no flux occurring in the system. No IL-2 or chemokine is externally supplemented or removed at any point in the

simulations.

Combining these components and conditions, we have an off-lattice agent-based model with movable rods.

4 Results

The model was first used to simulate T cell expansion for 7 days of growth to represent close to a typical experiment. The experiment by Cheung *et al.* expanded T cells for 14 days [7]. However, the model proved to be computationally expensive, taking 1 minute to run 1 hour of simulation on a laptop with 10 cores and 12 logical processors. Thus, we were limited in the length and number of runs per simulation we could complete. Only four runs per simulation have been completed for all results, and average and standard deviations shown. The 7 day simulation allows us to see a slightly extended cell growth under standard experimental conditions, seen in Figure 6.

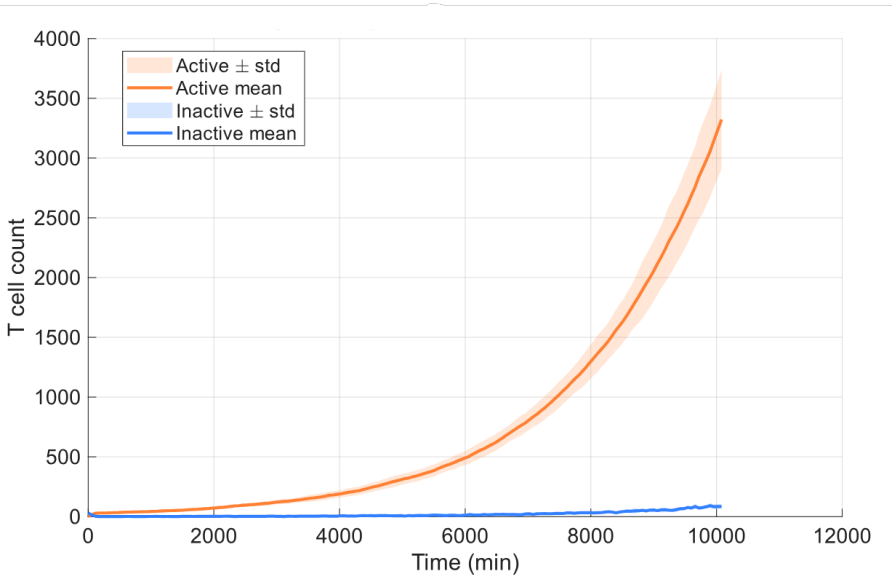

Figure 6: Number of activated and naive (inactive) T cells over 7 days of simulated cell expansion *ex vivo* with APC-ms for adoptive cell transfer. Average and standard deviation of 4 runs shown.

It is observed that activated T cell population increases exponentially over the 7 days while naive T cell population remains relatively low. This indicates rapid T cell activation, leading to rapid proliferation. This behaviour is seen in experiments, where T cells cluster around micro-rods forming cell-rod clusters promoting an increased rate of expansion. In animations produced from this model, the micro-rods are seen forming these clusters as they are moved by T cells. This could contribute to rapid expansion.

As mentioned above, this is a computationally expensive model. The 7 day simulation run 4 times took 14 hours to complete. Hence, to explore the changes to T cell behaviour and growth only 24 hour simulations were completed. This gives allows us to understand the initial rate of activation and expansion, and open to further simulation in future studies if significant results arise.

4.1 Changing the Maximum Rate of Proliferation

To understand the most impactful parameters and their potential affects, we simulated T cell expansion with a range of parameter values. We began with the maximum rate of proliferation. Simulating for 5 different values, a ten-fold increase, two-fold increase, standard, two-fold decrease, and ten-fold decrease.

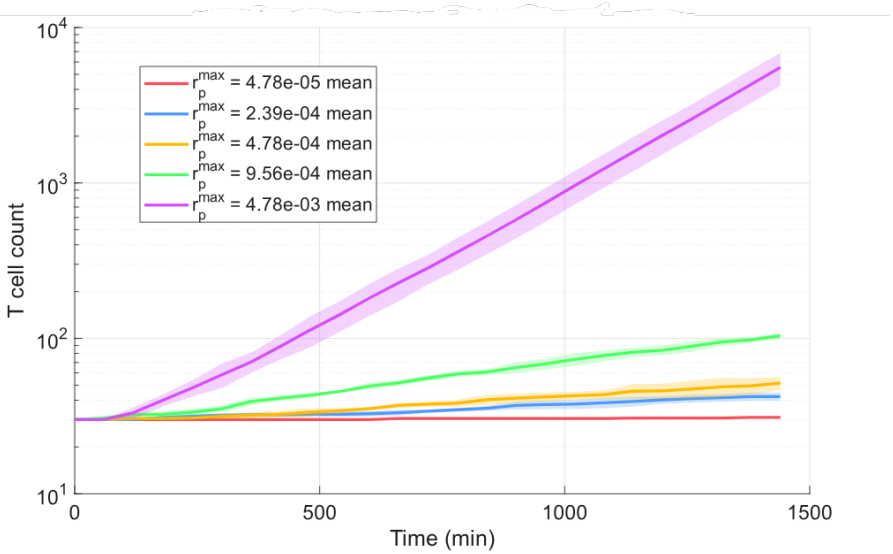

Figure 7: Number of total T cells generated over 24 hours of simulated cell expansion *ex vivo* with APC-ms for adoptive cell transfer with varying maximum rates of proliferation. Average and standard deviation of 4 runs shown.

A significant difference in cell growth after one day is noted. A ten-fold increase in the proliferation rate resulting in thousands of cells being generated and standard maximum proliferation resulting in only tens of cells. The exponential relationship seen in Equation 3 is evident in Figure 7, from increasing the r_p^{max} seen in Equation 4.

4.2 Impact of Presence of chemokine

We next investigated the impact of introducing biased movement through a chemokine secreted by micro-rods to attract T cells. While this chemokine is not something that exists physically in the experiment, T cell attraction to micro-rods was experimentally observed. To understand if this chemokine has an impact on total cell growth and recreates expected behaviours, we simulate with and without the chemokine present, seen in Figure 8.

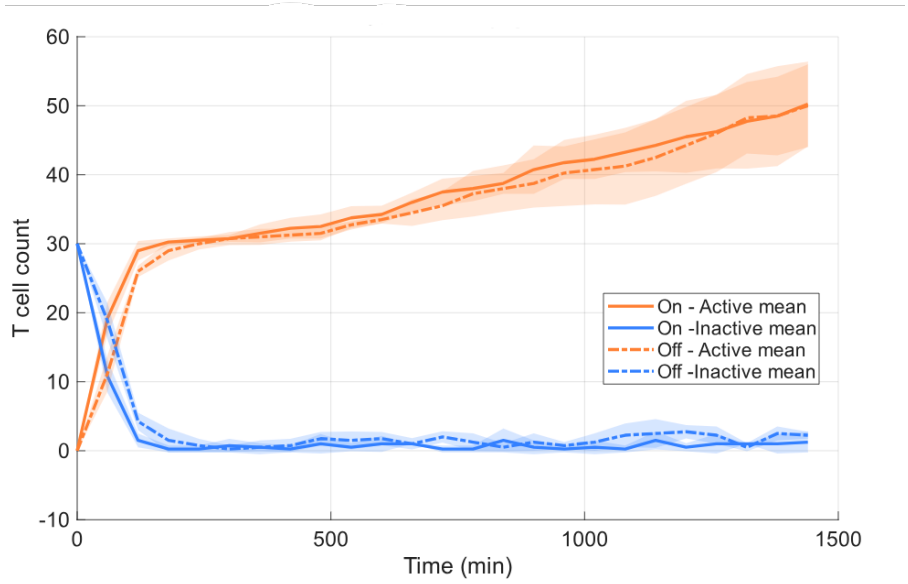

Figure 8: Number of activated and naive (inactive) T cells generated over 24 hours of simulated cell expansion *ex vivo* with APC-ms for adoptive cell transfer with chemokine present (on) and not present (off). Average and standard deviation of 4 runs shown.

No discernible difference is noted in the total amount of activated and naive T cells. This suggests that the expansion of T cells is not impacted by the presence of this chemokine attractant. However, there is a small increase in the rate of activation when the chemokine is present. This increase can be considered negligible as it is by a matter of minutes and has no lasting impact on the number of T cells generated after one day of growth.

4.3 Changing Amount of Initially Loaded IL-2

The impact of mass of IL-2 loaded into the micro-rods, the q value from Equation 6, was examined. This was completed in a similar to investigating the affect of maximum rate of proliferation, by completing simulations that differ the q value by a 10-fold increase, a two-fold increase, standard, a two-fold decrease, and a 10-fold decrease, seen in Figure 9.

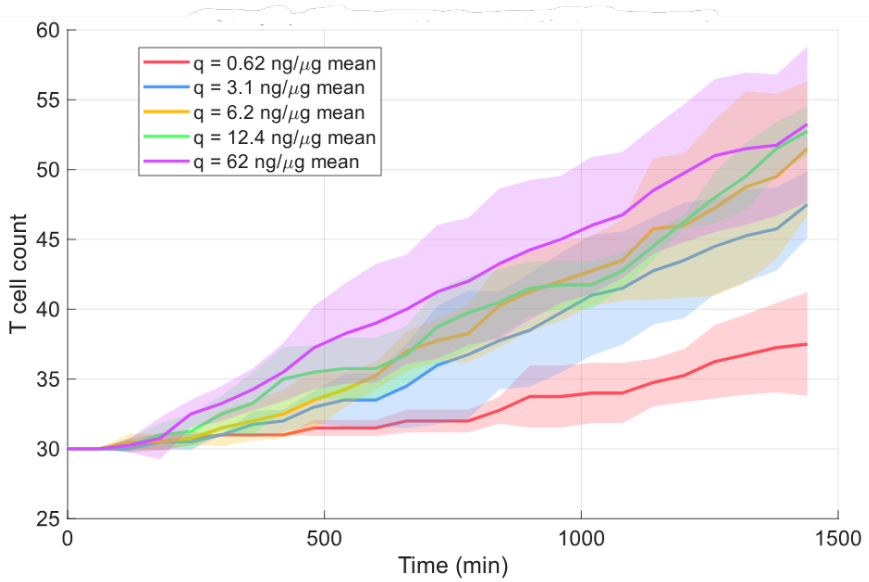

Figure 9: Number of total T cells generated over 24 hours of simulated cell expansion *ex vivo* with APC-ms for adoptive cell transfer with amounts of loaded IL-2 in micro-rods. Average and standard deviation of 4 runs shown.

While the standard, two-fold and ten-fold increase result in a similar amount of total T cells generated, with mild variability, the two-fold and ten-fold decrease show a decrease in the amount of T cells. The ten-fold decrease especially demonstrates a significant decrease in expansion after one day of growth. This was further investigated by mapping the diffusion of IL-2 after one day of simulation for ten-fold increase and decrease, seen in the top left and top right images respectively in Figure 10.

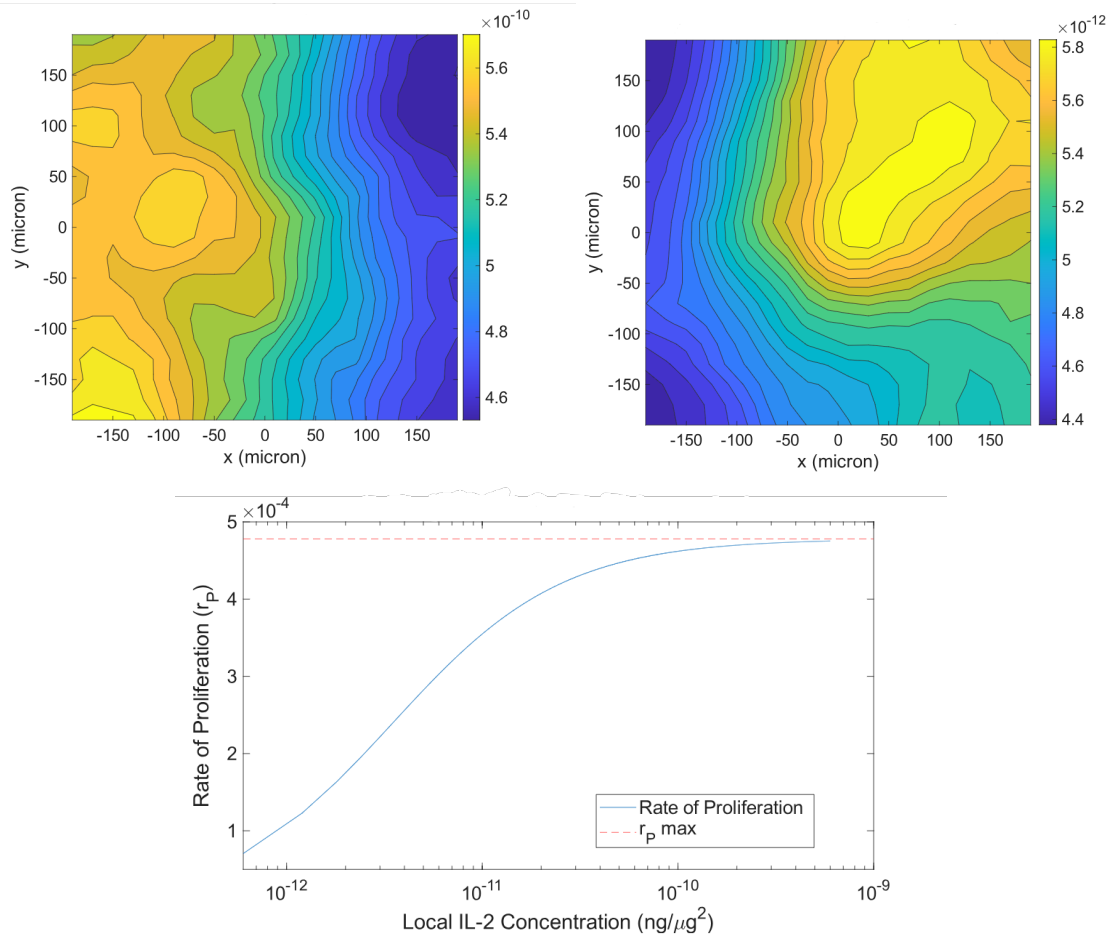

Figure 10: Concentration of loaded IL-2 across simulation domain and affect on rate of proliferation of T cells. [Top left] Distribution of IL-2 when ration of loaded IL-2 mass to rod cell mass is $q = 62$ ng/μg. [Top right] Distribution of IL-2 when ration of loaded IL-2 mass to rod cell mass is $q = 0.62$ ng/μg. [Bottom] Relationship between the local concentration of IL-2 to the rate of proliferation of a cell.

A factor of 100 difference in concentration across the domain between the increase and decrease is noted. When graphing the relationship from Equation 4, seen in the bottom image of Figure 10, it can be seen that the low concentration noted in the ten-fold decrease diffusion map leads to a significantly lower rate of proliferation. The ten-fold increase comparatively reaches close to the maximum rate of proliferation across all parts of the domain leading to the increase number of T cells after one day of growth.

5 Discussion and Future Works

In this study we show that changing the maximum rate of proliferation has a significant impact on the predicted efficiency of T cell expansion in ACT. While the general change in efficiency is expected as per Equation 3 and 4, the magnitude of this change has been shown to be very large. This suggests that the

calibration of the parameter r_p^{max} is a crucial step in designing this model, and must be done so precisely as to not greatly over or under-estimate the potential population of T cells generated in ACT. Furthermore, it has also been demonstrated that T cells have natural variability in their proliferation rate between donors when expanded *ex vivo* [15]. To account for this, the model could be calibrated to individual patient data given the rate of proliferation for their T cells is known. In addition, a wide range of maximum proliferation rates could be simulated in a virtual cohort to obtain a range of expansion efficiency for a general population. This will allow for more accurate predictions and potential optimisation of T cell growth, allowing biologists to determine new protocols and treatments more effectively.

It is observed that the inclusion of a chemokine secreted from micro-rods to attract the T cells has no discernible impact on one day of T cell expansion. As the domain has a high concentration of homogeneously distributed micro-rods it is likely the T cells will interact with the micro-rods due to their close proximity. Therefore, they do not need to be attracted towards the cells to increase the likelihood of contact. This indicates that the micro-rods do not need to be loaded with attracting chemokines when working with dense micro-rod scaffolds. The micro-rods could instead be loaded with other important chemical messengers, such as IL-2. It is possible if the micro-rod scaffold was more sparse the chemokine attractant would have a more significant impact on the rate of activation.

A decreased concentration of IL-2 loaded into the micro-rods was seen to cause a decrease in number of cells generated after one day of growth *ex vivo*. In particular, a significant decrease was only observed with 0.62ng/μg (ten-fold decrease) while 3.1ng/μg (2-fold decrease) had similar populations of T cells generated to simulations of 6.2ng/μg (standard) and higher concentrations. This suggests that the minimum amount of IL-2 needed for this most efficient expansion over one day is only 3.1ng/μg. While the true amount of IL-2 would likely need to be more over a 14 day simulation, the true time-frame for T cell expansion in ACT, this result proves the model can be used to find the true minimum amount of IL-2 required. Then, a recommendation could be made to the biologists to use this concentration of IL-2, potentially creating more room for other chemical messengers to be loaded into the micro-rods. Current methods of T cell expansion in ACT include the use of several other substrates, including IL-7, IL-15 and IL-21 which have been shown to increase the proliferation rate and decrease terminal differentiation [16].

The model contains limitations that must be considered, including its significant computational time, the exclusion of cell death, the bend in the micro-rods, and that the model is currently two-dimensional. As mentioned in the Methods section, the model takes 1 minute to run 1 hour of simulation on a laptop with 10 cores and 12 logical processors. This limited the length of simulations we were able to run, limiting the observations we were able to obtain. As such, results obtained provide more of a proof of concept for this model and the recommendations that can be made when longer simulations are completed. Several assumptions were made regarding the biological processes of cell behaviours, but potentially the most

impactful was that there is no cell death. Due to the short time frame of simulations completed in this project, cell death would have no significant impact, but over longer time-frames cell death could impact the total populations generated. The micro-rods featured in this model bend quite significantly when interacted with, often curving and creating arcs instead of staying straight. This is not a shape that can be formed physically in experiments and thus may change the overall behaviour of the system. Finally, the model is two-dimensional. In practice, T cells can climb over micro-rods and micro-rods can overlap one another creating different scaffold shapes and a more sparse domain. Our model currently does not account for either of these scenarios. It should be considered that the rate of expansion in a 3D model will likely be affected by different interactions between T cells and micro-rods.

Going forwards, we would like to expand this model to three-dimensions. The PhysiCell framework is designed to handle three-dimensional models and as such only a small amount of change would need to be applied to the current model to accommodate this. Initial work towards this, however, does show there is an increased computational load. Furthermore, we would like to completed longer simulations to gain a better idea of behaviours and expansion efficiency observed in a full scale experiment under different conditions. More concrete recommendations for biologists could be made regarding initial investigations explored in this report and mentioned above. More accurate cell interactions could be included in this model as well, including cell-to-cell and cell-to-rod adhesion. This would allow for better investigation into the effects of T cell clustering and a better model of T cell activation to be developed, improving the validity of our results.

6 Conclusion

In this project we successfully developed an agent-based model of *ex vivo* T cell expansion in ACT that included off-lattice agent movement and movable micro-rods. We utilised the pre-existing framework of PhysiCell to represent biomechanical interactions between T cells and micro-rods in two-dimensions. Simulating one day of growth, the impact of changing key parameters including maximum rate of proliferation, presence of a chemokine attractant, and amount of IL-2 loaded into micro-rods was explored. It was concluded that a chemokine attractant had no significant impact on cell activation or proliferation over one day, while rate of proliferation and IL-2 concentration did. It is recommended that the maximum rate of proliferation is carefully calibrated to biological data, and that modelling to patient specific data should be considered. It was shown that this model can be used to find the minimum amount of IL-2 needed to achieve optimal expansion. Longer simulations should be run to determine this value for 14 day long experiment. There are many directions this model can be taken in future work, including three-dimensional simulations and including more biological processes. Our model will allow for further exploration of T cell behaviour to understand how to optimise T cell expansion in ACT for better therapeutic outcomes for cancer patients.

7 Data Accessibility

Algorithms used to create the model and simulations are available on Github at `https://github.com/divyajoy365/vres2026`.

8 Acknowledgements

I would like to acknowledge the contributions of Divya Joy, a Vacation Research Experience Scheme student at the Queensland University of Technology. Divya completed significant work on the modelling of micro-rods in PhysiCell. I would also like to acknowledge my supervisors, Mason Lacy, Dr Adrianne Jenner, and Associate Professor Pascal Buenzli at the Queensland University of Technology for all their invaluable feedback and assistance during this project.

9 References

[1] Chakraborty, J & Rahman, T 2012, 'The difficulties in cancer treatment', *Ecancermedicalscience*, 6, ed16, `https://doi.org/10.3332/ecancer.2012.ed16`

[2] Vinay, DS, Ryan, EP, Pawelec, G, et al, 2015, 'Immune evasion in cancer: Mechanistic basis and therapeutic strategies', *Seminars in Cancer Biology*, vol35, pp S185-S198. `https://doi.org/10.1016/j.semcancer.2015.03.004`

[3] McCune, JS, 2016, 'Immunotherapy to Treat Cancer', *Clinical Pharmacology & Therapeutics*, vol100, no.3 , pp 198-203, `https://doi.org/10.1002/cpt.404`

[4] Zhang, DKY, Cheung, AS, & Mooney, DJ, 2020, 'Activation and expansion of human T cells using artificial antigen-presenting cell scaffolds', *Nature Protocols*, vol15, pp773-798. `https://doi.org/10.1038/s41596-019-0249-0`

[5] Veatch, JR, Lee, SM, Fitzgibbon, M, et al, 2018, 'tumour-infiltrating BRAFV600E-specific CD_4+ T cells correlated with complete clinical response in melanoma', *Journal of Clinical Investigation*, vol128, no. 4, `https://doi:10.1172/JCI98689`

[6] Biorender.com, 2026 *Biorender.com* [online] Available at: https://www.biorender.com/ (Accessed: 24 February 2026).

[7] Cheung, AS, Zhang, DKY, Koshy, ST, & Mooney, DJ, 2018, 'Scaffolds that mimic antigen-presenting cells enable *ex vivo* expansion of primary T cells', *Nature Biotechnology*, vol36, no2, `https://doi:10.1038/nbt.4047`

[8] Pishesha, N, Harmand, TJ & Ploegh, HL, 2022, 'A guide to antigen processing and presentation', *Nature Reviews. Immunology*, vol22, pp. 751-764, `https://doi.org/10.1038/s41577-022-00707-2`

[9] Flies, DB & Chen, L, 2013, 'Molecular Mechanism of T cell co-stimulation and co-inhibition', *Nature reviews. Immunology*, vol13, no. 4, pp. 227 - 242, `https://doi.org/10.1038/nri3405`

[10] Cantrell, DA & Ross, Sh, 2018, 'Signaling and Function of Interleukin-2 in T Lymphocytes'. *The Annual Review of Immunology*, vol36, pp. 411-433, https:\\doi:10.1146/annurev-immunol-042617-053352

[11] Meas, H, Logan, M, Boschen, M, et al, 2025, 'An Automated Solution for Efficient Isolation and Gentle Activation of T cells Using CTS Detachable Dynabeads CD4, CD8 and CD3/CD38 for CAR-T Manufacturing', *Cytotherapy*, vol27, no.5, pp. S213-S214, https://doi.org/10.1016/j.jcyt.2025.03.436

[12] Lacy, MS, Jenner, AL, Buenzli, PR, (2025), 'A stochastic model of T cell expansion in micro-rod scaffolds and its continuum limit: Importance of IL-2 loading and scaffold structure', *bioRxiv.org*.

[13] Macklin, P, Ghaffarizadeh, A, Heiland, R, Friedman, SH, Mumenthaler, SM, 2018, 'Supplementary Materials for: Ghaffarizadeh et al., PhysiCell: an Open Source Physics-Based Cell Simulator for 3-D Multicellular Systems', *PLoS Comput. Biol.*, doi:10.1371/journal.pcbi.1005991

[14] Ghaffarizadeh, A, Friedman, SH & Macklin, P, 2015, 'BioFVM: an efficient, parallelized diffusive transport solver for 3-D biological simulations', *Bioinformatics*, vol32, no. 8, pp. 1256-1258. https://doi.org/10.1093/bioinformatics/btv730

[15] Burnham, RE, Zoine, JT, Story, JY, et al, 2020, 'Characterization of Donor Variability for $\gamma\delta$ T cell *ex vivo* Expansion and Development of an Allogeneic $\gamma\delta$ T cell Immunotherapy'. *Frontiers in Medicine*, vol7, https://doi.org/10.3389/fmed.2020.588453

[16] Zhang, H, Passang, T, Ravindranathan, S, et al, 2023, 'The magic of small-molecule drugs during *ex vivo* expansion in adoptive cell therapy', *Frontiers in Medicine*, vol14, https://doi.org/10.3389/fimmu.2023.1154566