

## REVIEW

# Generation and evaluation of input values for computational analysis of transport processes within tissue cultures

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## Abstract

Techniques for tissue culture have seen significant advances during the last decades and novel 3D cell culture systems have become available. To control their high complexity, experimental techniques and their Digital Twins (modelling and computational tools) are combined to link different variables to process conditions and critical process parameters. This allows a rapid evaluation of the expected product quality. However, the use of mathematical simulation and Digital Twins is critically dependent on the precise description of the problem and correct input parameters. Errors here can lead to dramatically wrong conclusions. The intention of this review is to provide an overview of the state-of-the-art and remaining challenges with respect to generating input values for computational analysis of mass and momentum transport processes within tissue cultures. It gives an overview on relevant aspects of transport processes in tissue cultures as well as modelling and computational tools to tackle these problems. Further focus is on techniques used for the determination of cell-specific parameters and characterization of culture systems, including sensors for on-line determination of relevant parameters. In conclusion, tissue culture techniques are well-established, and modelling tools are technically mature. New sensor technologies are on the way, especially for organ chips. The greatest remaining challenge seems to be the proper addressing and handling of input parameters required for mathematical models. Following Good Modelling Practice approaches when setting up and validating computational models is, therefore, essential to get to better estimations of the interesting complex processes inside organotypic tissue cultures in the future.

**ABBREVIATIONS:** CHO, chinese hamster ovary cells; CPPs, critical process parameters; CQA, critical quality attributes; DTs, digital twins; ECM, extracellular matrix; MOC, multi-organ-on-a-chip; OC, organ-on-a-chip; OTR, oxygen transfer rate; PAT, process analytical technologies

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**KEYWORDS**

computational models, experimental techniques, fluidics, organotypic tissue cultures, transport processes

## 1 | INTRODUCTION

'Organotypic tissue culture' refers to a micro-structured cell culture under *in vitro* conditions that consider the three-dimensionality (3D) and the physiology of the tissue. The ultimate goal is to create a vital tissue with organ-specific structure and functionality, mimicking the complexity of the cellular microenvironment *in vivo*. Areas of application mainly cover basic research (e.g., stem cell research, tumour models), pharmacology and toxicity tests, regenerative medicine (tissue engineering for implantation), and *in vitro* meat, among others [1, 2]. Techniques for tissue cultivation have seen significant advances during the last decades and novel 3D cell culture systems have become available, for example, 3D organoids or organ/body-on-chip systems [3–6].

A tissue culture construct is mainly composed of cells, biomaterials/carrier systems and solutes, which are cultivated in a bioreactor. Bioreactors allow to control micro-environmental parameters and recapitulate tissue-specific cell–cell contacts or cell–matrix interactions, mimic gradients with respect to oxygen, nutrients, waste products or pH, diffusion of regulatory molecules, internal and external mass transfer (e.g., oxygen, nutrient, and waste materials transfer), as well as different kinds of stimulation (mechano-, electro-, thermos- and magneto-transduction) [1, 7]. The function of the cells in a tissue culture is strongly governed by fluidics and transport processes. Therefore, understanding the principles behind these transport phenomena is necessary for the targeted development of the tissue culture. To describe the internal actions of the cell in the tissue culture, as accurately as possible, biological and biochemical aspects, as well as engineering parameters such as internal and external mass transfer, fluid velocity, shear stress, have to be considered [8].

Due to the high complexity of such culture constructs, long lasting experimental techniques, modelling, and computational tools have to be combined to link different parameters to process conditions and critical process parameters (CPPs). If successful, this can allow a rapid evaluation of the product quality by means of Critical Quality Attributes (CQAs) [9]. Furthermore, modelling and computational tools can be integrated into Digital Twins (DTs), a virtual replica of a real process, service, or system [10]. DTs can help to anticipate potential issues and curb risks by testing new functionalities before rolling

them out or they can be used in real-time in parallel to the actual product for internal control. Hence, DTs are valuable tools for describing and understanding cellular reactions (e.g., mechanistic kinetic DTs), setting up meaningful and time-saving experiments (e.g., digital twin-assisted Design-of-Experiment), or designing and optimizing bioprocesses by digital twin-based control strategies. This approach is already well established for cell-based production of biopharmaceuticals [10, 11]. For tissue engineering, the potential has been recently uncovered [12, 7].

The results of both mathematical simulations and DTs are critically dependent on correct input parameters. Errors in input parameters can lead to dramatically wrong conclusions. However, acquiring accurate input parameters requires challenging techniques. Experimental techniques comprise analytic methods including on-line and off-line sensors have to be meticulously implemented. These techniques are used for characterization of culture systems (oxygen transfer, diffusion, permeation, flow, shear, mechanical load, etc.), and determination of cellular parameters (oxygen uptake rate, substrate uptake rate, metabolite production rates, etc.).

This review provides an overview, how transport processes within tissue cultures can be measured and summarizes state-of-the-art techniques and remaining challenges for input generation to be used in comprehensive computational analysis.

## 2 | RELEVANT ASPECTS OF TRANSPORT PROCESSES IN TISSUE CULTURES

Tissue cultures—like many other systems that deal with cell cultivation—entail several phenomena in which transport processes, mainly mass and momentum transfer, represent a key element in tissue development and maintenance (an overview of respective techniques is given in Table 1). The mass transfer controls how nutrients and growth/stimulating factors are transported to the tissue, and how waste products are removed and cells function in the tissue.

To create a functional tissue, the maintenance of mass transport and the precise and reproducible control of environmental conditions are essential [1]. Cells, especially stem cells, are known to respond to a variety of regulatory

**TABLE 1** General overview on techniques for organotypic tissue culture (adapted from [7])

Application	Techniques and devices
Cell expansion (2D, monolayer)	<ul style="list-style-type: none"> <li>Well-plates</li> <li>Flasks/roller bottles/multi-tray systems</li> <li>Microcarrier for suspension culture (spinner, shake flask, stirred bioreactor)</li> </ul>
Tissue generation and maturation (3D)	<ul style="list-style-type: none"> <li>Non-scaffold-based (e.g., hanging drop microplates, spheroid microplates, magnetic levitation, Multi-Organ-Chips, aggregate culture in suspension culture)</li> <li>Scaffold-based, for example, synthetic and biological macroporous carriers, hydrogels or de-cellularized matrices (e.g., micropatterned surface microplates, transwell membrane systems, Multi-Organ-Chips (“micro-bioreactors”), tissue specific bioreactors (“macro-bioreactors”))</li> </ul>

peptides/proteins in different developmental stages (e.g., differentiation, proliferation), different fate decisions (e.g., self-renewal vs. lineage specification) as wells as to gradients of biomolecular concentrations and cell–cell and/or cell–matrix interactions (for references see [7]). These regulatory proteins are also produced and secreted by the cells, generating feedback loops. The required tissue architecture to allow for these regulatory loops can be achieved by scaffold-free or scaffold-based techniques. Scaffolds are especially used in more sophisticated bioreactor systems (reviewed in [7]), which create an appropriate biomimetic microenvironment, recapitulate tissue-specific cell–cell contacts or cell–matrix interactions, mimic gradients of oxygen, nutrients, waste products or pH, and modulate diffusion of regulatory molecules. One of the major challenges in this regard is controlling the diffusion rates of growth factors, proteins, and other molecules for cell regulation and a careful exchange of nutrients and gases under spatial control [13–15].

Besides mass transfer, other transport processes like momentum and fluid flow are also important as they influence cell development. Shear stress below detrimental levels stimulates cells mechanically and can affect cell differentiation and proliferation [16, 17]. Compared to static conditions, perfusion can, for instance, enhance calcium and collagen production [18, 19], or increase mineralized matrix production by bone marrow cells [20–22] while decreasing chondrocyte-driven cartilage production [23, 24]. Mullender et al. [25] demonstrated that the perfusion rate can change cell responsiveness as well. Furthermore, it was shown that the cell response to gradients of shear stress differs from that of the magnitude of shear stress

[26, 27], as well as temporal and spatial variations [28]. Therefore, it is necessary to determine the precise shear stress stimuli that the cells actually experience to understand complex cell responses to it.

For appropriate design and operation of organotypic tissue cultures, not only a profound biological understanding of the tissue/organ of interest is required, but also an extensive “engineering” knowledge is necessary on special materials that enhance cell immobilisation (biocompatibility, biodegradability), special culture systems (bioreactors), including flow dynamics, mass transfer [29], shear effects (as discussed above), as well as control and cultivation strategies [8]. This creates diverse intercellular signaling networks and a complicated process, which is also manifested in *in vitro* culture of tissue constructs, most of them comprise dynamic non-linear interplays between several components. This makes it very difficult to distinguish individual mechanisms in order to control the local microenvironment and the functional development of the tissue construct.

### 3 | COMPUTATIONAL METHODS

Due to the high complexity of the microenvironment and flow dynamics, especially in micro-bioreactors, it is very difficult to design controlled experiments. Computational models are therefore valuable tools to gain a comprehensive understanding of this complex biological systems. *In silico* models and DTs of the real system can be used for cell culture system design (bioreactors), simulation and analysis of mass transfer (nutrient supply, removal of metabolites deposits). Furthermore, this can also result in replacement or reduction of *in vivo* experiments complying with the reduction, refinement, and replacement principles [30, 4–33].

Developing an *in silico* model often starts with general formulations and interpretations of previous experiments and hypotheses, intending to figure out fundamental mechanisms and their interactions. The more key parameters and important partial processes can be identified the more representative the model setup comprises of. The outcome is a set of equations that describe how the quantity of interest (e.g., metabolite concentration) changes with time and environmental process parameters (e.g., temperature and pH). Different mechanisms (e.g., physicochemical phenomena) can then be studied to design or optimize tissue engineering bioreactors. Furthermore, mathematical modelling and computational tools can be used to discover the interconnection of complex regulatory processes, investigate the systematic influence of perturbations for optimal engineering design, develop new hypotheses, and ultimately evaluate the functionality

**TABLE 2** Aspects addressed by mathematical modelling with respect to tissue culture (adapted from [7], for references see [7])

Aspects addressed by mathematical modelling	
Biological	Physico-chemical
Cell growth <sup>a</sup> , morphology, heterogeneity, dynamics	Culture conditions <sup>a</sup> , cell handling, cell seeding
Cellular kinetics <sup>a</sup>	Biomechanical effects (e.g., shear stress) <sup>a</sup>
Signaling networks, regulatory networks, cell–cell and cell–matrix interactions	Penetration <sup>a</sup> , permeation <sup>a</sup> , diffusion <sup>a</sup>
Tissue specific effects	Fluid dynamics <sup>a</sup> , mass transfer <sup>a</sup> (bioreactors, scaffolds)
Pharmacology	Chemical, physical and mechanical stimulation <sup>a</sup>

<sup>a</sup>Related to computational analysis of transport processes.

of specific molecules for therapeutic applications [34, 35]. The simulation approach provides us with an opportunity to probe different scenarios and perform parametric studies of the hypothesized model. The results of such computational experiments are then compared to known experimental facts for validation of the model. Afterwards, the validated model may be used to optimize certain aspects of the studied system. Some demonstrated examples of this approach relevant to tissue engineering include optimizing construct and scaffold geometries as well as fluid flow patterns in bioreactors [36–39]. Additionally, dynamic modelling can be a predictive tool for improving experimental design protocols.

Since different affecting variables, such as shear stress, are nearly impossible to measure due to the complex geometry of bioreactors, computational fluid dynamics (CFDs) emerged as a remedy. CFD can simulate fluid flow in freely definable scaffolds and bioreactor geometries [40, 8–44]. Given enough computer resources, high-resolution simulation on micro-computed tomography ( $\mu$ CT) reconstructed geometries can achieve precise flow distribution throughout the microstructure [45, 46]. Computational modelling is becoming the core of the development of the next generation of bioreactors and scaffolds, as it allows an enhanced combined consideration of biological and engineering effects for characterization of the system and the cellular response [9]. To complete the simulation using CFD, dynamic cellular parameters, such as cell growth and proliferation, are required to capture their effects on shear stress distribution. Different aspects that can be addressed by mathematical modelling of tissue cultures are reviewed in [7] and summarized in Table 2.

In the field of computational biomechanics, the finite element method (FEM) is by far the most common

numerical discretization and solution technique. The main strengths of FEM are its ability to work with complex geometries, its well-developed mathematical background that provides a consistent way to treat material inhomogeneities and different constitutive equations for the material behavior, as well as the high availability of solvers [35, 47–49]. Although there are several examples of *in silico* studies, there are still challenges for using their full potential. One of the critical challenges of biological systems is the connection of simultaneous interrelated phenomena in different scales and unknown system importance. The ensuing multi-disciplinarity makes it difficult to derive mathematical equations and find solutions. Therefore, multi-scale methods are used to take care of individual cell interactions in micro-scales and holistic behaviors in macro-scales. There also exist meso-scale methods, for example, Lattice Boltzmann method (LBM) [50], which work in between the scales mentioned above [51, 52]. Due to the kinetic nature of the LBM, microscopic interactions in the system can be handled in porous media and other complex geometries [50]. Moreover, the inherently local dynamics used in LBM allow an efficient implementation to exploit the computational power of present and emerging supercomputing architectures [53]. Besides such algorithmic advantages, LBM shows high versatility for multi-physics simulation and integration with tomographic measurement techniques as evident by works on perfusion flow in scaffold bioreactors [54] and even by patents for medical image analysis [55].

In general, model generations should follow Good Modelling Practice approaches and explicit workflows [56]. As a good example, the USA Food and Drug Administration (FDA) has provided standard technical guidelines [57] that need to be followed for digital tools to be used as medical devices. Several additional steps are necessary to utilize the model as a digital tool: verification, validation, and uncertainty quantifications [12]. Here, verification refers to the agreement between the results and the mathematical formulations, while validation is the establishing agreement with the experimental results. Uncertainty quantification should also be applied to make sure that the method is based on correct assumptions, is built correctly, and has already considered the effect of input uncertainty on the results. Other remaining challenges in the widespread application of *in silico* models in bioprocess technologies include; user-friendliness of tools and user acceptance, precise documentation for a unified process modelling implementation, and generation of valid input variables for *in silico* models. The latter has been surprisingly overlooked in research as will be highlighted in Section 4, most likely due to the common misconception that the iterative nature of mathematical models can eventually generate accurate results. Therefore, there is an

ever-increasing necessity to emphasize and discuss different techniques for precise data generation.

## 4 | TECHNIQUES FOR GENERATION OF INPUT VARIABLES FOR COMPUTATIONAL MODELS

### 4.1 | Sensor techniques

Sensors are essential tools for marker-free, continuous, on-line or at-line monitoring and control of cell metabolism and microenvironment parameters (e.g., temperature, pH, pressure, fluid flow, oxygen tension, metabolites, impedance, regulatory molecules, short-lived reactive species, shear stress, electrical pacing, mechanical properties). They deliver information beyond microscopy techniques and end-point tests [58–60], and have to be ‘able to detect events or changes in their environment at low concentrations under a complex matrix, which also permits multi-parametric analyses at the same time with continuous monitoring and fast response at low cost’ [61].

Whereas these techniques help to investigate tissue cultures in the first place, they are indispensable in standardizing techniques that are routinely required for CQAs. Furthermore, they provide the data required for the determination of cellular metabolic parameters (Section 4.2) and physicochemical process parameters for the characterization of culture systems (Section 4.3). Therefore, sensor readings are closely coupled and correspond with good mathematical modelling practices of fluid dynamics and mass transfer effects in culture devices (Section 3). An overview of sensor techniques is given in Table 3. For a detailed explanation of the working principles, pros and cons, and specific applications, see [58, 59, 62, 63].

In recent years, the focus has been on integrating sensors in microfluidic systems (e.g., multi-organ chips) to access parameters of cellular metabolism as well as morphological effects [58, 60, 62, 64–65], so far limited to extracellular readings providing indirect information about the metabolic state of the cells [58]. Future directions can focus on characterization of complex cell–cell and cell–matrix interactions and real-time quantification of small molecules uptake and release as well as protein expression, as these are essential input parameters for computational models (compare Section 4.2) [58, 59]. Furthermore, probing and monitoring of relevant key organ functions using tissue-/organ-related biosensing strategies has to be established [62]. Limitations that have to be overcome for establishing routine applications include high complexity of fabrication technology and operation, as well as incompatibility with standard procedures [58, 59, 65, 66].

### 4.2 | Cellular metabolic parameters relevant for modelling

Cell-specific metabolic parameters such as uptake rates for oxygen and substrates, production rates for metabolites and carbon dioxide, as well as growth and death rates, are essential variables for computational models addressing fluid dynamics, mass transfer effects and metabolic activities in tissue cultures. Whereas methods for determining such variables are well-established for biopharmaceutical cell culture using mostly established cell lines [57], this is hardly the case for tissue cultures for several reasons. An essential difference is that tissue cultures are mostly generated from primary cells and not well-established cell lines. For the latter (e.g., Chinese Hamster Ovary [CHO] cells), a stable metabolism can be assumed over a large number of passages. Furthermore, most of these cell lines have been adapted to growth in suspension. Therefore, a sufficient number of cells can be produced to perform experiments under appropriate, reproducible culture conditions, for example, in well-controlled bioreactor systems. Hence, it is possible to determine reliable data and investigate the impact of culture conditions on these parameters, for example, the relationship between metabolic rates and concentrations of oxygen, substrates or metabolites. From such data, thorough metabolic models can be formulated [67].

In the case of primary cells, however, determination of reliable data is more challenging. In most cases, just a small number of primary cells can be obtained from a biopsy of interest. Protocols for the proliferation of tissue cells have mainly been established only for adherent cells. This, in principle, allows to generate a large number of cells within several passages, even though it makes cultivation in controlled bioreactors a lot more challenging. More importantly, cellular properties and metabolic activities change significantly during the first passages. Therefore, data generated for cells in a higher passage number hardly reflect freshly isolated primary cells or even cells *in vivo*. Furthermore, cellular and metabolic parameters are donor-specific [68–71]. Due to the problems described for isolated primary cells, cell-specific metabolic parameters have often been determined using immortalized cell lines such as HepG2, HepaRG, HaCaT, hMSC-tert cells, etc. (see below).

To make matters worse, tissue engineering is often aimed to obtain functional organotypic tissues that consist of several cell types within an appropriate extracellular matrix (3D, organoid, etc.). Under such conditions, cellular properties and metabolic activities are different to those in monolayer flask cultures. Owing to the difficulty of detecting the exact cell number required to calculate

TABLE 3 Overview on sensor techniques for tissue culture devices (adapted from [58, 62], modified)

Parameter	Sensor type
<i>Cell culture supernatant</i>	
Oxygen	Direct amperometric sensors Clark-type sensors Optical sensors (luminescence/fluorescence)
pH	Light addressable potentiometric sensors (LAPS) Ion-selective field effect transistors (ISFET) Metal oxide-based potentiometric sensors Optical sensors (luminescence/fluorescence)
Glucose	Electrochemical (enzyme based) biosensors Optical biosensors Electrochemical multi-electrode arrays (MEA)
Lactate	Electrochemical (enzyme based) biosensors Optical biosensors
Glutamate	Electrochemical (enzyme based) biosensors
Pyruvate	Electrochemical (enzyme based) biosensors
Reactive oxygen (ROS) and reactive nitrogen species (RNS)	Microsensor approaches in combination with electrochemical sensing techniques (ultramicroelectrode (UME) arrays, planar sputtered gold/cytochrome c electrodes, pyramid-shaped Pt tip electrodes)
Proteins	Immunosensors
<i>Cellular properties</i>	
Cell density, cell morphology, marker secretion	Electric Cell-Substrate Impedance Sensing (IDEs (Interdigitated Electrodes)), FETs (field-effect transistor), ISFETs (ion-sensitive field-effect transistor (ISFET))
Tissue morphogenesis and maturation	Pillar deformation (fluorescence microscopy)
Mechanical strain of cellular barrier	Resistivity changes in impedimetric coplanar electrodes.
Barrier function and integrity, tight junction formation, cell growth and differentiation	Transepithelial/transendothelial electrical resistance (TEER) measurements
Barrier integrity	Transconductance measurements
Myokine secretion	Myokine concentration measurement by functionalized gold electrodes
Cardiac beat rate	Multi-electrode array and atomic force microscopy measurements Voltage and displacement current measurement by large area electrodes Cantilever array

cell-specific parameters in 3D or organoid cultures, cell-specific metabolic parameters can often be determined as volume-specific parameters only. For determination of volumetric rates, for example, in organoid cultures or multi-organ chips, specific methods have to be established due to the small volumes [8]. Furthermore, gradients of, for example, oxygen or substrates/metabolites, have to be foreseen, making it more difficult to investigate the impact of culture conditions on these parameters. Often, these variables are determined as adaptation parameters in mathematical models for flow/metabolism (see below).

In the following example, aspects related to the determination of cell-specific metabolic parameters are explicitly examined to determine cell-specific oxygen uptake rates. Further metabolic rates are only considered briefly.

Oxygen has a tremendous impact on the cellular and metabolic activity of cells. Therefore, it is essential to gen-

erate an appropriate microenvironment with regard to cell-specific oxygen concentrations. For example, in cartilage, liver or stem cells, lower oxygen concentrations seem to be advantageous with respect to the metabolic activity [72, 73]. With respect to mass transfer effects within 3D or organoid cultures, oxygen is usually regarded as more relevant than other medium compounds such as glucose or glutamine. This is often attributed to the poor solubility of oxygen in the culture medium, which restricts the diffusive penetration depth of oxygen within tissues in vivo in the range of only 100–200  $\mu\text{m}$  [8]. In vitro, this is even worse, as artificially generated tissues are usually not vascularized, calling for anticipating severe oxygen gradients or even oxygen limitations. Therefore, the oxygen concentration adjustment is a critical matter in the design of any tissue culture system. But as exact sensory determination of the oxygen concentration within tissue constructs is still

difficult and not well-established (compare Section 2), oxygen profiles can often only be estimated through mathematical modelling, which itself depends on reliable values for cell-specific oxygen uptake rates.

Methods used for the determination of parameters related to oxygen consumption are summarized in Table 4. These are mostly related to liver/HepG2 cells, due to the intensive research on cell-based extracorporeal liver support systems and oxygen distribution within the respective bioreactors. Selected examples for other tissue types have been added. The values themselves are deliberately not provided, as the focus of this review is on the respective methods. Furthermore, values reported in the literature often show a considerable deviation. As an example, for the uptake rate of liver cells, reported values fluctuate by a factor of 25–30 [74]. This is probably due to different methods and environmental conditions (cell density, growth phase, substrate concentrations, 2D/3D) that have been applied. Most references referring to bioreactors have applied macro-bioreactors. Only a few studies used the terminology to refer to micro-bioreactors or organoid cultures, even though techniques for monitoring and controlling oxygen within such systems have been described [75–79].

In respect to the relationship between the volumetric oxygen uptake rate and the oxygen concentration most studies cited in Table 4 refer to the work of Haselgrove et al. in 1993 [80], where an equation following Michaelis-Menten-Kinetics was used:

$$\text{OUR} = q_{\text{cell}} \cdot N_{\text{cell}} \cdot \frac{c_{\text{O}_2}}{k_m + c_{\text{O}_2}} \quad (1)$$

OUR: oxygen uptake rate ( $\text{mol L}^{-1} \text{h}^{-1}$ );  $q_{\text{cell}}$ : cell-specific oxygen uptake rate ( $\text{mol cell}^{-1} \text{h}^{-1}$ );  $N_{\text{cell}}$ : cell density ( $\text{cells L}^{-1}$ );  $c_{\text{O}_2}$ : oxygen concentration ( $\text{mol L}^{-1}$ );  $k_m$ : Michaelis-Menten-constant ( $\text{mol L}^{-1}$ ).

Even if the assumptions of Equation (1) are reasonable, actual verification is missing. As the exact determination of  $N_{\text{cell}}$  is often difficult for 3D culture,  $q_{\text{cell}} \cdot N_{\text{cell}}$  has been determined as the volumetric parameter instead ( $\text{mol L}^{-1} \text{h}^{-1}$ ). This problem in particular applies to microfluidic cell culture devices with respect to real-time monitoring of cell densities and oxygen concentration [77, 81].

With respect to cell-specific metabolic rates as input parameters for growth, substrate consumption or metabolite production in mass transfer models, mostly glucose as the substrate and lactate as the metabolite are considered, with some focus on the relevance of other compounds such as glutamine, ammonia or carbon dioxide [8]. Determination of cell-specific substrate uptake and lactate production rates is usually based on time-dependent analysis of the concentrations of the respective compounds, as well as the

cell numbers. The latter can further be used for calculation of cell-specific growth rates.

Even though the relevance of the abovementioned parameters and their tremendous impact on culture conditions is well known from established cell lines, extensive studies on tissue cells addressing these effects are rare. To some extent, such studies have been performed for monolayer cultures [82, 83], but hardly for 3D and organoid cultures. Techniques for determination (compare Section 2) and long-term monitoring [75, 84] of glucose and lactate for such complex systems have been established, and challenges related to the precise 3D determination of cell number for organoid culture have been discussed before.

Several studies exist on modelling of cell growth, glucose uptake and lactate production in tissue cultures [37, 83, 85–90], in most of which values for cell-specific growth, uptake and production rates were either taken from literature or determined by parameter estimation (similar to oxygen uptake rates; see above). Particularly, noteworthy in this context is the work of Higuera et al. [83], as they compared, measured and simulated values.

In conclusion, the above discussion reflects the problems in determination of reliable cell-specific metabolic parameters. This has to be taken into account if these parameters are applied in mass transfer models. Likewise, sensitivity analyses should be included, and discussion on how required simplifications and assumptions in the modelling have an inevitable influence on the adjustment parameters.

### 4.3 | Physico-chemical process parameters relevant for modelling

Several engineering parameters are relevant for modelling of mass transfer effects and fluid dynamics in tissue culture. For transport mechanisms, oxygen transfer rate, permeability, and diffusion coefficients are addressed. Fluid flow is mainly influenced by shear distribution, scaffold structure, and porosity of the scaffold. Respective techniques will be discussed in the following.

#### 4.3.1 | Oxygen transfer rates

As already highlighted in Section 4.2, oxygen transfer is a matter of utmost importance for tissue cultures due to the low solubility of oxygen in the culture medium and the tremendous impact of the oxygen concentration on the physiology of tissue cells [76, 8, 91, 92]. Naturally, the oxygen requirement of cells (volume-specific oxygen uptake rate, OUR) must be in accordance with the oxygen supply (oxygen transfer rate; OTR). For aeration of tissue cul-

**TABLE 4** Comparison of methods used for determination of parameters related to oxygen consumption based on suggestions by Weise et al. [74], extended; “*n*”: Specification of the number of samples, if this is evident from the publication

<b>Refs.</b>	<b>purpose</b>	<b>Cell type</b>	<b>Method for determination of OUR and/or <math>k_m</math></b>	<b>Verification</b>	<b>Comment</b>
[124]	Kinetics of oxygen metabolism in brain and liver tissues	Isolated tissue (liver, brain)	Whole organ consumption (volume specific), comparison of different kinetics, parameter adaptation and calculation of cell-specific rates	Mean values + standard deviation	The results imply that the apparent kinetics for oxygen consumption in tissue differs from the kinetics for mitochondrial suspensions.
[125]	Gradients of O <sub>2</sub> concentration in hepatocytes	Isolated rat hepatocytes (primary)	Polarographic assay (compare [126]), measured 1 h after preparation	Mean values + standard deviation cell-specific uptake rate: $n = 6$ ; $k_m$ : $n = 3 - 20$	Cell-specific oxygen uptake rates and $k_m$ determined
[127]	Oxygen uptake rates in cultured rat hepatocytes	Rat hepatocytes	OUR experimentally determined. Perfusion system based on T-75 tissue culture flasks. DO determined at inlet and outlet flow. Calculation of OUR from a simple mass balance depending on flow rate. $k_m$ n.d.	<i>t</i> -test at a significance level of 0.05; experiments were performed twice with two to three measurements per experiment	OUR of hepatocytes in culture may vary depending on the phase of culture (i.e., early vs. late) and on the extracellular environment
[128]	Metabolic activity of Hep G2 cells and primary rat hepatocytes were compared during in vitro application of a gel entrapment bioartificial liver.	HepG2 Rat hepatocytes (primary)	DO determined at inlet and outlet flow of a modified hollow fibre bioreactor with cells embedded in gel. Calculation of OUR from a simple mass balance depending on flow rate. $k_m$ n.d.	Mean values + standard deviation Hep G2: $n = 4$ ; rat hepatocytes: $n = 16$	Data system specific
[129]	Method for determining oxygen consumption rates of static 2D-cultures	Rat hepatocytes	Device to determine the dependence of OUR on oxygen partial pressure for anchorage-dependent cells cultured in standard culture dishes.	Mean values + standard deviation $n = 4-6$	Proof-of-concept for the technique, further studies of the effect of factors such as extracellular matrix composition, metabolic substrate, and drugs on the dependence of OUR on oxygen partial pressure suggested

(Continues)



TABLE 4 (Continued)

Refs.	purpose	Cell type	Method for determination of OUR and/or $k_m$	Verification	Comment
[130]	Techniques for measurement of oxygen consumption rates of hepatocytes during attachment and post-attachment	HepG2 Rat hepatocytes	Oxystat apparatus for HepG2 cells immobilized on Cytodex 3 microcarriers, flow cell for rat hepatocytes immobilized on single collagen layers, two-compartment oxystat for rat hepatocytes during the attachment phase.	n.d.	OUR decreased with increasing cell density. The OUR measured for rat hepatocytes during and post-attachment are significantly higher than those reported elsewhere (compare e.g., [74])
[131]	Oxygen uptake rate (OUR) of hepatocytes as parameter for the design of bioartificial liver assist (BAL) devices.	Porcine hepatocytes	Special culture chamber for monolayer culture with integrated DO-sensors. Measured oxygen tension versus time, parametric data was processed off-line to generate OUR versus oxygen tension curves.	Mean values + standard deviation $n = 12$	Examination of the $OUR_{max}$ and $k_m$ revealed different cellular metabolic states for the initial attachment phase and the subsequent steady-state culture condition.
[132]	Investigation of oxygen transfer to cultured hepatocytes in microchannel parallel-plate bioreactors with and without an internal membrane oxygenator with a mathematical model	Rat hepatocytes	Model calculations for different values of Pe and Da-number. Da includes volume specific oxygen uptake rate. Cell-specific oxygen uptake rate not given. $k_m$ taken from [129]	Results were confirmed by fluorescence imaging experiments.	Model calculation verified qualitatively.
[133]	Investigation on the role of oxygen in modulating cellular functions under steady state oxygen gradients in cell culture.	Rat hepatocyte (primary)	Model of oxygen transport in a flat-plate reactor to estimate oxygen distribution at the cell surface. Modelling parameters for $OUR_{max}$ and $k_m$ taken from [129]	Experimental measurements of outlet oxygen concentration from various flow conditions were used to validate model predictions	Model calculation verified quantitatively.

(Continues)

TABLE 4 (Continued)

Refs.	purpose	Cell type	Method for determination of OUR and/or $k_m$	Verification	Comment
[94]	Development of a method for determining oxygen consumption rates of static cultures from microplate measurements of pericellular dissolved oxygen concentration	Primary rat hepatocytes	Protocol based on a non-invasive oxygen-sensing microplate and a simple mathematical model derived from Fick's Law. Data for a large number of mammalian cells and microbes. $k_m$ n.d.	Cell-specific oxygen uptake rate determined from measured volumetric oxygen uptake rate and cell density. $n = 5$ for method development, standard deviation indicated graphically	Method development. OUR data for hepatocytes compared to own studies (not given)
[103]	Numerical model for investigation of fluid flow and oxygen transport and consumption in the AMC Bioartificial Liver	Hepatocytes, not specific	Values for OUR and $k_m$ taken from [131]. Those were determined for 2-D culture	Not verified experimentally.	Parameter studies for different configuration
[74]	Analysis and comparison of oxygen consumption of HepG2 cells in a monolayer and three-dimensional high density cell culture	HepG2	Measurement of DO by optical sensors in 2D culture and within a hydrogel Model for oxygen transfer and consumption	Model validation by experimental data	Comparison of evaluated data in the literature with own measurement data
[99]	Analysis of oxygen transport in microfluidic bioreactors	Intended for culture of hepatocytes and cardiomyocytes	Mathematical model to determine oxygen transport within microchannel parallel plate bioreactor with and without an internal oxygen-permeable membrane was developed. Oxygen diffusion in both directions, including parallel to the flow direction and perpendicular to the flow direction due to the cellular oxygen uptake were considered. $OUR_{max}$ from [127], $k_m$ neglected	Model not verified experimentally. No sensitivity analysis regarding $OUR_{max}$ .	Values for $OUR_{max}$ have been experimentally determined for a different culture system.

(Continues)

TABLE 4 (Continued)

Refs.	purpose	Cell type	Method for determination of OUR and/or $k_m$	Verification	Comment
[100]	Investigating oxygen transport efficiencies in precision-cut liver slice-based organ-on-a-chip devices	Mouse liver slices	The transport of nutrients in the simulation was described by the generic diffusion. The consumption of oxygen was calculated with Michaelis-Menten reaction kinetics. Furthermore, to account for cellular processes such as necrosis, a factor was implemented to eliminate the reaction term for the cellular consumption by going to zero below a critical DO. $OUR_{max}$ and $k_m$ taken from [124]	Model not verified experimentally. No sensitivity analysis regarding $OUR_{max}$ .	The optimal device architecture derived from the modelling was then fabricated and its operation confirmed with an LDH assay.
[80]	Computer modelling of the oxygen supply and demand of cartilage cells	Isolated chicken chondrocytes	OUR experimentally determined and plotted versus DO, parameter estimation for Michaelis-Menten-kinetic	Qualitatively discussed	OUR decreases at low oxygen tensions. The metabolism of chondrocytes is not controlled simply by the available oxygen supply [134].
[135]	Modelling of oxygen uptake with three-dimensional chondrocyte pellets	Cartilage cells from mini-pig (knee and elbow)	Oxygen uptake rate of a single pellet measured in a special designed reaction chamber. Mass balance equations for determination of oxygen profile in chondrocyte pellets	Diffusion coefficient as fitting parameter	Cell-specific oxygen uptake rate determined for suspended chondrocytes
[136]	Real-time monitoring of specific $o$ in a microfluidic cell culture	Embryonic stem cells	Monitoring of cell growth from phase contrast microscopy images, and of respiration using optical sensors for dissolved oxygen connected to inlet and outlet of the microfluidic culture device. <b>Calculation of OUR from a simple mass balance depending on flow rate.</b>	n.d.	Label-free and real-time monitoring of oxygen concentrations and cell proliferation

ture systems, one or a combination of the following methods can be applied, depending mainly on the size of the culture system: surface aeration, direct sparging, indirect and/or membrane aeration (diffusion), medium perfusion, increasing the partial pressure of oxygen, and increasing the atmospheric pressure [93]. Oxygen dissolved in the medium is mainly transported by diffusion within the bulk fluid phase, from the bulk to the surface of the organoid through the boundary layers, and subsequently reaches the bulk of the organoid. As the spatial distribution of oxygen within the boundary layer and within the bulk of the organoid can hardly be measured so far, mass transfer models that are applied here are based on diffusion (e.g., Fick's law).

Several techniques for determination of oxygen transfer rates are at hand in the case of surface aeration or direct sparging, which include the sulphite oxidation method, the dynamic method, the gassing out method (reviewed by [8]), and the membrane aeration [93] among others. Most of these methods have been developed and standardized for the stirred tank and similar bioreactors [74] or adapted to flask type cultures (well plates, T-flask) or shake flasks with surface aeration [94].

The exact experimental determination of OTR for tissue culture systems (mainly micro-bioreactors) has proven to be extremely difficult owing to the small size and the complexity of such systems, as well as the limited availability of techniques for measuring the oxygen concentration, as outlined in Section 4.1 [74, 95–98]. Hence, OTR is often considered within mass transfer models [46, 74, 92, 99–103].

#### 4.3.2 | Diffusion/permeability coefficients

As mass transfer in *in vitro* tissue organoids relies mostly on diffusion/permeation, proper determination of the respective transport coefficients is important. Diffusion is a transport process in which molecules move via Brownian motion in a volume or area, driven by the concentration gradient in the direction from higher to lower concentrations. Mathematically, it is usually described by Fick's law [104, 105]. Permeation is merely an aspect of diffusion, and while diffusion is related to the movement of molecules in a system, permeation describes how fast molecules move through a system, and is characterized by the permeability coefficient. Both parameters depend on the molecular weight of the compound and the properties of the medium or tissue, among others [104].

Within mass transfer models, mostly low-molecular-weight compounds such as oxygen, glucose, and lactate are considered for diffusion within the bulk medium surrounding a tissue organoid and within the organoid. In most cases, diffusion coefficients determined for diffusion

in water at medium temperatures are applied. For the bulk medium, this seems to be a sensible approach. For the tissue organoid itself, however, the respective properties should be considered. When hydrogels are applied as the scaffold material, this problem should be examined even more closely, as hydrogels consist of a water-containing polymer network, where the material transport takes place predominantly in the aqueous phase. For hydrogels, the polymer network turns out to be the actual barrier for molecular movement. Decisive factors for the diffusion of substances through a hydrogel are the nature and density of the cross-linked network and the shape and size of the particles. The water solubility and the surface charge also play major roles. In general, molecules migrate better and faster through a large-meshed, flexible network than through a small-meshed, rigid network. Likewise, molecules with a smaller hydrodynamic radius diffuse faster through hydrogels than large ones [106]. For larger molecules (approx. 4 kD), anomalous diffusion (subdiffusion) has to be expected, which cannot be described by Fick's law. This effect could be confirmed by Hsu et al. for *in vivo* skin models [107].

Aside from skin models/biopsies and cartilage, high molecular weight compounds such as growth factors or substances applied in penetration tests (e.g., skin models or drug testing) have hardly been considered in mass transfer models. Due to the high relevance of skin permeation following different mechanisms, mainly the transcellular, the intercellular and the follicular pathway [108], several methods have been developed to determine skin permeability, which will be summarized briefly in the following.

The Franz diffusion cell is a well-known device to measure the permeation of a substance through a tissue. Due to the usual size (height) of Franz diffusion cells, they are well established for skin biopsies but can hardly be applied for small tissue constructs [109]. Fluorescence recovery after photobleaching (FRAP) is a method to measure molecular diffusion in tissues or gels, mainly for high molecular weight compounds. For this, the substance must be labelled with a fluorochrome. Mostly fluorescence-labelled proteins or FITC-dextrans (fluorescein isothiocyanate-dextrans) with different molecular weight/sizes are used [110, 111]. Further imaging methods for the determination of diffusion of molecules in the skin are Fourier-transform-infrared (FTIR) spectroscopy, two-photon fluorescence correlation spectroscopy in combination with fluorescence correlation spectroscopy (FCS) and optical coherence tomography (reviewed by [15]). These methods are non-invasive and non-destructive but require expensive equipment.

As mentioned before, most methods for determining diffusion and permeability parameters have been developed for large biopsies and can hardly be applied for small-scale

tissue cultures. But this is indispensable if either multi-well test systems with several samples run in parallel or multi-organ chips are applied. Furthermore, most methods require treatment of the sample in one or the other way. Therefore, it is quite difficult to determine the time-dependent changes of diffusion and permeation. Hsu et al. developed a method based on fluorescence measurements to determine and simulate the permeation of high molecular weight compounds through a gel matrix in a multi-organ-chip and applied this to in vivo cultivated skin models [105, 112, 15].

### 4.3.3 | Porosity

The porosity of the scaffold—defined as the sum of the volumes of the pores divided by the sum of the volume of the scaffold material—can influence mass transport effects within the scaffold and by this the biological functionality and mechanical durability and is therefore important to be evaluated. Assessment techniques include theoretical approaches (e.g., unit cube analysis and mass technique), scanning electron microscopy (SEM), mercury porosimetry, gas pycnometry, gas adsorption, and flow porosimetry, all of which are associated with several limitations and pitfalls. A lack of sensitivity to characterize inconsistent/irregular architectures, destructiveness, and the necessity to perform complementary experiments are some of the more common drawbacks of the aforementioned techniques (reviewed by [113] and [114]). On the other hand, micro-CT has a key advantage over other approaches since it is a single, non-destructive technique that can be used in high-resolution modes to scrutinize the intricate micro-architecture of scaffolds, even if they contain irregular spatial layouts.

### 4.3.4 | Scaffold permeability

Successful tissue regeneration depends on the scaffold's ability to simultaneously allow nutrient diffusion, waste removal and to provide a sufficient mechanical environment. This is particularly mandatory to promote regeneration of bone tissue, where bone scaffolds should have a set of properties in terms of pore size and distribution, porosity ratio, tortuosity, interconnectivity, and specific surface area, among others. However, many of these parameters are interdependent and exhibit complex or even ambiguous results when it comes to their relation to mass transport and the biological performance of the scaffold [115]. For instance, even though larger pore sizes and higher porosity ratios in scaffolds are often ascribed to improved bone growth, some studies have reported

limited to no significant difference in bone regeneration capacity of scaffolds with varying porosity ratios, while the influence of pore size has been similarly a matter of debate [116].

To address these conflicting reports regarding the role of the design parameters, scaffold permeability has been introduced as a macroscopic parameter that indirectly accounts for all the aforementioned geometrical features. Methods of permeability evaluation are often classified into two general categories: experimental test benches and computational approaches (reviewed extensively in [117]). In experimental methods, the permeability is quantified in the system either by measuring the pressure drop under a constant flow rate or by measuring the volumetric flow rate for a given pressure difference applied to the scaffold. In purely viscous flows, that is, Darcy regime (negligible inertial losses; Reynolds number  $< 1$ ), the permeability  $K$  can be calculated by Darcy's law:

$$K = (Q\mu L)/(A\Delta P) \quad (2)$$

In Equation (2),  $Q$  is the volumetric flow rate,  $\Delta P$  is the pressure drop through the scaffold,  $A$  and  $L$  are the cross-sectional area and the thickness/length of the scaffold in the direction of flow, respectively, and  $\mu$  is the dynamic viscosity of the fluid. Here the impact of the individual molecule involved (size, charge, hydrophobicity) has to be considered as well. Additionally, permeability can be estimated indirectly by measuring geometrical features of the scaffold (e.g., pore size, porosity, tortuosity, etc.) and using semi-empirical models such as the Ergun–Forchheimer correlation [117]; in particular, when the assumption of the Darcy regime cannot be met.

One main challenge in the experimental assessment of scaffold permeability is a lack of widely shared, inter-laboratory protocols, making it nearly impossible to compare values obtained in different laboratories. Moreover, as the permeability evaluation is based on simultaneous measurement of multiple quantities (i.e., upstream and downstream pressure and flow rate), several sensors/transducers have to be implemented, which introduce inaccuracy to the system [118]. Critically, the sensitivity and the range width of these transducers have to be selected as a function of the permeability range. Another difficulty is raised from the fact that most experimental methods require high fluid pressures, large time intervals, and several cycles of measurement to ensure reproducible results [119, 120]. This can lead to irreversible deformation in the scaffold structure and induce obstruction of small pores, which in turn causes fluid entrapment inside the scaffold, another possible source of error.

An alternative way of determining the permeability coefficient of scaffolds is the computational simulation of fluid

flow dynamics [116]. By numerically solving partial differential equations that describe the fluid motion in different flow regimes, the permeability of the scaffold can be computed. For simulation, the geometrical 3D models of the scaffold can either be created from computer-aided design (CAD), or by image-based techniques such as micro-CT [121]. Since even small variations between the designed architecture and the manufactured scaffold can strongly influence the flow field [122], micro-CT has been deemed the gold standard for generating realistic 3D models of scaffolds, as well as measuring their geometrical characteristics (e.g., pore size and porosity) as input parameters for iterative processes [123]. Nevertheless, insufficient spatial image resolution can influence the accuracy of the 3D model and increase the surface roughness, which in turn would cause an underestimation of the computed permeability coefficient. On a more fundamental level, making accurate boundary conditions at different scales as well as incorporating material properties is a crucial challenge of numerical simulations.

## 5 | CONCLUDING REMARKS

The complexity of an in-depth characterization of fluidics and transport processes within organotypic tissue cultures requires the combination of experimental techniques, modelling, and computational tools. Tissue culture techniques are well-established, modelling tools are at hand, and sensor technologies are increasingly being integrated in culture devices. The main current problem is that mathematical simulations need valid material property values and correct input parameters, because errors in input values can lead to completely wrong predictions, even if the intermediate model is accurate. As outlined in this review, there are still many areas, where validated and generally accepted measurement methods for determination of the input parameters are lacking. In these cases, values are either taken from the literature, sometimes neglecting limitations of the original source, or the values result as adjustment parameters in the modelling, with the risk of generating self-fulfilling prophecies. Care needs to be taken to critically discuss how realistic such values are. This includes discussion on how the required simplifications and assumptions in the modelling may influence such adjustment parameters. Furthermore, if the precision of input parameters is uncertain, sensitivity analyses would allow an estimation of the magnitude of possible effects. Following such Good Modelling Practice approaches when setting up and validating computational models will, therefore, lead to better estimations of the interesting complex processes inside organotypic tissue cultures in the future.

## NOMENCLATURE

OUR, oxygen uptake rate ( $\text{mol L}^{-1} \text{h}^{-1}$ )  
 $q_{\text{cell}}$ , cell-specific oxygen uptake rate ( $\text{mol cell}^{-1} \text{h}^{-1}$ )  
 $N_{\text{cell}}$ , cell density ( $\text{cells L}^{-1}$ )  
 $c_{\text{O}_2}$ , oxygen concentration ( $\text{mol L}^{-1}$ )  
 $k_m$ , Michaelis–Menten-constant ( $\text{mol L}^{-1}$ )  
 $K$ , permeability (Da)  
 $Q$ , volumetric flow rate ( $\text{m}^3 \text{s}^{-1}$ )  
 $\Delta P$ , pressure drops through the scaffold (Pa)  
 $A$ , Cross-sectional area ( $\text{m}^2$ )  
 $L$ , thickness/length of the scaffold in the direction of flow (m)  
 $\mu$ , dynamic viscosity of the fluid (Pa s)

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## CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

## AUTHOR CONTRIBUTIONS

Ehsan Fattahi, Ralf Pörtner, Arndt F. Schilling and Shahed Taheri conceived the idea and wrote the manuscript. Arndt F. Schilling and Thomas Becker revised and edited the manuscript. All authors have approved the final manuscript.

## DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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