Bioprinted tissue constructs simulated by the Lattice Boltzmann method

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Introduction

Understanding the principles of morphogenesis is indispensable for developing efficient strategies to build living tissues in the laboratory. In this endeavor, computational methods proved valuable in pointing out dominant morphogenetic mechanisms. **Tissue fusion is essential in tissue printing**, an emergent technique based on computer-controlled deposition of multicellular building blocks along with supportive hydrogels.

Differential Adhesion Hypothesis (DAH) explain morphogenetic rearrangements in multicellular systems. According to DAH, cells take advantage of their motility to give rise to the configuration with the lowest energy of adhesion; that is, cells seek to establish the largest number of firm bonds with their neighbors. DAH is consistent with the view that **morphogenesis is driven by interfacial tensions** generated by interactions between cells just as phase ordering in immiscible fluids is driven by interfacial tensions generated by interactions between molecules.

Lattice Boltzmann model was specifically designed and used to simulate a process that is relevant for tissue printing: the sidewise fusion of identical cylinders made of cohesive cells in a hydrogel initially in contact along a common generator.

This process is analytically tractable and may be used to find a biologically relevant range of model parameters and to establish the time scale of the simulations. Post-printing rearrangements of cells were the subject of computational studies motivated by the need to predict the shape and stability of the printed construct.

Tissue printing has been used to build ***sheet tissue*** constructs. In a recent development of tissue printing, multicellular cylinders have been used as bio-ink to fabricate branched **tubular structures** with the histological features of ***blood vessels***. A simple way of creating a ***perfusable tissue*** construct from several layers of contiguous multicellular cylinders placed on top of each other.

Based on the liquid analogy, **a multicellular system is characterized by a surface tension, which drives its evolution**, and **viscosity, which hampers evolution**.

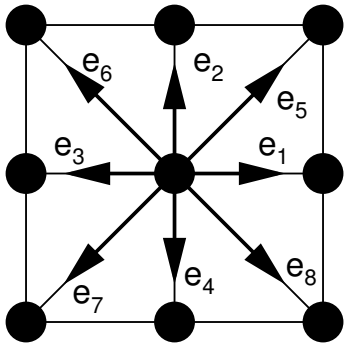
On a time scale of days, embryonic tissues and artificially assembled multicellular systems flow akin to an incompressible fluid that is about a billion times more viscous than water, inertia does not intervene in their evolution.

On a time scale of seconds, the multicellular system responds as an elastic solid, whereas on the time scale of hours or days (of interest in Tissue Engineering-TE) it responds as a highly viscous, incompressible fluid.

Lattice Boltzmann models on square lattices

The Lattice Boltzmann equations set:

$$\partial_t f_i^\sigma + \mathbf{e}_i^\sigma \cdot \nabla f_i^\sigma = -\frac{1}{\tau^\sigma}\left[f_i^\sigma - f_i^{\sigma,eq}\right] + \frac{\mathbf{F}^\sigma(\mathbf{r},t)}{m^\sigma \chi\,(c^\sigma)^2} \cdot \left[\,\mathbf{e}_i^\sigma - \mathbf{u}(\mathbf{r},t)\,\right] f_i^{\sigma,eq} \qquad (1)$$



isothermal $D2Q9$ ***model:***

According to the general approach for constructing high order schemes using ***flux limiters***, the updating rule is rewritten as a conservative quantity using two fluxes:

$$f_{i,j}^{\sigma,n+1} - f_{i,j}^{\sigma,n} + CFL^\sigma \left[F_{i,j+1/2}^{\sigma,n} - F_{i,j-1/2}^{\sigma,n} \right] =$$

$$= -\frac{1}{\tau^\sigma}\left[f_i^\sigma - f_i^{\sigma,eq}\right] + \frac{\mathbf{F}^\sigma(\mathbf{r},t)}{m^\sigma \chi\,(c^\sigma)^2} \cdot \left[\,\mathbf{e}_i^\sigma - \mathbf{u}(\mathbf{r},t)\,\right] f_i^{\sigma,eq} \qquad (2)$$

where $CFL^{\sigma} = c^{\sigma}\delta t/\delta s$ (δt - time step, δs - lattice spacing) is the Courant-Friedrichs-Levy number, and the fluxes $F^{\sigma,n}_{i,j+1/2}$, $F^{\sigma,n}_{i,j-1/2}$ are defined as follows:

$$F^{\sigma,n}_{i,j+1/2} = f^{\sigma,n}_{i,j} + \frac{1}{2}\left(1 - CFL^{\sigma}\right)\left[f^{\sigma,n}_{i,j+1} - f^{\sigma,n}_{i,j}\right]\psi(\theta^{\sigma,n}_{i,j}) \tag{3}$$

and

$$F^{\sigma,n}_{i,j-1/2} = F^{\sigma,n}_{i,(j-1)+1/2} = f^{\sigma,n}_{i,j-1} + \frac{1}{2}\left(1 - CFL^{\sigma}\right)\left[f^{\sigma,n}_{i,j} - f^{\sigma,n}_{i,j-1}\right]\psi(\theta^{\sigma,n}_{i,j-1}) \tag{4}$$

The flux limiter $\psi(\theta^{\sigma,n}_{i,j})$ is expressed as a function of the ***smoothness***

We consider a system with two types of particles: cells ($\sigma = 1$) and similar-sized volume elements of cell culture medium, or of a hydrogel soaked with cell culture medium ($\sigma = 0$). To describe the forces between particles of species σ and λ, in the present LB model we have four interaction parameters $\omega^{\sigma\lambda}$ ($\sigma = 0, 1$ and $\lambda = 0, 1$). For simplicity, here we have expressed them in terms of a single parameter, ω:

$$\omega^{00} = \omega^{11} = 0 \tag{5}$$

$$\omega^{01} = \omega^{10} = \omega. \tag{6}$$

The force $\mathbf{F}^{\sigma}$ that acts on a particle of species σ is expressed as

$$\mathbf{F}^{\sigma} = -\sum_{\lambda} \omega^{\sigma\lambda}\nabla X^{\lambda} + surface\,tension\,terms, \tag{7}$$

where X^{σ} are the mole fractions of the species $\sigma = 0, 1$:

Results and Discussion

We simulated the phase separation dynamics and self-assembly of biological tissue constructs currently used in tissue engineering (TE). More precisely, our D2Q9 model was employed to simulate the sidewise fusion of identical, contiguous multicellular cylinders. This process is interesting because it may be studied also experimentally, and a comparison between the simulated and experimental results would allow to calibrate the time scale of LB simulations.

Since the fusion of multicellular structures depends on their mechanical properties (surface tension and viscosity), we performed several LB simulations with similar parameters except for the values of the dynamic viscosity $\eta^\sigma = \tau^\sigma n^\sigma T$ of each component of the system.

Surface tension as an equilibrium quantity is included in the force term (7).

Viscosity, on the other hand, is a dynamic quantity, controlled via the relaxation time, τ.

For a given value of the surface tension, smaller viscosity leads to a quicker fusion.

Simulations were done on a lattice with the lattice spacing set to $\delta s = 0.001$; the time step was $\delta t = 0.0001$. The phase separation has been assured by a proper value of the nondimensionalized temperature, $T = 0.70$.

To study the time course of sidewise fusion of identical cylinders, we follow and describe the evolution of the area of contact between the cylinders by an exponential function of time. More precisely, we approximate the contour of the transversal cross section of the system by two arcs of circle of radius (R) that increases as fusion proceed and monitor $2h$, the width of the stripe of contact between the fusing cylinders. We normalize the area of contact ($2h$ times the length of the cylinder) by dividing it with the area of the longitudinal cross-section of a cylinder before fusion ($2R_0$ times its length).

Volume conservation yields the asymptotic value of the normalized area of contact ($h/R_0 \to \sqrt{2}$). Thus, a time constant of fusion, t_{f}, may be defined by fitting the plot of the normalized area of contact by the expression

$$\frac{h}{R_0} = \sqrt{2}\left[1 - \exp\left(-\frac{t}{t_{\mathrm{f}}}\right)\right]. \tag{8}$$

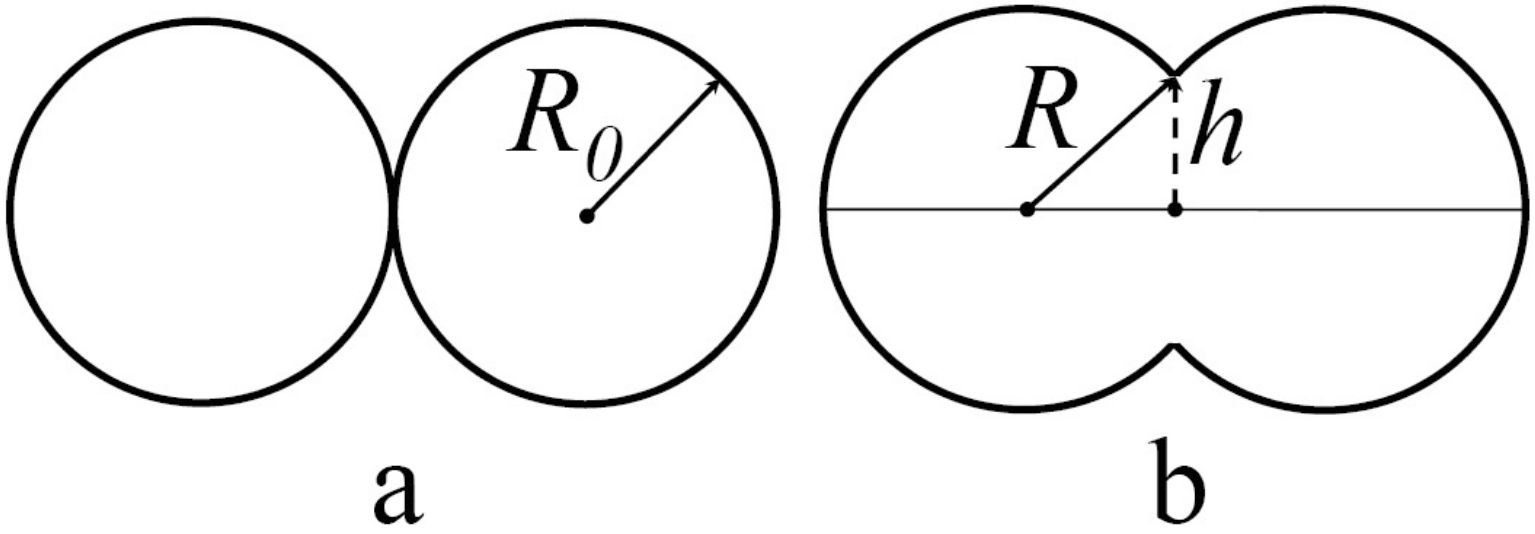


Figure 1: Transversal cross section of two multicellular cylinders (viewed as cylinders made of a highly viscous incompressible fluid) of radius R_0 in the initial state (a) and during sidewise fusion (b).

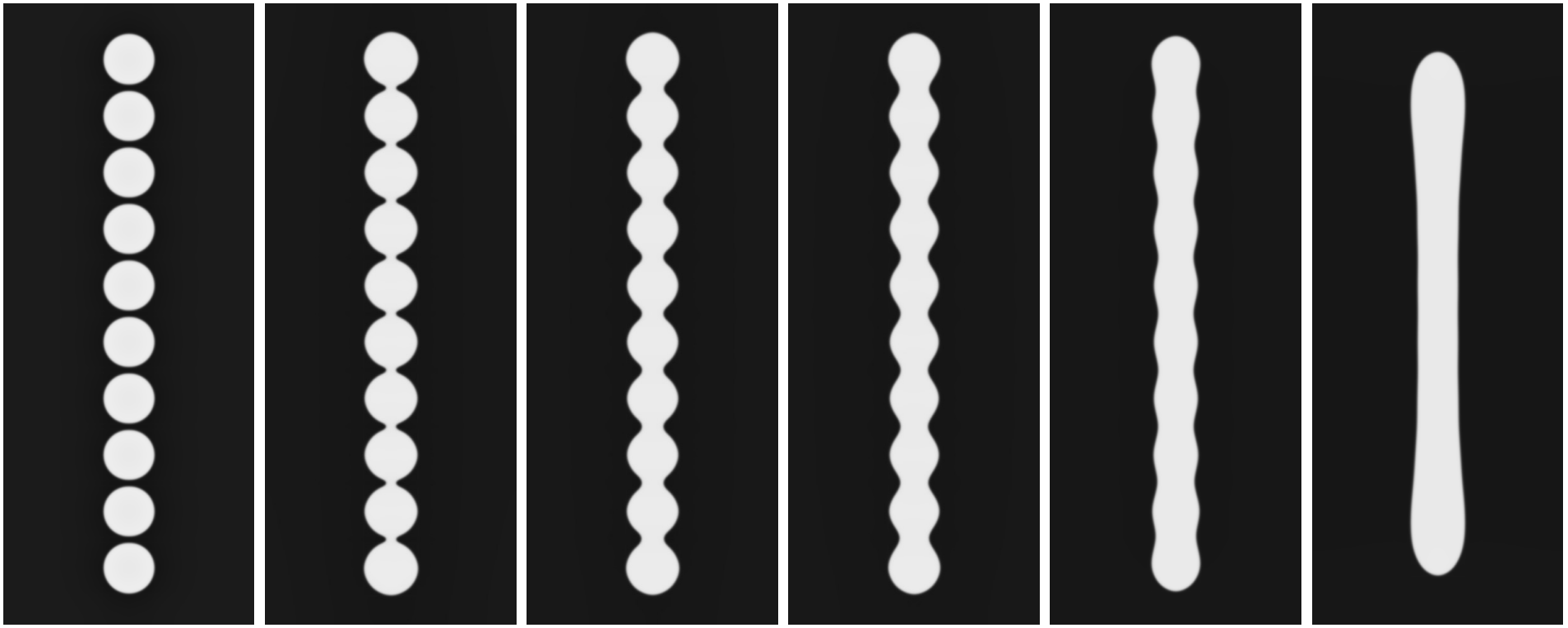


Figure 2: Fusion of multicellular cylinders sheet-like tissue fusion (***skin graft***). with various values of the relaxation time, τ. The shown configurations were obtained during 5×10^3, 5×10^4, 7×10^4, 1×10^5, 2×10^5 and 5×10^5 time steps.

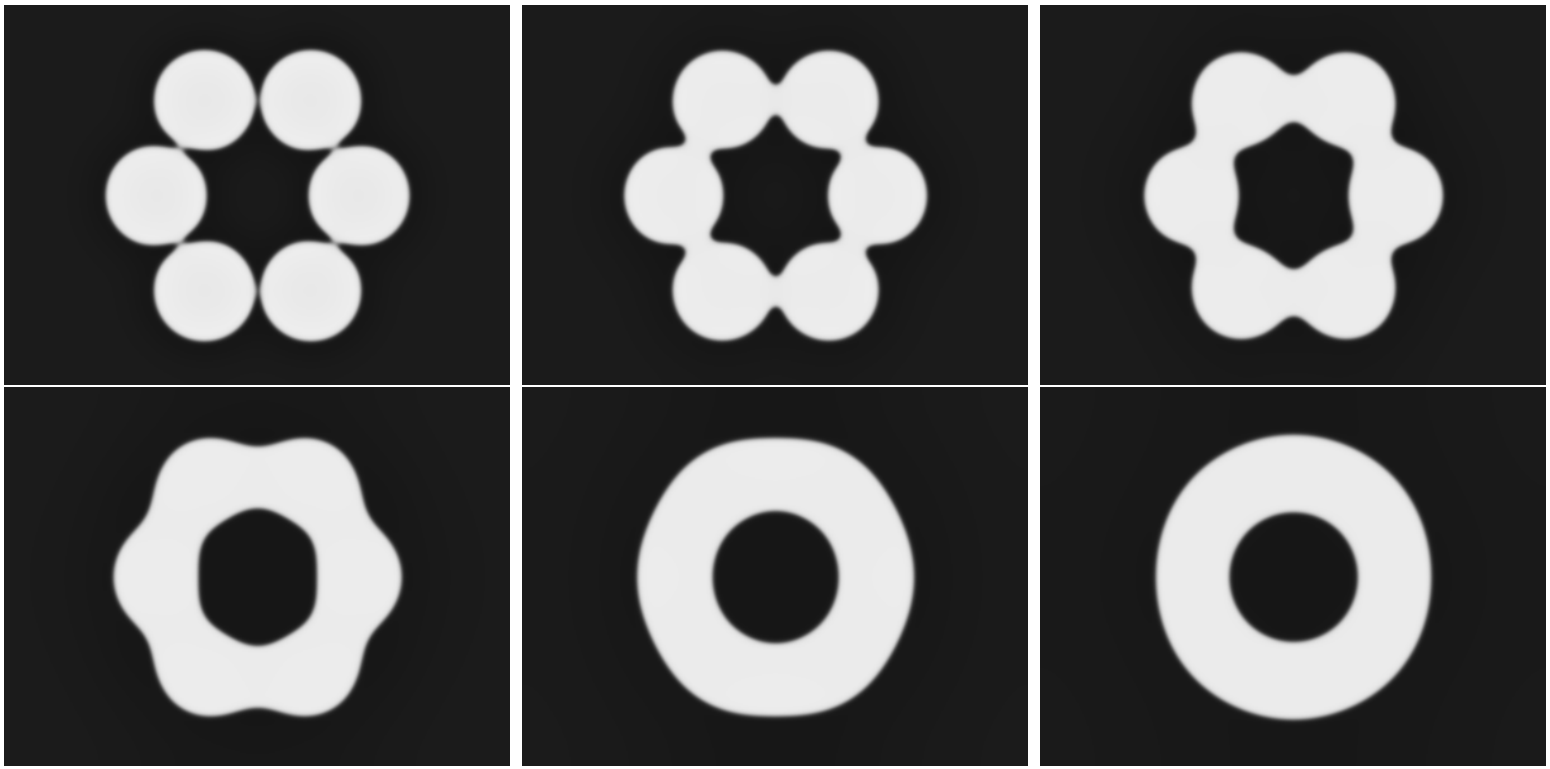


Figure 3: Fusion of multicellular cylinders tubular-like tissue fusion (***blood vessels***). The shown configurations were obtained during 5×10^3, 5×10^4, 7×10^4, 1×10^5, 2×10^5 and 5×10^5 time steps.

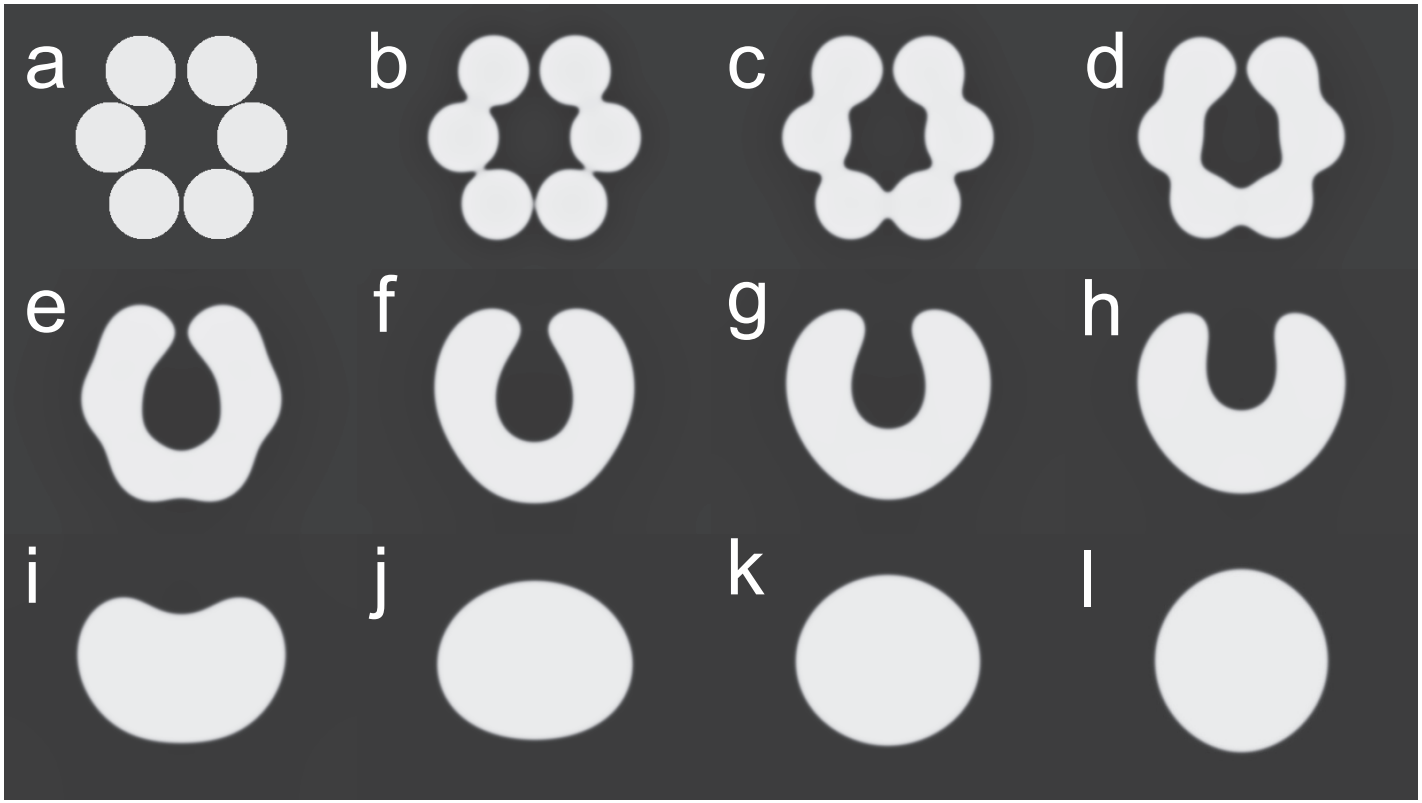


Figure 4: The evolution of a ***printing defect***. This figure shows successive snapshots of a 2D simulation that represents the transversal cross-section of a 3D structure obtained by printing multicellular cylinders in a hexagonal arrangement in which two cylinders are slightly misplaced.

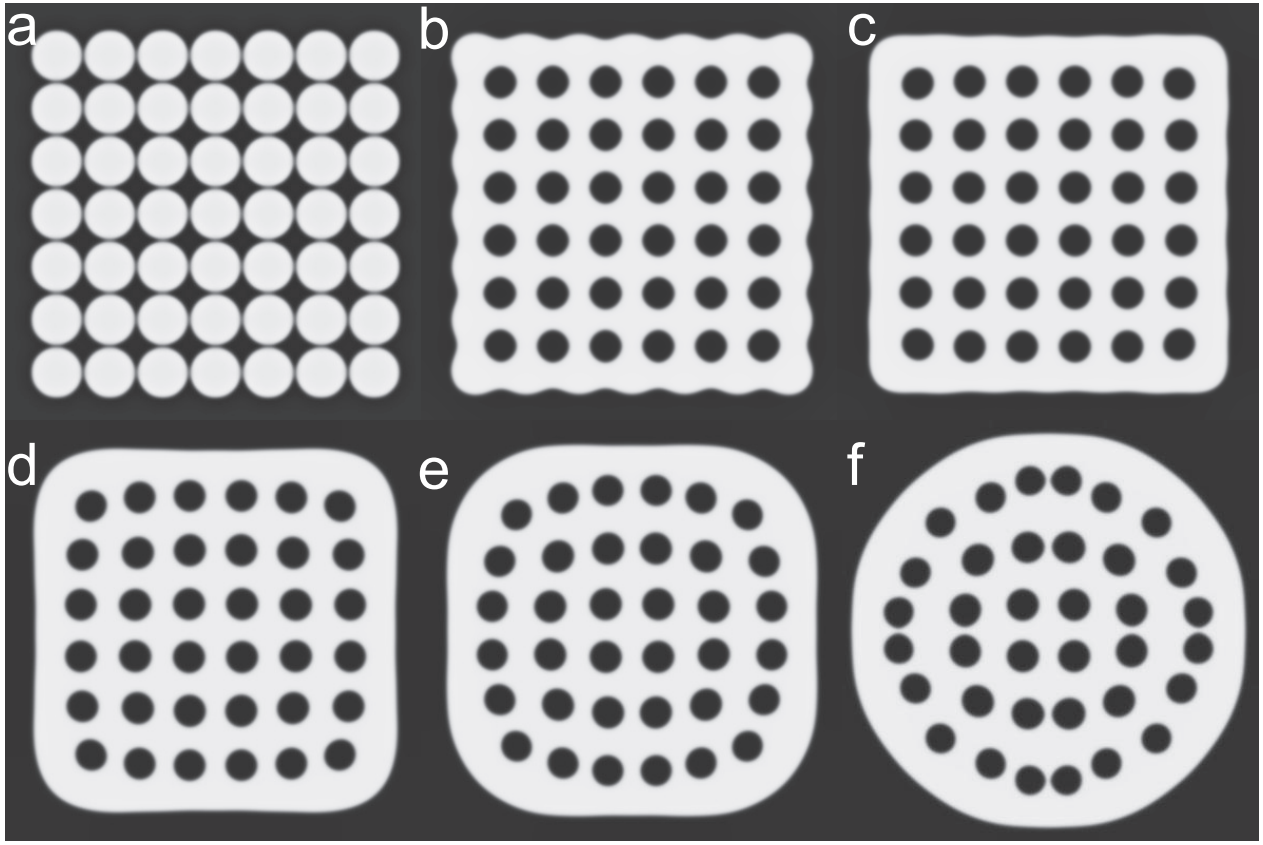


Figure 5: Emergence of a ***perfusable tissue*** a construct via fusion of stacked cylinders. This picture shows snapshots of a 2D simulation that represents the evolution of the transversal cross-section of a model tissue construct obtained by the fusion of 7×7 multicellular cylinders arranged contiguously in a square lattice.

Conclusions

Finite difference Lattice Boltzmann models are based on the physics at the mesoscopic scale and provide an alternative to current computational fluid dynamics methods. Expressing the system's evolution via distribution functions, LB methods avoid the limitations imposed on the number of constituent particles of the system. In this respect the LB approach is more versatile than particle-based simulation techniques, such as Monte Carlo or Particle Dynamics methods. Due to their local nature, ***Lattice Boltzmann models are suitable for parallel computing, using MPI / PETSc libraries or nVidia CUDA GPU techniques***.

Flux limiter techniques proved to be efficient for reduction of numerical effects of the model (e.g. spurious velocities in the interface region). ***Higher order numerical schemes are recommended***.

Lattice Boltzmann method are appropriate to describe the time evolution of multicellular living systems of interest in ***tissues engineering.***

Based on several simulations, we identified a domain of model parameters for which the model system behaves in qualitative agreement with experimental results reported in the literature for the fusion of multicellular spheroids. ***Software development (3D) still needed for tissues engineering purposes***.