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Acoustic manipulation of microparticle in a cylindrical tube for 3D printing

Abstract

Purpose – The capability of microparticle/objects patterning in the 3D printing structure could improve its performance and functionalities. This paper aims to propose and evaluate a novel acoustic manipulation approach.

Design/methodology/approach – A novel method to accumulate the microparticles in the cylindrical tube during the 3D printing process is proposed by acoustically exciting the structural vibration of the cylindrical tube at a specific frequency and subsequently focusing the 50-µm polystyrene microparticles at the produced pressure node towards the center of the tube by the acoustic radiation force. To realize this solution, a piezoceramic plate was glued to the outside wall of a cylindrical glass tube with a tapered nozzle. The accumulation of microparticles in the tube and printing structure was monitored microscopically, and the accumulation time and width were quantitatively evaluated. Furthermore, the application of such technology was also evaluated in the L929 and PC-12 cells suspended in sodium alginate and gelatin methacryloyl (GelMA).

Findings – The measured location of pressure and the excitation frequency of the cylindrical glass tube (172 kHz) agreed quite well with our numerical simulation (168 kHz). Acoustic excitation could effectively and consistently accumulate the microparticles. It is found that the accumulation time and width of microparticles in the tube increase with the concentration of sodium alginate and microparticles in the ink. As a result, the microparticles are concentrated mostly in the central part of the printing structure. In comparison to the conventional printing strategy, acoustic excitation could significantly reduce the width of accumulated microparticles in the printing structure ($p < 0.05$). In addition, the possibility of high harmonics (385 kHz and 657 kHz) was also explored. L929 and PC-12 cells suspended in the hydrogel can also be accumulated successfully.

Originality/value – This paper proves that the proposed acoustic approach is able to increase the accuracy of printing capability at a low cost, easy configuration, and low power output.

Keywords Additive manufacturing, Extrusion-based printing, Particle manipulation, Acoustic excitation

Introduction

Nowadays, additive manufacturing (AM) has been developed and used widely for various applications (Vaezi et al. 2013, Wong and Hernandez 2012). Its advantages include the generation of the free-form structure, scalable of component size, and cheap equipment compared to the other manufacturing techniques. Under the classification of the American Society for Testing and Materials (ASTM), most of material jetting (inkjet) and extrusion (fused deposition and extrusion-based bioprinting) printers use the nozzle as a main component. All of these printers drop ink on the substrate. The solid microparticles/objects are mostly suspended in the fluid as the printing ink. Spatial manipulation of microparticles/objects in the AM may improve the performance and functionalities of

printing structure. Proper alignment and orientation of the fibers in a polymer matrix as fiber reinforced polymer composite can transfer the loads away from critical locations for the improved performance (Holmes and Riddick 2014). Hierarchically ordered materials at the nano- and micro-scale levels that exploit material composition and capabilities at a variety of length scales can expand the applications of AM techniques. Ability to print 3D scaffolds with a controlled hierarchical structure could enhance mechanical strength, which is desirable for load-bearing bone defect repair and regeneration (Fu et al. 2011). Decorating the surface of carbon nanotubes with particular antibodies enables the detection of specific antigens as functional materials (Fattah et al. 2017). Application of multifunctional nano-composites with respective printing media may have common limitations, such as nozzle clogging (Campbell and Ivanova 2013). Focusing the particles in the microchannel could delay the accumulation on the channel wall (Sriphutkiat and Zhou 2017).

Recently, a few studies attempted to apply the external forces in assisting the printing process. For instance, magnetic force (Ahn et al. 2015, Fattah et al. 2017, Kokkinis et al. 2015) could align the microparticles orientation at the interface (Wang et al. 2015). But this method requires the use of microparticles/objects with specific electromagnetic properties or labeling the cells/proteins with magnetic nanoparticles, which is usually time-consuming and may cause some toxicity to the human organisms (Nel et al. 2006). Similarly, using electricity to manipulate conductive and/or dielectric microparticles also requires certain electrical charge property (Holmes and Riddick 2014, Kim et al. 2004, van der Asdonk et al. 2016). However, the high electric field may induce heating, which may affect the viability of the mammalian cells. Acoustic manipulation has also been applied for various applications, such as microparticle patterning (Ding et al. 2012, Raeymaekers et al. 2011, Shi et al. 2009), focusing (Piyasena et al. 2012, Suthanthiraraj et al. 2012), and cell sorting (Ding et al. 2012, Franke et al. 2010). This approach relies on the relative density, compressibility, and size of the microparticles. Some attempted to use the acoustic waves to pattern the microparticles during or after the printing (Friedrich et al. 2017, Llewellyn-Jones et al. 2016). However, the use of a rectangular nozzle, which is uncommon and needs complicated manufacturing at a high cost, and manipulation of microparticles after the printing in a rectangular container may limit the practice of such technology. The acoustic focusing of microparticles at the center of the cylindrical tube was possible (Goddard and Kaduchak 2005), but the effect on the printing structure was not shown and only fundamental vibration mode was explored. Importantly, the effects of experimental parameters (e.g., the concentration of microparticles and hydrogel) for printing (e.g., accumulation time and width) have not been investigated to completely evaluate the potential of this approach.

In this work, we studied a low-frequency bipolar mode of the structural vibration of a cylindrical tube and then utilized it to concentrate the microparticles at the center of the tube and subsequently the printing structure. The effects of experimental parameters, such as the concentration of microparticles and alginate, on the printing were studied. The fluid viscosity of ink with included microparticles is an important factor for extrusion printing (Crowson and Folkes 1980, Ley and Bruus 2016). The numerical simulation was first carried out to predict the excitation frequency, structural vibration, the distribution of acoustic pressure in a cylindrical tube, and the corresponding accumulation of microparticles. The experimental

excitation frequency of locating the pressure node of the structural vibration and accumulation of microparticles at the center of the cylindrical tube was similar to the simulation results. The time to accumulate microparticles to the center and its width in the tube were measured. The effect of concentration of sodium alginate and microparticles in the ink on microparticle accumulation in the tube and the printing structure was studied. The printing capability without and with acoustic excitation was compared statistically. Furthermore, the ability of higher harmonics was also evaluated. Various patterns of microparticles in the printing structure could be controlled by adjusting the excitation frequencies. Such accumulation capability was further validated using the bioink with the L929 and PC-12 cells suspended in sodium alginate and GelMA.

Material and Methods

Numerical simulation

A model was established for the numerical simulation using the finite element method (FEM) software (COMSOL 5.2, Stockholm, Sweden). A plate of piezoceramic (11×11×2 mm³) was attached to the outer side of a cylindrical glass tube, whose inner and outer diameter are 6.4 mm and 7.0 mm, respectively. The electrical signals were supplied to the piezoelectric material to excite the longitudinal mechanical vibration which is perpendicular to the surface of the glass tube and then coupled into the liquid inside the glass tube. Triangle meshes were used in the FEM, and there were in total 7531 meshes in the domain of piezoceramic, glass tube, and fluid. The average mesh growth rate was 1.521. The smallest mesh size was 2 μm at the interface between piezoceramic and glass. A total of 124 microparticles in the diameter of 50 μm were distributed uniformly inside the tube initially as shown in Figure 1. Numerical simulation was carried out using the modules of solid mechanics, electrostatic, acoustics, and particle tracing. Initially, eigen-frequency of the glass tube was calculated to determine the excitation frequency and stress-strain response. The outer boundaries of the glass tube were freely bound. Then the electrical signal was applied to the piezoceramic plate. With the piezoelectric effect, the electricity was converted to the stress and strain in the piezoceramic and then transferred to the glass which was described as the linear elastic material. At the interface between glass and fluid, the mechanical waves propagated into the fluid domain. Trajectories of microparticles in the fluid were calculated in the time domain at a step size of 1 ms. The primary radiation force applied to the microparticles pushes them towards the pressure node under the acoustic excitation. The parameters and governing equations used in this simulation are listed in Table S1 and Appendix.

Figure 1 Cross-section diagram of subdomains and boundary conditions in the FEM simulation

Experimental setup

A piezoceramic plate (355, 11×11×2 mm³, APC International, Mackeyville, PA, USA) was glued (Insta-Flex+, Bob Smith Industries, Atascadero, CA, USA) to a cylindrical glass tube (Glass Pasteur Pipet, Corning, NY, USA). A diagram of the experimental setup is illustrated in Figure 2. The sinusoidal signal at a certain frequency was generated by a function generator (AFG3000, Tektronix, Beaverton, OR, USA) and then undergone a power amplifier (240L, ENI, Rochester, NY, USA). The power input to the device was 0.71 W as measured by an impedance analyzer (R3272, Advantest Corp, Tokyo, Japan). To maximize the electrical power transferred to the piezoceramic plate, a matching unit was built in the lab to adjust the output impedance to the power amplifier at around 50 Ω . The vibration pattern of the glass tube was measured using a laser Doppler vibrometer (PSV-500, Polytec GmbH, Waldbronn, Germany). Trajectories of the microparticle along the glass tube were observed by an industrial camera (55326, Edmund Industrial Optics, Barrington, NJ, USA) with a 25-mm focal length lens under the illumination of a LED light source (V-LSL666, Valore, Singapore). In addition, cross-sectional images of microparticles in the cylindrical glass tube were captured using the light sheet. Fiber optic illuminator (MI-150, Edmund Optics) and single branch light line guide (#53-986, Edmund Optics) was used to produce the intensive flat light. Then, a cylindrical lens with a focal length of 25 mm (LOCPCXB22-25, Lighten Optics, Beijing, China) focused the light beam to the glass tube. The camera was then aligned vertically for photography. Temperatures of piezoceramic and glass tube were monitored noninvasively by a laser thermometer (AR320, Arco Science & Technology Ltd, Dongguan, Guangdong, China). The schematic diagram of the setup is shown in Figure 2.

Figure 2 Schematic diagram of the experimental setup to observe the motion of microparticles (a) along and (b) in the cross-section of the glass tube, and (c) representative photo of the accumulated microparticles in the glass tube under the acoustic activation.

Printing evaluation

In bioprinting application, 1%–4% of sodium alginate, a common hydrogel in the biological studies, in deionized (DI) water is widely used to construct the 3D structure for cells (Sheeran et al. 2012, Sun and Tan 2013). However, the addition of sodium alginate would increase the viscosity of the medium from 2.54 cPs to 37.5 cPs as measured by a rheometer (DHR-2, TA Instruments, New Castle, DE, USA), which is similar to the previously reported value (Sigma-Aldrich). In addition, 10% GelMA was also prepared with the established protocol (Loessner et al. 2016). A freeze-dried foamy GelMA was dissolved in the HyClone™ Dulbecco's modified eagle's medium (DMEM, GE Healthcare Life Sciences, HyClone Laboratories, Logan, UT, USA) first, mixed with 0.2 g of a photo-initiator (Irgacure 2959, Sigma-Aldrich, Singapore), and then kept in a dark chamber at 37°C till use. Various concentrations (e.g., 0.25%, 0.5%, 1.0%, 1.5%, and 2.0% w/w) of polystyrene microparticles (50 µm in diameter, Phosphorex, Hopkinton, MA, USA) suspended in the alginate solution (180947, Sigma-Aldrich) at the concentration of 1%, 2%, 3%, and 4% w/w were used as the printing medium (ink). Prior to each printing, the solution was spun by vortex (Maxi Mix III, Barnstead/Thermolyne, Dubuque, IA, USA) and degassed in a vacuum chamber (3608-1CE, Thermo Scientific, Waltham, MA, USA). The suspension was printed through an extrusion-based bioprinter (TechnoDigm, Singapore) on a petri dish (4", Corning, Sigma-Aldrich). The distribution of microparticles in the printed structures was observed under a light microscope (CKX-41, Olympus, Tokyo, Japan) with 4× magnification, and then the captured images were quantitatively analyzed using digital processing software (ImageJ, National Institute of Health, Bethesda, MD, USA) and calculation software (Matlab, MathWorks, Natick, MA, USA).

Distribution of microparticles in the glass tube was recorded, and the light intensity across the tube was used to analyze and quantify the characteristics of microparticle accumulation under the acoustic excitation. The change of measured peak light intensity, which is calculated from the obtained RGB color image as $0.299R + 0.587G + 0.114B$, in the course of the acoustic excitation is shown in Figure 4e. When the variation is within $\pm 1\%$ of the maximum value, the microparticle accumulation is assumed to reach its stabilization. The corresponding time is defined as the accumulation time of microparticles. The full width at half maximum (FWHM) of the light intensity distribution at the stabilized stage was used to determine the accumulation width of microparticles (see Figure 4d).

In the printing structures, the histogram of deposited microparticles was calculated from the captured images after determining the edge of all microparticles and then fitted using the Gaussian function

$$f(x) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (1)$$

where μ is the mean value, σ is the standard deviation. The corresponding FWHW in the Gaussian curve is given by

$$FWHW = 2\sigma \cdot \sqrt{2\ln 2} = 2.355 \cdot \sigma \quad (2)$$

FWHW is used to evaluate the microparticle distribution and compare the performance of acoustic excitation in the printing process. It's well known that 95% of the microparticles are within 2 standard deviations ($[\mu-2\sigma, \mu+2\sigma]$) in the Gaussian distribution curve.

Cell culture and harvest

L929 cells (mouse fibroblast, CCL-1, ATCC, Manassas, VA, USA) and PC-12 cells (rat adrenal pheochromocytoma, ATCC) were cultured in the DMEM, which contains 10% fetal bovine serum (FBS, Gibco, Waltham, MA, USA) and 1% antibiotic-antimycotic solution, including 10,000 units/mL of penicillin, 10,000 µg/mL of streptomycin, and 25 µg/mL of amphotericin B (Gibco). They were harvested with the standard trypsinization at ~90% confluence. Briefly, the cells were incubated with 0.25% trypsin-1mM EDTA solution (Lonza, Basel, Switzerland) at 37°C for 3 minutes. The reaction of trypsin was terminated with the cell culture medium for 5 minutes at room temperature (25°C). The cells were then washed with phosphate buffered saline (PBS, Sigma-Aldrich) and centrifuged at 1,000 revolutions per minute (RPM) for 5 minutes. The initial cell concentration was enumerated using the standard hemocytometry (79001-00, Cole-Parmer, Vernon Hills, IL, USA).

Statistical analysis

Student's *t*-tests were carried out to determine the statistical significance (95% confidence interval or *p*-value below 0.05) between different experimental conditions using SigmaPlot (Systat Software, San Jose, CA, USA). In each group, at least 6 data were included for the analysis.

Results

Vibration modes

Acoustic excitation aims to accumulate the microparticles during the printing process. The eigen-frequency of the glass tube produced by the piezoceramic plate was simulated by the FEM, and there are 3 main vibration modes at 168 kHz, 393 kHz, and 563 kHz, respectively (see Figure 3). The use of fundamental mode which gathers microparticles to the center of the tube was first investigated (see Figure 4). The vibration direction is perpendicular to the glass tube. The predicted frequency in the numerical simulation is 168 kHz. The vibration on the surface of the glass tube was measured by the laser Doppler vibrometer and compared to the simulation results. In the scanned contour, two regions with high positive vibration velocity were observed at 83.2° (0.070 mm/s) and 277.8° (0.061 mm/s), close to the piezoceramic and its opposite side, which is similar to the previous studies (Goddard and Kaduchak 2005). However, a slight difference of the excitation frequency was observed (168 kHz in the simulation and 172 kHz in the experiment), which may be due to the discrepancies of material properties and inconsistent thickness of the glass tube. In addition, the microparticles assembled due to the secondary Bjerknes force (attractive inter-particle force), gradually grew to lumps, and then moved towards the pressure node in the cross-section of the glass tube (Garcia-Sabaté et al. 2014, Sepehrirahnama et al. 2015). The measured distribution of 50-µm microparticles in the cross-section with the acoustic excitation at 172 kHz is found similar to the simulated pattern. Overall, there are good agreements between the simulation and measurement.

Figure 3 (a) The eigen-frequency simulation using the finite element method, and the structure vibration of glass tube produced by the piezoceramic plate at the frequency of (b) 168 kHz, (c) 393 kHz, and (d) 563 kHz.

Figure 4 (a) The simulated radial stress of glass tube at the excitation frequency of 168 kHz in kPa, (b) comparison of simulated (168 kHz) and measured (172 kHz) normalized vibration velocity in the polar plot, (c) time-averaged acoustic pressure in kPa at 168 kHz, (d) the locations of 50- μ m microparticles after 0.2 seconds of excitation in the simulation, and comparison of the distribution of 50- μ m microparticles in the cross-sectional of glass tube (e) without the acoustic excitation where the microparticles are quite uniformly distributed and (f) with the acoustic excitation at 172 kHz where the microparticles are concentrated mostly at the center in a similar pattern as the numerical prediction.

Accumulation of microparticles in the glass tube

Initially, the microparticles were distributed randomly in the tube, and the light intensity across the tube was quite uniform, whose profile may be associated with the laminar flow for extrusion. Under the acoustic excitation, most of the microparticles gradually moved toward the pressure node at the center of the tube so that the light intensity distribution had a sharp peak (see Figure 5). However, some microparticles may attach to the inner wall of the glass tube due to the surface tension. During the microparticle accumulation, the peak light intensity (mostly at the center of the glass tube) exponentially rose to its maximum value. With the increase of microparticle concentration, the peak light intensity in the stabilized state increased correspondingly but at a longer microparticle accumulation time. It is noted that there are some pressure nodes close to the wall boundary and subsequently, a small fraction of microparticles may accumulate there as predicted numerically (see Figures 3c-d).

Figure 5 The representative photo of microparticles in the glass tube with 1% sodium alginate and 0.25% microparticle (a) before and (c) after the acoustic excitation, and the corresponding distributions of the normalized light intensity in (b) and (d), and (e) the change of the peak light intensity during the microparticle accumulation in the fluid with 1% alginate and varied microparticle concentrations.

The concentration of alginate and microparticles in the fluid plays a significant role in the hydrodynamics of microparticles, which subsequently determines the efficiency and effectiveness of microparticle accumulation (see Figure 6). The microparticle accumulation width (FWHM) increased in 3.3 fold from 0.19 ± 0.07 to 0.64 ± 0.18 mm at the microparticle concentrations from 0.25% to 2% with 1% alginate in the fluid. The corresponding increase was 2.4 fold from 0.30 ± 0.07 mm to 0.73 ± 0.12 mm with the increase of alginate from 1% to 4% with 0.5% microparticles in the fluid. The accumulation time increased almost linearly in 4.1 fold from 29.3 ± 3.5 s to 121.2 ± 16.1 s with the increase of alginate from 1% to 4%.

However, there are fewer influences on the accumulation time by the concentration of microparticles than that of alginate. The corresponding value increased slightly in 1.3 fold from 30.3 ± 3.6 s to 38.1 ± 5.2 s with the increase of microparticle concentration from 0.25% to 2.0%. Overall, the accumulation time is more sensitive to the concentration of alginate than that of the microparticle, which may be due to the fluid viscosity.

Figure 6 Accumulation time and width of microparticles in the solution with (a) 1%, 2%, 3%, and 4% sodium alginate and 0.25% microparticles and (b) 0.25%, 0.5%, 1.0%, 1.5%, and 2.0% microparticles and 1% sodium alginate ($n = 6$ for each condition).

Microparticle distribution in the printing structure

The printing structure by the extrusion-based bioprinter was straight lines on the petri dish. The distribution of microparticles inside the printing structure was observed under the light microscope. It is found that microparticles distributed quite uniformly without an acoustic activation, but mostly at the center after the printing with the acoustic excitation due to the in prior accumulation in the glass tube (see Figure 7).

Figure 7 (a) The printing structures with 2% sodium alginate and 0.5% microparticle on the petri dish, and zoomed photos illustrating the distribution of microparticle distribution inside them (b) without and (c) with an acoustic excitation during the printing (scale of 1 mm).

Microparticle distribution in the printing structure was represented in the histogram quantitatively and then fitted by the Gaussian curve to compare the accumulated microparticle width (see Figure 8). The acoustic excitation could accumulate the microparticles mostly at the center. The percentage in the three central bins of the histogram was $46.5\pm3.7\%$, $41.8\pm4.1\%$, and $43.4\pm4.9\%$ at the alginate concentration of 1% and the microparticle concentration of 0.25%, 0.5%, and 1%, respectively. However, the corresponding value significantly dropped to $32.8\pm5.2\%$ at 2% microparticle. In comparison, the percentage of accumulated microparticles in the three central bins fairly dropped from $41.8\pm4.1\%$ to $35.6\pm5.7\%$ with the increase of alginate concentration from 1% to 4% at the microparticle concentration of 0.5%. In comparison to the conventional printing without the acoustic excitation, the values of FWHM were always larger than those of acoustic excitation at all experimental conditions ($p < 0.05$) and increased with the concentration of alginate and microparticles with the acoustic excitation, from 0.31 ± 0.13 mm to 1.13 ± 0.17 mm (3.7 fold) for 0.25% and 2% microparticle and 1% alginate and from 0.73 ± 0.11 mm to 1.39 ± 0.22 mm (1.9 fold) for 1% and 4% alginate and 0.5% microparticle, respectively. The discrepancy between the accumulated microparticle width in the glass tube and the printing structure is due to the streamline through the nozzle. Another reason may be the difference between the counted microparticles in the printing structure and the detected light intensity in the glass tube.

Figure 8 Histogram (solid line) and fitted Gaussian curve (dashed line) of microparticle distribution in the printing structure using the ink with 1% sodium alginate and 0.5% microparticle (a) without and (b) with the acoustic excitation, and comparison of the distributed microparticle width (c) at the various sodium alginate concentration from 1% to 4% and microparticle concentration of 0.5% and (d) at the sodium alginate concentration of 1% and various microparticle concentrations from 0.25% to 2%, *: statistically different between the experimental data without and with the acoustic excitation ($p < 0.05$).

High orders of structural vibration

The higher orders of structural vibration were also investigated here (see Figure 9). In the numerical simulation, the driving frequencies of two high orders were found to be 393 and 563 kHz. The microparticles will accumulate at the pressure nodes (between the acoustic peaks). At 393 kHz, there are 4 symmetric beam patterns distributed evenly in the polar coordinate of the average acoustic field and subsequently four accumulation regions in the cross-section of the glass tube. In comparison, there are six symmetric acoustic beams at 563 kHz. Microparticle accumulation at the center is more significant than that at 393 kHz. The resonant frequencies of high orders found in the experiment were 385 kHz and 657 kHz, which are slightly different from the simulation as the fundamental mode. The patterns of accumulated microparticles in the cross-section at these two frequencies were found similar to the simulation. There were two accumulated streamlines along the glass tube (~ 1.8 mm away from each other with the microparticle accumulation width of 0.70 ± 0.24 mm) although not very straight at 385 kHz. In comparison, three main streamlines were observed (one at the center with the width of 0.25 ± 0.02 mm, and the other two ~ 1.3 mm away from the center with the width of 0.46 ± 0.12 mm) at 657 kHz. The accumulation time is 24.67 ± 4.2 s and 6.88 ± 1.54 s at 385 kHz and 657 kHz, respectively, with the statistical difference ($p < 0.05$). After the printing, the histogram of microparticle distribution could be fitted by different Gaussian curves at the accumulation positions. Accumulation widths were 1.08 ± 0.34 mm at 385 kHz, and 0.70 ± 0.21 mm (at the side streamlines) and 0.37 ± 0.09 mm (at the central streamline) at 657 kHz.

Figure 9 Comparison of the (a) simulated acoustic pressure field in kPa, and (b) location of accumulated microparticles in the cross-section at 393 kHz (left column) and 563 kHz (right column), representative photos of accumulated microparticles (c) in the cross-section with the scale bar of 2 mm, (d) along the glass tube with the scale bar of 2 mm, (e) in the printing structure with the scale bar of 1 mm, (f) the histogram and fitted Gaussian curves for each accumulation lines under the acoustic excitation at 385 kHz (left column) and 657 kHz (right column).

Cell accumulation

The proposed technology was further evaluated using the bioink. Two types of biological cells (L929 and PC-12) were suspended in hydrogel (sodium alginate and GelMA, see Figure 10). With the acoustic excitation during the printing, most of the cells were accumulated at the center of the printing construct. In comparison, the accumulation width seems larger than that of the microparticles, which may be due to the low stiffness of biological cells and resultant weak acoustic radiation force applied to them. In addition, cells are also found to form clumps easily because of the interaction between them.

Figure 10 Comparison of the printed L929 in the (a) 2% and (b) 4% sodium alginate, and (c) PC-12 in the 10% GelMA without (left column) and with (right column) the acoustic excitation, scale bar presenting 50 μm .

(b)

(c)

Discussion

The performance of proposed acoustic excitation in assisting the printing process was evaluated both numerically and experimentally in this study. It is found that the structural vibration produced by a piezoceramic plate simply attached to the cylindrical glass tube at a specific frequency could generate the pressure node(s) in the cross-section to accumulate the microparticles. Such capability of microparticle accumulation could not only enhance the printing functionality but also reduce the risk of clogging. The motion of microparticles is also dependent on the hydrodynamic properties of streamlines, such as the concentrations of microparticles and sodium alginate. Moreover, the proposed method can accumulate the cells successfully and has the potential in biological applications. It is noteworthy that this acoustic method is non-invasive and has low heat accumulation, 24-26°C over 10-15 minutes of acoustic excitation at room temperature around 24°C, which may pave the way to the use of temperature-sensitive biological samples in maintaining their morphologies and viabilities. In the near future, the investigation of the motion of cells, their distribution in the printing structure, viability, and proliferation after the printing will be carried out. Although 2D construct was printed and evaluated in this study, the formation of a more complicated object is possible using the 3D bio-printer (see Figure A1). The cell proliferation in the development and differentiation in the 3D printed structure, as well as the functionalities of the constructed tissue sample will be investigated later. The cell density and spatial distribution are critical to

the morphogenetic development of engineered tissue, including proliferation, differentiation, and migration (Li et al. 2001). Because of the smaller size of mammalian cells (e.g., 15-30 μm for Hela) and lower stiffness in comparison to the microparticles used here (e.g., ~ 120 kPa for Hela and ~ 3 GPa for polystyrene) much slower motion speed is expected. In addition, the optimum viscosity of bio-ink should be explored, hindering the motion of biological cells at the high medium viscosity while spreading abundantly at the low medium viscosity (Šikalo et al. 2005, Yarin 2006). The printability of hydrogels mainly depends on extrusion force, the viscosity of hydrogel, temperature, and nozzle diameter (He et al. 2016, Kyle et al. 2017).

Our numerical simulation of the excitation frequency and the location of microparticles in the cylindrical tube agreed quite well with the experiment results. When the tube is driven for a long time or at the high power, the accumulation of microparticles will break into discrete nodes, which is mostly due to a weakly coupled standing wave along the cylinder central axis of the glass tube and the formation of vortices until the introduction of thermal convective currents and eventual fluid boiling by the heating of the transducer (Goddard 2004). The distance between these nodes along the glass tube is moderately constant (~ 8 cm) which is similar to the acoustic wavelength (8.63 cm). The formation of these nodes is affected by the tube symmetry, length, and edge conditions. For example, when an O-ring is placed at the nodal position, several neighboring nodes partially disappear. In contrast, there are no changes when placing the O-ring at the anti-nodal position.

The motion of microparticles is governed by the acoustophoretic (the acoustic radiation force given by Eq. A5) (Bruus 2012, Glynne-Jones and Hill 2013), Stokes drag (resistance of microparticles in the medium given by Eq. A4) (Batchelor 2000), and hydrodynamic forces (Bruus 2011). The acoustic method is capable of manipulating a variety of materials, such as plastic beads, biological cells (Ding et al. 2012, Xu et al. 2011), microfiber (Schwarz et al. 2015, Yamahira et al. 2000), and anisotropic objects (Lim et al. 2007). However, because of discrepancies in the material stiffness the acoustic power delivered should be adjusted for the satisfactory accumulation. At the high excitation frequency, higher acoustic radiation force could speed up the particle motion and reduce the accumulation width as shown by the high order vibration modes (Israelachvili 2015, Shi et al. 2008). However, the accumulation pattern will be more complicated and sensitive in the cross-section. For example, there are 8 symmetric acoustic beams in the cross-section polar space at 736 kHz in the simulation, and two acoustic beams at 0° and 180° in the polar coordinate reduce their magnitudes by 20% at 395 kHz (data not included). In contrast, the effect of longitudinal convection streaming and bubble cavitation becomes significant at the low excitation frequency. The medium viscosity increases with the microparticle concentration and subsequently decreases the mobility of microparticles in the fluid (Ladd 1990). In the highly viscous medium, the increased Stokes drag force pushes the microparticles in the opposite direction of the acoustic radiation force so that the focusing of microparticles is hindered (Sriphutkiat and Zhou 2017). As a result, the accumulation is prolonged with the large accumulation width at the high concentrations of alginate and microparticle (Ley and Bruus 2016). However, the microparticle distribution in the glass tube is not exactly same as that in the printing structure, which is due to several factors, such as the size and shape of the nozzle tip, extrusion pressure, scanning speed of the printer, and the

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medium viscosity of the ink. Thus, extensive work is required to achieve the desired microparticle accumulation width in the printing structure at each experimental condition. The design of the nozzle tip could be optimized. A short convergent constriction bends fluid streamline inward suddenly, which might shift the microparticle accumulation toward the center. This streamline bending effect is subsided by using a symmetric long convergence constriction and orifice slightly smaller than the tube (Chow and Soda 1972).

To improve the focusing efficiency and reduce the accumulation time, several strategies are suggested. Firstly, the higher excitation power applied to the piezoceramic is straightforward to increase the acoustic pressure and subsequently acoustic radiation force to the microparticles. But the risk of overheating is increased without appropriate thermal diffusion or cooling. Secondly, it is to increase the energy transmission efficiency from the piezoceramic to the glass tube. Using the piezoceramic plate whose poling direction is perpendicular to the glass tube surface or new piezocomposite materials with larger mechanical quality factor and lower dissipation factors may be the solutions. Finally, other vibration modes, such as thickness, thickness shear, longitudinal, cross-section shear, and a torsional wave, will be explored (Araz 2010). By utilizing harmonic flexural vibration of the capillary, subharmonic acoustic pressure standing waves in the fluid can be generated inside the cylindrical tube. Flexural modes are the most important in terms of pressure variation inside the fluid because it enables relatively high normal velocities.

Conclusions

We demonstrated a practical application of acoustic manipulation to assist the additive manufacturing (extrusion-based printing). Structural vibration of a cylindrical tube with a tapered tip was produced, and the acoustic wave was coupled into the ink to accumulate the microparticles to the position of induced pressure node(s). The prediction of the excitation frequency and location of microparticles inside the glass tube in the numerical simulation agrees quite well with the experimental results. Acoustic excitation has a statistically significant effect on the microparticle accumulation in the glass tube. The time and width of microparticle accumulation under the acoustic excitation increase with the concentration of alginate and microparticles in the ink. Although the microparticle concentration has a slightly higher effect on the accumulation width than the alginate concentration, its effect on the accumulation time is much less. In the printing structure, the distribution of microparticles could be fitted well in a Gaussian curve, whose FWHM is usually larger than that in the glass tube due to the printing process through the tapered tip. However, the dependence of microparticle accumulation in the printing structure on the microparticle and alginate concentrations is similar to that in the tube. High orders of structural vibration could not only reduce the microparticle accumulation time but also produce more complicated accumulation patterns. The performance of accumulating the L929 and PC-12 cells suspended in the hydrogel is also satisfactory. Overall, this acoustic technology excitation could improve the patterning of microparticles in the AM and may be applied in the future 3D bioprinting.

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Appendix A.

The linear behavior of the piezoelectric material is presented in the stress-charge and strain-charge forms

$$T = c_E S - e^T E, \quad D = eS + \varepsilon_S E \quad (A1)$$

$$S = s_E T - d^T E, \quad D = dT + \varepsilon_T E \quad (A2)$$

where T is the stress, S is the strain, E is the electric field, D is the electric displacement, c_E is the elasticity matrix, e is the coupling matrix, ε_S is the permittivity matrix. Then the propagation of an acoustic wave in the liquid is expressed using the Helmholtz equation,

$$\nabla \cdot \left(-\frac{1}{\rho} \nabla p \right) - \frac{\omega^2 p}{\rho c^2} = 0 \quad (A3)$$

where the acoustic pressure (p) is a harmonic quantity ($p = p_0 e^{i\omega t}$), ρ_c is the density, ω is the angular frequency, and c is the speed of sound in the fluid.

Because of the different traveling velocities of microparticle and fluid, the Stokes drag force from the fluid acting on the microparticles is commonly described as (Glynne-Jones and Hill 2013)

$$F_{Drag} = 6\pi\mu r (v_{fluid} - v_{particle}) \quad (A4)$$

where r , v_{medium} , and $v_{particle}$ refer to the radius of the microparticle, the velocity of the fluid and the microparticle, respectively.

In the acoustic field, monopole and dipole scattering from oscillation and pulsation of the microparticle result in the acoustic radiation force that is described using the Gauss's theorem (Bruus 2012).

$$F^{rad} = \frac{4}{3}\pi r^3 \nabla \left[f_{mono} \frac{1}{2} k_0 p_{prop}^2 - f_{dip} \frac{3}{4} \rho_0 v_{prop}^2 \right] \quad (A5)$$

$$f_{mono} = 1 - \frac{k_p}{k_f}, \quad f_{dip} = \frac{\rho_p - \rho_f}{\rho_p + \rho_f/2} \quad (A6)$$

where ρ_p and ρ_f are the density of particle and fluid, k_p and k_f are the compressibility of particle and fluid, f_{mono} and f_{dip} are the dimensionless scattering coefficients for monopole and dipole, respectively, and k_0 is the acoustic wave number. In the viscous fluid, Prandtl–Schlichting and acoustic boundary layer could be taken into account by adding the viscosity-dependent correction into the dipole scattering coefficient (Settnes and Bruus 2012).

$$f_{dip} = \frac{2(1 - \gamma)(\rho_p - \rho_0)}{2\rho_p + \rho_0 \left(1 + \frac{9}{2} [1 + i(1 + \frac{\delta}{r})] df(\frac{\delta}{r}) \right)} \quad (A7)$$

where δ is the distance to the boundary layer, and i is the complex unit. The motion of microparticles was governed by Newton's second law.

FEM was used to find the approximate solution of partial differential equations (PDEs). The main components consist of piezoelectric material and a cylindrical glass tube filled with fluid. The electrical signal is applied to the piezoceramic plate attached to the glass tube. One side of the piezoceramic was defined as the free boundary and the other side was attached to the glass tube. The boundary of the glass tube was considered as a hard wall and assumed to be reflective

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$$\vec{n} \cdot \left(-\frac{1}{\rho} \nabla p + q \right) = 0 \tag{A8}$$

where $\vec{n}$ is the normal vector pointing inward the center of the tube. Hence the acoustic standing waves could be formed in the fluid surround by a hard wall.

Table A1. Material properties used in the numerical simulation

medium	parameter	value
water	density, ρ_w	997 kg/m ³
	speed of sound, c_w	1497 m/s
	viscosity, μ_w	0.890 mPa·s
	compressibility, κ_w	448 TPa ⁻¹
microparticle	density, ρ_w	997 kg/m ³
	speed of sound, c_w	1497 m/s
	viscosity, μ_w	0.890 mPa·s
	compressibility, κ_w	448 TPa ⁻¹
glass tube	density, ρ	7600 kg/m ³
	Young's modulus, E	70 GPa
	Poisson's ratio, ν	0.23
piezoceramic	density, ρ	7600 kg/m ³
	speed of shear wave, v_T	2005 m/s
	speed of longitudinal wave, v_L	1700 m/s
	electromechanical coupling factors, k_{33} and k_{31}	0.68 and 0.33

Figure A1. Printed 10% of GelMA in a three-dimensional structure using the acoustic nozzle from (a) isometric and (b) top views.

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