In-silico analysis on biofabricating vascular networks using kinetic Monte Carlo simulations

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Abstract. We present a computational modeling approach to study fusion of multicellular aggregate systems in a novel scaffold-less biofabrication process, known as “bioprinting”. In this novel technology, live multicellular aggregates are used as fundamental building blocks to make tissues or organs (collectively known as the bio-constructs,) via the layer-by-layer deposition technique or other methods; the printed bio-constructs embedded in maturogens, consisting of nutrient-rich bio-compatible hydrogels, are then placed in bio-reactors to undergo the cellular aggregate fusion process to form the desired functional bio-structures. Our approach reported here is an agent-based modeling method, which uses the kinetic Monte Carlo (KMC) algorithm to evolve the cellular system on a lattice. In this method, the cells and the hydrogel media, in which cells are embedded, are coarse-grained to material’s points on a three-dimensional (3D) lattice, where the cell-cell and cell-medium interaction are quantified by adhesion and cohesion energies. In a multicellular aggregate system with a fixed number of cells and fixed amount of hydrogel media, where the effect of cell differentiation, proliferation, and death are tactically neglected, the interaction energy is primarily dictated by the interfacial energy between cell and cell as well as between cell and medium particles on the lattice, respectively, based on the Differential Adhesion Hypothesis (DAH). By using the transition state theory to track the time evolution of the multicellular system while minimizing the interfacial energy, KMC is shown to be an efficient time-dependent simulation tool to study the evolution of the multicellular aggregate system. In this study, numerical experiments are presented to simulate fusion and cell sorting during the biofabrication process of vascular networks, in which the bio-constructs are fabricated via engineering designs. The results predict the feasibility of fabricating the vascular structures via the bio-printing technology and demonstrate the morphological development process during cellular aggregate fusion in various engineering designed structures. The study also reveals that cell sorting will perhaps not significantly impact the final fabricated products should the maturation process is well-controlled in bioprinting.
1. Introduction

Tissue fusion is an ubiquitous phenomenon during embryonic development and morphogenesis, in which tissues and organs are formed by living cells through cell-cell and cell-extracellular matrix interactions [1]. In the past, bioengineering processes have been devised to fabricate tissues under controlled conditions using cell self-assembly on well-designed scaffold, in which one seeds cells into biodegradable polymer scaffolds or gels, which are then cultured in bioreactors for several weeks and finally implanted into the recipient organism, where the maturation of the new organ continues [2, 3, 4, 5]. In a novel biomimetic scaffold-less or solid scaffold-free biofabrication process, known as “bioprinting”, multicellular tissue spheroids or other shapes of aggregates are used as fundamental building blocks to construct the 3D tissue or organ [6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. The multicellular aggregates are first prepared in the form of tissue spheroids, rods or other stable configurations that consist of cells blended with bio-compatible hydrogels. They are then directly deposited or laid by computer-aided design tools [17, 18] into desired 3D tissue or organ constructs via the layer-by-layer deposition technique or other plausible deposition methods. The printed bio-constructs immersed in a hydrogel are then placed in bioreactors for further maturation, during which the multicellular aggregates undergo the fusion process to form the desired functional tissue or organ products by following the natural rule of histogenesis and organogenesis [8, 9, 10, 14, 19, 20]. Tissue fusion driven biofabrication is a fundamental biophysical and biochemical process in emerging organ bioprinting technology, whose main mechanisms dictating final fused bioconstructs warrant a thorough experimental as well as theoretical investigation.

In bioprinting, the bio-constructs ranging from the ones comprised of tissue spheroids or other stable cellular constructs to functioning tissues or organs all exhibit fluid behavior during tissue fusion processes (in the time and length scale of engineering interest). Numerical simulations of the morphogenesis phenomenon in bioprinting using the Monte Carlo method and experimental evidences [8, 9, 19, 21, 22] reveal a strong influence of surface tension to the multicellular aggregate fusion in the biofabrication process when the biomaterials (tissue spheroids and hydrogel matrices) are regarded as viscous fluids. A separate study using experimental methods and numerical simulations with the cellular Potts model [23, 24] also explored the effect of surface tension and hydrodynamics to the rounding of cellular aggregate [25]. Recently Yang et al. developed a coarse-grain phase field model for studying fusion of cellular aggregates at a coarse-grain length and time scales featuring a long range multicellular aggregate-aggregate interaction in addition to the surface tension impact [26, 27].

In this paper, we present a multicellular lattice model which describes the interactions between cells as well as between cells and media based on the Differential Adhesion Hypothesis (DAH) proposed by Steinberg [28] to study cellular aggregate fusion in the process of fabricating vascular constructs via 3-D bioprinting. DAH implies that early morphogenesis is a self-assembly process, whereby mobile and interacting cells
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spontaneously give rise to the morphological structure [29, 30, 31]. This hypothesis states that (i) cell adhesion in multicellular systems depends on energy differences between different types of cells and (ii) an aggregate of cells is motile enough to reach the configuration which minimizes the interfacial energy of the system. In light of DAH, embryonic tissues mimic the behavior of highly viscous, incompressible liquids and their liquid-like phenomena can then be interpreted using the theory of ordinary viscous liquids, consistent with the multiphase fluid theory adopted by Yang et al. [26, 27]. Many embryonic tissues have been characterized in terms of effective tissue surface tension, which were measured and consistent with the mutual sorting behavior of these tissues [32]. As one of the most important principles of developmental biology, DAH provides a connection between the tissue surface tension and the strength of adhesion between cells constituting the tissue. The implications of DAH have been confirmed not only in vitro and in silico [23, 24], but also in vivo [33, 34].

DAH provides a useful mechanism for predictions on equilibrium tissue configurations using Metropolis Monte Carlo (MMC) methods [35], but this direct Monte Carlo implementation cannot address the question of how these configurations are developed in time since the time used in the MMC is not the real time. Instead, in our numerical experiments, we employ the kinetic Monte Carlo (KMC) algorithm [36] to simulate the stochastic dynamics of the cells based on the Arrhenius law [37, 38]. The reason to choose KMC instead of the MMC method is that the KMC method can provide the transition rates which are associated with possible configurational changes of the multicellular system, and then the corresponding time evolution of the system can be expressed in terms of these rates while the traditional MMC cannot. Our goal of developing the KMC simulation tool is to describe the self-assembly process of cells within tissue constructs during cellular aggregate fusion. The KMC method treats each cell as a point on the lattice and likewise a comparable sized medium chunk as a particle point on the lattice as well. It can thus deal with various types of particles (i.e., cells, including different cells, or hydrogel medium) present on each lattice site rather than following cell shape changes.

Our research reported here is a part of the South Carolina Project for Organ Biofabrication [16], in which scientific progress includes computational modeling of vascular trees and experimental testing of natural and engineered constructs. As the mathematical/computational thrust, we focus on the development of necessary predicative tools, including mathematical models, to support testing of vascular constructs and prefabrication engineering design. Our research goals are to develop computer-aided design or simulation tools for naturally branched vascular trees and predict structure formation results to aide bioengineering design and fabrication. Our KMC simulation tool is thus developed under this backdrop and tested against experiments and benchmarking simulations, with which some preliminary simulation results can be found in [38]. In this paper, we extend our simulations to engineering designed vascular networks in 3-D bioprinting to show the morphological development of vascular veins and networks when they are fabricated using solid as well as uniluminal
multicellular spheroids. These simulations explore not only the feasibility of bioprinting 3-D vascular network constructs using multicellular spheroids, but also demonstrate the potential to aide engineering design in biofabrication, which will be an integral part of the 3-D biofabrication technology.

We organize the rest of the papers into three sections. In Section 2, we describe the lattice model and discuss the KMC algorithm and its implementation for multicellular systems. In Section 3, we present numerical results involving cellular aggregate fusion in bioprinting of bifurcating and networked vascular constructs and discuss their biological implication in terms of developmental biology. We conclude the study in Section 4.

2. Kinetic Monte Carlo algorithms for multicellular system

We first describe the kinetic Monte Carlo method. In the lattice model, we discretize the space and present the multicellular system on a 3D cubic lattice, in which each site is occupied either by a cell or a similar-sized volume element of hydrogel medium or extracellular matrix (ECM) [8, 21, 22]. The configuration at each site $\mathbf{r} = (x, y, z)$ is defined by an integer type index $\sigma_\mathbf{r} \in \{0, 1, 2, \ldots, N_{\text{type}} - 1\}$, where $N_{\text{type}}$ is the number of particle types in the system. For instance, in a binary particle system ($N_{\text{type}} = 2$) formed by cells of type-1 embedded in a hydrogel medium, we take $\sigma_\mathbf{r} = 0$ if medium occupies the site or $\sigma_\mathbf{r} = 1$ if a cell is at the site. The total pair interaction energy of the system is given by:

$$E = \sum_{\langle \mathbf{r}, \mathbf{r}' \rangle} J(\sigma_\mathbf{r}, \sigma_{\mathbf{r}'})$$  \hspace{1cm} (1)

where $J(\sigma_\mathbf{r}, \sigma_{\mathbf{r}'}) = -E_{ij}$ is the contact interaction energy between two particles, located at neighboring sites $\mathbf{r}$ and $\mathbf{r}'$, of type $\sigma_\mathbf{r} = i$ and $\sigma_{\mathbf{r}'} = j$, respectively, and $E_{ij}$ is a positive quantity to represent the mechanical work needed to break the bond between them. Each pair is counted only once in the summation $\langle \mathbf{r}, \mathbf{r}' \rangle$ over neighboring sites.

In the binary particle system, each term $J(\sigma_\mathbf{r}, \sigma_{\mathbf{r}'})$ can take the values $J(0,0) = -E_{mm}$, $J(1,1) = -E_{cc}$, $J(0,1) = J(1,0) = -E_{cm}$, where $E_{mm}$, $E_{cc}$, and $E_{cm}$ represent the interaction energy strengths between medium-medium, cell-cell, and cell-medium pairs, respectively [39].

By separating interfacial and bulk terms in the sum in (1), the total interaction energy can be rewritten into [21]

$$E = \sum_{i,j=0, i < j}^{N_{\text{type}}-1} \gamma_{ij} N_{ij}^b - \frac{1}{2} n_n \sum_{i=0}^{N_{\text{type}}-1} N_i E_{ii}$$  \hspace{1cm} (2)

where $\gamma_{ij} = \frac{E_{ii} + E_{jj} - E_{ij}}{2}$ are the interfacial tension parameters between different particle types $i$ and $j$, and $N_{ij}^b$ are the total numbers of bonds between them [39, 8]. Clearly, there are $N_{\text{type}}(N_{\text{type}} - 1)/2$ independent $\gamma_{ij}$ parameters and the corresponding numbers $N_{ij}^b$ of bonds between different particle types. The parameter $n_n$ denotes the number of significant neighbors. In our 3D simulations, we consider interactions between first, second, and third nearest neighbors, for which $n_n = 26$, and we assume that the
interaction strengths between a particle and all of its $n_n$ neighbors are the same. During simulations that do not include cell proliferation, differentiation and death, the numbers of particles of each type, $N_i$, are constant so that the second term in (2) is constant as well; therefore, it can be omitted. The conformational evolution of the multicellular system is energetically driven to the configuration which minimizes the interfacial free energy (2) of the system, i.e., the first term is minimized after the second one is omitted.

We have applied the kinetic Monte Carlo (KMC) simulations to study the fusion of the cellular clusters when they are embedded in a host hydrogel medium, i.e., the time evolution of the whole multicellular system arranged in a designer’s pattern for tissue and organ generation [38]. We note that Metropolis Monte Carlo (MMC) simulations [35] using (2) yielded results in qualitative agreement with experiments on living tissue self-assembly [8, 19, 21]. However, in the MMC method, trial steps are sometimes rejected because the acceptance probability is small, in particular when a system approaches equilibrium, or is in a metastable state. The KMC method that we adopt here is related to the method proposed by Bortz, Kalos, and Lebowitz as a speed-up to the MMC method for simulating the evolution of the Ising model [36]. A main feature of the KMC algorithm is that it is “rejection-free”. In each step, the transition rates for all possible changes from the current configuration are calculated and then a new configuration is chosen with a probability proportional to the rate of the corresponding transition. Since the interaction is short-ranged, there is only a small number of local states that need to be changed due to the previous transition. The other feature of KMC is its capability of simulating the dynamics of the system in real time. Since the corresponding time evolution is expressed in terms of these rates, KMC method should provide a more accurate description of the time evolution of a multicellular system than the MMC method.

The dynamics of the cells depends on the transition rates of swapping cells with adjacent cells of different type and/or with medium particles [37, 38]. In the spin-exchange Arrhenius dynamics, the simulation is driven based on the energy barrier two particles have to overcome exchanging with each other. We perform a swap only if the potential energy of the cells is higher than a given threshold. The swap is taken with its rate given by the Arrhenius relation:

$$r = w_0 \exp(-E_b)$$  \hspace{1cm} (3)

where the prefactor $w_0 = 1/\tau_0$ corresponds to the cell swapping frequency with $\tau_0$ the characteristic or relaxation time unit. The energy barrier $E_b$ is assumed to depend only on the local state of the swapping cells/particles. For instance, in the binary particle system, the energy barrier of swapping a cell $i$ and a medium particle $j$ is given by

$$E_i^{i,j} = E_s + 2\gamma_{cm}(n_n - n_{cm}^{i,j})$$  \hspace{1cm} (4)

where $E_s$ is the energy associated with the site binding of the particle which could vary in both space and time to account for spatial and temporal flow situations (in this study we set $E_s = 0$). The parameter $\gamma_{cm}$ is the interfacial tension parameter, and $n_n = 26$ is
the number of significant neighbors. As shown in Figure 1, \( n_{cm}^{ij} \) is the average number of cell-medium bonds connected to cell \( i \) and medium particle \( j \), which is

\[
n_{cm}^{ij} = \frac{1}{2}(n_{im} + n_{jc}). \tag{5}
\]

Here \( n_{im} \) is the number of bonds connecting cell \( i \) with the neighboring medium particles and \( n_{jc} \) is the number of bonds connecting medium particle \( j \) to the neighboring cells. Then all swapping events can be classified into at most \( 2n_n \) folds according to the value of \( n_{cm}^{ij} \). To summarize, the following parameters need to be given for KMC simulations:

(i) the characteristic or relaxation time unit \( \tau_0 \); (ii) the interfacial tension parameter \( \gamma_{ij} \) which depends on the interaction energy strengths \( E_{ii}, E_{jj}, \) and \( E_{ij} \).

\[\begin{align*}
(a) & \quad n_{im} = 6 \quad n_{jc} = 4 \\
(b) & \quad n_{im} = 6 \quad n_{jc} = 5 \\
(c) & \quad n_{im} = 6 \quad n_{jc} = 6 \\
(d) & \quad n_{im} = 6 \quad n_{jc} = 7 \\
(e) & \quad n_{im} = 6 \quad n_{jc} = 8 \\
(f) & \quad n_{im} = 7 \quad n_{jc} = 7
\end{align*}\]

**Figure 1.** Schematic representation of swapping a cell \( i \) and a medium particle \( j \) (for 2D, \( n_n = 8 \)). Six examples are shown. \( n_{im} \) is the number of bonds connecting cell \( i \) with neighboring medium particles and \( n_{jc} \) is the number of bonds connecting medium particle \( j \) to neighboring cells. Therefore, the average number of cell-medium bonds connected to cell \( i \) and medium particle \( j \) given in (5) are (a): \( n_{cm}^{ij} = 5 \); (b): \( n_{cm}^{ij} = \frac{11}{2} \); (c): \( n_{cm}^{ij} = 6 \); (d): \( n_{cm}^{ij} = \frac{13}{2} \); (e): \( n_{cm}^{ij} = 7 \), and (f): \( n_{cm}^{ij} = 7 \).

The KMC algorithm is built on the assumption that the model features \( N \) independent Poisson processes (corresponding to \( N \) swapping events) with transition rates \( r_i \) that sum to give the total rate \( R = \sum_{i=1}^{N} r_i \). The general KMC algorithm at each step is given as follows.

**Algorithm 2.1 (The KMC algorithm)**

**Step 1:** Generate a uniform random number, \( \xi_1 \in (0, 1) \) and decide which process will take place by using a binary search to choose the event index \( s \) such that

\[
\sum_{i=1}^{s-1} \frac{r_i}{R} < \xi_1 \leq \sum_{i=1}^{s} \frac{r_i}{R} \tag{6}
\]
Step 2: Perform the selected event leading to a new configuration.

Step 3: Use $R$ and another random number $\xi_2 \in (0,1)$ to decide the time it takes for that event to occur (the transition time), i.e., the nonuniform time step $\Delta t = -\log(\xi_2)/R$.

Step 4: Update the total rate $R$ and any rate $r_i$ that may have changed due to that event.

In simulations with a finite number of distinct processes it is more efficient to consider the groups of events according to their rates [40, 41, 42]. This can be done by forming groups of the same kinds of events according to the number of cell-medium bonds $n_{cm}^{i,j}$ in (5). A faster algorithm at each step of the KMC simulation based on the grouping of events can be found in [38]. The KMC simulations are implemented with fixed boundary conditions so that the cells are constrained to move within the hydrogel medium and the medium boundary is treated as a fixed physical boundary of the system. It is only when a significant portion of the cells migrates away from the cell ensemble in the model construct and reaches the system boundary that finite-size effects may interfere with the energetically-driven rearrangement of cells and medium particles. However, this is usually not the case in our simulations since only a few cells can detach from the construct formed by the ensemble.

3. Results and Discussions

We demonstrate the numerical methods described in the previous section by investigating cell self-assembly and multicellular aggregate fusion in several cases relevant to biofabrication with different and complex geometries, namely directed self-assembly of cells into complex bio-constructs of controlled shape by engineering design. We first study an example of fusion of single type cellular aggregates. Then we focus on the cellular aggregates formed by multiple types of cells with distinct adhesivities.

3.1. Tube construct formation via fusion of single cell type cellular spheroids

Here, we present an example to simulate a tubular construct formation. As shown in Figure 2(a) and (e), initially, spherical aggregates (red) are deposited layer-by-layer in the axial direction on the circumference of a hexagon supported by agarose rods (gray), which are formed in situ and deposited by the bioprinter automatically, rapidly and accurately. Since agarose is an inert and biocompatible hydrogel that cells neither invade nor rearrange, the rods can keep their integrity during post-printing fusion, and can be removed to release the fused multicellular construct [14]. There are total 480 identical spheroidal aggregates stacked into 20 layers in the axial direction. Each aggregate contains 280 cells and its diameter is about 8 cell diameters. Thus, the length of initial construct is 160 cell diameters.

Since cohesion between medium-medium $E_{mm}$ and adhesion between cell-medium $E_{cm}$ are considered to be negligibly small compared with $E_{cc}$, we set them to zero,
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Figure 2. Time evolution of tube formation via fusion of 480 identical cellular aggregates in a KMC simulation. Initially, each aggregate contains 280 cells of the same type (in red) and the aggregates are surrounded by agarose rods (in gray). The snapshots are taken at $t = 0$, $2 \times 10^7$ steps ($\approx 30\tau_0 \approx 1.4h$), $2 \times 10^8$ steps ($\approx 422\tau_0 \approx 20h$), and $6 \times 10^8$ steps ($\approx 2250\tau_0 \approx 105h$), respectively. The cross-sectional views in panels (a)-(d) show that the cellular aggregates are embedded in the structure of agarose rods. The panels (e)-(h) depict only the tube construct, which is shrinking. We set the interaction energy strength $E_{cc} = 1.0$. 

(a) $t = 0$ steps

(b) $t = 2 \times 10^7$ steps

(c) $t = 2 \times 10^8$ steps

(d) $t = 6 \times 10^8$ steps

(e) $t = 0$ steps

(f) $t = 2 \times 10^7$ steps

(g) $t = 2 \times 10^8$ steps

(h) $t = 6 \times 10^8$ steps
Figure 3. Time evolution of the total interfacial energy $E$ (left-top) and the tube length (left-bottom) calculated for tube formation in the KMC simulation shown in Figure 2. (Right): the relationship between the time (in the unit of $\tau_0$) and the number of KMC steps. The insets in the right panel show that the times at $2 \times 10^7$ and $2 \times 10^8$ KMC steps are about $30\tau_0 \approx 1.4h$ (square) and $422\tau_0 \approx 20h$ (diamond), respectively. All panels show the results up to $t = 6 \times 10^8$ steps ($\approx 2250\tau_0 \approx 105h$). In the long time regime (the number of steps $> 4 \times 10^8$), the actual KMC time scales linearly with the number of steps.

which makes $\gamma_{cm} = \frac{E_{cc}}{2}$. The interaction energy strength is set to $E_{cc} = 1.0$. We remark that we nondimensionalize the interaction energy by a characteristic energy value $E_0$, which is the average biological fluctuation energy of a cell, the analog of the energy of thermal fluctuations $k_B T$ ($k_B$ is Boltzmann’s constant and $T$ is the absolute temperature) [44, 45]. In the simulations, only the dimensionless combinations $E_{ij}/E_0$ appear. In [38] we have calibrated the parameter $\tau_0 = 168s$ and the other model parameters in the KMC code by simulating fusion of two identical cellular aggregates and compared with the existing experiments and published Monte Carlo results. The relationship between the KMC time (the accumulated transition time $\Delta t$ of each KMC step in the unit of $\tau_0$) and the number of KMC steps is shown in Figure 3 (right panel).

Once the aggregates start fusing, the tubular wall thins first upon fusion shown in Figure 2(b) and (f) at $t = 2 \times 10^7$ KMC (nonuniform) steps ($\approx 30\tau_0 \approx 1.4h$, see the inset of Figure 3 (right panel)). As fusion goes on, the tube substantially shrinks its length in the axial direction to maintain the total volume of the aggregates. Meanwhile, the thickness of the tubular wall recovers and the cells fill the gap between the internal and external layers of agarose rods (Figure 2(c,d) and (g,h)). As the free energy of the multicellular system is minimized after $5 \times 10^8$ steps ($\approx 1600\tau_0 \approx 75h$), the tube length also approaches its steady state value at about $107 \sim 109$ cell diameters (Figure 3 (left panels)). The shrinkage rate during fusion is approximately $107/160 \approx 2/3$. This is consistent with the height shrinkage rate that a sphere with the volume $\frac{4}{3}\pi R^3$ (the
radius \( R \) is changed into a cylinder with the same volume. If the sectional area of the latter is \( \pi R^2 \), its height is \( h = \frac{4R}{\pi} \). Thus, the shrinkage rate is \( \frac{h}{2R} = \frac{2}{\pi} \). This is a potential way to fabricate a vascular tube if the stability of the initial packing can be achieved. The simulation gives a fairly accurate description for the final fused tube product.

### 3.2. Branched tube formation via fusion of uniluminal cellular spheroids

Next, we study the fusion process of uniluminal cellular spheroids which have outer layers of vascular smooth muscle cells (SMCs) and a contiguous inner layer of endothelial cells (ECs) lining a central lumen [43]. This is perceived as a potential means to fabricate vascular veins via engineering designs. To simulate the fusion process with the 3D lattice model and the KMC method, we take the type index \( \sigma \) to account for all the four experimental materials in the system: the external hydrogel medium (\( \sigma = 0 \)), SMCs (\( \sigma = 1 \)), ECs (\( \sigma = 2 \)), and the internal hydrogel medium/lumen (\( \sigma = 3 \)). The summation in the first term in (2) includes six terms that account for the interaction energies along the six interfaces between the four phases. After many tests for tuning the interfacial tension parameter \( \gamma_{ij} \), we choose the following values that are related to the ones used in [43]: \( \gamma_{01} = 0.25 \), \( \gamma_{02} = 0.6 \), \( \gamma_{03} = 0.9 \), \( \gamma_{12} = 0.2 \), \( \gamma_{13} = 0.6 \), and \( \gamma_{23} = 0.25 \). We simulate the fusion process using the 3D lattice model and the general KMC Algorithm 2.1.

Figure 4 shows the KMC simulation results of fabricating a branched tube with interior and exterior views of the spheroids, respectively. Initially, six uniluminal cellular spheroids are positioned side by side along the \( x \)-axis and another three spheroids are positioned along the \( z \)-axis to form a T-shaped joint (Figure 4(a) and (e)). Each aggregate has a radius of about 7 cell diameters and contains 982 cells, which includes 680 SMCs (red) in the outer layer and 302 ECs (green) in the inner layer that engulfs 436 lumen particles (blue). Once the spheroids make contact, partial fusion of the SMCs in the outer layers ensues. At \( t = 5 \times 10^5 \) steps (\( \approx 126\tau_0 \approx 6h \)), the ECs from several spheroids have begun merging (Figure 4(b) and (f)). At \( t = 1 \times 10^6 \) steps (\( \approx 244\tau_0 \approx 11h \)), the lumens inside several spheroids have begun merging (Figure 4(c) and (g)). At the final \( t = 2 \times 10^6 \) steps (\( \approx 500\tau_0 \approx 23h \)), the lumens inside all spheroids have joined together and the fused branched tube has a fairly smooth outer surface (Figure 4(d) and (h)). The biological analogues of such structures are branched blood vessels, which are contractile due to smooth muscle cells and endothelial cells are lining their interior wall. To arrive at lumens, the internal hydrogel medium would have to be removed in the final tubular structure.

Figure 5 depicts another set of KMC simulation results. Initially, each of the nine spheroids has a radius of about 7 cell diameters and contains a total of 1418 randomly intermixed SMCs, ECs and internal lumen particles. It consists of 50% SMCs (red), 20% ECs (green), and 30% lumen particles (blue) shown in Figure 5(a) and (e). Cell sorting in these three-component aggregates takes a major role at the beginning of the fusion process, which sorts the cells and the lumen particles such that each spheroid
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![Figure 4](image)

**Figure 4.** Time evolution of T-shaped branched tube formation via fusion of nine uniluminal cellular spheroids in a KMC simulation. Initially, each spheroid contains 982 cells, which include 680 SMCs (red) in the outer layer and 302 ECs (green) in the inner layer that engulfs 436 lumen particles (blue). The four snapshots of external views (a)-(d) are taken at $t = 0$, $5 \times 10^5$ steps ($\approx 126 \tau_0 \approx 6h$), $1 \times 10^6$ steps ($\approx 244 \tau_0 \approx 11h$) and $2 \times 10^6$ steps ($\approx 500 \tau_0 \approx 23h$), respectively. For better visualization of cell dynamics, the panels (e)-(h) show the corresponding cross-sectional views by cutting the spheroids through the plane normal to direction $[0 1 0]$. We set the interfacial tension parameter $\gamma_{ij}$ as follows: $\gamma_{01} = 0.25$, $\gamma_{02} = 0.6$, $\gamma_{03} = 0.9$, $\gamma_{12} = 0.2$, $\gamma_{13} = 0.6$, and $\gamma_{23} = 0.25$, where the subscripts correspond to the four phases (see text).
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Figure 5. Time evolution of T-shaped branched tube formation via fusion of nine three-component spheroids in a KMC simulation. Initially, each spheroid contains a total of 1418 randomly mixed cells and internal lumen particles, including about 50% SMCs (red), 20% ECs (green), and 30% lumen particles (blue). The four snapshots of external views (a)-(d) and the corresponding cross-sectional views (e)-(h) are taken at $t = 0$, $5 \times 10^5$ steps ($\approx 112\tau_0 \approx 5h$), $1 \times 10^6$ steps ($\approx 234\tau_0 \approx 11h$) and $2 \times 10^6$ steps ($\approx 500\tau_0 \approx 23h$), respectively. We take the same values for the interfacial tension parameter $\gamma_{ij}$ as the ones in the previous example.
has outer layers of SMCs and a contiguous inner layer of ECs lining a central lumen, just like the case with initially uniluminal spheroids simulated in the previous example. At $t = 5 \times 10^5$ steps ($\approx 112\tau_0 \approx 5h$), the ECs from all spheroids have joined and the lumens inside most spheroids have begun merging (Figure 5(b) and (f)). Then, at $t = 1 \times 10^6$ steps ($\approx 234\tau_0 \approx 11h$), the lumens continue merging and there are still some SMCs that have not been sorted out (Figure 5(c) and (g)). Finally, at $t = 2 \times 10^6$ steps ($\approx 500\tau_0 \approx 23h$), the lumens inside all spheroids join together and the fused tube has a fairly smooth outer surface made up of SMCs and inner surface made up of ECs (Figure 5(d) and (h)). This simulation is hypothetical. However, if we can find the right hydrogel, biodegradable to be removed from the tube interior after the formation of the well-separated tube structure, this would be a viable way to fabricate a bifurcating vascular vein.

Figure 6 shows the time evolution of the total interfacial energy during the fusion process in Eq. (2) for both initially uniluminal and mixed cases above. As shown in Figure 6(a) of the simulation up to $t = 2 \times 10^5$ steps, cell sorting in the initially mixed aggregates dominates at the beginning of the fusion process, which results in a very quick drop of the energy until $t = 8 \times 10^4$ steps. Then the energy decreases much slower in both mixed and uniluminal cases as shown in Figure 6(b) when lumenized multicellular spheroids start fusing. At the time the simulation terminates, the energy is still decaying slowly though. This indicates that the branched tube formation structure is in a quasi steady state. The relationship between the KMC time (the accumulated transition time $\Delta t$ of each KMC step in the unit of $\tau_0$) and the number of KMC steps are shown in the left and right panels in Figure 7 for the initially uniluminal and mixed cases, respectively, in which they scale linearly.

Figure 8 depicts the KMC simulation result of fabricating a Y-shaped branched tube with interior and exterior views of the spheroids, respectively. Initially, six uniluminal cellular spheroids are positioned side by side along the $x$-axis and another four spheroids
are positioned slantingly in the $xz$-plane (Figure 8(a) and (e)). The components in each spheroid at initial time and the values of interfacial tension parameters $\gamma_{ij}$ are the same as the ones used in Figure 4. Once the spheroids make contact with each other, partial fusion of the SMCs in the outer layers ensues. At $t = 5 \times 10^5$ steps ($\approx 124\tau_0 \approx 6h$), the ECs from most spheroids have begun merging and the lumens in the bottom two spheroids have joined together (Figure 8(b) and (f)). At $t = 1 \times 10^6$ steps ($\approx 243\tau_0 \approx 11h$), the lumens inside the three spheroids on the left in the horizontal line have connected with those from the slanting branch (Figure 8(c) and (g)). At the final $t = 2 \times 10^6$ steps ($\approx 495\tau_0 \approx 23h$), the lumens inside all spheroids have joined together and the fused branched tube has a fairly smooth outer surface (Figure 8(d) and (h)). We also performed a similar KMC simulation with ten spheroids formed by randomly mixed cells as the ones used in producing the result shown in Figure 5 and the final result at $t = 2 \times 10^6$ steps is similar to the one shown in Figure 8(d) and (h).

Figure 9 shows the KMC simulation result of fabricating a “trident”-shaped branched tube with interior and exterior views of the spheroids, respectively. Initially, six uniluminal cellular spheroids are positioned side by side along the $x$-axis and another two branches, each of which containing four spheroids, are positioned slantingly in the $xz$- and $xy$-plane, respectively (Figure 9(a) and (e)). The components in each spheroid at the initial time and the values of interfacial tension parameters $\gamma_{ij}$ are the same as the ones used in producing the result shown in Figure 4. Once the spheroids make
Figure 8. Time evolution of Y-shaped branched tube formation via fusion of ten uniluminal cellular spheroids in a KMC simulation. The components in each spheroid at initial time and the values of interfacial tension parameter $\gamma_{ij}$ are the same as the ones in Figure 4. The four snapshots of external views (a)-(d) and the corresponding cross-sectional views (e)-(h) are taken at $t = 0$, $5 \times 10^5$ steps ($\approx 124\tau_0 \approx 6h$), $1 \times 10^6$ steps ($\approx 243\tau_0 \approx 11h$) and $2 \times 10^6$ steps ($\approx 495\tau_0 \approx 23h$), respectively.
Figure 9. Time evolution of “trident”-shaped branched tube formation via fusion of 14 uniluminal cellular spheroids in a KMC simulation. The components in each spheroid at initial time and the values of interfacial tension parameter $\gamma_{ij}$ are the same as the ones in Figure 4. The four snapshots of external views (a)-(d) and the corresponding cross-sectional views (e)-(h) by cutting through the planes normal to the directions $[0 \ 1 \ 0]$ and $[0 \ 0 \ 1]$ are taken at $t = 0$, $1 \times 10^6$ steps ($\approx 246\tau_0 \approx 11h)$, $2 \times 10^6$ steps ($\approx 494\tau_0 \approx 23h)$ and $5 \times 10^6$ steps ($\approx 1280\tau_0 \approx 60h)$, respectively.
contact with each other, partial fusion of the SMCs in the outer layers ensues. At $t = 1 \times 10^6$ steps ($\approx 246\tau_0 \approx 11h$), the ECs from most spheroids have begun merging and the lumens in each branch have joined together, respectively (Figure 9(b) and (f)). At $t = 2 \times 10^6$ steps ($\approx 494\tau_0 \approx 23h$), the lumens inside the left part of the horizontal line have connected with those from the lower branch in the $xz$-plane, (Figure 9(c) and (g)). At the final $t = 5 \times 10^6$ steps ($\approx 1280\tau_0 \approx 60h$), the lumens inside all spheroids have joined together and the fused, branched tube has a fairly smooth outer surface (Figure 9(d) and (h)). We also performed a similar KMC simulation with 14 spheroids formed by randomly mixed cells as the ones used in Figure 5 and the final result at $t = 5 \times 10^6$ steps is similar to the one shown in Figure 9(d) and (h) although the time taken to reach the final state is longer.

3.3. Large branched tube/network formation via fusion of uniluminal and mixed cellular spheroids

Here we simulate large branched tube formation by using the same four-phase materials as the ones reported in the previous subsection, but with more complex engineering designs in the construction. To form the main vessel tube, instead of using three-component spheroids, we take two-component spheroids made up of mixtures of SMCs and ECs, and pack them along a column of internal hydrogel medium which gives rise to the lumen upon removal. But the smaller branching vessels are still consisted of three-component uniluminal cellular spheroids shown in previous subsection. Then the whole construct is immersed in the external hydrogel medium for maturation. Again, we simulate the fusion process with the 3D lattice model and the general KMC Algorithm 2.1. The values of interfacial tension parameters are the same as we used in the previous subsection.

Figure 10 depicts the KMC simulation results with interior and exterior views of the construct. As shown in Figure 10(a) and (e), the main vessel tube initially consists of three rings of eight spheroids across the rings. Each two-component spheroid is made up of randomly mixed SMCs (80% in red) and ECs (20% in green). Another two smaller vessels are branched out from the main vessel, each of which containing five three-component uniluminal cellular spheroids positioned side by side along the $x$-axis, respectively. Each three-component spheroid includes SMCs (35%) in the outer layer and ECs (20%) in the inner layer that engulfs lumen particles (45% in blue). Each spheroid has a radius of about 6 cell diameters and contains a total of 908 cells/particles. The inside column contains 10800 internal hydrogel particles (blue). We conducted many tests to adjust the percentages of different cells so that there are sufficient ECs lining the central lumen in the final fused tube structure. In the two-component spheroids of the main vessel, cell sorting takes a major role at the beginning of the fusion process such that each spheroid has outer layers of SMCs which engulf the ECs. Meanwhile, the lumens inside three-component uniluminal cellular spheroids of the branching vessels have begun merging and joining together at $t = 4 \times 10^5$ steps ($\approx 106\tau_0 \approx 5h$) (Figure
Figure 10. Time evolution of large branched tube formation via fusion of uniluminal and mixed cellular spheroids in a KMC simulation. Initially, each two-component spheroid of the main vessel tube contains a total of 908 randomly intermixed cells, including about 80% SMCs (red) and 20% ECs (green). The three-component uniluminal spheroids of the branching vessels includes 35% SMCs in the outer layer and 20% ECs in the inner layer that engulfs lumen particles (45% in blue). The values of interfacial tension parameter $\gamma_{ij}$ are the same as the ones in Figure 4. The four snapshots of external views (a)-(d) are taken at $t = 0$, $4 \times 10^5$ steps ($\approx 106\tau_0 \approx 5h$), $1.2 \times 10^6$ steps ($\approx 351\tau_0 \approx 16h$) and $2.4 \times 10^6$ steps ($\approx 855\tau_0 \approx 40h$), respectively. For better visualization of cell dynamics, the panels (e)-(h) show the corresponding cross-sectional views by cutting the spheroids through the plane normal to direction [0 0 1].
10(b) and (f)). At $t = 1.2 \times 10^6$ steps ($\approx 351\tau_0 \approx 16h$), while the SMCs in the outer layers start fusing, the SMCs and the ECs also begin sorting such that the ECs move towards the internal hydrogel column (Figure 10(c) and (g)). At the final $t = 2.4 \times 10^6$ steps ($\approx 855\tau_0 \approx 40h$), the ECs are completely sorted out forming a thin layer separating the SMCs from the internal hydrogel in both main and branched vessels. Eventually, the construct shrinks to a solid fused tube which has a fairly smooth outer surface (Figure 10(d) and (h)). To arrive at lumens in the tube, the internal hydrogel medium have to be removed in the final structure afterward.

Figure 11 shows the KMC simulation result with interior and exterior views of a longer branched tube construct. As shown in Figure 11(a) and (e), the main vessel tube initially consists of five rings of eight spheroids across the rings and the inside column contains 18000 internal hydrogel particles. Another four smaller vessels are branched out from the main vessel, each of which containing five three-component uniluminal spheroids positioned side by side along the $x$-axis, respectively. The components of uniluminal or mixed spheroids are the same as the ones used in the previous example. Here, we observe the similar result. At the beginning, cell sorting makes the SMCs engulf the ECs in the mixed two-component spheroids of the main vessel while the lumens inside three-component uniluminal spheroids of the branching vessels have begun merging and joining together with the internal hydrogel column at $t = 8 \times 10^5$ steps ($\approx 207\tau_0 \approx 10h$) (Figure 11(b) and (f)). At $t = 2 \times 10^6$ steps ($\approx 568\tau_0 \approx 26h$), the SMCs and the ECs sort such that the ECs move towards the internal hydrogel column (Figure 11(c) and (g)). At the final $t = 3.2 \times 10^6$ steps ($\approx 1026\tau_0 \approx 48h$), the SMCs and ECs are completely sorted out forming a thin EC layer separating the SMCs from the internal hydrogel in both main and branched vessels, where the final construct has a fairly smooth outer surface (Figure 11(d) and (h)).

Finally, Figure 12 shows another set of KMC simulation results of tube network formation via fusion of uniluminal and mixed cellular spheroids. This time, each of the two main vessel tubes initially consists of seven rings of eight spheroids across the rings and the inside column contains 25200 internal hydrogel particles. Another six smaller vessels are branched out from the main vessel. Moreover, the two main vessel tubes are connected through branching vessels between them, which form a network. Like the results in the previous examples, cell sorting takes a major role at the beginning of the fusion process such that each spheroid has outer layers of SMCs which engulf the ECs. Then the lumens inside three-component uniluminal spheroids of the branching vessels begin merging and joining together with the internal hydrogel column at $t = 1 \times 10^6$ steps ($\approx 242\tau_0 \approx 11h$) (Figure 12(b) and (f)). At $t = 5 \times 10^6$ steps ($\approx 1388\tau_0 \approx 65h$), the SMCs and the ECs sort such that the ECs move towards the internal hydrogel column (Figure 12(c) and (g)). At the final $t = 1 \times 10^7$ steps ($\approx 3289\tau_0 \approx 153h$), the ECs are completely sorted out forming a thin layer separating the SMCs from the internal hydrogel and the networked construct has a fairly smooth outer surface (Figure 12(d) and (h)).
Figure 11. Time evolution of large branched tube formation via fusion of uniluminal and mixed cellular spheroids in a KMC simulation. Initially, the components of uniluminal or mixed spheroids are the same as the ones used in previous example shown in Figure 10, but the main vessel tube is longer and has four branching vessels. The values of interfacial tension parameter $\gamma_{ij}$ are the same as the ones in Figure 4. The four snapshots of external views (a)-(d) and the corresponding cross-sectional views are taken at $t = 0$, $8 \times 10^5$ steps ($\approx 207\tau_0 \approx 10h$), $2 \times 10^6$ steps ($\approx 568\tau_0 \approx 26h$) and $3.2 \times 10^6$ steps ($\approx 1026\tau_0 \approx 48h$), respectively.
Figure 12. Time evolution of tube network formation via fusion of uniluminal and mixed cellular spheroids in a KMC simulation. Initially, the components of uniluminal or mixed spheroids are the same as the ones used in previous example shown in Figure 10, but the two main vessel tubes are longer and have six branching vessels. Moreover, the two main vessel tubes are connected through branching vessels between them, which forms a network. The values of interfacial tension parameter $\gamma_{ij}$ are the same as the ones in Figure 4. The four snapshots of external views (a)-(d) and the corresponding cross-sectional views (e)-(h) are taken at $t = 0$, $1 \times 10^6$ steps ($\approx 242\tau_0 \approx 11h$), $5 \times 10^6$ steps ($\approx 1388\tau_0 \approx 65h$) and $1 \times 10^7$ steps ($\approx 3289\tau_0 \approx 153h$), respectively.
4. Conclusion

We have used the kinetic Monte Carlo (KMC) method with a 3D lattice model developed in [38] to study fusion of cellular aggregates during their morphogenesis process where cell sorting, mixing and biomechanical relaxation takes place. Our work is motivated by the growing need to understand morphological changes and biomechanical properties of engineered tissue constructs during their fabrication processes that have practical applications in bioengineering and regenerative medicine, in particular, the emergent field of 3D bioprinting [9, 14, 15].

The 3D lattice model using the KMC method is based on the differential adhesion hypothesis (DAH) on the liquid-like nature of embryonic tissues consisting of engineered cellular aggregates proposed by Steinberg [28], which provides useful predictions on quasi-equilibrium tissue configurations. As DAH cannot address the question of how these configurations are developed in time, we use the KMC method to simulate the time evolution of the multicellular system, in which the self-assembly of cells in the systems is described in terms of the transition rates corresponding to possible configurational changes of the system and then the corresponding time evolution of the systems can be expressed in terms of these rates. After a careful calibration on the time scales and other energetic model parameters on experiments and benchmarking computations [38], we use KMC simulations to describe and predict the time evolution of the morphology and dynamics of the simulated multicellular tissue systems. These simulations explore the feasibility of 3-D bioprinting of vascular networks using engineering designed multicellular spheroids either in solid spheroids or uniluminal spheroids, providing a powerful simulation tool for the novel 3-D bioprinting technology.

The time for fusion of the cellular systems depends on the initial packing of the cellular spheroids, their structural stability, and bio-compatibility with the supporting ambient materials. Tight packing will certainly result in shorter fusion time. However, the time-saving in tight packing is negligible compared to the system fusion to quasi-steady state, which we explored in our simulations but did not show any figures in the text. The KMC simulation tool used here can certainly be employed to test various packing options which may provide an optimal strategy for biofabrication using 3-D bioprinting technology. Bio-compatibility issue is largely ignored in this study, which we will look into in the future research. This simulation tool developed for cellular aggregate fusion can be extended to aide design of tissue constructs via tissue fusion technology. A computer aided design and monitoring tool is therefore plausible in the near future to virtually simulate the entire biofabrication process and provides guidance to the bioprinting process in precision.

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