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A framework for the design, modeling and optimization of biomedical systems

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Abstract

We present an overview of the key building blocks of a design framework for modeling and optimization of biomedical systems with main focus on leukemia, that we have been developing in the Biological Systems Engineering Laboratory and the Centre for Process Systems Engineering at Imperial College. The framework features the following areas: (i) a three-dimensional, biomimetic, *in vitro* platform for culturing both healthy and diseased blood; (ii) a novel, hollow fiber bioreactor that upgrades this *in vitro* platform to enable expansion and continuous harvesting of healthy and diseased blood; (iii) a global optimization-based approach for the design and operation of the aforementioned bioreactor; (iv) a pharmacokinetic / pharmacodynamic model representing patient response to Acute Myeloid Leukemia treatment; (v) an experimental framework for cell cycle modeling and quantitative analysis of environmental stress. This manuscript recapitulates the progress made in the different areas as well as the way in which these areas are connected, finally leading to a hybrid *in vitro/in silico* platform which allows the optimization of the *ex vivo* expansion of healthy and diseased blood.

Keywords: Biomedical design framework; Red blood cell production; Bioreactor design; Chemotherapy modeling and optimization; Cell cycle; Environmental stress

1. Introduction

One of the most challenging features of modeling biomedical systems is bridging the gap between phenomena occurring at multiple scales. Between molecular, cellular, patient and population scales, an appropriate translation is needed to evaluate the effects small scale processes have at large scale and vice-versa. The study of normal and abnormal blood production faces these challenges and many others related to the complexity of the underlying biological system and the heterogeneity observed in hematological malignancies. Deriving patient data directly is not always possible, thus making *ex vivo* observations and studies imperative. For the latter to be accomplished it is essential to develop appropriate experimental setups that reproduce *in vitro* the biological characteristics and behavior of the *in vivo* system.

Blood cell production takes place in the bone marrow (BM) which is a highly porous three dimensional organ of high complexity, wherein hematopoietic stem cells (HSCs) reside. HSCs are unique due to their ability to: (i) self-renew (ii) mature/differentiate towards functional cellular units of the immune and oxygen-carrying systems (Quesenberry et al., 2001).

BM failure is characterized by the inability of HSCs to produce healthy blood cells at an acceptable rate and quality, leading to a variety of health issues and diseases, among which lies leukemia. Leukemia is a cancer of the hematopoietic system characterized by the incapability of blood progenitors (HSCs) to mature normally, leading to the accumulation of immature white blood cells in the bone marrow (Williams, 2001). *Acute Myeloid Leukemia (AML)* is one of the most common types of leukemia; it affects only cells from the myeloid blood lineage. According to Cancer Research UK, approximately 8,000 cases of AML occur annually in the UK alone.

The most frequent treatment for AML is *chemotherapy* which aims at eliminating the cancerous population in the BM through attacking highly proliferative cells (immature blasts) (Cancer Research UK, American Cancer Society). This represents a reactive approach to the disease initiating after the disease symptoms appear. Current chemotherapy treatment protocols are designed based on pre-clinical animal experiments and on empirical clinical trials as well as the acquired experience of subspecialist physicians. However, a very high heterogeneity in the leukemia characteristics between patients but also within a specific patient exists (Preisler et al., 1995), consequently leading to unpredictable treatment outcomes. Clinical treatment protocols could, therefore, benefit from a more rational and personalized treatment scheduling strategy.

In addition to receiving drug treatment, these patients (as well as those undergoing other diseases of the blood or severe accidents) require frequent blood transfusions. Despite the success of blood donations in covering the needs in most of the countries (92M blood units collected globally [WHO, 2011]), there is a shortage in rare types of blood and a raising demand for regular availability. This is increasingly becoming a problem for health services; rare blood is extremely expensive, especially for patients requiring frequent transfusions (Tahhan et al., 1994; Meny et al., 2013). A promising solution to cover blood shortages either in time or in type is to produce it artificially. However, the techniques currently available are extremely expensive at $8,330 per unit of artificial blood, compared to $225.42 on average per donated unit of non-rare blood (Timmins and Nielsen, 2009) or up to $3,025 per donated unit of rare blood (Meny et al., 2013). Clearly, a more cost-effective solution needs to be implemented in order to shift towards artificial blood supply.

The current trends and developments in genomics, proteomics and metabolomics open the possibility for obtaining specific information related to the genetic characteristics, together with the proteomic and metabolomics profiles of an individual patient, which can then be used towards *personalized medicine*. In this context, personalized healthcare is expected to deliver a *step change* in quality and value of care, through more precise and personalized diagnostics as well as cost-effective and targeted therapies. Some of the challenges in the delivery of personalized medicine lie in (a) *In vitro*: the *fidelity and validity of current experimental systems used to investigate human diseases*; (b) *In silico: the integration of patient-specific and disease-specific datasets and the development of validated predictive adaptive models*; and (c) *In vivo:* the application of these models to identify *simple targets* and *more efficient, yet less toxic therapies and drugs* for a specific condition.

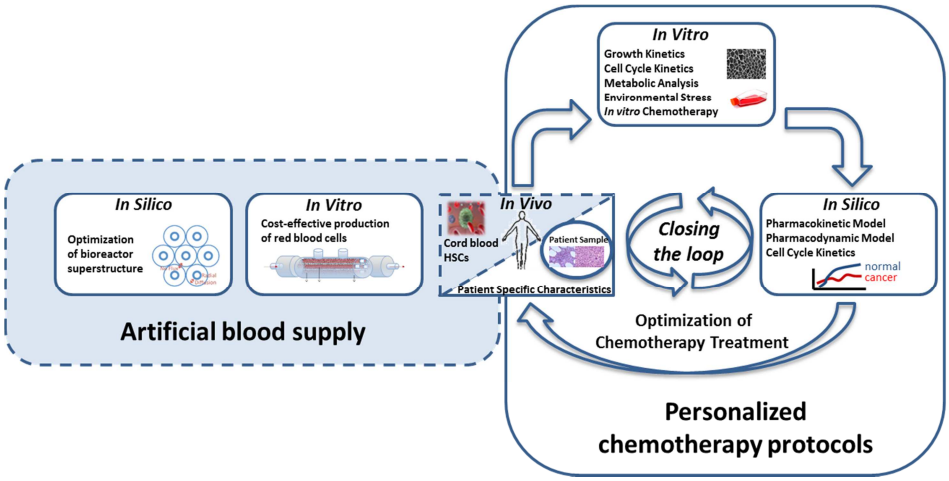

Figure 1: A framework for the optimization of blood production and personalized leukemia treatment in AML

Here, we present the fundamental features of an integrated framework which aims to address (some of) these challenges - with main focus on leukemia.

2. Design framework.

Fig.1 presents key building blocks of the integrated design framework under development at the BSEL in the Imperial College CPSE. It involves (i) a suitable platform for on-demand production of artificial blood (regularly needed for transfusions in critical patients); and (ii) an optimization strategy for personalized chemotherapy treatment design through closing the loop: from *in vivo* to *in vitro* and *in silico*. More specifically, hematopoietic cells donated from AML patients are cultured ex vivo via appropriately-designed in vitro platforms (Sections 3, 4 & 6) which expose to measurement a variety of parameters crucial for the cancer evolution: cell growth; cell cycle kinetics; metabolic evolution under appropriate environmental conditions. The parameters derived from the *in vitro* studies are incorporated in advanced mathematical tools (Section 5) that enable the prediction of patient response to chemotherapy. In parallel, umbilical cord HSCs are cultured in a biomimetic, cost-effective, 3D bioreactor, expanded and differentiated into red blood cells by careful signaling to externally control the same process of blood production that is diseased in leukemia (Sections 3 & 4).

3. A novel 3D bioreactor for ex-vivo culture of healthy and diseased blood

As mentioned in Section 1, both normal and abnormal hematopoiesis take place in the BM. A first step towards understanding and further optimizing chemotherapy for AML treatment is executing *in vitro* studies that recapitulate the *in vivo* micro-environment.
Blood cell production naturally occurs in the bone marrow, where stem cells receive the appropriate signals to proliferate and specialize. These signals consist of both chemical (nutrients, oxygen and growth factors, which are signaling proteins that provide extracellular stimuli to the cells) and mechanical (adhesion, cell-cell contact) stimuli that are unique to the 3D microenvironment (Panoskaltsis et al., 2005). However, most of current research is still performed in under 2D culture systems, wherein the mechanical stimuli received by the cells are nonnative and thus the cellular proliferation

is reduced. This limitation is typically overcome by increasing chemical stimulation in the form of the expensive, specialized growth factor proteins (Timmins and Nielsen, 2009).

Taking into consideration the architecture of the BM microenvironment, we describe, in the sequel, the development of two 3D *in vitro* platforms which serve as an *in vitro* bone marrow mimicry allowing the expansion of normal and diseased blood.

3.1. A high throughput 3D micro-bioreactor for ex vivo normal and diseased blood expansion

A 3D micro-bioreactor was developed by Mortera et al. (2010, 2011), consisting of highly porous Polyurethane (PU, pore size approximately 100μm), of dimensions 5x5x5 mm, as shown in Fig.2, which allows perfusion of nutrients and oxygen within the matrix. The adhesion signals of the ECM are recapitulated by coating this PU cube with collagen type I. This micro-bioreactor successfully supported the long-term expansion of three leukemic cell lines (K-562, HL-60 and Kasumi-6), resembling three different leukemia sub-types, i.e., human erythromyeloblastoid, acute promyelocytic and acute myeloid leukemia respectively, for over 6 weeks. Moreover, it successfully supported expansion and differentiation of Umbilical Cord Blood Cells (blood cells with high proliferation/differentiation potential that are extracted from the cord which arises from the navel that connects the fetus with the placenta) without any exogenous cytokines for a time frame of 4 weeks, in contrast to traditional 2D culture systems that allowed Umbilical Cord Blood Cells expansion only for a few days in absence of exogenous growth factors. This 3D *ex vivo* BM mimicry enabled the formulation and long-term maintenance of a dynamic culture population consisting of erythroid and myeloid precursors as well as progenitor and myeloid maturing cells. The 3D micro-bioreactor provides an ideal laboratory high throughput technical platform for screening several environmental factors and identifying those that are crucial for the successful *ex vivo* expansion of normal and leukemic blood. In order to produce blood at quantities sufficient for transfusion purposes, it is essential to scale up the *ex vivo* blood expansion (Rousseau et al., 2014). Moving on that direction, we developed a 3D hollow fiber reactor which enables red blood cell expansion at higher and continuous rate.

3.2. A 3D bioreactor for scaling up ex vivo normal and diseased blood expansion

Based on our 3D micro-bioreactor we scaled up the system and incorporated circulation of oxygenated nutritious medium, resulting in a 3D perfusion bioreactor capable of producing artificial blood (see Fig.1) which was patented by Panoskaltsis et al. (2012). It recapitulates the architectural and functional properties of blood formation and thereby reduces the need for expensive growth factors by more than an order of magnitude. The red blood cells (RBC) produced comply with the clinically required properties in terms of oxygen-carrying capacity, surface markers, and shape (Macedo, 2011).

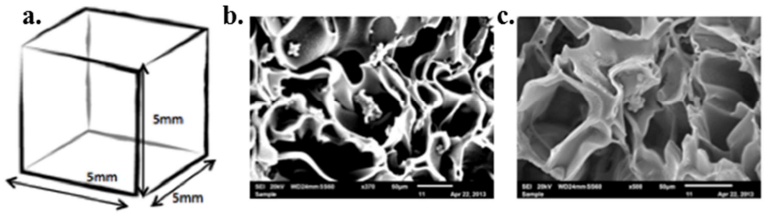

Figure 2: (a): Geometry of the 3D micro-bioreactor (b)-(c): Scanning Electron microscopy (SEM) images of the highly porous 3D micro-bioreactor including seeded leukemic cells.

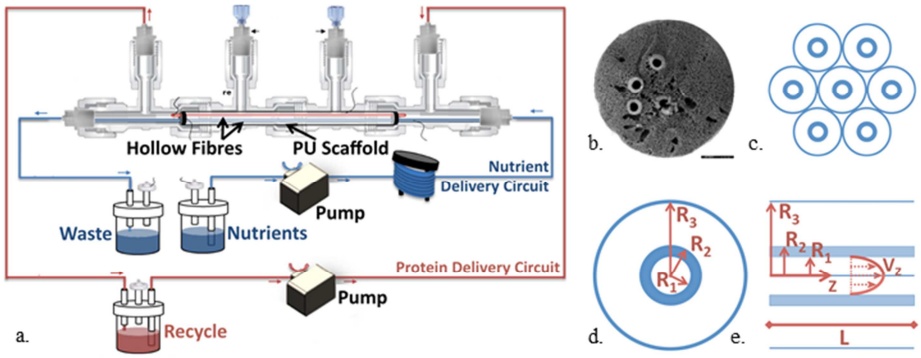

Figure 3: (a) Diagram of blood-producing bioreactor (Macedo 2011); (b) Cross-section of a bioreactor (SEM); (c)(d) Krogh Cyclinder approximation; (e) Poiseuille flow

In comparison to other perfusion bioreactors for HSCs expansion (Engelhardt et al, 2011), our solution costs 10x less per cell produced with a 28x reduction in bioreactor size; a cell output 18x superior was also achieved (Chaudhuri and Al-Rubeai, 2005). Similarly, 2D cultures producing RBCs require 5x more growth factors (6x higher cost) and 4000x larger volumes (Neildez-Nguyen et al., 2002).
From an architectural point of view, the bioreactor is composed of a 3D polyurethane scaffold traversed by two different circuits as shown in Fig.3a, (i) a high-uptake ("nutrient delivery") circuit which delivers nutrients and oxygen and removes waste through a plastic hollow fiber with very narrow pore size, (ii) a low-uptake ("protein delivery") circuit which circulates the growth factors required for cell differentiation towards RBCs through a ceramic hollow fiber with larger pore size, allowing the exit of mature RBCs only.

The current bioreactor has also been efficiently applied for *in vitro* leukemia cultivation, therefore serving as an ideal platform for the *ex vivo* expansion and study of diseased blood (Rende et al., 2013).

4. Bioreactor design optimization

Experiments in the bioreactor described in 3.2. are typically cost- and labor- intensive; *in silico* optimization strategies for the design and operation of the bioreactor can be highly beneficial. While different optimization approaches have previously improved individual degrees of freedom in hollow fiber bioreactors (Davidson et al., 2010, Shipley et al., 2011), our proposed bioreactor design and bioprocess optimization considers multiple design choices and explicitly incorporates uncertainty into the framework (Misener et al., 2014).
Our modeling approach for the bioreactor design reduces the cost of producing one unit of RBC while maintaining enough nutrients/growth factors to satisfy the quality requirements; operating choices include: (i) external diameter and length (aspect ratio) of the cylindrical bioreactor (R_4, L); (ii) number of hollow fibers for delivering reactants and extracting products and by-products (N_{HF}); (iii) flow rate of nutritious medium through the bioreactor (U_z); (iv) medium inlet composition in terms of glucose and growth factors ($C_{k,IN}$), (v) ambient oxygen concentration ($C_{oxygen,IN}$):

$$\min_{C_{k,IN},L,R_4,N_{HF},N_R,U_Z} U_Z \times N_R \times N_{HF} \times \sum_k p_k \times C_{k,IN} \quad (1)$$

where p_k represents the price of the material entering the reactor at concentration $C_{k,IN}$ and flowing at rate U_Z through each of the N_{HF} hollow fibres in each of the equivalent N_R parallel reactors. Modeling occurs across different scales: cellular growth, metabolism, fluid dynamics and chemical diffusion. More specifically, perfusion of nutrients, oxygen and proteins in the bioreactor is modeled as axial flow within the fibers (Fig.3e) following Poiseuille flow, while diffusion in the scaffold occurs radially (Fig.3d). In addition, mass exchange is reduced to five representative species: glucose corresponds to cellular nutrients; lactate models waste; oxygen stands in for cellular metabolism; stem cell factor (SCF) represents cellular expansion; erythropoietin (EPO) is mapped to cellular differentiation. The model for cellular growth, proliferation, and differentiation is derived from Ma et al. (2012) and Colijn and Mackey (2005). The model is implemented in GAMS 24.1 and solved using the MINLP solver ANTIGONE 1.1 (Misener and Floudas, 2013a; 2013b). The optimized bioreactor would produce RBCs at a competitive price compared to rare blood ($1k-3k).

This approach uses superstructure optimization applied to hollow fiber bioreactors for the first time; previous attempts varied individual parameters instead of using deterministic global optimization for a simultaneous choice of design and operation.

5. Design of optimal personalized treatments for AML

As described in Section 1, chemotherapy in the case of AML involves the use of cytotoxic drugs, which interact with cells that are proliferating. More specifically, only cells that are in one of the phases of the cell cycle (the process by which cells duplicate) will be eliminated. Since healthy cells also proliferate in order to renew the cellular material, they will equally be affected; it is very important to keep a balance between the number of cancer cells killed and the loss of healthy cells. However, clinical treatment protocols ignore the mechanisms behind drug action on the normal and abnormal population, which can lead to over- or under- treatment. Here, a more rational approach for the design of clinical treatment protocols based on the personalization of the chemotherapy schedule for each patient (Pefani et al., 2013) is presented (see also Fig.1).

5.1. Model overview

As shown in Fig.4, the model is composed of two main sections: pharmacokinetics (PK), which describe the elimination of the drug by organs, and pharmacodynamics (PD), which account for the effects of the drug on the cells in the BM, where the tumor resides.

The main input to the system is the treatment inflow; it is calculated according to the administration route and the injection rate. The resulting drug concentration then reaches the body through the blood streams, delivering it to the organs, which absorb it at different rates. Mass balances are performed in each of these organs, giving the drug concentration profiles. The drug concentration profiles calculated in the PK model are the main input for the PD model, in which the effect of the drug on the normal and the cancer cells is computed according to cell cycle kinetics of each population. Two separate models are used for each of them.

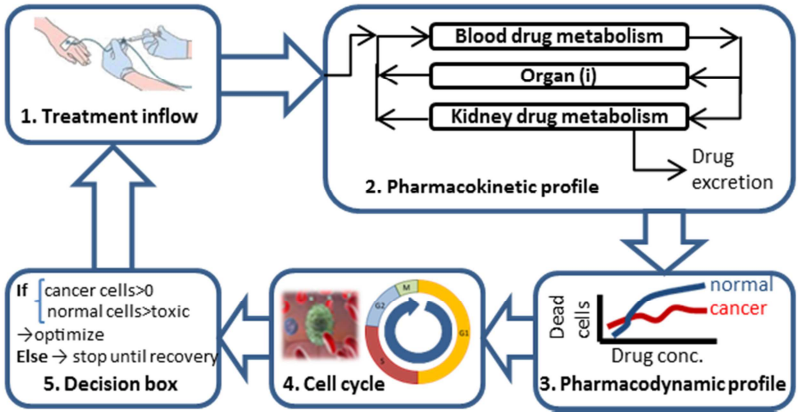

Figure 4: Design of clinical treatment protocols

Because most of the cancer cells are proliferating, the cell cycle model in this case incorporates 3 compartments in which the cells are non-resting. Each of them is described by the mass balance between compartments (including cell death by drug action if applicable). The transition rates are dependent on cell cycle times and natural apoptosis rates in each of the phases (Basse et al., 2003). The normal cell population model considers a proliferative population and a resting population that can move into a proliferative state. In both cases, the cell cycle kinetics are modeled through a set of Ordinary Differential Equations (ODEs) (one per compartment *y*):

$$\frac{dP_y}{dt} = k_{y-1}(T_{y-1}) \cdot P_{y-1} - k_y(T_y) \cdot P_y - effect_j \cdot P_y \tag{2}$$

where P_y and P_{y-1} are the number of cells in compartments *y* and *y-1*, $k_y(T_y)$ and $k_{y-1}(T_{y-1})$ are the transition rates from compartment *y* and *y-1* respectively (dependent on the duration of the corresponding phases, T_y and T_{y-1}) and $effect_j$ is the effect of drug *j* in the compartment.

A more refined cell cycle model based on the work of García Münzer et al. (2013, 2014) has been developed consisting of a multi-stage population balance model (MS-PBM). It is distributed on cell cycle progress-related events (s_y: protein expression, DNA). Cell cycle kinetics are tracked not only between compartments but also within them. The transition rates $k_y(s_y)$ are now dependent on the state variable level (the drug effects are not considered):

$$\frac{\partial P_y}{\partial t} + \frac{\partial P_y}{\partial s_y} = \int_{s_{y-1,0}}^{s_{y-1,\max}} k_{y-1}(s_{y-1}) \cdot P_{y-1}\, ds_{y-1} - k_y(s_y) \cdot P_y \tag{3}$$

The MS-PBM is discretized in the state variable space using a fully stable upwind scheme; it has been proven to accurately predict experimental data (Section 6.1.).

5.2. Model analysis and optimization

The output of the optimization section is an improved determination of patient-specific drug dosage and infusion duration. The decision box ensures that clinically mandatory constraints are satisfied, informing a new chemotherapy cycle of the effect of the

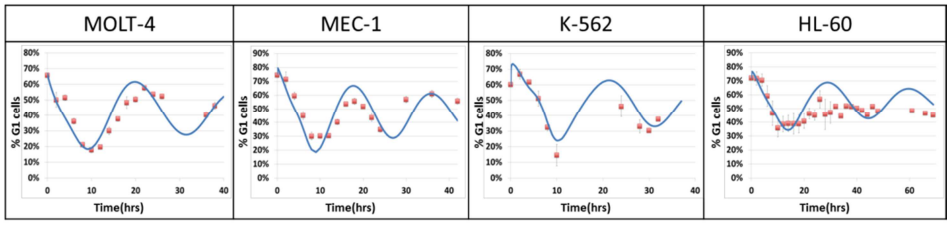

Figure 5.: Comparison of experimental (squares) and predicted (lines) kinetics of the G1 phase % population over time (x-axis: time in hours; y-axis: % G1 phase cells) for four different cell lines.

previous one and suggesting a better treatment. Remarkably, treatments suggested by the tool use similar drug dosages to those used clinically but, for instance, the scheduling is different; based on the predicted patient response to both protocols given by the model, the optimized treatment would have much better outcomes (Pefani, 2014). Global sensitivity analysis was performed on the original model following the method described in Kiparissides et al. (2009); the most critical parameters affecting the model output were found to be the cell cycle times (Pefani, 2013).

6. Model parameters derived from experiments

Sensitivity analysis identified cell cycle kinetics as one the key factors affecting treatment outcomes (Section 5.2). Hence, it was deemed necessary to direct our experimental efforts towards (i) the determination of the duration of each of the cell cycle phases, (ii) the impact of environmental factors, such as oxygen and glucose concentration as these factors highly affect the leukemic kinetics and, consequently, the pharmacokinetic profile of the BM compartment of our model (Section 5.1) as well as the cell cycle evolution.

6.1. Obtaining parameters for an MS-PBM of the cell cycle

The cell cycle times of four different leukemia cell lines (K-562, HL-60, MEC-1 and MOLT-4) were determined experimentally by following the timings of entry and exit events of a subpopulation of cells to and from each of the phases under 2D conditions at a first step. In parallel, protein and DNA production rates were recorded and assumed to be constant. The kinetics of the subpopulation counterpart were then simulated and compared to the experimental (independent) results (Fuentes-Garí et al., 2014). Good agreement was observed in all four cell lines, especially in the first cycle (Fig.5: G1 (gap 1) phase; the kinetics of the other phases matched similarly well with experimental data (not shown)).

6.2. Tackling parameters that incorporate the impact of environmental stress

Fluctuations of the oxygen and glucose concentration in the different body compartments (in the BM) and the peripheral blood or the liver, and between AML patients (individual cases of hypoglycemia, hyperglycemia) may lead to a different stress adaptation of the leukemic population. The latter will most likely affect the cancer growth and inactivation kinetics as well as the response to a chemotherapeutic drug *in vivo*.

We monitored and compared *in vitro* the proliferation, cell cycle and metabolic evolution of an AML model system in our 3D micro bio-reactor (Section 3.1) and in a conventional 2D culture for different oxygen and glucose conditions close to physiological (*in vivo*) levels (Velliou et al., 2013; 2014a; 2014b). More specifically, K-562 cell line was cultivated in the 3D micro bio-reactor as well as in 2D suspension cultures in 5% (hypoxia) as well as 20% (normoxia) oxygen and for three different

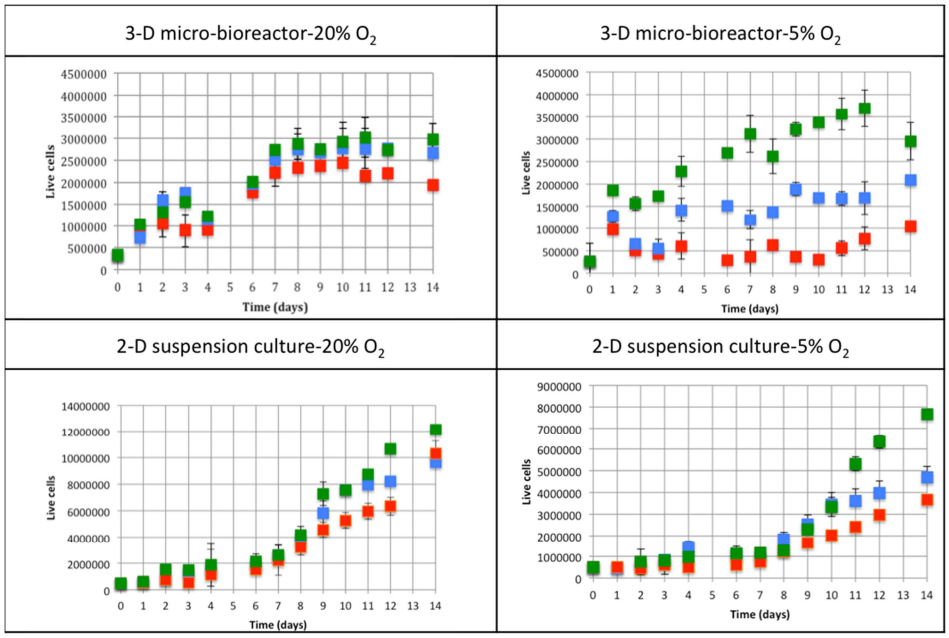

Figure 6: K-562 growth in the 3D micro-bioreactor and the 2D suspension culture, at different oxygen levels, i.e., 20% and 5% O_2. Different colors represent different glucose levels: (■) 4.3 g/L (■) 1.6 g/L, (■) 0.3 g/L.

glucose levels, i.e., 4.3 g/L (optimal level generally applied in laboratory growth media), 1.3 g/L (highest human physiological level *in vivo*) and 0.6 g/L (lowest human physiological level *in vivo*) for 2 weeks.

The experimental results presented in Fig.6 show that there are significant differences in the K-562 proliferation in the 3D micro-bioreactor and the 2D culture. The lower proliferation in the 3D system can be attributed to (1) possible nutrient and oxygen transfer limitations in certain parts of the micro-bioreactor, especially after the formation of cell bulks and (2) the consumption of cellular energy for the interaction with the collagen matrix, i.e., production of signaling molecules such as shock proteins and chemokines. In both the 2D and the 3D system, glucose is identified as the limiting factor that highly affects the kinetic evolution of K-562 only under hypoxic conditions.

These observations are of importance when applying chemotherapy *in vitro*. Most chemotherapy agents generally applied for the treatment of patients with AML are targeting highly proliferative cells. Therefore, under oxidative or glucose stress or in a 3D microenvironment, cells with a slower proliferation may be less susceptible to chemotherapeutics.

This quantitative information can be readily incorporated in the pharmacokinetics/pharmacodynamics and the cell cycle part of our model.

7. Conclusions and future perspectives

An integrated framework was presented for the optimal design of chemotherapy treatment strategies in leukemia, featuring *ex vivo*, *in vivo*, *in vitro* and *in silico* components. A predictive tool for the optimization of chemotherapy delivery was developed which *a priori* suggests patient-specific treatments with outcomes better than those resulting from current clinical protocols. The application of our established GSA

framework emphasized the need to determine accurately cell cycle parameters. Experimental monitoring of the cell cycle kinetics *in vitro* provided the most significant parameters needed *in silico* to predict growth kinetics in leukemia. With regards to the production of healthy blood, a self-contained bioreactor with promising RBC expansion capabilities *in vitro* was constructed. In preparation for large scale artificial blood production, optimization of the bioreactor superstructure defined the optimal physical bioreactor layout in order to minimize the cost of producing one unit of blood.
Future work will focus on elucidating key mechanisms/factors of genetic or proteomic/metabolomics that affect the evolution of normal and abnormal blood expansion. Quantitative information on these key mechanisms will serve as an appropriate input for the construction of more detailed predictive models for the *in silico* description of healthy and diseased blood evolution. Quantification of appropriate intra-cellular biomarkers that are related to the blood *in vitro* kinetics can enable the combination of macroscopic kinetics with microscopic information leading to the construction of more detailed models of grey or white box nature.

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