Cultivated Meat Modeling Consortium: Inaugural Meeting Whitepaper

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Abstract

The broad environmental, societal and animal welfare issues associated with large-scale animal protein production are well-documented, and cultivated meat represents a potentially more sustainable alternative protein form to conventional production methods. Some believe cultivated meat is years away from commercial success; however, computational modeling has real potential to reduce the timeline for commercial roll-out. In June, 2019, a diverse group of stakeholders attended the inaugural Cultivated Meat Modeling Consortium meeting in Seattle, Washington to develop a multiscale modeling framework for cultivated meat manufacturing. This whitepaper was created from the extensive brainstorming that occurred during the meeting and afterwards. The early stage framework presented here is meant only as a beginning, and our hope is that this paper will drive future discussions and generate more collaborations that maximize the utility of computational modeling in the areas outlined here and beyond.

1. Introduction

There is a critical and immediate need for alternatives to conventional meat and seafood due to the many environmental issues and externalities associated with animal protein production [1]. One alternative that could offer sustainable production is the nascent field of cultivated meat [2]. The field advances technologies culturing animal cells for human consumption in a medium that contains nutrients and energy sources required to support cell growth [3]. Though still pre commercial, cultivated meat has attracted millions of dollars in investment [4]. While diverse process designs are under development, approaches are generally composed of four steps.

The first necessary step in cultivated meat production is selecting and sourcing a starter cell, typically via a non-lethal biopsy. Starter cell types range from embryonic stem cells to differentiated muscle cells, although few companies are starting off with fully differentiated muscle cells [5].

In the second step, the selected starter cell goes through an expansion bioprocess consisting of many rounds of division in a bioreactor. A medium composed of amino acids, vitamins, inorganic salts, lipids and growth factors [5] fuels the bioprocess until the desired mass of cells has been produced.

The third step differentiates the proliferated starter cells into muscle cells, fat cells, or connective tissues. A media formulation containing morphogens drives this differentiation by dictating specific cell lineages.

Finally, when skeletal tissue is desired, the fourth step structures the differentiated cells by seeding them into a scaffold, which facilitates proper assembly, development and, ideally, hypertrophy [5].

Ambitious timelines for cultivated meat product release have suggested commercialization is only 5-10 years away [6], while other complementary and reputable reports predict cultivated meat growth will not eclipse plant-based alternative market share until 2030 [7]. Given the possible extended time horizon before longer term commercial cultivated meat success and the immediate need to realize associated sustainability goals, any tools or techniques that can expedite roll-out would be of considerable value. Computer modeling is one such powerful tool that has numerous applications in cultivated meat process and product development.

2. What is computer modeling?

Computational modeling is the use of computers to reproduce some aspects of the behavior of a real world complex system and to subsequently use the model for making predictions under new, as of yet unobserved, conditions. Computational modeling in science and engineering is typically used in situations where the researcher or engineer is either unable to do physical experiments (e.g. star formation in distant galaxies, or climate predictions), where experiments would be very dangerous and unethical (e.g. crowd control during disasters or human brain surgery experiments) or would be very costly or time consuming (e.g. car crash tests, ecological systems and lab experiments). A computer model typically takes several parameters as input and produces quantitative data as output that can be validated against measurements in the physical system. Once a model has been formulated and implemented it can be run for any number of parameter combinations to explore and quantify a system's behavior (science) or it can be optimized for a certain outcome (engineering) (Fig. 1). For this reason, models provide a crucial nexus between the scientific method and virtual prototyping.

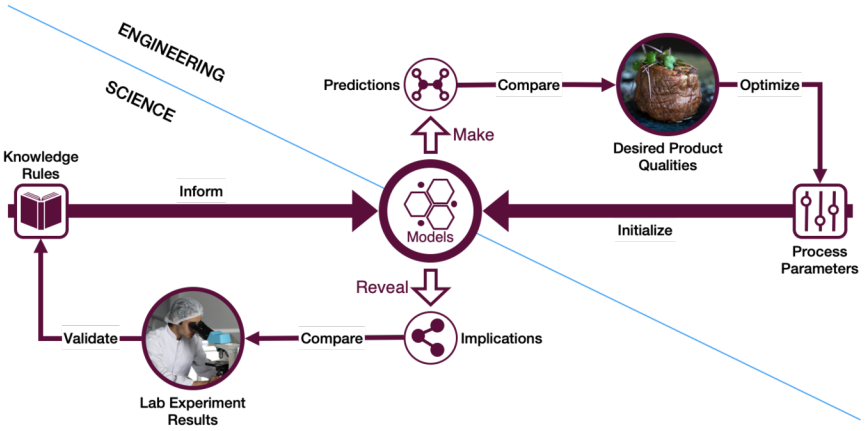

Figure 1. Schematic demonstrating the utility and power of computational modeling approaches in Science and Engineering.

Besides saving costs and time and enabling otherwise impossible experiments, a model abstracts scientific knowledge from messier, physical manifestations creating unambiguous, actionable encapsulations that facilitate reuse and interaction. This in itself is a valuable trait. How this knowledge is encapsulated depends for a large part on the operational definitions or, practically speaking, on the format of the available data, whether the behavior of the system changes discretely or continuously in time and/or space and the required output of the model.

2.1 Numerical mathematical modeling

When the dynamics of a system are well known and one can formulate a mathematical equation for its behavior, numerical modeling can be used to run the system in otherwise unexplored conditions or settings, changing parameters to investigate their influence on the system's behavior. Good examples for this are weather and climate models. These models often consist of equations derived from known physical processes

that occur in the atmosphere for which mathematical formulations exist. Examples are fluid dynamics, evaporation and condensation, chemical reactions and radiation. Although each of these processes is well understood in isolation, their interaction makes the system unpredictable. Combining them in a computer model enables us to calculate how the system might behave in the future based on specific initial conditions. Comparing the model outcome with historical and present day observations shows the accuracy of the model and how well we can trust its predictions of future climate observations.

2.2 Agent/cell-based models

A different kind of modeling is that of agent-based models. This kind of modeling is typically used for systems where it is easy to discern and divide a system into sub-parts or subsystems (called agents) that follow explicit behavioral rules. Since there are numerous discrete subsystems and a potentially vast number of interactions among such subsystems, it becomes challenging or unfeasible to write down a mathematical set of equations governing the entire system. The agents in such a system can typically be represented as relatively simple entities. Examples of this are individual people in a crowd, birds in a flock, or cells in a Petri dish. In each case, every agent interacts with other agents following some specified set of rules, although such interactions typically involve only a small number of "neighbors" of an agent, such as birds interacting only with nearby birds.

3. Cultivated meat modeling consortium

We have articulated (1) an urgent need for alternatives to animal agriculture, (2) the potential of cultivated meat to satisfy that need, (3) the steps common to most cultivated meat processes and (4) the capacity of computational modeling to accelerate the optimization of process steps and product outcomes, thus mitigating efficiency challenges.

To connect the dots, Biocellion SPC hosted a meeting on June 5-7, 2019, in Seattle, Washington, where attendees included the Biocellion team, cultivated meat company representatives, academic researchers from related fields, students from multiple disciplines, investors, and industry experts. The meeting intention was to begin the process of developing a computer model platform for use by cultivated meat companies and researchers. The platform would enable companies to bring cultivated meat to market more quickly and at a lower cost by reducing the need for laboratory experiments. The on site meeting was preceded by a series of virtual meetings (starting in May, 2019) with individual participants and the Biocellion team. The goal of the June meeting was to initiate a conversation between stakeholders in various fields, and to begin to establish an early stage consortium. The purpose of the nascent group is to i) develop a multiscale modeling framework for cultivated meat manufacturing, and ii) outline the first steps of developing the model framework that require immediate action.

3.1 Defining scope and challenges

On day one of the meeting, the group started with a dialogue on the cultivated meat industry and its potential impact on the world, both positive and negative. This was followed by a small group activity where participants envisioned the ideal impact of the cultivated meat modeling consortium (CMMC) on the cultivated meat field by 2022. Participants identified various goals of the consortium, such as the development of open source tools for a highly collaborative cultivated meat modeling effort, financial support from public sector institutions such as the USDA and NSF, endorsement from respected public figures such as political leaders, and the early debut of cultivated meat products on the market.

In order to achieve these goals, the CMMC would have to address current technical ("science" and "process"), product quality ("product") and commercial challenges in the cultivated meat industry. The group brainstormed the broad challenge types and identified those that could be most easily addressed by computer modeling. The "science" challenges identified as "low hanging fruit" for the consortium included data collection, understanding relevant metabolic pathways, and medium composition/optimization. "Process" challenges, or challenges in developing a manufacturing process for cultivated meat that were identified as relevant to the consortium include optimal cell density, the use of plant-based media for sustainable cell

culture, and efficient channeling of cell differentiation. "Product" challenges most relevant to the consumer included nutritional value of the product and its sensory qualities such as texture, taste and flavor. The group also identified the issue of getting funded to develop a modeling framework for 2-3 years before a fully mature product is available to users.

3.2 Open space

After identifying a number of actionable challenges, the group moved to Open Space, where small groups of participants discussed individual challenges and subsequently shared their findings with the broader membership. These notes consisted of key insights into the problem, and a list of what is needed if the group is to move forward with addressing that particular challenge. Open Space sessions on day one were devoted to technical challenges, and sessions on day two were devoted to consortium formation. Technical challenge areas included finding or generating data for modeling, differentiation, metabolic processes, automating tissue production, growth kinetics, medium composition and modeling fluid dynamics in thick tissues/scaffolds. Issues that were central to consortium formation were funding, communicating this meeting + who else needs to be here, demonstrating how can models be useful for cultivated meat, the next 30 days and organizational aspects/communications platform.

3.3 Design for wiser action

On day two, a Design for Wiser Action session was held, in which small groups chose topics from the Open Space session and developed roadmaps for addressing the problems at hand. These roadmaps included a desired outcome (such as a tool for media optimization), what info or resources we already have, what info or resources are missing, next steps, key questions, and a timeline for future work on the problem. Key topic areas included:

-Generating and finding data for modeling

-Metabolic modeling

-Media optimization

-Bioreactor modeling

-Optimization of scaffold design

3.4 Next steps

At the end of day two, the group identified a series of topics to be discussed by the consortium in the future and a time frame for when these conversations need to happen. These included:

-Writing a white paper

-Open data types/formats/metadata

-Communication within the consortium

-Media – virtual experiments

-CMMC membership, and what it means

3.5 Post-meeting progress

Considerable progress has occurred since the inaugural June, 2018 meeting, and many follow up conversations have already happened, including the formation of sub-groups to pursue the roadmaps established in the Design for Wiser Action session. Additionally, a volunteer working group with diverse interests was formed and has been meeting weekly to continue to drive the CMMC agenda forward. Researchers within the CMMC network have also applied for the 2019 GFI Competitive Research Grant program, and CMMC representatives attended cultivated meat conferences in the Netherlands and the US. New collaborations have also formed

as a result of the CMMC June meeting, notably, one between Andrew Stout (Tufts University) and Patrick Suthers (Pennsylvania State University).

4. Computer modeling applied to cultivated meat

In the broadest sense, the core technical development areas in cultivated meat R&D and commercialization, as defined in the CMMC June meeting, can be grouped as basic science, process challenges and product challenges. Herein, we define important data-based prerequisites that are generally required to implement computer modeling approaches and then we briefly explore several projects in some of the core technical development areas where computer modeling techniques may be applied. To explore these projects, we start by identifying the problem and/or optimization objective, describe the problem's relevance to the cultivated meat field, and then demonstrate how computational modeling may be used to address these challenges.

4.1 Data-based prerequisites

A preliminary yet critical challenge in applying computer modeling techniques to any biological system is having access to sufficient data to construct the foundational models. Repositories are well-established for common research species, such as *Mus Musculus* ,*Drosophila melanogaster* , *Xenopus laevis* and *Danio rerio* [8,9]; however, similar databases do not exist for the diversity of species consumed by humans. Therefore, one of the first areas information curation approaches must be applied in cultivated meat R&D is to generate databases for those species which are the focus of academic and industrial cultivated meat research. Here, modelers can work with stakeholders to create plans, protocols and workflows to transition data into Systems Biology Markup Languages (SBML).

4.2 Metabolic pathway models

An important objective in bioprocess design is achieving highly efficient conversion of inputted nutrients into biomass, a metric known as the yield coefficient [10]. In cultivated meat production this is particularly important as the cost of cell culture media is currently very high and because of the enormous scaling requirements to support conventional meat demand. Computer models of metabolic pathways should prove to be advantageous in this process optimization challenge, facilitated, in part, by the depth of intercellular information that can be obtained with "omics" approaches. Indeed, constraint-based modeling techniques have proved very useful in understanding and optimizing CHO cell growth in a bioreactor environment [11] and metabolic network models have been used with bone marrow-derived stem cells [12].

4.3 Experimental design and surrogate models for growth media

A major problem in cultivated meat is that industrially relevant cells are grown with media with upwards of $30 - 50$ different metabolites [13]. Additionally, cellular responses to metabolites not only depend on reactor conditions like temperature, pH and mixing, but on metabolite-metabolite interactions, causing response nonlinearity. Lacking verified system-level and mathematical approaches to optimization, tools for experimental methods that can be used in the lab quickly are highly valuable. A traditional way that modeling has addressed the issue of high dimensionality (many inputs) is through combining statistical design of experiments (DOE) with linear or polynomial regression in a family of methods called response surface methods (RSM) [14]. However, both DOE and sequential RSM tend to become infeasible at input dimensions > 5 [15] and linear and low-order polynomial models often are unable to capture nonlinear effects of responses in design problems.

Nonlinearity has been overcome in the past by using heuristic optimizers such as genetic algorithms [16,17] (dim ˜ 10). Higher dimensional problems in media optimization have been solved using neural networks [18] (dim ˜ 20) and are especially useful in their ability to capture nonlinear and high dimensional responses. Combining many of these separate ideas is the field of meta-modeling. Usually (i) a DOE or other space-filling design of experiments is conducted then (ii) a polynomial, neural network, kriging model [19], SVR and/or regression/classification method [20] maps input-output responses and (iii) a nonlinear solver, trust-region algorithm, or search rule chooses a new set of experiments in sequence based on an objective function. Finally, information theoretic criteria for function approximation has been applied to media design [18] and

process optimization [21,22]. As the purpose of these methods is to leverage modeling techniques to discover better media designs while minimizing cost/experimentation, model agreement is important so researchers should consider liberal use of cross-validation, hyperparameter optimization, and model ensemble methods.

4.4 Bioreactor process optimization

Producing large numbers of cells efficiently is a key step in the process of creating cultivated meat. Several bioreactor configurations have been suggested [10], but the jury is out as to whether any of them will be practical at the much larger scale and lower cost required to support commercial production. Building out large-scale bioreactor prototypes is an expensive and time-consuming proposition, so making a judgement by empirical means is hampered by cost. Developing a single model specific to one configuration is also likely to be expensive and time-consuming; however, once created, a model can be iteratively refined and tested at a far lower cost than redesigning and testing physical bioreactors. In addition, the methodology used to model any one bioreactor configuration is likely applicable to others with incremental modifications. Indeed, computational modeling approaches have been applied to bioreactor use in cell therapy development [23-28], and these approaches should translate to cultivated meat production.

Here, we briefly detail five bioreactor configurations that have potential utility in cultivated meat - i) suspension growth (w/o microcarriers) in a stirred tank bioreactor, ii) adherent growth (w/ microcarriers) in a stirred tank bioreactor, iii) hollow fiber bioreactors, iv) a continuous flow bioreactor design relying on coated surface and v) a rocking bioreactor design. We also describe instances where computational modeling has been applied to study these reactors. In the future, novel and more holistic modeling of these systems must combine simulation of the cell biology with modeling of physio-mechanical properties (e.g. adherence, elasticity, susceptibility to shear forces) and computational fluid dynamics to get a better understanding of which reactor design is best-suited to achieve the high output requirements of cultivated meat production.

4.4.1 Suspension growth (w/o microcarriers) in a stirred tank bioreactor

In the case of stirred tank set-ups w/o microcarriers, Bayrak et al. [29] uses a hybrid methodology combining flux balance and agent-based modeling which can predict quantitative changes in nutrient and metabolite concentrations in a fed-batch set up. The model uses the measured dissolved oxygen and sodium data as input together with initial cell culture conditions and predicts viable cell density, viability and concentrations of glucose and lactate. The model shows good agreement with experimental trials. However, the application is low density ($< 10^7$ cells/mL) and, as the authors explain, assumes ideal mixing, with no allowance for spatial gradients. In cultivated meat, the output required demands high density and/or large-scale bioreactors, in which gradients are inevitable. To capture spatial gradients and heterogeneities will require three-dimensional modeling with cells or aggregates of cells represented by distinct agents.

The review paper by Hutmacher and Singh [30] on the application of computational fluid dynamics to three-dimensional modeling of tissue engineering-related bioreactors concludes that fluid flow processes have direct implications on cellular responses such as attachment, migration and proliferation. For cultivated meat, we propose to extend these principles to modeling of suspension growth in a stirred tank bioreactor, introducing a high-density of cell-clusters, each represented by an agent, to correspond to a higher density of cells.

4.4.2 Adherent growth (w/ microcarriers) in a stirred tank bioreactor

Use of microcarriers increase the efficacy of stirred tank bioreactors for a number of reasons, including reducing media requirements [31,32]. Yet, microcarrier utilization does have the drawback that attached cells become particularly susceptible to damage from agitation [32]. To model the utility of microcarriers of varying geometries will require representing individual cells as agents adhering to a microcarrier also represented as a distinct agent. In the literature, work by Croughan et al. [32] identified and mechanistically modeled some of the hydrodynamic effects in microcarrier cultures and sought to quantitatively correlate these with fluid dynamic properties. The rationale underpinning this work was that successful scale up of a microcarrier-based stirred bioreactor system would require an in depth knowledge of the mass transport and hydrodynamic phenomena. The critical parameters to assess hydrodynamic effects on animal cells grown

on microcarriers in suspension cultures are the Reynolds number, Kolmogorov eddy length, maximum mean aggregate size and maximum shear stress [33].

In this work by Croughan et al. [32], the researchers found individual cells on microcarriers are most likely damaged by small intense eddies that can affect individual cells, but are too small to move individual microcarriers. Overall, these researchers made scale up recommendations/predictions for microcarriers in suspension system based on their thorough analyses - i) if scale up at constant power input per unit volume is employed with vessels that are not geometrically similar, one should consider the role of vessel and impeller geometry in terms of the power requirements for suspension and regional distribution of power generation and ii) scale up at constant time-averaged outer radial shear rate should not lead to detrimental effects from hydrodynamic forces.

Additional relevant research detailed a high aspect ratio vessel (HARV) that was developed to study tissue and cellular engineering in a low-shear, non-turbulent, simulated microgravity environment [34]. While not a microcarrier-based stirred tank system per se, microcarrier beads are co-injected with cells into the HARV system and rotation is initiated at a desired angular velocity; therefore, the computational modeling of microcarriers in this study is of considerable value. Here, computational modeling of microcarriers is used to recapitulate observed behaviors where microcarriers with density greater than the surrounding fluid medium follow a circular motion relative to the culture medium combined with a persistent migration and eventual collision with the outer wall of the reactor; whereas microcarriers with a density less than the fluid medium follow a circular motion migrating toward the central region of the reactor. When multiple microcarrier beads that are lighter than water are inserted into the reactor, the centrally directed migration results in the formation of clusters that are stabilized by tissue bridges formed by osteoblasts seeded onto the microcarriers. We propose to follow a similar modeling paradigm to understand the effects of high-density on both flow and bridging behaviors.

4.4.3 Hollow fiber bioreactors

In the stirred tank bioreactor systems, cells are taken from high density environments with little variability in nutrient and oxygen supply, and they are adapted to low-density suspension growth. While these systems are widely used, they are not necessarily representative of the *in vivo* physiological conditions that many cell types of interest experience [35]. A more recent innovation that better mimics *in vivo* growth conditions are hollow fiber bioreactors [35]. With this technology, many semipermeable fibers are arranged in parallel within a generally cylindrical cartridge that is equipped with inlet and outlet ports. During operation, cells can be grown on the inside of the fiber, outside, or both and culture medium is pumped through the hollow fibers, allowing nutrients and other metabolic products to diffuse both ways.

Mohebbi-Kalhori et al. [36] models hollow-fiber membrane bioreactors intended to grow human bone tissue, in part motivated by the fact that these systems are difficult to sample in real-time during cell and tissue growth. The model developed treats the hollow-fiber scaffold as a porous domain consisting of two interpenetrating porous regions separated by a membrane through which nutrients and waste products pass. In this conception, the hollow-fiber membrane scaffold is perfused with culture medium on the lumen side and cells are grown at the exterior of the fibers. The model was used to show how nutrient gradients impact the cell volume fraction distribution as cells proliferate inside the bioreactor. Predictions agreed with experimental results from the literature. Notably, recent work by Allan et al. [37] presented at the 5th International Conference on Cultured Meat explored the applicability of hollow fiber bioreactors for large-scale production of skeletal muscle stem cells. This represents an excellent collaboration opportunity to apply computational modeling methods.

4.4.4 Continuous flow bioreactors with a special smart coating

Continuous bioreactors constantly convert raw material inputs into intermediate or final products. These systems can have the advantage of lowering manufacturing costs, reducing the footprint of the production facility and overall improved quality, reproducibility and standardization of outputs. Miotto et al. [38] recently reported the

first successful proof-of-concept design for a closed, continuous system for therapeutic cell production using fibroblast-like cells [39]. The key technological element in the system is a smart enzyme-sensitive coating comprising a fully synthetic, multifunctional peptide amphiphile. With this special coating material, it was possible to achieve a steady-state condition, where the number of cells detached/produced for downstream applications was matched by their proliferation. These researchers have only demonstrated their technology at the very small scale on coverslips in 6-well plates, but have formed a company, CellulaREvolution Ltd, to examine its utility in the cultivated meat space [39]. Undoubtedly, computational modeling techniques can be applied to further examine this novel technology, and assess whether it can be applied to the future design of an actual at-scale, closed, continuous bioreactor system. Reports of continuous bioprocess systems for viral vaccine production using different cell lines [40] should inform the design process for similar bioprocesses in cultivated meat.

4.4.5 Rocking bioreactor design

Turbulent flows and shear forces can induce spontaneous generation of stem cells [41], so reactor configurations which produce less of these forces are of interest in cultivated meat production. Wave bioreactors, designed to provide excellent mixing and gas transfer with reduced shear stress, are well-suited to the culture of cells highly sensitive to hydrodynamic conditions. These systems consist of a bag on a rocking tray that agitates the cell culture with a back and forward wave-like movement, and they are available at working volumes up to 500 L [42]. Computational fluid dynamic modeling approaches have been applied to study fluid flow within wave bioreactor designs, with a focus on an improved understanding of the effects of agitation and rocking angle [42]. Interestingly, in their work, Zhan et al. [42] found that the lowest rocking speed (15 rpm vs. 22 and 30 rpm) produced the highest fluid velocity, mixing and shear forces due to a resonance phenomenon. We intend to use these techniques coupled with other approaches to inform a biological model, describing what happens to the cells, how their proliferation is slowed or how apoptosis is induced for some due to the forces they endure. These novel models should be particularly useful to assess the suitability of a rocking bioreactor design from *Celltainer Biotech BV* which has been promoted for use in cultivated meat production.

4.5 Modeling flow through scaffolds

One of the preeminent challenges in tissue engineering is developing a suitable support material to facilitate the attachment and subsequent development of anchorage-dependent cells into full tissue [43]. This material, called a scaffold, must at least partially mimic the *in vivo* properties of the extracellular matrix of the tissue of interest [43], and should offer i) sufficient mechanical strength, ii) a network of interconnected pores, iii) adequate transport of oxygen and nutrients and iv) easy removal of waste products [44]. Scaffold dynamics and functionality have been well-studied in tissue engineering applications [43], but novel scaffold systems are required in cultivated meat research and development. Additionally, cultivated meat scaffolds will need to be considerably larger than those currently in use in tissue engineering if this nascent technology has a chance to off-set conventional meat demand. Optimization and scaling of scaffolding design is another area where computer modeling will be an invaluable tool in cultivated meat research and development.

Computational modeling has been broadly utilized in tissue engineering scaffold characterization and design [44,45]; however, we will focus on three specific examples that should also be applicable in cultivated meat research. One foundational way that modeling techniques can be useful is in developing scaffold geometries. Here, it is particularly important that the physical scaffold architecture provides adequate porosity, and three-dimensional computer-aided design models have been used to examine 119 polyhedrons as base units for scaffold construction and to assess the performance of these designs [46]. Beyond the physical architecture of the scaffold, it will be fundamentally important to understand cell-scaffold material interactions to achieve a controlled and reproducible cultivated meat growth system. In this regard, computer modeling should be useful, as numerous mathematical and computational models have focused on, for example, modeling the active mechanosensing behavior in cell-matrix interactions [47].

In general, computational analysis of scaffold properties should consider two phases: a solid bulk scaffold

and a fluid medium inside the pores [44]. Indeed, computational fluid dynamics are an important part of computer modeling of scaffolds. Application of these techniques may be used to determine optimal pore size, branching and flow rates within the scaffold and how these may change as a scaffold becomes cell-laden, and the influence of these parameters on the overall cultivated meat bioreactor design [48].

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