Chapter 15

Agent-Based Numerical Methods for 3D Bioprinting in Tissue Engineering

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15.1. STATE OF THE ART IN 3D BIOPRINTING

In 3D bioprinting, a computerized numerical control system accurately places biomaterials and living cells with the potential to create tissue, organs, and body parts. The computerized printing system can use cell-free biopolymers, cell-seeded microcarriers (bioink), and living cell spheroids. Depending on the bioprinting material, 3D bioprinting is classified into scaffold-based and scaffold-free methods. Scaffold-based methods involve jetting, extrusion, or photopolymerization of a liquid bioink containing living cells, nutrients, and an artificial extracellular matrix (ECM) known as the scaffold (Butcher et al., 2014; de la Puente and Ludeña, 2014; Singh et al., 2016). Scaffold-free methods consist of the direct placement of living cell spheroids on a preexisting support structure in an arbitrary three-dimensional array (Pataky et al., 2012). An indirect approach to bioprinting is the fabrication of a porous structure from a cell-free biopolymer. The cell-free structure allows the introduction and proliferation of living cells shaping the desired tissue or organ. By this method, prevascularization can be employed to promote the development of the vasculature in nutrient-demanding tissue constructs (Naderi et al., 2011). In all methods, the successful implementation relies on the interaction between biomaterials and living cells, which can be predicted by numerical models described in the succeeding sections.

15.1.1 Scaffold-Based Methods

The objective in scaffold-based 3D bioprinting is to produce an artificial ECM or scaffold that mimics a natural ECM, allowing cellular proliferation, differentiation, and migration and, ultimately, supporting the survival and functioning of the living tissue. Artificial scaffolds are produced with biocompatible polymers that can be biologically degraded, absorbed, and replaced by the natural ECM produced by living cells. Biodegradable scaffolds produced from a bioabsorbable polymer have been investigated and utilized in fabrication of various tissues or organs such as skin, cornea, cartilage, bladder, and vessels (Shimizu and Matsuura, 2015).

Polymer hydrogels are the most commonly explored materials for fabricating complex 3D cellular microenvironments, as they can be tuned for ideal degradability, mechanics, and the ability to incorporate biomolecules of interest (Bajaj et al., 2014). Natural hydrogels are commonly utilized as scaffolds in tissue engineering; however, because of potential immunoreaction or infection, synthetic alternatives are preferred (Chen and Hunt, 2007). Alternatively, synthetic hydrogels offer low levels of immunoreaction or infection (Shim et al., 2011). They can also be jetted, extruded, and, in some cases, photopolymerized (Censi et al., 2011; Gudapati et al., 2016).

15.1.2 Scaffold-Free Methods

Another example of a support structure is a Cartesian needle array or kenzan in which the cell spheroids can be aggregated (Moldovan et al., 2017). These methods (scaffold-based and scaffold-free methods) can be used to construct complex biological materials with a high degree of control. This ability provides the potential to fabricate material architectures that match the ones of endogenous tissues and organs. Engineering these complex tissues requires an interdisciplinary approach, which combines material science innovations with cell biology development and growth factor chemistry by exploring the steps involved in organ development and morphogenesis.

One of the fundamental properties of cells is their propensity to self-organization. This is essential in all biological processes, from bacterial colony formation to mammalian embryology, organogenesis, and tissue repair (Neagu et al., 2005). Remarkably, although the molecular constituents are encoded in genetic information, there is no genetic “blueprint” for their assembling at any dimensional scale. Supramolecular assemblies are emergent structures basically driven by physical principles, such as energy minimization, operating under entropic constraints. For example, this explains the round shape of cell clusters, from embryos to tumors (Shirinifard et al., 2009), also called for this reason cell “spheroids.” Moreover, if there are no additional barriers (such as layers of ECM), then when two such spheroids come in contact, they will fuse and collapse into a larger spheroid (Yang et al., 2012). This is possible because within the spheroids the cells perform continuous random movements similar to the Brownian motion of molecules within a lipid droplet (Mombach and Glazier, 1996). All implications of this similarity hold true for fusing spheroids, such as surface tension (Beysens et al., 2000), contact angle, and “neck” diameter (McCune et al., 2014). In addition, energy minimization obliges different cells with various adhesiveness to assume a layered distribution within the spheroids (Beysens et al., 2000). This spontaneous process called “cell sorting” is of fundamental importance in embryogenesis and beyond (Zhang et al., 2011).

These phenomena are now exploited for rational design of biosimilar constructs with tissue engineering applications (Mironov et al., 2003; Yang et al., 2012), representing the foundation of the emerging “scaffold-free” biofabrication (Mironov et al., 2009). Moreover, cell spheroid fusion, either in dual or in larger constructs, has been modeled using a variety of methods, as described in subsequent sections of this chapter.

15.2. MODELS IN 3D BIOPRINTING

To further the capabilities of tissue engineering applications, various models have been proposed to predict the dynamics of cellular aggregates. These models seek to predict morphogenetic phenomena such as proliferation (Mueller-Klieser and Sutherland, 1982), self-assembly (Jakab et al., 2008; Surapaneni et al., 1997), and cell sorting (Mombach et al., 1995; Steinberg and Takeichi, 1994), or application-specific metrics such as spheroid fusion (Shafiee et al., 2015) or viability (Freyer, 1988; Freyer and Sutherland, 1980; Haji-Karim and Carisson, 1978; Mueller-Klieser et al., 1986; Sutherland et al., 1986). Models in tissue engineering typically fall within one of three methods: lattice Boltzmann (LB) methods, cellular particle dynamics (CPD), and kinetic Monte Carlo (KMC) methods. However, other models have been proposed, including cellular automaton (CA) (Picioreanu et al., 1998) and phase-field methods (Yang et al., 2012).

15.2.1 Lattice Boltzmann Methods

On some scale, living tissue functions like a fluid. For example, the tendency of cells to sort themselves into distinct regions is comparable to the ordering of fluids (Beysens et al., 2000), and spheroid fusion has been compared to the merging of liquid drops (McCune et al., 2014). By this paradigm, the dynamics of a population of cells can be modeled according to the description of fluids in statistical mechanics. Specifically, LB methods have emerged as a discretized form of the Boltzmann equation to simulate the macroscopic dynamics of large populations of microscopic entities. These methods neglect individual cellular interactions and instead seek to efficiently model macroscopic behavior of cellular populations over extended periods of time. In LB methods, cell populations and the medium in which they are placed are

described by distributions of particles with a particular velocity, which generally evolve to some equilibrium state. In this way, a system of cellular aggregates is modeled as a multiphase system, where aggregates tend to evolve according to principles such as surface tension and barycentric velocity. LB methods have been applied to provide insight into the fusion of single cell-type spheroid pairs (Cristea et al., 2011) and complex constructs (Cristea and Neagu, 2016), as well as the characteristics of multicellular populations (Krafczyk and Berrios, 1998).

15.2.2 Cellular Particle Dynamic Methods

On a smaller scale, CPD methods model cells as pointlike particles, called cellular particles (CPs), in a continuous domain, or as consisting of a collection of CPs that are modeled to obey classic descriptions of motion with the introduction of stochastic processes in some overdamped Langevin equation. The Langevin equation of a system of CPs is constructed as a system of differential equations that consists of a summation of potential energy expressions. These potential energy expressions model the influence of intercellular, environmental, and, for cells consisting of multiple CPs, intracellular interactions. CPD methods have been applied to the modeling of aggregate fusion (Flenner et al., 2008; Kosztin et al., 2012; Shafiee et al., 2015) and cell sorting of multicellular systems (Flenner et al., 2012).

15.2.3 Kinetic Monte Carlo Methods

On a still smaller scale, KMC methods model cells as occupying points in a discretized lattice and evolving according to simple, stochastic rules. The modeling description of cellular dynamics is type specific and uniformly applied to all cells of a particular type. In this way, KMC methods most closely resemble an agent-based model (although certain aspects of LB and CPD methods accomplish the same). The rules that govern the evolution of the cellular system generally enforce a tendency of the system to some lower energy state, where events that increase the system energy still may occur, but with a decreasing probability proportional to the increase in system energy. The likelihood of events is then considered at randomly selected sites, which produces characteristics like Brownian motion (Mombach and Glazier, 1996). The dynamics of cellular activity then takes the form of the occupancy of lattice sites being traded among cells in the population, or by the medium in which they are situated, which has been successful at predicting cell sorting (Flenner et al., 2012; Graner and Glazier, 1992; Zhang et al., 2011) and fusion of aggregates (Fleming et al., 2010; Flenner et al., 2008, 2012; Jakab et al., 2008). A very popular KMC method in literature is the focus of subsequent sections of this chapter, the implementation of which is most popularly used in the software CompuCell3D (Swat et al., 2012).

15.3. AGENT-BASED MODELS IN SCAFFOLD-FREE 3D BIOPRINTING

This section introduces the basic concepts and relevance of agent-based modeling, namely the paradigm by which complex systems may be reduced to elementary, predictable components. A widely used agent-based model in the modeling of cellular dynamics is then presented, called the cellular Potts model (CPM), which is the focus of subsequent sections in this chapter.

15.3.1 Abstract Description of an Agent

It is not mere musing to propound that both a human and a block of iron are the consequences of a (practically) uncountable number of active constituents and their perpetual activities. Even if either macroscopic entity has the appearance of being stationary, at some smaller scale there are a great number of components that are very much not stationary at all. This is, of course, referring to the consistency of matter at the atomic scale, which, for most practical macroscopic modeling applications, can be represented by macroscopic descriptions.

Considering the block of iron, if subjected to some stimulus, there are many sufficiently accurate descriptions to predict how the block responds. The same is true for a whole population of blocks, whether they be colliding or joined together into some structure. But subject a human to stimuli—whether caffeine or conversation—and what will the human do? Depending on the context, the behavior of a human can be well predicted through empirical means and probabilistic descriptions: subject a human to hunger and, with a very high probability, the human will seek out and consume food. But subject a population of humans to hunger who have access to multiple sources of food, and what will the population do? And how will they go about acquiring their respective choice, given their respective level of hunger, or how they know each other, or how each source dispenses food? Introduce more humans, or more sources, or more behavioral considerations, and the scenario very quickly presents a sort of insurmountable complexity to traditional mathematical de-

scriptions for modeling global behavior. Therefore, in the case of the human (as the sociologist or city planner would ask), how does one model the collective behavior of a population of humans? The same can be asked about cells, or about nations, or about a great many other complex systems. Except, like humans, a particular cell or nation tends to exert a particular response to a particular stimulus or to a set of stimuli.

This is all to say, although the behavior of a population of decision-making entities may be too complex to predict with mathematical precision, on some smaller scale the behavior of the individual entities is reasonably predictable. These entities are called agents, and the modeling and simulation of a population of interacting agents in an environment is called an agent-based model (Railsback and Grimm, 2011). An agent is a discrete, autonomous entity that makes decisions in response to interactions. It makes decisions according to local, rule-based behavior, which is affected by the elementary attributes of the agent. Attributes may be specific to each agent in a population, or to a subset of agent type, and each agent may modify its own attributes as part of its behavioral processes. Typically, each agent has a limited knowledge of its environments and is confined to make decisions based on local conditions, called the interaction of the agent. Agents decide according to environmental conditions (agent–environmental interactions), internal mechanisms (intraagent interactions), and neighboring agents (interagent interactions). Agents are connected through some interaction topology, whether it be by proximity in a Euclidean space or discretized lattice, or otherwise.

15.3.2 From Cells Come Biological Patterns: Scales and Emergence

The intraagent interactions of each agent can either be modeled as some set of analytic expressions, or the attributes of each agent can result from some population of agents on a smaller scale. In the aforementioned case of a human, every agent can consist of a population of organs, or of cells, or of some other population of biological “subagents,” which then constitutes the internal mechanisms of the human. Generally, the intraagent interactions of an agent on some particular scale can be the consequence of a population of agents on some smaller scale, and the global behavior of the population of agents on that particular scale can govern the behavior of a single agent on some larger scale, and so on—e pluribus unum.

Whether modeling a single population, or multiple populations at various scales, resulting macroscopic phenomena emerge from microscopic activity, broadly referred to as emergence, which is a central goal of agent-based modeling: given a population of discrete, autonomous agents, what global phenomena emerge? In the case of the block of iron, the material properties of iron emerge from a population of iron atoms. The societal constructs in which humans live emerge from a population of humans, each of which emerges from a population of cells, which emerge from populations of atoms, and so on (Deutsch et al., 2007). In biological applications, the distinction is probably less ambiguous: the terminal emergent phenomenon of a population of cells is the organism, during which various intermediary phenomena emerge.

15.3.3 Generalized Cellular Potts Model

15.3.3.1 Qualitative Description

Imagine a discretized regular domain containing a collection of integers that have aggregated by value. The integer value of each aggregate is unique so that each aggregate can be readily identified, and all of the aggregates are surrounded by another unique integer that fills the rest of the domain. Each aggregate is an agent in a medium, and the unique integers are their identification. A population of agents then exists in a medium, in which they carry out their various activities. Fig. 15.1 shows the agents (cells) and the medium.

The agents mingle and jostle each other with the characteristics of Brownian motion. On inspection of some particular agent, it is found that the agent continues to overtake some points of the domain from a neighboring agent, and surrenders others points to the neighbor, in a seemingly random manner. The agent happens to have multiple neighbors, and similar phenomena occur at its interface with each of them. Yet, despite all of the capturing, surrendering, and retaking of territory, the agent tends to maintain a particular shape, as do all of the agents of the population. In fact, some agents tend to maintain the same particular shape.

Those same similar-looking agents also appear to exert some other similar characteristics. Imagine that the agent population was initially randomly distributed and that these similar-looking agents, on meeting each other, tend to stay together; eventually, they begin to form an aggregate of similar-looking agents. Some of these agents continue to mingle within the population of other agents (who also happen to look alike); however, over time, the similar-looking agents eventually all cluster together. Occasionally one of the agents leaves the group and ventures out into the population of

FIGURE 15.1 Discrete, two-dimensional Cellular Potts Model (CPM) lattice. The medium is labeled with 0. The cells are labeled with unique numbers (1, 2, 3, and 4). The two different agent (cell) types are shaded light and dark gray.

other agents, where it then seems more obtuse than before. Imagine that those other agents have formed a sort of perimeter around the aggregate, rather than forming their own; they must not prefer to be in contact with each other and prefer the medium even less.

These are some of the emergent phenomena of a simulation produced by the generalized *CPM*, which, in its original proposal (Graner and Glazier, 1992), was intended to produce this imaginary scenario: cell sorting. In the CPM, every agent is assigned a unique *identification number*, and each is one of a set of agent types. CPM modeling characteristics are described by the agent type, and every agent of a type is assigned the same attributes such that two agents of the same type are only distinguishable by the identification number, hence the similar-looking agents with similar characteristics.

The territorial exchanges of the domain, called the *lattice*, by the agents are actually the accepted subset of randomly selected simulation events, called *copy attempts*. For each copy attempt, a lattice site is randomly selected, called the *source site*, as is one of its nearest neighbor sites, called the *target site*. The CPM considers the likelihood that the identification number at the target site becomes the identification number at the source site.

Just as the selection of the site of a copy attempt is a stochastic process, so too is its acceptance or rejection. Acceptance and rejection are dictated by a *transition rule* that expresses the probability of the occurrence of the copy attempt, which is influenced by a virtual Hamiltonian of the agent population. The transition rule dictates that if the copy attempt, at most, does not increase the Hamiltonian, then the copy attempt is accepted. If the copy attempt does increase the Hamiltonian, then the probability of occurrence decreases and continues to decrease with increasing additional virtual energy due to the copy attempt.

The Hamiltonian is a modeling description that is universally applied to the agents, but how the agents affect the Hamiltonian is contextual. Among an arbitrarily complex set of other agent-based modeling attributes, a CPM agent type is necessarily modeled with attributes that dictate how the agent type influences the Hamiltonian. The relationship between the agents and the Hamiltonian is subject of subsequent elaboration, but, for the present discussion, agents generally obey the rule of reducing the system energy, and occasionally they disobey. The CPM then models random microscopic activity such that, given the occurrence of a sufficient number of microscopic events, emergent phenomena occur because of some lower macroscopic energy state. The following sections outline the mathematical formulation of the CPM as described here, along with select applications so to demonstrate both some emergent phenomena predicted by the model as well as its usefulness.

15.3.3.2 Mathematical Formulation

Let $\mathcal{L}$ be a regular d -dimensional lattice occupied by agents of unique identification $\sigma_i \in \mathcal{J} \subset \mathbb{N}$. Let $\sigma(r, t) \in \mathcal{J}$ denote the identification number of the occupied site r at time t ,

$$\sigma : \mathcal{L} \rightarrow \mathcal{J}, \quad (15.1)$$

where each σ_i occupies the lattice sites $\mathcal{V}^{\sigma_i} \subset \mathcal{L}$. Each σ_i has a corresponding agent type $\tau = \tau(\sigma) \in \mathcal{F}$ that defines the behavior of agent σ ,

$$\tau : \mathcal{J} \rightarrow \mathcal{F}, \quad (15.2)$$

such that for some σ_i and σ_j , if $\tau(\sigma_i) = \tau(\sigma_j)$, then σ_i and σ_j are phenotypically indistinguishable.

Let $r_s \in \mathcal{L}$ denote a randomly selected lattice source site, and let $r_t \in \mathcal{N}(r_s)$ denote a randomly selected nearest neighbor target site in the neighborhood $\mathcal{N}(r_s)$ of r_s . The copy attempt $\sigma(r_t, t') \rightarrow \sigma(r_s, t)$ at time $t' > t$ occurs as a stochastic function of the system Hamiltonian $\mathcal{H}$,

$$\begin{aligned} \sigma(r_t, t') \\ = \sigma(r_s, t) \text{ with probability } e^{-\max\{0, \frac{\Delta\mathcal{H}}{\mathcal{H}^*}\}}, \mathcal{H}^* > 0, \end{aligned} \quad (15.3)$$

where $\mathcal{H}^*$ is a coefficient and $\Delta\mathcal{H}$ is the change in system Hamiltonian due to the copy attempt. This is to say, the probability that the copy attempt occurs is a Boltzmann acceptance function of the system Hamiltonian, where events that, at most, do not increase the Hamiltonian always occur. $\mathcal{H}^*$ can be thought of as a sort of system temperature, because greater $\mathcal{H}^*$ increases the probability of a copy attempt and so produces more agent motility.

15.3.3.3 Methods and Models

What, then, is a CPM agent in a biological process? Cells interact, take particular shapes, and perform particular functions, so perhaps an agent is a cell, and so do cell aggregates, organs, and organelles. One may construct a simulation that produces an agent with the phenotype of a certain cell, another with agents resembling the proteins within that cell, and then another with agents that act like a population of that cell. The mathematical framework of the CPM is ambiguous in this context (and powerfully so) in that only the tendency of the random events associated with each agent is described. That is, the agents of a CPM simulation are assigned physical meaning by their emergent attributes, which may be a product of, or contribute to, the intraagent interactions of some other agent on some other scale.

The CPM has many characteristics of a traditional CA. Like the CA, the CPM consists of a lattice of states, and the states evolve according to a transition rule that considers neighboring states. However, contrarily to the CA, where the transition rule is applied globally and simultaneously, the copy attempts in the CPM occur at randomly selected lattice sites and are not simultaneously considered. For example, say some two copy attempts are to be accomplished in a simulation while modeling a volumetric constraint, and both happen to involve a particular cell. If the first copy attempt is accepted, then the volume of the cell changes, which then may affect the probability of the occurrence of the second copy attempt. And so a given set of copy attempts can only be computed independently if each attempt is sufficiently far from all other attempts, which, for a finite domain and sufficient events to produce emergence, is never the case. In this way the CPM has many of the dynamic characteristics of a CA, but is, in principle, a Monte Carlo method. More specifically, because changes in state occur in a probabilistic manner, the CPM is a KMC method and of the class of Potts models (the identification number is the Potts model spin state).

In the spirit of both CA and the general Monte Carlo method, the virtual time unit of the CPM is called a *Monte Carlo step* (MCS) and is typically demarcated as the accomplishing of a number of randomly selected copy attempts equal to the number of sites in the lattice. Therefore, similarly to the simultaneous application of a transition rule at all lattice sites in the CA, in one MCS every lattice site has equal probability of being selected once as a source site r_s and once as target site r_t in a copy attempt $\sigma(r_t, t') \rightarrow \sigma(r_s, t)$. And if T and T' denote the current and next virtual times, then T' takes the form $T' = T + |\mathcal{L}|(t' - t)$. However, it should be noted that the demarcation of an MCS with respect to the number of copy attempts is arbitrary and has no intrinsic relationship with physical time.

15.3.3.4 The Hamiltonian

In general, the Hamiltonian $\mathcal{H}$ is a summation of energy components $\mathcal{H}^i$, each of which models various emergent phenomena,

$$\mathcal{H} = \sum_i \mathcal{H}^i. \quad (15.4)$$

Hamiltonian expressions are formulated to model an agent-type attribute such that the attribute produces the minimum value of the Hamiltonian expression. Therefore if, say, an agent is to dissociate into fragments, then a corresponding Hamiltonian expression would contribute energy because of the coincident elements of the agent in the lattice. That is to say, the lowest energy state of a dissociated agent is one where no elements of the agent in the lattice are in contact.

If agent σ_i of type $\tau(\sigma_i)$ tends to occupy $v_c^{\tau(\sigma_i)}$ lattice sites, then let $\mathcal{H}^v$ be the energy contributed to the system because of deviations of $|\mathcal{V}^{\sigma_i}|$ from $v_c^{\tau(\sigma_i)}$ for all $|\mathcal{J}|$ agents:

$$\mathcal{H}^v = \sum_{i=1}^{|\mathcal{J}|} \lambda_v^{\tau(\sigma_i)} \left(|\mathcal{V}^{\sigma_i}| - v_c^{\tau(\sigma_i)} \right)^2, \lambda_v^{\tau(\sigma_i)} > 0, \quad (15.5)$$

where $\lambda_v^{\tau(\sigma_i)}$ is the Lagrange multiplier for the spatial occupation constraint of type $\tau(\sigma_i)$. $\mathcal{H}^v$ then contributes to the system such that copy attempts tend to maintain each σ_i at the spatial constraint $v_c^{\tau(\sigma_i)}$ of its type $\tau(\sigma_i)$.

In the same manner, if $\tau(\sigma_i)$ tends to maintain some number of boundary sites $b_c^{\tau(\sigma_i)}$, then let $\mathcal{B}^{\sigma_i} \subseteq \mathcal{V}^{\sigma_i}$ denote the set of lattice sites that form a closed boundary of $\mathcal{V}^{\sigma_i}$, and let $\mathcal{H}^b$ be the energy contributed to the system because of deviations of $|\mathcal{B}^{\sigma_i}|$ from $b_c^{\tau(\sigma_i)}$:

$$\mathcal{H}^b = \sum_{i=1}^{|\mathcal{J}|} \lambda_b^{\tau(\sigma_i)} \left(|\mathcal{B}^{\sigma_i}| - b_c^{\tau(\sigma_i)} \right)^2, \lambda_b^{\tau(\sigma_i)} > 0, \quad (15.6)$$

where $\lambda_b^{\tau(\sigma_i)}$ is the Lagrange multiplier for the boundary constraint of agent type $\tau(\sigma_i)$. When paired with $\mathcal{H}^v$, $\mathcal{H}^b$ contributes to the system such that copy attempts tend to maintain each σ_i at a particular shape. For example, for $b_c^{\tau(\sigma_i)} = 0$ and any $v_c^{\tau(\sigma_i)}$, the minimum surface area in three-dimensional space is produced by a sphere, but for very large $b_c^{\tau(\sigma_i)}$, an agent in three-dimensional space would tend to resemble a line.

For each pair of agent types τ and τ' , if there is some contact energy $J(\tau, \tau')$ due to the interface between two agents, then let $\mathcal{H}^h$ describe the contact energy of all agent interfaces:

$$\mathcal{H}^h = \sum_{i=1}^{|\mathcal{J}|} \sum_{j=1}^{|\mathcal{N}(r^i)|} \left(1 - \delta_{\sigma(r^i), \sigma(r^{j,i})} \right) J(\tau(\sigma(r^i)), \tau(\sigma(r^{j,i}))), \quad (15.7)$$

where $r^{j,i} \in \mathcal{N}(r^i)$ is the j th element of the neighborhood of the i th element $r^i \in \mathcal{L}$ and δ is the Kronecker delta. $\mathcal{H}^h$ then contributes to the system such that copy attempts tend to rearrange the agents to minimize their contact energy. The construction of $\mathcal{N}(r^i)$ can be the same as those from the CA (e.g., Neumann, Moore) or of some other formulation.

15.3.3.5 Implementation

Numerical implementation of the CPM can be accomplished in a number of ways (Swat et al., 2012), although some basic, requisite features of the formulation will always appear. Neglecting design considerations of the lattice, initial cellular configuration, and specific energy expressions (e.g., adhesion), any implementation of the CPM must maintain the elementary components of the unique identification, agent type, and, for any specific energy expression, spatial configuration of each agent. Although identification and type labels are not necessarily restricted to any class, positive integer labels are probably most advisable for efficient programming considerations such as index mapping, where identification and type labels can be used to construct complex lookup tables.

As a simple example, consider the case of modeling adhesion. Rather than allocating an array of adhesion coefficients for each simulation agent, a symmetric matrix of adhesion coefficients can be constructed, where each element in the adhesion matrix corresponds to the adhesion coefficient of a pair of agent types. When considering a copy attempt, a programming call can efficiently retrieve the relevant adhesion coefficient by using the two agent types of the attempt as the indices into the adhesion matrix. Further programming efficiencies can include maintaining the list of agent types as a one-dimensional array, where the order of each element corresponds to the agent identification number (e.g., element two

is the type of agent two). This effectively eliminates the need to maintain a separate array of all identification numbers by relying on whatever structure that maintains the lattice to store information about the existence of currently present agents in the simulation. If the lattice is a multidimensional array of integers, then one can simply use the integer label at a site as an index to retrieve the agent type, which can then be used as an index into the various agent models, etc.

It should also be noted that, while calculating the total energy of the system may be useful for validation of an implementation, e.g., to demonstrate the minimization of energy (Neagu et al., 2005) or, perhaps, to formulate some convergence criterion, which is not commonly reported in literature, the only relevant information to calculating the probability of a copy attempt is the change in system energy due to its occurrence. This is particularly relevant because the copy attempt is the fundamental action of the dynamical system of a CPM simulation, which may consist of even billions of copy attempts. Considering this computational aspect, significant improvements in simulation performance may be accomplished by merely neglecting to maintain a transient history of the system energy.

The same is true for seemingly insignificant modifications such as algebraic simplifications, as well as for programming considerations such as program structure, preallocation, and parallelization (Chen et al., 2007). For an algorithm that performs the copy attempt, a mathematical expression that produces the same result with one less numerical calculation results in one less calculation per copy attempt per MCS, potentially reducing the total computational cost of a simulation by billions of calculations. While the fundamental ideas of agent-based modeling may beg the programmer to construct an object-oriented programming structure, meticulous analysis must be performed to ensure that additional communication overhead does not severely diminish computational efficiency, even to striving to reduce the time to complete an MCS on the scale of microseconds. Aforementioned attempts at parallelization of the CPM must devise an algorithm that can determine the independence of a series of copy attempts, send and receive all necessary information about the copy attempts to separate processors, and compile results from all processors, and all in less time than what would have been required to simply process them in a serial implementation. Any implementation must also account for various sizes of simulations for memory considerations, perhaps by the perpetual activity of asking, at what simulation size would this structure crash an ordinary computer?

With all these in mind, the following pseudocode presents a general implementation of one MCS for a simple simulation, using some of the aforementioned examples of index mapping and preallocation (Algorithm 15.1). In this algorithm, Δr is a previously allocated array of relative indices to a neighborhood $\mathcal{N}(r)$ of lattice site r . The function `draw_a_source( $\mathcal{L}$ )` returns a randomly selected index in $\mathcal{L}$, `draw_a_target( $r$ )` returns a randomly selected site in $\mathcal{N}(r)$, and `rand()` returns a uniformly distributed number in $[0, 1]$. According to aforementioned efficiencies, the pseudocode assumes a symmetric adhesion coefficient matrix $J(\tau_1, \tau_2)$ where (τ_1, τ_2) is the index to the adhesion coefficient between types τ_1 and τ_2 , as well as a one-dimensional array of agent types $\mathcal{T}$, where the identification number of an agent is the index to its type in $\mathcal{T}$. Comments are annotated in parentheses and all mathematical simplifications are neglected, for clarity.

Fig. 15.2 shows an example of the application of this algorithm on a small agent population. This particular simulation is designed to model a volumetric constraint (intraagent interactions) and intercellular adhesion (interagent interactions) and to simulate a number of copy attempts per MCS equal to the number of lattice sites $|\mathcal{L}|$. Fig. 15.2 illustrates the initial configuration, the configuration after one completed MCS, and configuration after 100 MCS.

15.4. APPLICATIONS

This section first presents two traditional applications for spheroid-based fabrication, followed by three applications of a “hybridized” CPM: cell sorting, fusion, diffusion, chemotaxis, and hypoxia.

15.4.1 Cell Sorting

In its original publication (Graner and Glazier, 1992), the CPM was presented for the application of modeling cell sorting in aggregates. From a qualitative perspective, modeling cell sorting in the CPM can be accomplished by the following thought experiment. Say an aggregate is initially populated with a random distribution of two types of cells, type 1 and type 2. If the contact energy at the interface of two cells of type 1 is greater than the energy at the interface of two cells of type 2, then what would be the distribution of the aggregate with the least system energy? The answer is a population distribution such that type 2 cells are most in contact with each other and type 1 cells are least in contact with each other. That is, type 1 cells migrate to the perimeter of the aggregate. In the CPM, agent types that are more strongly bonded are modeled as contributing less energy to the system so as to increase the probability of their maintaining an interface.

Algorithm 15.1 (Monte Carlo Step of Copy Attempts) To Find the Resulting Lattice Configuration After One MCS, Given the Current Lattice Configuration

```

For copy_attempt = 1 to  $|\mathcal{L}|$  (number of lattice sites)
  (Get the next copy attempt site information)
   $r_s = \text{draw\_a\_source}(\mathcal{L})$ 
   $r_t = \text{draw\_a\_target}(r_s)$ 
   $\mathcal{N}(r_t) = r_t + \Delta r$ 
  (Get the identification and type labels)
   $\sigma_s = \mathcal{L}(r_s)$ 
   $\sigma_t = \mathcal{L}(r_t)$ 
   $\tau_s = \mathcal{F}(\sigma_s)$ 
   $\tau_t = \mathcal{F}(\sigma_t)$ 
  (Calculate the energy contribution due to the volumetric constraint)
   $\Delta \mathcal{H}^v = \lambda_v^{\tau_s} \left[ (|\mathcal{V}^{\sigma_s}| + 1 - v_c^{\tau_s})^2 - (|\mathcal{V}^{\sigma_s}| - v_c^{\tau_s})^2 \right]$ 
   $\Delta \mathcal{H}^v = \Delta \mathcal{H}^v + \lambda_v^{\tau_t} \left[ (|\mathcal{V}^{\sigma_t}| - 1 - v_c^{\tau_t})^2 - (|\mathcal{V}^{\sigma_t}| - v_c^{\tau_t})^2 \right]$ 
  (Calculate the energy contribution due to adhesion)
   $\Delta \mathcal{H}^h = \sum_{r_n \in \mathcal{N}(r_t)} \left( 1 - \delta_{\sigma_s, \mathcal{L}(r_n)} \right) J(\tau_s, \mathcal{F}(\mathcal{L}(r_n)))$ 
   $\Delta \mathcal{H}^h = \Delta \mathcal{H}^h - \sum_{r_n \in \mathcal{N}(r_t)} \left( 1 - \delta_{\sigma_t, \mathcal{L}(r_n)} \right) J(\tau_t, \mathcal{F}(\mathcal{L}(r_n)))$ 
  (Calculate the probability of occurrence of the copy attempt)
   $\Delta \mathcal{H} = \Delta \mathcal{H}^v + \Delta \mathcal{H}^h$ 
   $P = \exp \left( -\max \left\{ 0, \frac{\Delta \mathcal{H}}{\mathcal{H}^*} \right\} \right)$ 
  (Check for occurrence of the copy attempt and implement if accepted)
  If  $\text{rand}() \leq P$ 
     $\mathcal{L}(r_t) = \mathcal{L}(r_s)$ 
  End If
Next copy_attempt

```

FIGURE 15.2 Two-dimensional simulation demonstrating the Monte Carlo step algorithm. The medium is depicted in white. The two different cell types are shaded light and dark gray.

Fig. 15.3 shows results of a two-dimensional cell sorting simulation performed in CompuCell3D. In this simulation, a spheroid was initialized with a randomly distributed population of two cell types, referred to as NonCondensing and Condensing, which are less and more adhesive, respectively. By the first 20 MCS, all initiation artifacts vanished and nearby cell types collected together. Distinct regions of high- and low-adhesion cells formed by 880 MCS, as well as the completion of a monolayer of low-adhesion cells at the perimeter of the spheroid. By 10,000 MCS, cell sorting completed with a distinct core of high-adhesion cells being surrounded by a region of low-adhesion cells. Further morphological development of the spheroid would likely produce an approximately uniform thickness of the low-adhesion region, as the two cell-type regions evolve to minimize their interface.

FIGURE 15.3 Snapshots of the cell-lattice configurations for the cell-sorting simulation in Listing 1. The boundary energy hierarchy drives NonCondensing (light gray) cells to surround Condensing (dark gray) cells. The white background denotes surrounding medium (Swat et al., 2012).

15.4.2 Spheroid Fusion

If a domain is randomly populated with cells that contribute more energy by being in contact with the medium than with each other, given sufficient time and Brownian motion, the cells will tend to minimize the total interface of all cells with the medium: cell aggregation. Because the lowest energy state of the system has the smallest interface with the medium, the cells will tend to form a circular aggregate in two-dimensional space and a spheroid in three-dimensional space. Placing two spherical aggregates in contact, they then have the opportunity to further decrease their combined energy by further decreasing their interfaces with the medium: spheroid fusion. Fig. 15.4 demonstrates on such application, where 10 spheroids are arranged in a circle and permitted to fuse, producing a toroidal structure. Further simulation of this structure would likely produce a sorted configuration.

15.4.3 Diffusion

The CPM can be extended to modeling in a continuous domain $\mathcal{G}$, where every lattice point $r = r(X)$ has a corresponding coordinate $X \in \mathcal{G} \subset \mathbb{R}^d$ and every MCS $m = m(t)$ has a corresponding time $t \in \mathbb{R}_{\geq 0}$. For example, let $C = C(X, t)$ be the concentration of a chemical species in a homogenous domain of diffusion coefficient D . If each agent is described with a chemical source rate $s = s(\tau(r, m)) \in \mathcal{S}$ (production or consumption),

$$s : \mathcal{F} \rightarrow \mathcal{S}, \quad (15.8)$$

then a corresponding source field $S = S(X, t) = s(r(X), m(t))$ can be assigned to an expression for the diffusion of C in $\mathcal{G}$

$$\dot{C} = D\partial_{ii}C + S. \quad (15.9)$$

The simultaneous updating of $\mathcal{S}$ and solutions to differential equations in $\mathcal{G}$ are readily accessible because, in numerical applications, differential equations are often discretized in a similar manner to that of the CPM and solved, whether by the finite difference method, finite element method, or otherwise. By relating the lattice of the CPM to continuous fields, the CPM can be extended to modeling the effects of discrete agents on their environment, and vice versa, such as the consumption of nutrients, cell signaling, chemotaxis, and cell state changes.

FIGURE 15.4 KMC simulation of toroidal structure formation through the fusion of 10 cell aggregates. Top view of the fusing aggregates at (A) the beginning ($t = 0$) and (B) the completion of fusion. (C) Cross section through the median plane of the fused toroidal structure shown in (B). Otherwise identical cells, initially located in adjacent aggregates, are colored differently to emphasize the degree of mixing during fusion (Flenner et al., 2012).

15.4.4 Chemotaxis

If an agent moves along a chemical gradient, then let $\mathcal{H}^c$ describe the energy contributed to the system because of the influence of the chemical species C on the agent:

$$\mathcal{H}^c = \sum_{i=1}^{|\mathcal{L}|} \lambda_c^{\tau(\sigma(r^i))} C(X(r^i)), \quad (15.10)$$

where $X(r^i)$ is the coordinate in $\mathcal{E}$ corresponding to r^i in $\mathcal{L}$ and $\lambda_c^{\tau(\sigma(r^i))}$ is the Lagrange multiplier for the chemotactic response of agent type $\tau(\sigma(r^i))$. Note that if $\lambda_c^\tau > 0$, type τ agents are inclined to move toward lower field values in $\mathcal{E}$, and conversely type τ agents will move toward higher field values for $\lambda_c^\tau < 0$. For $\lambda_c^\tau = 0$, type τ agents are unaffected by C .

15.4.5 Hypoxia

Additional transition rules can be applied to a CPM simulation that model the influence of field solutions in $\mathcal{E}$ on the agents in $\mathcal{L}$. Such rules may seek to model phenomena such as cell splitting, differentiation, or death. Generally speaking, transition rules of this class further capture the interactivity of CPM agents and their corresponding environmental conditions.

Fig. 15.5 demonstrates one such transition rule, which models the death of an agent due to insufficient field values in $\mathcal{E}$ (i.e., hypoxia). In this simulation, a spheroidal cross section was initiated with a population of high-adhesion, low-consumption agents.

Let $\mathcal{T}^d$ be a stochastic transition rule that describes the transition of agent type τ to its dead state τ^d :

$$\tau(\sigma_i(t')) = \mathcal{T}^d(\tau(\sigma_i(t)), C(X^{\sigma_i})), \quad (15.11)$$

where $X^{\sigma_i} = X(\rho_{\sigma_i}^j) \in \mathcal{E}^{\sigma_i}$ for the subdomain $\mathcal{E}^{\sigma_i} \subseteq \mathcal{E}$ that corresponds to the subdomain $\mathcal{V}^{\sigma_i} \subseteq \mathcal{L}$ of agent

FIGURE 15.5 Hypoxic cell death in a randomly distributed cell population.

σ_i . Agent σ_i transitions to its dead state as a function of the mean concentration C_{σ_i} in $\mathcal{E}^{\sigma_i}$:

$$C_{\sigma_i} = \frac{1}{|\mathcal{E}^{\sigma_i}|} \sum_{j=1}^{\mathcal{E}^{\sigma_i}} C \left(X \left(\rho_{\sigma_i}^j \right) \right). \quad (15.12)$$

The transition occurs with the probability

$$P \left(\tau \left(\sigma_i \left(t' \right) \right) = \tau^d \right) = pe^{-\left(\frac{C_{\sigma_i}}{\gamma} \right)^2}, \quad (15.13)$$

where p and γ are coefficients. Another agent type is then introduced to the simulation, with corresponding attributes. In this example, the corresponding dead state was modeled with a 25% reduction in volumetric constraint, no consumption and no change in adhesion attributes. As cells at the core of the spheroid in Fig. 15.5 are subjected to lower concentration field values, the probability of cell death then increases. As more MCS transpire, more cells at the core die, which has been observed in experimental observations (Freyer and Sutherland, 1980; Mueller-Klieser et al., 1986; Mueller-Klieser and Sutherland, 1982).

15.5. DISCUSSION AND OUTLOOK

Comparison of different models. The process of choosing a model involves many intrinsic and extrinsic considerations. Compared with other methods, the mathematical formulation of the CPM and other KMC methods is relatively straightforward, making them more accessible for implementation. However, both LB and CPD methods are well-suited for computational parallelization, making them much more efficient for in silico experiments. This is due to that LB and CPD methods involve solving systems of discretized differential equations for each simulation step, whereas KMC methods utilize an algorithm that, as previously mentioned, performs a series of serial state changes per simulation step. Although it is possible to construct a parallel implementation of the CPM by partitioning the lattice into subdomains (Chen et al., 2007) each of which is simulated on individual cores or machines, significant effort must be put toward overcoming difficulties such as cell migration into different subdomains, minimizing communication overhead, etc. This, of course, is also subject to technical considerations such as available software and computational resources.

Computational considerations. Each method seems (at least for now) best suited for applications at particular scales. At the tissue level, where billions of cells contribute to morphology, CPD and CPM methods introduce computational requirements that are currently infeasible. In this case, the continuous distribution functions of LB methods provide a useful means to represent the activity of many cells at various locations; yet, at the cellular level, the activities of discrete, individual agents become significant. This difference in spatiotemporal resolution is apparent in the relating of physical and simulation time by comparing emergent events.

Time calibration. In simulations using each of these three methods, spheroid fusion was simulated and compared with in vitro results to determine a time correlation of the amount of time per simulation step. This calculation is performed by recording the time to accomplish fusion in vitro and then dividing that time by the number of simulation steps to accomplish fusion in silico. Those correlations were calculated as 5.184 s/MCS for an LB method simulation (Cristea and Neagu, 2016), 36 s/MCS for a CPD simulation, and 1.6×10^{-5} s/MCS for a KMC simulation (Flenner et al., 2012). Although these correlations are specific to model parameters used in each simulation, they are generally demonstrative of the scale at which each method is best applied, where KMC methods can probably be considered the most meticulous, and also the most demanding, of the three.

Capturing global/multiscale behavior. This observation presents a fundamental challenge to biological modeling: how does one predict the emergent behavior of billions of discrete agents at multiple scales, when those agents exhibit such complex behavior? True, the structure of tissue does exhibit behavior like liquid drops, except that, in this case, the drop consists not only of relatively predictable agents (e.g., a water molecule), but rather of lots of types of agents that communicate, intentionally migrate, consume, generate waste, split, change type, and die. Yet the very concept of a computer simulation allocating, simulating, and recording the behavior of a billion individual agents seems daunting, in the least. Perhaps some characterization of the CPM at the cellular level may produce analytic descriptions that can then be incorporated into CPD and LB methods, whether modeling cell migration, metabolism, or population dynamics. What

can be confidently asserted is that there is a clear need for feasible and reliable predictive capabilities at both the tissue and cellular levels to progress bioprinting technologies into medical applications.

Relevance for scaffold-free 3D bioprinting. All the methods discussed have benefited, in one way or another, the modeling needs of the emerging field of scaffold-free bioprinting, which revolves around cell spheroids biology. Their constrained size (around 20,000 cells per “building block”) gives hope that, with improvements in software and computational optimizations, the capturing of even deeper aspects of their behavior and metabolism (possibly cell differentiation also) is a feasible endeavor. The rewards will be substantial in better understanding these objects of the model, in optimizing their biotechnological manipulation, and in simplifying the human–instrument interfaces (such as the operation of the Regenova bioprinting robot).

Concluding remarks. Computational modeling is the actual process of creating “virtual objects,” as members of the ever-increasing realm of “virtual reality.” “Virtual tissue engineering” (Sun et al., 2004) and “virtual biofabrication” (Mironov et al., 2011) are very serious candidates for citizenship in this emerging metareality, with all its advantages and challenges. Expectedly, the inhabitants of this virtual world are subjected to the same “evolutionary” constraints (i.e., validation) and selection (i.e., optimization) as the biological objects themselves. Our overview highlighted several approaches toward the creation, validation, and optimization of objects in this category, with the anticipation of an increasing number of new and (evolutionary speaking) “better fitted” examples to occur soon.

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