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COMPUTATIONAL SIMULATION OF INTERSTITIAL FLOW IN BIOPRINTED 3D TISSUE CONSTRUCTS

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ABSTRACT: Organ printing is a robotic computer-aided layer by layer additive biofabrication of 3D tissue and organ constructs using tissue spheroids as building blocks. It has been demonstrated that intraorgan branched vascular tree could be bioprinted inside 3D tissue and organ constructs. However, maturation of built-in branched vascular tree suitable for perfusion needs some time. In order to buy time necessary for maturation of branched vascular tree and maintain viability of bioprinted 3D tissue constructs an interstitial perfusion with special irrigation dripping bioreactor could be used. Computational simulations with using Surface Evolver software and Computational Fluid Dynamics software demonstrated that short term viability of bioprinted 3D tissue and organ constructs by interstitial flow is feasible.

INTRODUCTION

Organ printing is a computer-aided robotic layer by layer additive biofabrication of 3D human tissues and organs constructs using tissue spheroids as building blocks (Mironov et al., 2003; Mironov et al., 2008; Mironov et al., 2009; Mironov et al., 2011; Rezende et al., 2013a). Tissue spheroids closely placed layer by layer in supporting hydrogel can fuse in horizontal and vertical directions and form 3D tissue and organ constructs. Tissue fusion is a natural ubiquitous process occurring during embryonic development of tissues and organs (Pérez-Pomares and Foty, 2006). In organ printing technology tissue fusion is a fundamental biological process which enables biofabrication of 3D tissue and organ constructs. Mathematical modeling and computer simulation methods have been successfully used for predictive modeling of postprinted tissue fusion process (Yang et al. 2012; Sun et al., 2014; Flenner et al., 2012; McCune et al., 2014).

Bioprinted 3D tissue and organ constructs must be vascularized in order to maintain their viability (Visconti et al., 2010; Auger et al., 2013). It has been demonstrated that bioprinting of build in intraorgan branched vascular system could be accomplished using solid and lumenized vascular tissue spheroids [Kasyanov et al., 2011; Gentile et al., 2008) which also undergo process of tissue fusion. The branched vascular tree is suitable for intravascular perfusion.

However, due to certain kinetics of tissue fusion process the bioprinted 3D tissue and organ constructs with build in branched vascular tree are not possible to perfuse immediately after finishing printing. Special type of irrigation dripping perfusion bioreactor has been proposed for interstitial (inter-spheroidal) perfusion (Rezende et al., 2013b). During bioprinting process tissue

spheroids are placed close to each other but until they fuse completely there is inter-spheroidal space which forms interstitial microchannels suitable for interstitial perfusion.

However, the geometry of these inter-spheroidal or interstitial microchannels at different tissue spheroids packing patterns at different stage of tissue fusion process as well as flow dynamics have not been subject of special investigations.

Here, we present results of computational simulations of geometrical change of microchannels during post-printed tissue fusion process using Surface Evolver software and associated flow dynamics using computational fluid dynamics software. Our data indicates that during post-printed tissue fusion there is certain period (window of time) when inter-spheroidal or interstitial perfusion is feasible. Interstitial perfusion maintains viability of bioprinted 3D tissue and organ construct, buys necessary time for maturation of intraorgan branched vascular tree and enables sequential switching to intravascular perfusion of bioprinted 3D tissue and organ constricts. Thus, using interstitial (inter-spheroidal) perfusion with using irrigation dripping perfusion bioreactor it is possible to buy time necessary for maturation of intraorgan vascular tree.

MATERIAL AND METHODS

1. Mathematical modeling and computer simulation of inter-spheroidal microchannels

In order to visualize geometry and estimate post-printed kinetics of evolution of inter-spheroidal microchannels in bioprinted 3D tissue constructs the open source Surface Evolver software originally developed by Ken Brakke (Brakke, 1992) has been used [<http://www.susqu.edu/brakke/evolver/evolver.html>]. It has been assumed that tissue spheroids have regular size and undergo complete fusion process. Two tissue spheroids packing pattern have been analysed.

2. Visualization of inter-spheroidal microchannels using rapid prototyping

The inter-spheroidal space has been visualized using rapid prototyping. Original file in STL format has been developed using Surface Evolver software where is generated a negative image and fabricated using rapid prototyping machine SLS (Selective Laser Sintering) using polymer poliamide. In order to visualize 3D geometry of microchannel the negative image of tissue spheroids together has been enlarged in several times. Different stages of tissue fusion process as well as different packing patterns of tissue spheroids have been visualized using fabrication of inter-spheroidal microchannels in physical form.

3. Computational Fluid Dynamics software

Computational Fluid Dynamics software developed by ANSYS CFX (version R15.0) has been used in our simulations. Different stages of fusion of tissue spheroids such as 1%, 5%, 10%, 20% and 40% have been considered in order to verify which conditions of flow each level of fusion implies. It was built a cylinder with many layers. Each layer is compounded by spheroids put side by side. The analogy with fusion is done taking into account that the distance between two neighbor spheroids is 100 μ m. Then, as far as the fusion starts this distance is decreased according to the percentage analyzed. For example, 1% fusion means a distance between center equals to 99 μ m and so on.

RESULTS

Computational simulation (using software) and physical visualization (using rapid prototyping) allow visualizing 3D architecture and geometry of interstitial (inter-spheroidal) microchannels during their post-printed evolution (Figure 1a-b). It has been demonstrated that with advancing tissue fusion process in bioprinted 3D tissue and organ constructs the interfacial area of adjacent tissue spheroids increase and inter-spheroidal space progressively reduced.

Figure 1 - (a) Virtual and (b) printed models of interstitial microchannels during post-printing.

Figure 2a-c shows a unit cell from a cubic lattice of spheroids with varying degrees of inflation obtained by software Evolver Surface. The spheroids are completely non-wetting with each other. The "Border volume" is the fraction of volume in the channels between the spheres. The "Neck area" is the area of the cross-section of the thinnest part of the channel, and is reported as the fraction of area of a face of the unit cell. Figure 3 illustrates the tendency of decreasing of the border volume and neck area met during the fusion process of adjacent spheroids.

Figure 2. Channels between squeezed spheres in a cubic lattice. Bare channel version. Different stages of fusion: (a) initial, (b) intermediate and (c) final.

Figure 3. Decreasing in border volume and neck area during fusion process

Using Computational Fluid Dynamics software, each spheroid represented in the simulation was considered as a sphere of alginate. In this work, it was chosen an alginate concentration of 3%, where $k = 6 \text{ Pa.s}$ and $n = 0.84$ with density equals to 1.4 g/cm^3 and a shear rate range from 0.01 to 100 s^{-1} and an average viscosity in the range of 2.8 to 12.5 Pa.s (Rezende et al., 2009). Water is the flow that contours the interstitial microchannels among the alginate spheres.

In order to discrete the governing equations the software ANSYS CFX® makes use of an element-based finite volume method, which firstly involves to discrete the spatial domain using a mesh. The mesh is used to construct finite volumes, which are used to conserve relevant quantities such as mass, momentum, and energy. A control volume is constructed around each mesh node and these equations are integrated over each control volume (Rezende et al., 2011).

As much the fusion percentage occurs the less interstitial microchannels are present and therefore there is a natural decreasing in the flow as shown in Figure 4a. There is no interstitial microchannels from about 40% of fusion as can be seen in Figure 4b .

Figure 4. (a) Decreasing on flow in terms of fusion and (b) Profile of flow for 40% fusion at last time step.

Initially inter-spheroidal microchannels are continuous and enable interstitial perfusion. However, at final steps of tissue spheroids fusion process inter-spheroidal microchannels became non-continuous and their geometry does not permit effective interstitial perfusion due to loss of percolation.

DISCUSSION

Computational simulation and rapid prototyping allows visualizing geometry of inter-spheroidal space at different stages of post-printed tissue spheroid fusion process with different packing patterns. Computational Fluid Dynamic and Software Surface Evolver have been increasingly developed during the past decade and are powerful tool to calculate flow fields, shear stresses and mass transport within and around 3D constructs, and fits very well to this work. The size of inter-spheroidal microchannel is reduced as function of increasing interfacial space of fusing adjacent tissue spheroids. Our data indicate that inter-spheroidal interstitial perfusion is possible and feasible but only during limited period or window of time. Thus, in order to maintain viability of bioprinted 3D tissue and organ constructs during certain limited period of time after finishing bioprinting it is possible to perform interstitial perfusion using previously proposed and described irrigation dripping tripled perfusion bioreactor.

CONCLUSIONS

With increasing level of tissue spheroids fusion the area of inter-spheroidal space is proportionally reduced. It is possible to perfused 3D bioprinted construct from tissue spheroids certain period (or window of time) until inter-spheroidal network of channels stay continuous. Interstitial or inter-spheroidal perfusion allows to "buy" necessary time for maturation of bioprinted build in 3D tissue or organ construct intraorgan branched vascular tree. When inter-spheroidal (interstitial) network of channel became non-continuous interstitial or inter-spheroidal perfusion is technically not possible and interstitial perfusion must switch to intravascular perfusion through the bioprinted in 3D construct intraorgan branched vascular tree.

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