

The use of mathematical modelling for improving the tissue engineering of organs and stem cell therapy

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Abstract: Regenerative medicine is a multidisciplinary field where continued progress relies on the incorporation of a diverse set of technologies from a wide range of disciplines within medicine, science and engineering. This review describes how one such technique, mathematical modelling, can be utilised to improve the tissue engineering of organs and stem cell therapy. Several case studies, taken from research carried out by our group, ACTREM, demonstrate the utility of mechanistic mathematical models to help aid the design and optimisation of protocols in regenerative medicine.

Keywords: regenerative medicine; tissue engineering; stem cell therapy; stem cells; mathematical modelling; computational modelling

1. INTRODUCTION

1.1. Background

Regenerative medicine is a rapidly progressing field of medical science that promises to improve and save the lives of countless numbers of people over the coming decades. Important milestones in the clinical application of tissue engineering were achieved with the first in-human transplantations of tissue engineered tracheas using donor [1] and artificial scaffolds [2]. Clinical and pre-clinical studies have shown great promise for the tissue engineering of a range of organs including heart [3], lung [4], and oesophagus [5].

The two major classes of therapies used in regenerative medicine, which are the subject of this paper, are the tissue engineering of organs [6, 7] and stem cell therapy [8, 9]. Tissue engineering refers to the methods by which a natural or artificial tissue engineering (TE) scaffold that serves as the extra cellular matrix (ECM) of an organ or tissue is implanted into a patient, with or without repopulating the scaffold with cells either *ex vivo* or *in vivo*. Stem cell therapy refers to the method of delivering cells directly to afflicted organs or tissues of the patient [10].

pluripotent cells. In the latter case the aim is to harness the reparative properties of the cells either through their ability to differentiate into adult cells, or as a means of boosting the endogenous repair mechanisms of the tissue. The types of stem cells that can be used are autologous stem cells harvested from the patient, e.g. mesenchymal stromal cells (MSCs) and mononuclear cells (MNCs) isolated from bone marrow, or non-autologous types of cells, e.g. embryonic stem cells (ESCs) [11].

1.2. New technologies for regenerative medicine

Despite the early promising successes, the field of regenerative medicine faces significant scientific and technical challenges to the goal of attaining widespread clinical use [12]. The key problems lie in the creation of effective natural or artificial scaffolds for complex organs such as the heart and lung, and of ensuring the engraftment of sufficient numbers and types of seeded cells to ensure that TE organs become functional after implantation [13].

Being a multidisciplinary field, regenerative medicine relies on the contribution from a diverse range of specialities within the medical, biological and engineering sciences. The result of this interdisciplinary collaboration has been the development of a raft of novel technologies including new materials [14] and fabrication techniques for TE scaffolds [15], the development and purification of stem cell lines [16], and methods for *in vivo* and *in vitro* cell tracking [17, 18]. Progress in the field of regenerative medicine will continue to benefit greatly from the incorporation of new technologies and techniques sourced from outside of traditional biomedical disciplines.

1.3. Mathematical modelling for regenerative medicine

Regenerative medicine, as indeed all of biomedical science, is increasingly making use of advanced quantitative

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Both scaffold seeding and cell delivery to organs can be carried out using either mature or differentiated cells, or

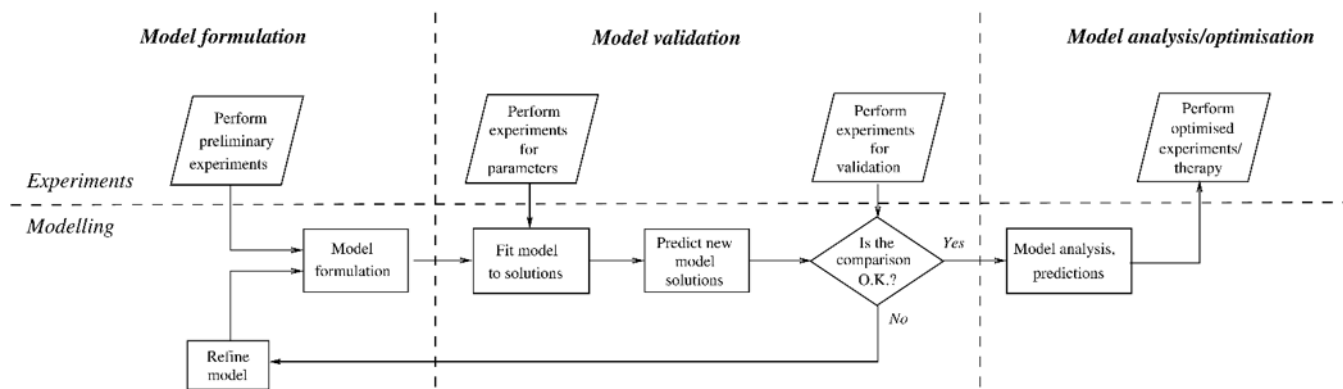


Figure 1: Workflow diagram of the formulation, validation and implementation of a mechanistic mathematical model.

methods. Beyond the traditional use of descriptive statistics and statistical hypothesis testing used for analysing experimental data, current research in stem cells and tissue engineering now routinely incorporates bioinformatics techniques [19] to infer the molecular profiles and interactions of cells and tissues *in vitro* and *in vivo* [20]. These methods use advanced statistics and sophisticated computer algorithms applied to the large volumes of data produced from experiments to infer biological mechanisms such as gene and protein interactions and signalling pathways within tissue samples [21].

Another powerful quantitative approach being increasingly used in regenerative medicine is the use of mathematical modelling. The method involves creating a mathematical formulation of the underlying mechanisms of a biological system based on *a priori* understanding and experimental results. A mathematical model can be used to simulate and analyse the workings of a real biological system, thereby making it a powerful means of replacing *in vitro* and *in vivo* models for therapies in regenerative medicine.

An expansive literature pertains to mechanistic mathematical modelling studies applied to different areas of biomedicine. Mathematical modelling has been applied extensively to study cancer growth [22], and has successfully been used to optimise chemotherapy [23, 24]. Because regenerative medicine is a relatively new area, which has hitherto progressed mainly by experimental means, exciting opportunities are opening up for the application of mathematical modelling within the field.

The aim of this paper is to demonstrate how mechanistic mathematical modelling can be successfully applied to regenerative medicine. In §2 an explanation of the general principles of a mechanistic mathematical modelling approach is given. In §3 several key applications are described where mathematical modelling has been used in studies of tissue engineering and stem cell therapy carried out by our research group, the Advanced Center for Translational Regenerative Medicine (ACTREM). These include (i) biological TE scaffold production, (ii) seeding of

TE organs, (iii) TE organ biomechanics, and (iv) stem cell delivery to the lung. This is followed in §3 by further discussion, and concluding remarks.

2. GENERAL PRINCIPLES AND METHODOLOGY OF MATHEMATICAL MODELLING

A comprehensive description of the techniques and methods used for building and evaluating mathematical models is beyond the scope of this article, and can be found elsewhere [25, 26], but a brief description is given as follows. The process of developing a mechanistic mathematical model is depicted in the flow chart in Fig. 1. The initial model formulation is based on inferences obtained from previous experiments and hypotheses, and usually begins with a schematic diagram encapsulating the key postulated mechanisms and their interactions. The nature of the included mechanisms can be diverse and may be chemical e.g. for describing the metabolism of nutrients by cells [27], or physical e.g. to account for fluid shear stress experienced by cells in bioreactors [28].

These mechanisms are used as the basis of the formulation of the mathematical model. The result is a set of equations describing how the modelled quantities, e.g. the concentration of a metabolite or density of cells on a seeded scaffold, change within the tissue or experimental system with time. Examples of model equations arising in applications to regenerative medicine are given in §3 (see Figs. 2, 3 & 4).

A key challenge to the derivation of mathematical models for biological applications is to account for the vastly disparate spatial scales involved, which range from the sub-cellular scale ($< 1 \mu\text{m}$) to the macroscopic scale ($> 1 \text{mm}$) [29]. The macroscopic-scale models presented in §3 do not directly take into account the behaviour of individual cells. However, some cellular-scale models used for tissue engineering directly simulate the motion and interactions of individual cells in tissue or in a bioreactor [30]. A topical area in mathematical modelling research is to formulate so called “multi-scale” models [31, 32] which can take into

account the different spatial and time scales inherent in tissue growth [33].

To obtain predictions from the mathematical model involves “solving” it to determine how the modelled quantities vary with time and space within the experimental system. The solution procedure can be done manually using pen and paper, which is called an “analytical solution method” or with the aid of a computer. Models that make significant use of computer algorithms and resources, such as models based on individual cells, are called “computational models”. Such computational approaches arise when performing computational fluid dynamics (CFD) simulations of the fluid flow within bioreactors [34], and in the finite elements techniques used to model the complex geometries of scaffolds and organs [35].

Owing to the inherent complexity of biological systems, even relatively simple mechanistic mathematical models can depend on a large number of parameter values. These parameters typically characterise intrinsic properties within the experimental system, such as the rate of a chemical reaction or the speed of cell migration. Model parameter values can be obtained by carrying out specific experiments, or by sourcing them from published studies. It is standard practice to determine unknown parameter values by “fitting” the model solutions to the experimental data [36]. Validating a mathematical model is carried out by comparing the model predictions against new experimental data not used for the fitting procedure. If the predictions do not agree satisfactorily with the data, or the model predicts spurious behaviour, the model should be refined by adding additional mechanisms, and then revalidated [37].

For the applications of mathematical modelling to regenerative medicine, such as those described in §3, analysis of the predictions of the validated model over

ranges of values of controllable experimental parameters can yield useful information on how to optimise an experiment or therapy.

3. CASE STUDIES OF THE APPLICATION OF MATHEMATICAL MODELLING TO REGENERATIVE MEDICINE

This section presents examples that demonstrate how mathematical modelling, using the methodology described in §2, is being applied to research in tissue engineering and stem cell therapy within ACTREM. Reference is given to related studies reported in the literature, and possible future directions for research.

3.1. Organ decellularisation

The production of biological TE scaffolds from donor organs requires the careful use of decellularisation protocols to remove immunogenic cellular components from the tissue while leaving the underlying ECM intact. Decellularisation involves alternatively rinsing the organ with detergents (e.g. deoxycholate), enzymes (e.g. DNAase) and purified water over a repeated series of cycles.

Decellularisation protocols have been developed for a variety of organs [38]. However, applying a pre-specified protocol, in terms of the concentration of reagents used and the duration of the cycles, may not necessarily be optimal for a given organ due to variability of properties such as size, donor age, and the initial state of degradation of the organ. Applying the protocol too rigorously to an organ may be detrimental to the ECM but, in contrast, inadequately applying the protocol may leave allogeneic remnants in the tissue. Thus the decellularisation protocol should ideally be adjusted to suit the particular organ being decellularised.

Such an approach calls for a way of non-destructively

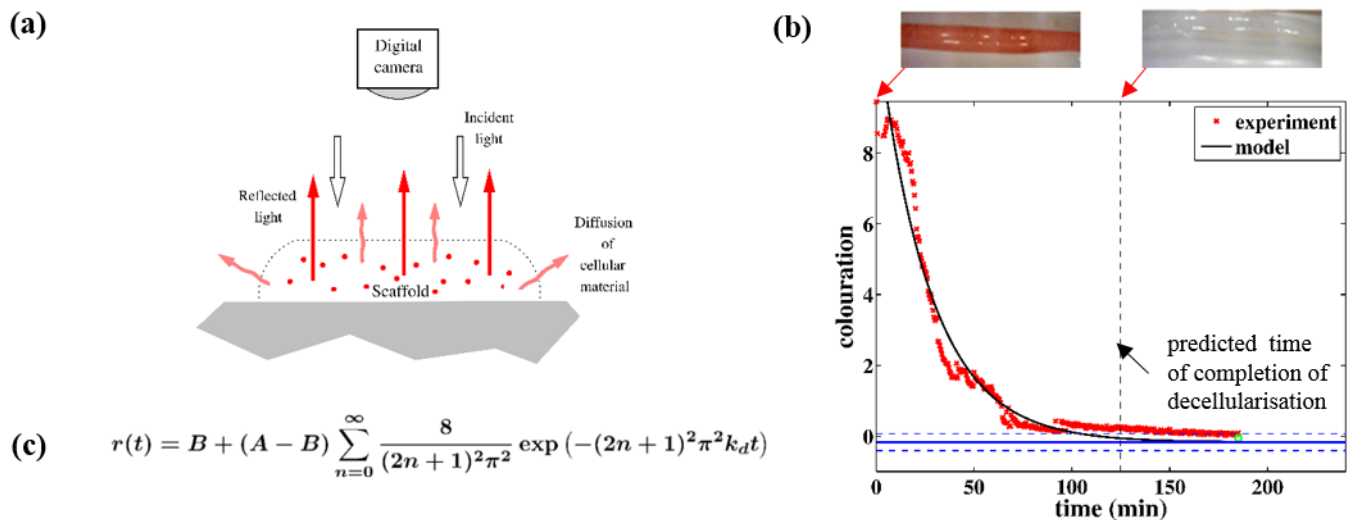


Figure 2: Customised decellularisation of biological scaffolds. During decellularisation the change in colouration of the scaffold is quantified (a) and compared (b) to the results from the mathematical model (c) to predict the optimal decellularisation time.

measuring the amount of cellular material in an organ [39]. Our approach is to quantify changes in the degree of red colouration of the organ from digital images taken during the decellularisation (Fig. 2). As decellularisation proceeds, and cellular debris is released from the tissue, the depth of the red colouration fades and the tissue becomes a white translucent colour (Fig. 2 (a), (b)).

Mathematical modelling was used to obtain a quantitative relationship between the amount of cellular material remaining in the scaffold and the degree of observed red colouration. The mechanisms used to build the model include an account of the diffusive transport of cellular remnants from the interior of the organ into the surrounding media, and how incident light on the scaffold is dispersed from the remaining cellular material thereby giving rise to the observed red colouration.

The analytical solution of the mathematical model (Fig. 2 (c)) is a formula for the predicted amount of red colouration of the organ with respect to time. Calculating the value of r for each value of t involves summing an infinite series of terms where $n = 1, 2, 3 \dots$ etc. (in practice, however, it is sufficient to terminate the summation at a very large value of n). The equation contains unknown parameters A and B which specify the initial and base levels of the colouration, and k_d which characterises the rate of diffusion of cellular

material through the tissue. Estimates for the three parameters are determined in real time during the decellularisation procedure, by fitting the values of the model to the colouration data as each new image is received by the camera. The predicted time taken for complete decellularisation is calculated based on how long it takes for $r(t)$ calculated from Fig. 2 (c) to remain within a small range of the final value (blue lines in Fig. 2 (b)). By continuing the decellularisation protocol until the time predicted by the model (vertical dashed line in Fig. 2 (b)), the organ is subject to the shortest possible protocol that removes sufficient cellular material without deleteriously affecting the ECM of the organ.

Such “customised” and automated decellularisation technologies, which are being successfully applied in our preclinical studies of the oesophagus, intestine and kidney of rats, are likely to feature prominently in future research and clinical translation as a means of rapidly facilitating the production of high quality TE scaffolds [40].

3.2. TE organ reseeded

A critical step in the production of TE organs is the seeding of artificial and biological scaffolds with cells. Scaffold seeding typically involves the use of large numbers of cells, and there is an urgent need to optimise the cells

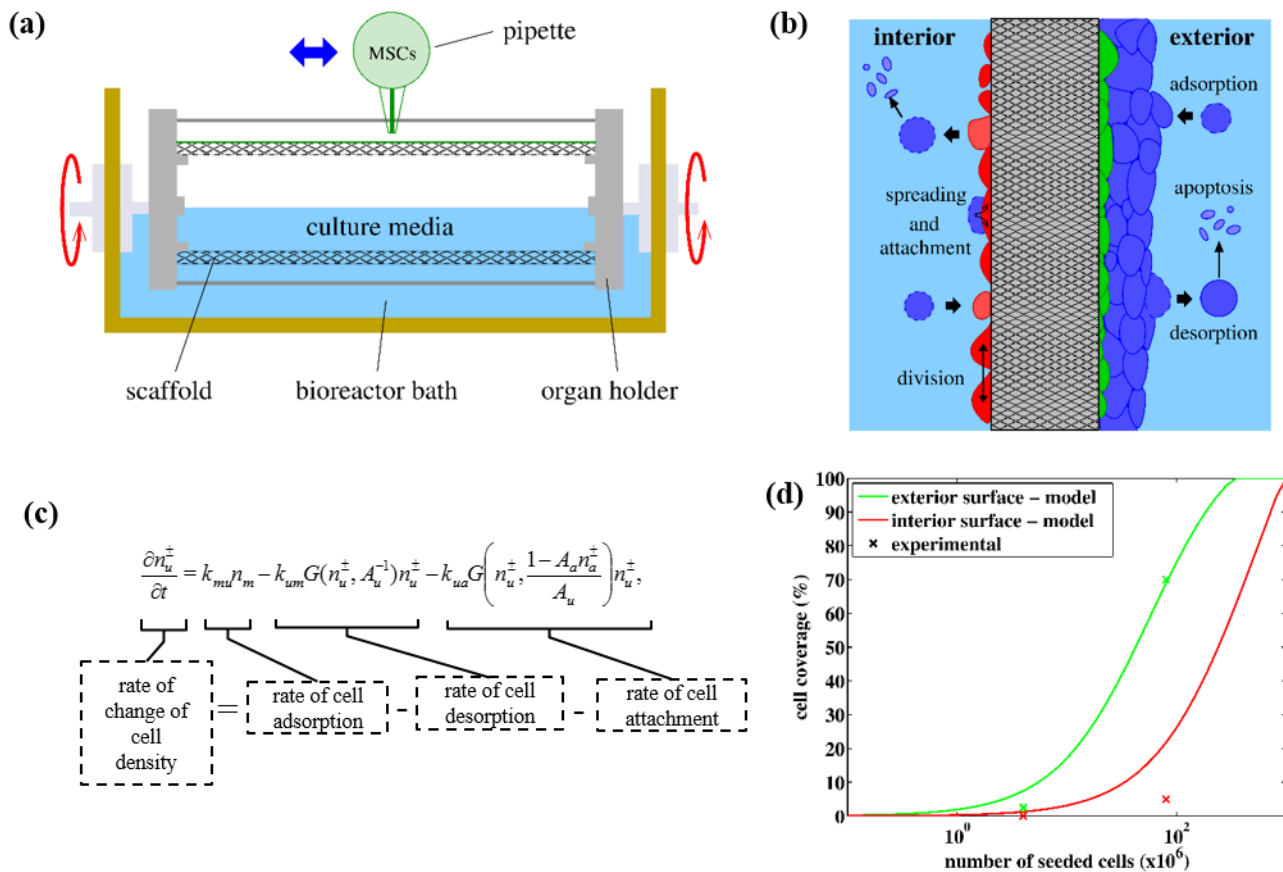


Figure 3: Mathematical modelling of seeding of a tissue engineered trachea (a). The different processes acting on the cells in the bioreactor (b) are used to formulate a mathematical model (c) that predicts the cell coverage at the end of the incubation (d).

harvested for clinical therapy and pre-clinical research [41]. Mathematical and computational modelling studies concerned with understanding and optimising cell seeding [42-44] and growth [45-47] in TE scaffolds can be put to good use for this purpose by informing of the minimum number of cells required for the seeding.

In our preclinical studies involving the static reseeded of decellularised rat oesophageal [5], diaphragmatic and intestinal scaffolds with rat MSCs the number of cells used for the seeding was determined based on the number of attached cells that would fully cover the external surfaces (due to the static seeding method and the incubation time being typically less than 50 hours there was negligible cell migration into the scaffold interior). For the case of the reseeded of a decellularised rat diaphragm, a typical value used for the scaffold area was $A_d = 9 \text{ cm}^2$, and the cell area was $A_a = 281 \text{ } \mu\text{m}^2$ which was obtained from studies of rat MSCs attached to electrospun fibres [48]. This gives $N_s = A_d/A_a = 3.2 \times 10^6$ as the number of cells required for the seeding.

Such a calculation provides an approximate estimate for the number of cells required, however it does not take into account effects such as cell spreading and proliferation, and distributions in cell size, all of which can significantly complicate the analysis [49]. Another important effect is the loss of cells from the scaffold, due to detachment and death, which can be particularly significant where dynamic seeding methods are used. The type of bioreactor currently used in the clinic to seed tracheal scaffolds with MNCs from patients is shown in Fig. 3(a). The cells are pipetted manually onto the scaffold, followed by incubation for approximately 50 hours to allow the cells to attach, spread and proliferate over the scaffold. A key problem is that the constant mechanical rotation of the scaffold (which is done to keep it moist while maintaining exposure of cells to the air) generates fluid shear stress that causes large numbers of cells to be lost to the bioreactor bath. To account for this loss in the estimate for the number of cells for the seeding, mechanistic mathematical modelling was carried out of the fate of cells in the bioreactor (Fig. 3).

The derivation of the mathematical model utilises understanding of the different physical and biological processes involving the cells. These include cell attachment, spreading, proliferation, and desorption and adsorption of cells due to contact with the fluid in the bath (Fig. 3 (b)). The resulting mathematical model is in the form of a set of ordinary differential equations (ODEs). Fig. 3 (c) gives the

specific equation describing the surface density of unattached cells, n_u , on the scaffold at a given time, t . The variables n_u and n_m respectively represent the densities of cells attached to the scaffold and of cells in the bioreactor bath. The superscript \pm is used to distinguish cells attached to the external and internal surfaces of the scaffold. The quantities A_a and A_u represent the specific areas of individual attached cells and unattached cells. The quantities of the form k_x are rate parameters for the transitions between the different cell compartments. The function G characterises the effect of the multi-layering of the cells on the rate of attachment.

For a given number of cells initially seeded onto the scaffold, the model equations are solved to obtain the total numbers of attached cells on the tracheal scaffold at the end of the incubation period (Fig. 3 (d)). The model allows the correct number of cells to be estimated for bioreactor seeding of any scaffold of a given size.

With the same kind of bioreactor as shown in Fig. 3 (a), mathematical modelling has been used to study nutrient consumption by cells seeded onto tracheal scaffolds [50, 51]. An implication of these studies, and others concerning nutrient depletion in scaffolds [52, 53], is that the depth of tissue penetration into a scaffold after implantation will be limited if it does not become sufficiently vascularised [54].

Such modelling studies are conventionally developed in parallel but not usually intimately connected with laboratory experimental work. There is however a need to implement the predictive ability of models within practical devices to aid laboratory and clinical procedures [55]. Similarly to the automated system for the production of biological scaffolds described in §3.1, the mathematical model for TE scaffold seeding described above could form the basis of a controller to produce optimal bioreactor seeding [56]. Such a system could incorporate non-invasive means of quantifying cell coverage, sensors for various environmental variables within the bioreactor, and be capable of controlling bioreactor inputs, such as actuators to deliver growth factors. Using the principles of model-based control [57], a feedback controller could be derived from the mathematical model to optimally guide the incubation of the scaffold, in real time, so as to achieve optimal cell coverage. The model-based controller would continuously monitor the bioreactor sensors and in response manipulate the control inputs to ensure that full coverage of attached cells is maintained, while minimising the amounts of cell detachment, apoptosis and aberrant cell differentiation.

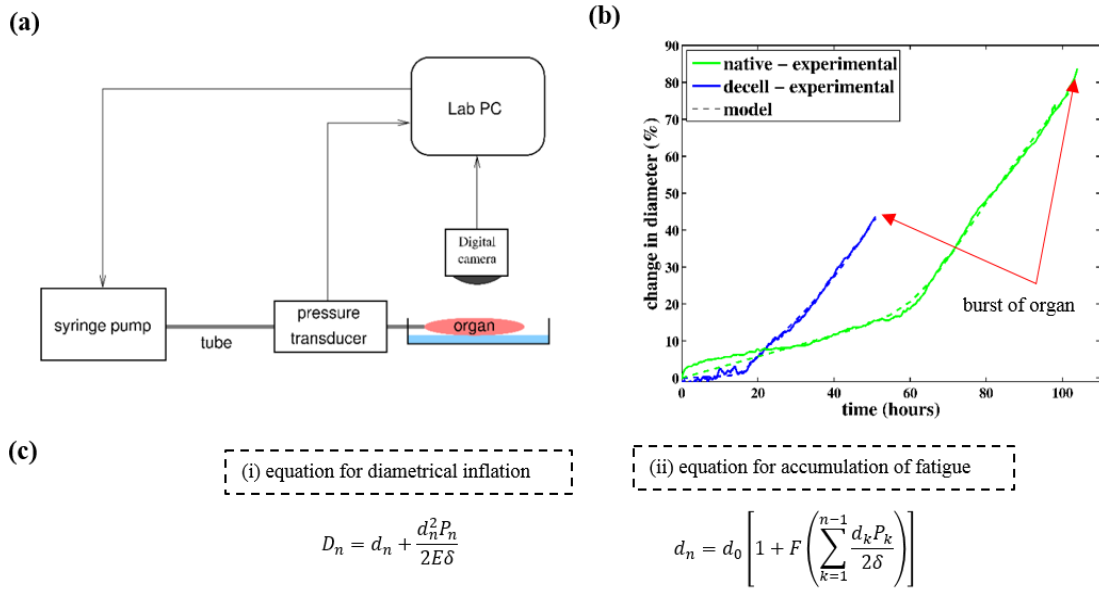


Figure 4: Fatigue testing of tissue engineered organs. Data acquired from image analysis (a) of organs subject to cyclical pressure is fitted (b) to a mathematical model (c) to determine model parameter values.

Hitherto the goal of bioreactor seeding has been to produce a TE scaffold having a highly confluent layer of viable stem cells attached to the surface. There remains a wider question of whether this constitutes a sufficient number of cells to bring about complete regeneration of the organ after implantation. To address this question mathematical modelling has been carried out of regeneration mechanisms of TE organs and tissues including bone [58], cartilage [59], skin [60] and MSC-seeded tracheal scaffolds [59]. The need for vascularisation of TE organs means that mathematical modelling of angiogenesis [62, 63] will play a key role in future modelling studies.

3.3. TE organ biomechanics

It is important to be able to measure the mechanical properties of TE organs so as to ensure that they can function robustly after implantation. Mathematical modelling can be used to predict the stresses and strains induced within implanted TE organs under normal physiological conditions. There is an extensive literature of mathematical and computational modelling studies investigating the mechanical behaviour of native organs, including trachea [64], lung [65] and heart [66], with an emphasis on understanding pathologies. There are, however, far fewer modelling studies which investigate TE organs.

There is a particular need to ensure mechanical viability of biological scaffolds [67] because directly after implantation they lack the full complement of cells which typically reside within native organs. These cells contribute significantly to the organ's mechanical properties,

particularly smooth muscle cells (SMCs) which are capable of active force generation.

By testing small portions of decellularised or artificial tissue using uniaxial or biaxial testing apparatus, quantitative data can be obtained of tensile strength, yield stress, elasticity, viscoelasticity and anisotropy properties [68]. Such data is used to derive constitutive laws [69] to relate the stress developed in response to strain within localised parts of a TE graft. Based on these constitutive laws, mathematical models can be developed to predict the stresses and strains generated throughout an entire TE organ or graft after implantation [70]. This is done to ensure that the graft is mechanically compatible with the host tissue, and that breaking stresses within the implant are not exceeded.

Another mechanical property of biological tissue, which is important within the context of tissue engineering, is its response to fatigue stress. Extended periods of being subject to repeated cycles of stretch, due to the normal cyclic processes that occur *in vivo*, can cause the accumulation of damage to the underlying ECM of tissues [71]. Without remodelling of the ECM by resident cells, this accumulated fatigue can lead to failure of the organ. Current research in ACTREM involves the evaluation of the fatigue properties of tubular biological scaffolds (Fig. 4) by subjecting them to cyclic luminal pressure waveforms (Fig. 4 (a)) and measuring the corresponding change in organ dimensions over time (Fig. 4 (b)).

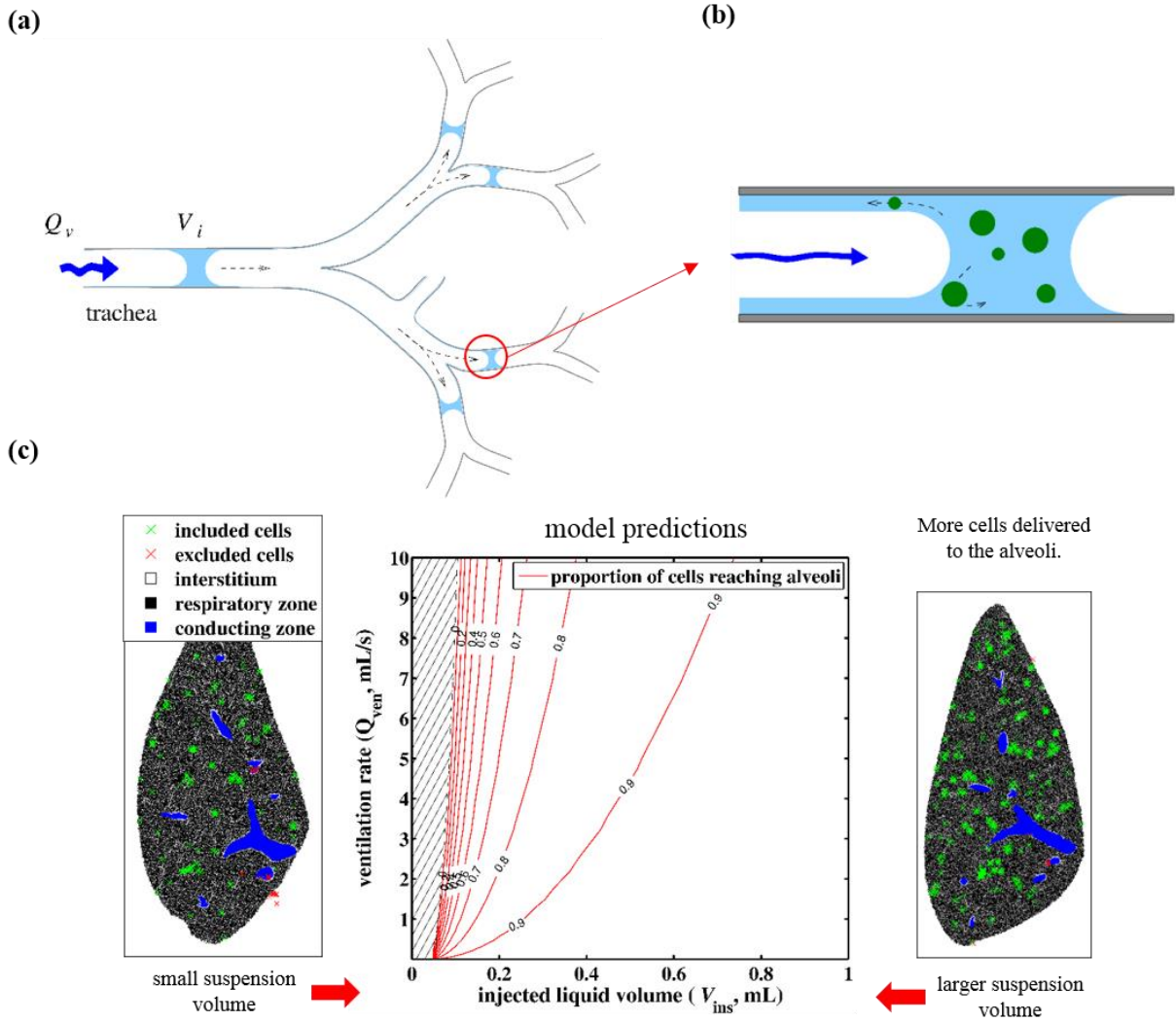


Figure 5: Mathematical modelling of intratracheal cell delivery. A suspension of MSCs is injected into the trachea and forced down in the airway using ventilation with air (a). Consideration of the physical mechanisms acting on the fluid (b) is used to derive a mathematical model to predict the proportion of cells reaching the alveolar regions (c).

To complement the experimental work, mathematical modelling is being used to characterise the changes in elastic properties of the organ wall due to accumulated fatigue, and how the fatigue response of the decellularised tissue differs from that of native tissue. The mathematical model shown in Fig. 4 (c) describes the response of an organ to a cyclical pressure waveform and comprises two parts: (i) the equation giving the diameter of the organ, D_n , at the maximum applied pressure, P_n , and (ii) the equation for how the unstretched (zero applied pressure) diameter, d_n , changes with the applied number of cycles, n . In these equations d_0 is the initial diameter, E is Young's modulus, δ , is the wall thickness, and F is a function that characterises the response of tissue to accumulated fatigue. The appropriate form of the function F is determined by fitting the mathematical model to the experimental data (Fig. 4 (b)).

3.4. Stem cell delivery

Mathematical modelling is also a useful tool for stem cell therapy, as a means of determining the optimal delivery protocols for targeting of cells to organs. We are using mathematical modelling to study the intratracheal delivery [72] of MSCs to the lung in a rat model of pulmonary hypertension (PHT) [10]. The delivery procedure involves injecting a suspension of cells into the trachea, followed by an injection of air behind the liquid to force it down into the alveolar regions where the delivered MSCs promote the repair of damaged pulmonary vessels.

Mathematical modelling is being used to guide the determination of a protocol that will deliver the maximum number of cells to the alveolar regions of the lung (Fig. 5). The physical principles used to construct the model are based on the physics of plugs of liquid propagating along straight tubes (Fig. 5 (a)). The model includes a morphometric description of the rat lung and accounts for effects such as lung asymmetry and the changes in the volume of the lung due to inflation. The model was adapted from similar

modelling approaches used to study the delivery of surfactant to the lungs of neonates [73, 74].

As the fluid plugs propagate through the conducting zone, they split at the bifurcations in the airway tree. The plugs deposit a thin layer of cell suspension on the walls of the airway tubes, the thickness of which depends on the rate of injection of air (Fig. 5 (b)). The mathematical model predicts the proportion of cells that are delivered to the alveolar regions in terms of experimental parameters such as the volume of the suspension, V_{ins} , and the rate of ventilation, Q_{ven} (Fig. 5 (c) – centre panel).

The model predicts that the optimal protocol comprises a rapid injection of the cell suspension into the trachea, so as to promote the formation of a stable plug of fluid in the upper airway, followed by a slow ventilation so as to minimise the thickness of deposited layer thereby minimising the loss of cells to the conducting zone. Also to minimise the cell loss, the volume of the cell suspension should be maximised thereby minimising the volume of the liquid film relative to the total volume of suspension (concomitant on the amount of delivered liquid not obstructing gas exchange in the respiratory zone). The predictions of the mathematical model will be validated using data of the numbers of green fluorescent protein (GFP)-labelled cells counted in digital images of cross-sections of excised rats lungs (Fig. 5 (c) – side panels).

One noteworthy aspect is that this model is not appropriate for lungs of adult humans because of the larger sizes of the airway tubes. In the case of the adult human lung stable fluid plugs cannot form in the upper airway and the injected cell suspension tends to flow down into the lung under gravity. To study cell delivery in this case would require CFD simulations, incorporating highly resolved representations of lungs, to accurately simulate the flow of the cell suspension through the airway tree. Such computational modelling approaches have been pursued extensively for the simulation of aerosol deposition in airways [75, 76]. Computational modelling involving CFD should also be useful for understanding how to seed complex biological TE scaffolds, such as the kidney and lung, by perfusion of a cell suspension through the organs' remnant vasculature.

As in the case of the model for bioreactor seeding described in §3.2, the cell delivery model does not provide information about the *number* of stem cells needed to be injected to successfully repair the lung. For this, information about the long-term fate of the delivered cells and the dose-response mechanisms of the MSCs would need to be included in the model. A barrier to creating mechanistic models of the reparative effects of MSCs is an imprecise knowledge of the mechanisms involved, and to what extent their reparative properties is due to differentiation into the phenotypes of the host tissue [77] as compared to paracrine and endocrine effects that stimulate endogenous repair [78].

This question is however a fertile area for research in mathematical modelling, and will allow hypotheses

concerning the mechanisms, such as modulation of inflammation [61, 79] and stem cell differentiation [80], to be explored. The understanding of systemic effects including the homing of endogenous stem cells to organs and the mechanisms of inflammation, could be aided using whole body pharmacokinetic models [81, 82]. Such models will also be informative for optimising stem cell delivery via systemic routes e.g. by intravenous injection.

Quantitative predictions of the dose response of stem cells obtained from such studies could be incorporated into mechanistic models for cell delivery and used to calculate the number and timing of the doses of stem cells, in a similar way that has been achieved with models that predict the optimal dosage in cancer treatments [24].

4. DISCUSSION

This paper has highlighted the use of mathematical modelling as a valuable tool for research in different areas of tissue engineering and stem cell therapy. In §3 a broad range of examples of such applications of mathematical modelling used by our group (ACTREM) were given. The list is not exhaustive but serves to illustrate the utility and scope of mathematical modelling techniques within regenerative medicine. Those examples also serve to motivate further work and model refinements.

The current trend with mathematical and computational modelling is to produce progressively more sophisticated and refined mathematical multi-scale models of tissues and organs which incorporate large volumes of “omics” data [83]. It is possible to envisage that eventually highly realistic computational models of whole organs will be built on which to perform experiments, instead of living tissue [84]. The idea of virtual or so called “*in silico*” organs and tissues has been pursued actively for the heart [85], liver [86], lung [87] and cancerous tissue [88].

There are, however, significant challenges to creating realistic *in silico* organs for the use in regenerative medicine. There is still a lack of complete understanding of the underlying mechanisms contributing to the growth and regeneration of engineered tissues, particularly those concerning the therapeutic action of stem cells [89] and systemic effects such as inflammation and cell homing. In addition, the need to resolve fine structural details in the tissue make *in silico* mathematical models of whole organs computationally demanding to solve [90]. This will, however, become more feasible with the relentless increase in the power of cheaply-available computer hardware. A more modest approach, and that which was pursued in the applications presented in §3, was to develop specialised models tailored for particular experiments and therapies. The methodology used was to intimately combine *in vitro* and *in silico* modelling approaches.

However, with all mathematical models a fundamental problem lies in being able to accurately determine the values of model parameters from available experimental data [91]. Whereas many modelling studies use parameter values that

are “typical” or “representative” of the tissue, the effective clinical translation of mathematical models requires the use of accurately determined patient-specific parameters [92, 93].

An appealing aspect of the use of mechanistic mathematical models for tissues lies in the potential time and cost saved through reducing the amount of laboratory work required. Also, research in regenerative medicine requires large numbers of animals to be sacrificed for the development of surgical techniques, the testing of therapies, and the harvesting of stem cells. *In silico* models can in principle be used as a substitute for laboratory and human subjects; experimentation and optimisation of therapies could be carried out painlessly on “virtual” tissues and organs. *In silico* models will also become an important tool for reducing the reliance on animal experimentation in regenerative medicine in the future [94].

CONCLUSION

Mathematical modelling is a highly effective research tool for tissue engineering and stem cell therapy. Mathematical modelling techniques should be well integrated with experimental work, with a continual interaction between experiments, theory and simulation. This will allow for the creation of more refined and accurate models for use in regenerative medicine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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