Ultrasonic manipulation of cells for alleviating the clogging of extrusion-based bioprinting nozzles

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Abstract. Extrusion-based bioprinting is one of the most common biomanufacturing methods. The bioink composed of biomaterials and living cells is extruded into cylindrical filaments. The filaments are deposited on the substrate and stacked layer by layer to form a three-dimensional structure. During the extrusion process, the ink tends to accumulate at the nozzle, which clogs the nozzle and increases extrusion pressure, resulting in the decrease of cell survival rate. Here, we propose a method of using ultrasound to manipulate cells to assist the bioprinting process, which can alleviate nozzle clogging. The ceramic piezoelectric plate(PZT) is used to drive the nozzle to generate structural resonance, and the ultrasonic standing wave generated by the vibration make the cells/particles in the ink gather at the center of the nozzle, thereby reducing the risk of clogging of the nozzle. In this paper, we first analyze the mechanism of the standing wave generated in the cylindrical elastic nozzle and drive the cells to move under the PZT drive, and then use the finite element software to simulate the standing wave drive process. The results show that this method can be solved by a simple and low-cost device. The results indicate that this method has the possibility of using simple and low-cost equipment to solve the problem of nozzle clogging in bioprinting.

1. Introduction
Tissue engineering is an interdisciplinary subject involving clinical medicine, cytology, molecular biology, materials science, mechanical engineering and so on[1]. Its purpose is to generate artificial tissues and organs in vivo or in vitro to repair the function of damaged tissues or organs. The key factors of tissue engineering research includes three elements: cells, scaffolds and bioactive molecule. However, current studies have shown that it is still difficult to localize and implant a variety of cells, biomaterials and bioactive molecules on preformed scaffolds[2].

In general, bioprinting is using a computer-controlled 3D motion device to accurately deposit cells and biomaterials into precise geometries with the goal being the creation of anatomically-correct biological structures[3]. D J Odde et al. captured and propelled a single cell through a nozzle onto a slide in 2000 with a laser pulse to align it in a straight line, and first proposed the concept of cell printing[4]. Three-dimensional cell printing is considered a promising technology due to its outstanding ability of precisely positioning multiple biomaterials and living cells in a layer-by-layer manner based on patient-specific designs acquired from medical imaging[5]. However, nozzle clogging is one of the fatal problems of extrusion-based bioprinting technology. The gradual accumulation of cells at the nozzle will choking the flow of bioink and cause clogging, which not only affects the accuracy and
reliability of cell printing, but also increases the extrusion force on the cells, cell survival is decreased with increasing pressure, nozzle gauge, and shear stress[6].

Inkjet printing can achieve high-precision drop-on-demand, but the lower suspension concentration (<10⁶ cells/ml) and the smaller viscosity selection range (<100 mPa·s) have become the biggest limitation of development. Studies have indicated that the extrusion-based cell printing technology can print high-concentration (10⁷ cells/ml) and high-viscosity (1 Pa·s) cell suspensions, but the nozzle tends to reduce the nozzle aperture in order to improve accuracy, which will cause clogging[7]. Most of the current cell printing experiments will consider the element of nozzle clogging, which will limit the types of bio-inks that can be selected, which in turn will affect the experiment.

The gradual accumulation of cells/particles will eventually hinder fluid flow and form blockages, which will seriously affect the accuracy and reliability of nozzle printing and cause damage to the nozzle. However, such low-viscosity and low-mechanical strength bio-inks are difficult to construct a stable free-form three-dimensional scaffold structure. Therefore, it is necessary to reduce the clogging problem of the nozzle printing system and improve its printability. In order to reduce the interaction force between the liquid and solid layers and the surface tension of the printing material, surfactants are usually added. However, surfactants can change the properties of cell membranes and reduce cell proliferation. Shabnam P et al. used Pluronic as a surfactant to slow down cell aggregation and blockage in cell suspension, but it may affect cell activity[8]. For the clogging issue, researchers usually optimize the experimental parameters, which limits the concentration of biomaterials and the density of cells[9]. The electromagnetic force generated by direct current or electromagnetic induction can change the turbulence in the nozzle inlet area, which reduces the turbulence area in the cylindrical tundish nozzle, thereby reducing the possibility of oxide cells blocking[10].

In order to alleviate the clogging problem, a method of applying ultrasonic manipulation cell assist the printing process was proposed. First, we explained the movement mechanism of cells/particles in the nozzle made of elastic material driven by the PZT, and then the finite element method was used to analyze the extrusion effect of the proposed ultrasonic-assisted cell printing nozzle structure. The results indicated that the ultrasound-assisted cell printing scheme is a promising application in alleviating the problem of nozzle clogging.

2. Mechanism of manipulating cell in cylindrical printing nozzle

2.1. Vibration of cylindrical tube

The ultrasonic-assisted extrusion-based cell printing process is shown in figure 1. This structure first appeared in 2004, the author applies the structure for cell concentration, separation and cell screening[11]. Here, the bioink with cells flows into the nozzle from the top, and the cells are evenly dispersed in the cylindrical tube. The nozzle resonates with the ink driven by the PZT, the acoustic pressure node is formed in the center of the lumen at a specific frequency and the cell tends to the low potential energy sound pressure node, which realizes the manipulation of the cell. The cells gathered in the center of the nozzle that reduces the contact with the inner wall of the nozzle when the bioink is extruded, which slow down the clogging of the nozzle. The flexible nozzle containing bioink mainly undergoes bending vibration under the PZT low frequency drive, Euler-Bernoulli beams theory can explain this vibration mode well. We decompose the bending nozzle into countless units. The basic elements of the bending nozzle are shown in figure 2.
Figure 1. Diagram of the mechanism of ultrasound-assisted cell printing to reduce nozzle clogging.

Figure 2. (a) Bending of the glass nozzle element (b) Radius of curvature in a bending nozzle.

From figure 2(a) we can write

\[ \Delta x' = (R - z)d\theta \]

(1)

\[ \Delta x = Rd\theta \]

(2)

So, the strain of element is defined by,

\[ \varepsilon(z) = \frac{(R - z)d\theta - Rd\theta}{Rd\theta} = -\frac{z}{R} \]

(3)

At point \( z = 0 \)

\[ M(x) = -\int_{-h/2}^{h/2} \frac{z^2}{R} dA \]

(4)

From figure 2(b) we can write

\[ \frac{\partial y}{\partial x} = \tan \theta = \theta \]

\[ \frac{\partial^2 y}{\partial x^2} = \frac{1}{R} \]

(5)

From equation (4) and (5) we can conclude,

\[ M(x) = -EI \left( \frac{\partial^2 y}{\partial x^2} \right) \]

where \( A \) is the cross section area of the nozzle, \( \sigma(z) \) is the stress of the nozzle element, \( E \) is the elasticity modulus, \( I \) is the moment of inertia.

We may use the separation of variables technique here. Assuming the solution has the form

\[ y(x,t) = Y(x)T(t) \]

(7)

Expand the Fourier transform of \( Y(x) \) and \( T(t) \).
\[ T(t) = Ae^{iat} \]
\[ Y(t) = C_1 \sin \beta x + C_2 \cos \beta x + C_3 \sinh \beta x + C_4 \cosh \beta x \]  \hspace{1cm} (8)

where

\[ A \text{ and } C_\beta \text{ are constant}, \quad \beta^2 = \frac{\omega^2}{a^2}, \quad \text{and} \quad a^2 = \frac{EI}{\rho A}, \]

therefore, the general form of the bending vibration mode of the flexible nozzle is obtained.

2.2. Acoustic pressure on cells

As described in section 2.1, after the ultrasonic pressure field is generated in the lumen, the cells will tend to the position of low potential energy. In the acoustic assist nozzle, the cells will be affected by gravity, buoyancy, acoustic radiation force, and drag force. Whether the cells can move to the center of the lumen depends on the diameter, density, compressibility of the cells and the viscosity and compressibility of the bioink and other factors.

The nozzle resonates with the ink driven by the PZT, the acoustic pressure node is formed in the center of the lumen at a specific frequency and the cell tends to the low potential energy sound pressure node, which realizes the manipulation of the cell. The cells gathered in the center of the nozzle that reduces the contact with the inner wall of the nozzle when the bioink is extruded, which slow down the clogging of the nozzle. For a dilute suspension in an arbitrary field, Gorkov’s\cite{12} theory for noninteracting cells provides a good description of the equilibrium cell distribution. The acoustic force \( F \) acting on a small spherical cell of radius \( r \) and density \( \rho_p \) in a fluid of density \( \rho_f \) is given by

\[ F = -\nabla \left( 2 \pi R^3 \left[ \frac{p^2}{\rho_f c_f^2} - \frac{p^2}{\rho_p c_p^2} - \frac{3(p_f (\rho_p - \rho_f))}{2\rho_p + \rho_f} \frac{1}{v^2} \right] \right) \]  \hspace{1cm} (9)

where the variables \( c_f \) and \( c_p \) are the acoustic velocities in the fluid and the cell, respectively, \( p \) and \( v \) are the pressure and velocity of the wave at the location of the cell. Based on equation (7) (8) and (9), we can figure out how the cells are controlled in the flexible nozzle.

3. Ultrasonic-assisted nozzle extrusion simulation

3.1. Simulation of nozzle clogging and Vibration modes

Some cells may attach to the inner wall of the glass tube due to the surface tension. It is noted that there are some pressure nodes close to the wall boundary, and subsequently, a small fraction of cells may accumulate there as our previous analysis. We used the finite element software COMSOL Multiphysics 5.4 to simulate the process of cells adhering to the tube wall and causing clogging. It can be seen from the figure that the cells have less adhesion on the tube wall at the beginning, but as the cross section area of the nozzle decreases, the adhesion increases.
In order to subsequently simulate the movement process of the cells in the nozzle driven by PZT, we calculated the eigenfrequency of the nozzle according to formulas (7) and (8), as shown in Figure 4. The eigenfrequency of the glass tube produced by the piezoceramic plate was simulated by the FEM, and there are three main vibration modes at 436, 492 and 506 kHz, respectively. And the results show that the driving effect is the best at a frequency of 492KHz.

### 3.2 Ultrasonic manipulation cells simulation

A 2D simulation is shown in figure 5, which is the top view of figure 1. Numerical simulation was carried out using the modules of solid mechanics, electrostatic, acoustics and cell tracing. A PZT (20×10×2mm, 492KHz) was attached to the outer side of a cylindrical glass tube, whose inner and outer diameter are 5.6 and 7.8mm, respectively. Other parameter settings can be seen in figure 5. The simulation results show that the ultrasonic-assisted cell printing method can gather about 10⁶ cells in the center of the nozzle (accounting for 20% of the cross section area of the nozzle) within 1 s, which effectively reducing the possibility of cells adhering to the tube wall. It will further reduce the extrusion pressure and increase the survival rate of cells.
4. Conclusions

During the extrusion process, the ink is easy to accumulate and block the nozzle, which in turn causes the cells to undergo destructive pressure and cause their death. This article proposes the use of ultrasound to manipulate cells to assist the bioprinting process. The ceramic piezoelectric plate is used to drive the nozzle to generate structural resonance, and the ultrasonic sound waves generated by the vibration make the cells/particles in the ink gather at the center of the nozzle, thereby reducing the risk of clogging of the nozzle. This article applies a technology of ultrasonically manipulating cells to solve the problem of clogging of the cell printing nozzle. First, the movement of cells in the glass print nozzle driven by the ceramic piezoelectric sheet is theoretically analyzed, and then the finite element method is used to analyze the extrusion effect of the proposed acoustic assisted cell print nozzle structure. The results show that the proposed ultrasound-assisted cell printing scheme has a good effect on alleviating the clogging problem of the nozzle.

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References

[1] Jammalamadaka U and Tappa K 2018 Recent Advances in Biomaterials for 3D Printing and Tissue Engineering J. Funct. Biomater. 9
[2] Gorabi A M, Tafti S H A, Soleimani M, Panahi Y and Sahebkar A 2017 Cells, Scaffolds and Their Interactions in Myocardial Tissue Regeneration J. Cell. Biochem. 118 2454-62
[3] Skardal A and Atala A 2015 Biomaterials for integration with 3-D bioprinting Ann. Biomed. Eng. 43 730-46
[4] Odde D J, & Renn, M. J. 2000 Laser-Guided Direct Writing of Living Cells Biotechnology and Bioengineering. 67(3) 312-18
[5] Gungor-Ozkerim P S, Inci I, Zhang Y S, Khademhosseini A and Dokmeci M R 2018 Bioinks for 3D bioprinting: an overview Biomater. Sci. 6 915-46
[6] Leberfinger A N, Ravnic D J, Dhawan A and Ozbulat I T 2017 Concise Review: Bioprinting of Stem Cells for Transplantable Tissue Fabrication Stem. Cells. Transl. Med. 6 1940-48
[7] Ferris C J, Gilmore K G, Wallace G G and In het Panhuis M 2013 Biofabrication: an overview of the approaches used for printing of living cells Appl. Microbiol. Biotechnol. 97 4243-58
[8] Parsa S, Gupta M, Loizeau F and Cheung K C 2010 Effects of surfactant and gentle agitation on inkjet dispensing of living cells Biofabrication. 2 025003
[9] Zhou Y 2016 The Application of Ultrasound in 3D Bio-Printing *Molecules*. 21
[10] Lavers J D and Kadar L 2004 Application of electromagnetic forces to reduce tundish nozzle clogging *Applied Mathematical Modelling*. 28 29-45
[11] Goddard G and Kaduchak G 2005 Ultrasonic particle concentration in a line-driven cylindrical tube *J. Acoust. Soc. Am.* 117 3440-7
[12] Gor’kov L P 1962 On the forces acting on a small particle in an acoustical field in and ideal fluid *Sov. Phys. Dokl.* 6 773-75