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Article

Function Representation for basic processes modeling in 3D bioprinting

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Abstract: We propose a non-invasive approach to control the quality of spheroids and their fusion into a complex bioconstruct. The proposed method is based on the union of the nutrient concentration change calculated using Fick's law and the "reaction-diffusion" equations, taking into account absorption coefficient with specifying the exact fusion geometry using implicit Function Representation (FRep) functions. The proposed approach allows us to analyze the viability of cells within the spheroid, predict the fusion of spheroids, and accurately model complex heterogeneous biostructures as a future task for our research. These results will significantly accelerate the development of such a promising field of additive biotechnologies as 3D bioprinting.

Keywords: Function Representation; implicit functions; geometry and topology, 3D bioprinting, tissue spheroids, modeling and simulation, diffusion-concentration calculations, cellular necrosis

1. Introduction

Additive biotechnologies could be divided into bioprinting from living cells and 3D printing for medical purposes. Bioprinting includes creating tissues or whole organs for surgery, pharmacological research and tests, transplantation. Printing heterogeneous structures is the primary subject of research in bioprinting nowadays. One of the reasons is the structure of most human organs, vascularized and most often heterogeneous. Considering the specifics of models in this area, the model should meet several requirements due to its creation and the simulated structure details.

The Functional Representation (FRep) is a promising method in heterogeneous volumetric modeling, contributing to the creation of a new scientific and applied field of knowledge - informatics in bioprinting. Because of the complexity of 3D printing from living cells, mathematical modeling studies at the stage of preparation of the experiment are necessary at the level of structural elements of technology, tissue spheroids. Tissue spheroids are multicellular aggregates, the natural phenomenon of fusion of which is the basis of 3D bioprinting technology.

Observation of spheroids' geometric characteristics and the viability of cells throughout the depth of the spheroid is necessary to ensure their quality. The classic method of growing multicellular spheroids is the "hanging drop" method. In this method, spheroids' growth involves the needed tracking of cells for necrosis due to the cessation of access to nutrients. With the growth of spheroids and compaction of the structure, cells located in the center are especially vulnerable. Since it does not have regeneration processes before the successful transplantation of the spheroid bioconstruct, necrotic regions are unacceptable in spheroids intended for use in an experiment or production. For the production and technological process of using spheroids, an automated system for monitoring the properties of spheroids is required. Without the use of non-invasive spheroid control methods, bioprinting 3D technology will not be widespread.

2. Materials and Methods

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Our solution is based on an efficient mathematical approach FRep (Function Representation) that allows us to describe processes inside a spheroid using mathematical functions. The approach of representing the biological structure and morphology in the form of mathematical functions helps to set the necessary parameters, analyze the fusion of spheroids, model complex heterogeneous biostructures, consider complex processes in living tissues, and work with compact and accurate models of spheroids oriented for use in bioprinting. The innovation of our approach lies in the fact that we offer a specialized solution for quality control of spheroids for their further use in bioprinting. There are no similar solutions in the field of bioprinter software.

At the beginning of our product development, the goal was to provide a method for non-invasive cell viability monitoring for spheroid manufacturers. Our approach is based on combining the results of the oxygen diffusion-reaction equations and boundary constraints presented as FRep functions. Knowing the type of cells and the type of medium, the size of the spheroids, we can determine whether this type of spheroid is viable for a given diameter for use in bioprinting [1].

Simulation using the Metropolis Monte Carlo method (SIMMMC) is the specialized application for predicting post-printing structure formation in bioprinted constructs, generating 3D models of various bioprinted constructs [2]. The application has been extended for bioprinting purposes, such as modeling and simulation of the development of bioprinted tissue constructs composed of living cells, hydrogels, and cell culture medium. SIMMMC software has a function for tissue evolution simulation. However, SIMMMC it is too large and complex for the targeted problem. The software tool CompuCell3D is currently used in tissue engineering. It is based on the Glazier-Graner-Hogeweg model and is open-source software developed, especially for simulating bioprinted constructs' evolution. The problem of this software is similar to SIMMMC. Surface Evolver was developed to predict, and model directed self-assembly in multicellular systems by examining each individual cell using the finite element method [2, 3]. In the field of bioprinting, Surface Evolver can be used to simulate the fusion of spheroids for vessel printing. This approach allows us to estimate the required number of concentric layers of vascular tissue spheroids. This program does not take into account the viability of spheroids when printing.

Currently, there is no software available to model all aspects of bioprinting with a large batch of cells (1 million - 1 billion). However, it is now common to develop control programs for bioprinting to provide information about changes in the shape of spheroids. In the future, bioprinters need an integrated computing structure that should include software modules for modeling and simulation. However, the most vital is operating with viable tissue spheroids of equal size. Therefore, this stage of spheroid production is described, where we come with our solution.

Our solution, as we mentioned above, is based on the equation of dependence of the diffusion reaction and the representation of a function describing the boundary conditions of the spheroid. The reaction-diffusion dependence allows you to realize the actual change in the concentration of the most essential nutrients for cell life, such as oxygen, glucose, and water. The most important thing in cell life is oxygen, so our first prototype focuses on it. Under laboratory conditions, the viability of spheroids is evaluated by the intensity of cell staining using fluorescence microscopy. The most accurate approach is to consider the rate of oxygen consumption by cells and estimate the drop in oxygen concentration caused by intracellular diffusion. A decrease in oxygen concentration indicates necrotic processes and cell death. We combine the diffusion-reaction equations with the innovative FRep approach, which allows us to consider all complex biological processes as implicit mathematical functions. FRep for biological things represents a complex natural structure to set all needed hydrogels and cells' parameters. It operates with compact and accurate models applicable for 3D bioprinting to analyze tissue spheroid fusion and to prototype complicated heterogeneous biological systems with complex processes in living tissues. On the surface, the function is constant. FRep can define an object by a continuous function

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$$f(x_1, x_2..., x_n) \geq 0 \quad (1)$$

where f is a real continuous function defined on n-dimensional Euclidean space En that must have positive values inside the object, negative values outside, and zero on the surface [4]. In the 3D space, the object boundary is named "implicit surface." Any algorithm or function can be used until it can return a real value. Functions in the FRep approach form a system where different materials and other parameters can be described [5]. The FRep approach is a suitable method to provide a heterogeneous representation of objects with high complexity. Besides, a mammalian cell colony was simulated using the FRep approach. A cell colony was modeled as a collection of deformable particles in contact with each other. A new pair of particles that simulate the cell division process can replace an existing particle. Real functions define the arbitrary shape of particles and model the behavior of "particles." A collision detection algorithm was used to describe the relationship between particles. In addition to looking at processes, the FRep approach allows us to visualize the result as an accurate 3D model [6, 7]. Our solution is focused on measuring the viability of cells distributed in tissue spheroids in order to produce spheroids and build an accurate model of spheroid printing.

2.1. Geometry modeling details

To model the fusion of spheroids, Blending operations are used in pair with Union operation [8]. Blending operations are based on R-functions, Eq.2:

$$\begin{cases} F(f_1, f_2) = R(f_1, f_2) + d(f_1, f_2) \\ d(f_1, f_2) = a_0 / (1 + (\frac{f_1}{a_1})^2 + (\frac{f_2}{a_2})^2) \end{cases} \quad (2)$$

To model the natural surface of the tissue spheroid, modified for this particular case FRep Gardner Noise (GN) function was used, Eq.3 [9]:

$$GN = (a \times \sin(q \times X) + (\frac{a}{1{,}17}) \times \sin(\frac{q \times X}{1{,}35} + p \times sin(q \times Z)) \times (a \times \sin(q \times Y) + (\frac{a}{1{,}17}) \times \sin(\frac{q \times Y}{1{,}35} + p \times sin(q \times X))) \times (a \times sin(q \times Z) + \frac{a}{1{,}17}) \times \sin(\frac{(q \times Z)}{1{,}35} + p \times sin(q \times Y))). \quad (3)$$

Parameters such as amplitude (a), frequency (q), and phase (p) were selected to bring the surface as close as possible to the observed in natural conditions. In Fig.(1), the unite volume of the homogeneous tissue is generated by a fusion of homogeneous tissue spheroids. The angle between every two spheroids equals 120 degrees because it is an ideal angle to maximize fidelity in a fusion, minimizing necrosis risk.

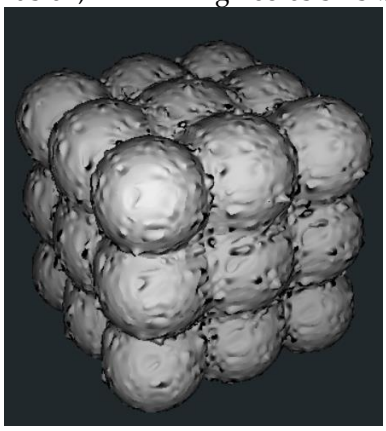

Figure 1. The unite volume of the homogeneous tissue generated by the FRep geometry

2.2. Processes modeling details

Developing a model of the biggest tissue spheroid without any necrotic processes, the diffusion-reaction task had been solved on the volume geometry described by the FRep approach. The concentration of oxygen in cells was considered depends on the time interval and coordinate to realize what diameter of the tissue spheroid is maximum without any necrotic processes in the center. We use upgraded diffusion-reaction equations

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with source members describing the nutrient consumption. It is needed to mention that the concentration changes are forced not only by natural diffusion processes but also by the consumption of cells. To survive, cells need several nutrients, and the main ones are glucose, water, and oxygen. The experience shows that the diffusion flow of any component. Fick's law describes a diffusible component passing per unit time through a unit area. It is perpendicular to the diffusion direction, proportional to the concentration gradient of this component, taken with the opposite sign, Eq.4:

$$I = -D\frac{\partial C}{\partial r}\ . \qquad (4)$$

Here I is the diffusion flow of the element of interest in the direction of the axis r. The minus on the right side shows that diffusion flow is directed towards decreasing concentration. The coefficient D in the equation is called the coefficient of diffusion. At not too high concentration of substances, D weakly depends on the concentration of the substance itself and is determined by the mobility of molecules degree. The mechanism reactions determine the functional dependence of the chemical reaction rate. In the general case, the function in the equation depends on both concentrations of reacting substances and clearly on the spatial coordinate r and time t. We consider precisely a multi-component system because biological objects are never simple. Due to the fact that, in our technology, we have "a mathematical experiment" for the limited time slot, we also set Neumann boundary conditions. Considering the diffusion process more precisely with nutrient consumption as a function, we have an equation (5):

$$\frac{\partial C(\vec{r},t)}{\partial t} = D\Delta C(\vec{r},t) - F(\vec{r},t), \qquad (5)$$

Where $F(\vec{r},t)$ describes oxygen consumption function, $\vec{r}$ is a vector $\vec{r}(x,y,z)$ for 3D space because tissue spheroid is a cellular volume structure and Δ is Laplace operator $\frac{\partial^2}{\partial x^2}+\frac{\partial^2}{\partial y^2}+\frac{\partial^2}{\partial z^2}$. When we upgrade our solution considering complex nutrient diffusion in tissue spheroids, there will be three substances diffusing with concentrations C_{O2}, C_{H2O}, C_{GL} for oxygen, water, and glucose diffusion, respectively. Thus, we will have a system of n equations:

$$\frac{\partial C_i(\vec{r},t)}{\partial t} = D_i\Delta C_i(\vec{r},t) - F_i(\vec{r},t), \quad i = 1,2,\dots n. \qquad (6)$$

For the described above case, n=3, but this model could be extended further if we will find out that there is a need to mention more diffused substances. The diffusion coefficient is determined not only by diffused substance properties but also by other system components' properties. Thus, instead of the self-diffusion coefficient, we need to use the cross-diffusion coefficient.

Considering the solving PDEs, an exciting method was proposed by Kassam [10]. The proposed approach unites Exponential Time Differencing (ETD) and 4-th order Runge-Kutta (RK4) methods for time-stepping, while the whole problem is solved on a Fourier space. Due to the fact that the resultant ETDRK4 method in its classical form is presented in Eq.7:

$$\begin{aligned}
a_n &= e^{\frac{Lh}{2}}u_n + L^{-1}(e^{\frac{Lh}{2}} - I)N(u_n,t_n),\\
b_n &= e^{\frac{Lh}{2}}u_n + L^{-1}(e^{\frac{Lh}{2}} - I)N(a_n,t_n+\frac{h}{2}),\\
c_n &= e^{Lh/2}a_n + L^{-1}(e^{\frac{Lh}{2}} - I)(2\times N(b_n,t_n+\frac{h}{2}) - N(u_n,t_n)),\\
u_{n+1} &= e^{Lh/2}u_n + h^{-2}L^{-3}\{[-4-Lh+e^{Lh}(4-3Lh+(Lh)^2)]N(u_n,t_n) +\\
&2[2+Lh++e^{Lh}(-2+Lh)](N(a_n,t_n+\tfrac{h}{2}) + N(b_n,t_n+\tfrac{h}{2})) + [-4-3Lh-(Lh)^2 +\\
&e^{Lh}(4--Lh)]N(c_n,t_n+h)\}, \qquad (7)
\end{aligned}$$

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Where L is the linear diffusion term, N is the nonlinear reaction term, and $-Dk^2$ is also a nonlinear reaction term used in a Fourier spectral method for spatial discretization (8):

$$\widehat{u_t} = -Dk^2\hat{u} + \widehat{N(u)} \quad (8)$$

In this form, ETDRK4 suffers from numerical instability. It needs some numerical stabilization to work correctly. Kassam and colleagues had handled this by computing specific coefficients by means of integrals around contours in the complex plane [10, 11]. Initially, ETDRK4 was developed by Cox and Matthews from explicit and implicit Exponential Tile Differencing (ETD) schemes [12]. To summarize, this numerical method is spectrally accurate in space, fourth-order accurate in time, and uses periodic boundary conditions. While nonlinear terms are evaluated in physical space, time-stepping is carried out in Fourier space, and one must be careful about de–aliasing. Due to such accuracy, ETDRK4 is expected to be effective for solving complex reaction-diffusion problems. Its effectivity for solution of Schnakenberg, Ginzburg-Landau, and Gray-Scott equations for such a task was proven in [10]. Regarding these cases, the Gray-Scott equation system could be considered solving our task because it has a wide application field, including biological systems.

3. Results

Approbation and validation of the initial simplified model were provided on bovine chondroblasts in cellular culture DMEM. The OCR was taken as a constant equal to 2.47*10-16g/cell/s [13, 14] divided on cell volume. Calculations were produced in the Wolfram Mathematica software toolkit. According to modeling results, the maximum diameter of tissue spheroid from bovine chondroblasts in cellular culture DMEM without any necrotic processes in the center equals 285 mcm. The validation of the model was provided at 3D Bioprinting Solutions on tissue spheroids on the third day after seeding. Tissue spheroids were seeded in concentrations of 29000 cells/ml and 16000 cells/ml. Half of the tissue spheroids from both two groups were painted by CellTox Green during seeding, and another half was painted by Live/Dead on the third day. Considering experimental results in comparison with modeling results, both have the same trend of concentration decreasing after 250 mcm, and in experiment results, the first necrotic processes start at 285 mcm. Therefore, the model passed the validation and required upgrading with extending geometry and diffusion details considering simulating more complex bioconstructs with various geometry. A possible way of development in the point of complex geometric forms could be vascular branching. Vascular branching bioprinting is a crucial bioconstruct processing because it is the first step of vessels conducting in the printed tissue. The second step of this process is providing angiogenesis from this printed branching by implantation of endothelial cells. Because angiogenesis requires fully viable bioconstruct to start from, necrotic areas are inadmissible in primary vascular branching. Therefore, studying the necessary conditions for the absence of necrosis in such a bioconstruct using diffusion-reaction-consumption equations is critical for future work.

4. Discussion

The deficiency of donor organs is a significant problem for transplantation surgery. According to the report 2018 of Global Observatory on Donation and Transplantation, living donors donate 36% and 19 % of transplanted kidneys and livers, respectively. The growth of transplantation cases increased to 6% for kidneys, 5 % for livers, 5% for heart, to 6 % for lungs in 2017-2018. It indicates the demand for tissue fabrication methods such as 3D bioprinting. The bioprinting process collides with the low printability of complex biological structures such as human organs. Printing could be optimized with software that simulates physical processes within the operation of 3D cellular building blocks that are called spheroids and a support matrix that is called a hydrogel. Tissue spheroids are in the study for tissue engineering, but there is no software tool to build a simulation to assess their bioprinting impact. Laboratories need a non-invasive tool to control the cell

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viability of 3D cultures. We suggest a solution that could implement spheroid technology in the new tissue engineering industry.

Tissue spheroids are building blocks to construct micromodels for pharmaceutical studies and tissue engineering approaches such as cell cultivation and 3D bioprinting [15]. Standard spheroids of the same size are required to reproduce living tissue. Three-dimensional tissue spheroids mimic the characteristics of native tissues. This biomimetic approach is better in both preclinical drug studies and biofabrication than the two-dimensional cell cultures traditionally used today [16], [17].

Besides, spheroids are used in developing therapy against the COVID-19 pandemic. That approach provides a regenerative cell-based treatment for pulmonary fibrosis [18, 19]. Thus, tissue spheroids are applicable to advanced regenerative medicine. The spheroids manufacturer must provide viable spheroids. The conventional method of growing multicellular spheroids is the "hanging drop" method. In laboratory conditions, fluorescence microscopy manages the assessment of the morphology, size, and viability of cells. The growth of spheroids is associated with the problem of tracking the state of cells for the presence of necrosis due to the lack of access to nutrients and oxygen [20]. There is a need for a non-invasive tool for standardization and control of up-scale production. Firstly there is an issue of scattering and light penetration for the fluorescence signal within complex three-dimensional multicellular structures. Secondly, the cost of dyes for producing fibroblast spheroids reaches 70,000 rubs for a vial of CellTox dye (Promega Corp.). The experiment requires time near a week (8 days in the medium) and an operator's additional work to analyze images and control the investigations. So this technology should be adapted for obtaining a large number of microscopic images of spheroids in mass production. As an alternative to fluorescence microscopy, it is proposed to use optical coherence tomography methods to observe the inner layers with high resolution [21].

Mass production of spheroids is a challenge taking into account laboratory technology. The ability to plan cell culture experiments and assess cell viability will reduce the risks of spheroids production. One of the most widespread hazards with spheroids production is that cells can lose their viability due to various factors. The main risk at this point is cellular necrosis. Before transplantation, constructs cannot have division processes and regeneration; therefore, necrotic processes could lead to total death. Consequently, we target necrotic tracing as the first goal of our solution. Mass production of spheroids is difficult in the laboratory, considering only conservative methods. Still, the ability to plan cell culture from a preliminary forecast and cell viability assessment will reduce the risk of costs in the mass production of spheroids. The production of spheroids requires an automated solution for modeling, analyzing, and controlling.

1.1. Bioprinting and tissue spheroids market analysis

According to the report on the global 3D bioprinting market from 2020 to 2026, it is expected to strengthen the status worldwide in key regions with growth and development prospects. The 3D bioprinting market trend research process includes the analysis of different factors affecting the industry, including the government policy, competitive landscape, historical data, market environment, present trends in the market, upcoming technologies, technological innovation, and the technical progress in related industry, and market risks, market barriers, opportunities, and challenges.

Top Leading Key Players nowadays are Organovo Holdings Inc. (US), Cellink AB (Sweden), Stratasys Ltd. (US), FUJIFILM Wako Automation Corporation (US), EnvisionTEC GmbH (Germany), Nano3D Biosciences Inc. (US), Allevi (US), Cyfuse Biomedical KK (Japan), REGENHU Ltd. (Switzerland), Aspect Biosystems Ltd. (Canada), Regenovo Biotechnology Co. Ltd. (China), and Poietis (France) [22]. It should be noted that Northern America prevails along the regions of selling.

The global organoids and spheroids market size was valued at $ 405.3 million in 2019, with a compound annual growth rate (CAGR) of 22.5% from 2020 to 2027. That is TAM market assessment, where another market research claims the increase from 18 billion euros in 2016 to 130 billion euros in 2025 for the regenerative medicine industry. Assuming

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that the spheroids market's size is about 50% of the need of both spheroids and organoids, we can estimate that the spheroids market is valued at about 202.1 million $ in 2019 and will grow to 2027. Usage of the spheroid and organoid models to reveal how the COVID-19 virus affects several intestinal cells is expected to drive organoids in research. Scientists have been using spheroids for the testing and identification of promising medications.

Companies are engaged in the development and launch of products well before the COVID-19 pandemic. For instance, Agilent Technologies has developed a spheroid microplate that facilitates cellular energy metabolism measurement in 3D cell culture spheroids with a Seahorse XF analyzer. The Seahorse XFe96 Spheroid Microplates measures respiration within the 3D cell cultures to assess their viability. Corning Incorporated also launched HTS Transwell tissue culture systems and spheroid microplates for high-performance cultivation and analysis. In the case of an estimate of SAM (Serviceable Available Market), we present data from the annual report of Thermo Fisher Scientific. For example, the yearly revenue of Thermo Fisher Scientific in 2019 was $ 25.5 billion, where revenue from sales of cell culture analysis systems is almost $ 6.4 million.

Technological developments in 3D spheroid technology are expected to drive the market at a significant rate. For instance, the Cultrex Organoid Progenitor Cells introduced by AMS Biotechnology (AMSBIO) can be optimized using different sets of extracellular matrices. Such developments facilitate the researchers to direct the activities of cells by controlling the culture microenvironment. Culture spheroid proliferation/viability and invasion assays stimulate cohesive spheroids even in low adhesion environments. The spheroids produced by this technique have been embedded in an invasion matrix and are used to model cell invasion from tumor spheroids. Researchers have further developed a 3D tri-culture model by adding vascular cells and human mesenchymal stem cells (hMSCs) to these spheroids. These efforts have led to developing a predictive in-vitro model such as the tri-cultures exhibit drug response and tumor morphology similar to xenografts. Such studies have propelled the adoption of spheroids and organoids. To summarize, the market for spheroids and the cell culture analysis segment is rapidly growing and developing.

55. Conclusions

The union of the complex geometric, physical, mathematical representations, and nutrients concentration calculations is a promising approach for the 3D bioconstruct viability prediction. Precise description using both FRep and ETDRK4 could help to model a bioprinted system and processes in it in detail. Considering Neumann boundary conditions can help understand the final geometric form of the bioconstruct and the existence of necrotic areas in it. Regarding the 3D bioprinting market situation, there are further conclusions:

- the proposed solution as a product is not an analogue of the listed software solutions;

- the listed software solutions and the proposed software product are aimed at the same consumer group, but for solving different tasks, which indicates the existing consumer niche for our software product;

- the high volume of increasing sales of the listed software solutions indicates a high potential demand for the proposed software product due to the fact that our software product and existing solutions are essentially complementary;

- the explosive growth in the number of scientific publications in the field of bioprinting over the past five years (the analysis was made on the basis of scientometric data Clarivate Analytics –WoS) indicates increased scientific interest in this area, supported by the prospect of rapid commercialization of research works, which suggests an additional factor in increasing demand for the proposed software product in the coming years.

Summarising all the above, such a solution is expected to be a promising basis for specialized 3D bioprinting software development.

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