Emerging acoustic bioassembly represents an attractive strategy to build cellular closely-packed organotypic constructs in a tunable manner for biofabrication. However, simultaneously assemble heterogeneous cell types into heterocellular functional units with spatially-defined cell arrangements, such as complementary and sandwich cytoarchitectures, remains a long-lasting challenge. To overcome this challenge, herein we present an acoustic differential bioassembly technique to assemble different cell types at the distinct positions of the acoustic field based on their inherent physical characteristics including cellular size and buoyant density. Specifically, different cell types can be differentially assembled beneath the nodal or the antinode regions of the Faraday wave to form complementary cytoarchitectures, or be selectively positioned at the center or edge area beneath either the nodal or the antinode regions of the Faraday wave to form complementary cytoarchitectures, or be selectively positioned at the center or edge area beneath either the nodal or the antinode regions of the Faraday wave to form sandwich cytoarchitectures. Using this technique, we assemble human induced pluripotent stem cell-derived liver spheroids and endothelial cells into hexagonal cytoarchitectures in vitro to mimic the cord and sinusoid structures in the hepatic lobules. This hepatic lobule model reconstitutes liver metabolic and synthetic functions, such as albumin secretion and urea production. Overall, the acoustic differential bioassembly technique facilitates the construction of human relevant in vitro organotypic models with spatially-defined heterocellular architectures, and can potentially find wide applications in tissue engineering and regenerative medicine.

1. Introduction

In nature, tissue-specific heterocellular units that can fully implement basic functions of corresponding tissues and organs are a ubiquitous biological phenomenon existing in a wide range of species not limited to human [1]. These heterocellular units are commonly formed by the self-organization of tissue-specific parenchymal cells and non-parenchymal cells into highly-ordered and closely-packed heterocellular architectures, which are essential to determine tissue’s and organ’s functions [2, 3]. For example, the hepatic lobule is a hexagon-shaped functional unit of the liver and consisting of hepatocytes and endothelial cells closely packed into hepatic cords and sinusoids radi ally arranged around a central vein [4]. The hepatic lobules implement most of the liver’s essential functions like carbohydrate metabolism, urea production, and albumin secretion. Thus, the ability to recapitulate the hepatic lobule with biomimetic heterocellular architecture is critical to enhancing hepatic functions in liver tissue engineering.

For a long time, a pivotal task of biofabrication is to generate biological products with tissue- and
organ-specific functions [5–7]. To fulfill this task, bioprinting or bioassembly are developed as two separate strategies for biofabrication [8–10]. However, it remains an unmet demand to faithfully reconstruct heterocellular tissue functional units. Conventional extrusion and inkjet bioprinting enable to deposit cells in a spatially-controlled manner, but these techniques generally cannot further precisely arrange living cells within the printed constructs to form closely-packed heterocellular architectures [11]. Recent emerging single-cell printing [12], and spheroid printing [13–15] techniques enable to precisely position single cell and cell spheroid by directly bioprinting in suspension baths. In addition to bioprinting, bioassembly has emerged as an increasingly-accepted technical alternative that has the advantage of constructing spatially-defined cytoarchitectures with physiologically relevant cell densities [16]. Up to now, a number of bioassembly techniques have been demonstrated to create tissue- and organ-mimics by exploring the interactions between physical fields and living cells [17], including gravitational field [18], magnetic field [19, 20], electric field [21], and acoustic field [22, 23]. Among these bioassembly techniques, acoustic bioassembly represents a promising approach that is increasingly explored to construct diverse cytoarchitectures since the acoustic field can be more flexibly tuned in a spatially-controlled manner compared to other fields [24].

Currently, three types of acoustic techniques have been explored for bioassembly including surface acoustic wave [25, 26], bulk acoustic wave [27], and Faraday wave [28–30]. These acoustic bioassembly techniques have been demonstrated to construct diverse types and shapes of tissue constructs including stripe-shaped cardiac tissues [31], multilayered neural tissues [32], and ring-shaped vascular networks [33, 34]. Especially, the acoustic holography technique opens the possibility for customized arbitrary pattern formation [35–37]. However, few of these techniques enable to simultaneously assemble heterogeneous cell types into heterocellular units with spatially-defined cell arrangements.

To address this challenge, herein we present an acoustic differential bioassembly technique based on Faraday wave to perform a precisely differential arrangement of heterogeneous cells in a spatially-defined cytoarchitecture. The technique enables to construct heterocellular architectures with complementary and/or sandwich cellular arrangements based on the differences in cellular size and buoyant density. We first exploit a mathematical physical model to analyze the force bearing of building blocks (e.g. microspheres, cell spheroids, cell-encapsulating microgels) in the acoustic field and the force potential distribution derived from the interplays between the acoustic field and the building blocks. Based on the theoretical model, we conduct finite element numerical simulation to predict the spatial distributions of building blocks in the acoustic field. Experimentally, building blocks with varied sizes are differentially located at distinct positions either beneath the nodal or the antinode regions of the Faraday wave. In addition, the building blocks can also be differentially positioned at the center or the edge area beneath either the node or the antinode by varying their densities. Using this technique, we can differentially assemble tissue-specific parenchymal cells and non-parenchymal cells into highly-ordered and closely-packed heterocellular architectures, thus reconstituting tissue-specific biological functions.

2. Materials and methods

2.1. Finite element analysis of Faraday wave

Finite element analysis was conducted to characterize the assembly of polystyrene (PS) microspheres under Faraday wave. The Faraday wave was caused by body force \( F \) and generated at the gas–liquid interface with a fixed boundary in a circular chamber. The body force \( F \) was generated by a sinusoidal acceleration signal applied to the exciter, which can be written as:

\[
F = a \ast \rho \quad (1)
\]

\[
a = a_{\text{max}} \ast \cos(4\pi ft) \quad (2)
\]

In which, \( \rho \) is the density of the fluid, \( a_{\text{max}} \) is the maximum acceleration applied to the vertical vibration exciter, and \( f \) is the working frequency of the vertical vibration exciter, respectively.

The Faraday wave was simulated by applying this external force when solving the governing equation of the dynamic flow motion. The governing equation refers to the Navier–Stokes equation, which can be written as [38]:

\[
\rho \frac{\partial V}{\partial t} = F + \rho g - \nabla p + \mu \nabla^2 V. \qquad (3)
\]

In which, \( V \) is the velocity vector of the flow field, \( F \) is the body force in equation (1), \( \mu \) is the dynamic viscosity of the fluid, and \( g \) is the acceleration of gravity, respectively.

For a microsphere at the bottom of the assembly chamber under Faraday wave, the force potential on the microsphere contains a positive contribution from the pressure of the exciter and a negative contribution from the flow field [39]. The pressure of driven fluid in the assembly chamber can be calculated as time-average forces acting on the microsphere divided by area. The flow rate can be calculated by the finite element analysis through equation (3). The force potential \( U \) can be described as follows:

\[
U = \frac{4}{3} \pi r^3 \left[ \frac{k_0}{2} \left( \frac{E_0}{S} \right)^2 - A_2 \frac{3\phi_0}{4} \langle V^2 \rangle \right]. \quad (4)
\]
In which, \( r \) is the radius of the microsphere, \( A_1 \) and \( A_2 \) are dimensionless coefficients, \( k_0 \) is an explicit expression for the compressibility, \( F_0 \) is the force generated by the exciter acting on the microsphere, the \( S \) is the projection of microsphere in the direction of force, \( \langle (\frac{F_0}{S})^2 \rangle \) and \( \langle V^2 \rangle \) are time average over a full oscillation period of a quantity.

All the simulations were performed in COMSOL Multiphysics. To reduce computational cost and built the mathematical physic model correctly, we simplified the experiment setup. ‘Laminar Flow’ physics were used to solve the governing equations of dynamic fluid motion, and the deformation of fluid motion was handled by adding a ‘dynamic mesh’ condition. The ‘volume force’ condition was applied to the 3D calculation domain of ‘Laminar Flow’ physics. The boundary conditions on the gas–liquid interface and solid–liquid interfaces were set to ‘free surface’ (surface tension of gas–liquid interface \( \sigma \)) and ‘no slip’, respectively. The problem was solved via a ‘Time-Dependent’ solver. The parameters of materials used in the calculations are given in table 1.

### 2.2. Experimental setup

The experimental apparatus for Faraday wave bioassembly was illustrated in figure S1(A). Both assembly chambers had 20 mm in diameter and 1.5 mm in height. The assembly chambers were fabricated from polymethylmethacrylate (PMMA), white paper, and double-sided adhesive (DSA) by fabricating from polymethylmethacrylate (PMMA), white paper, and double-sided adhesive (DSA) by using a water-cooling CO\(_2\) laser engraving system (350–50 W, Longtai laser, Liaocheng, China). The chamber was connected with the vertical vibration exciter (SA-JZ005T, Shiao, Wuxi, China) via a screw. The level of the assembly chamber was adjusted by using a bubble level. The vertical vibration exciter was electrically driven by an arbitrary function generator (AFG3052C, Tektronix, OR, USA) and a power amplifier (DTA-120, Dayton Audio, OH, USA). The PS and stainless-steel microspheres bought from Cospheric (CA, USA) were used in the assembly experiments.

### 2.3. Aggregation index calculation

First, the gray value of all pixels in an assembly pattern was measured by ImageJ software (NIH, Bethesda, MD, USA). Next, the variance in the gray value was calculated using the variance of an entire population function. The variance was normalized and defined as aggregation index.

### 2.4. Cell culture

Green fluorescent protein (GFP)-transfected human umbilical vein endothelial cells (HUVECs) were cultured in endothelial cell medium (ECM) (SciCell, CA, USA) supplement with 5% (v/v) fetal bovine serum, 1% (v/v) endothelial cell growth supplement, and 1% (v/v) penicillin/streptomycin. After confluence, HUVECs were digested with 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) (Gibco, CA, USA), and used for the fabrication of cell containing building blocks.

### 2.5. HUVEC-encapsulating microgels fabrication

The 10\(^6\) HUVECs were resuspended in 50 \( \mu l \) 6% porous GelMA (EFL, Suzhou, China). The circular droplets were fabricated by using a 2.5 \( \mu l \) pipette gun. Each droplet contained 0.8 \( \mu l \) GelMA. The immobilization of the circular droplets was accomplished by treating the GelMA hydrogel under a 405 nm light source for 15 s. Then, formed HUVECs-encapsulating microgels were collected using a tweezer and utilized in the Faraday wave bioassembly.

### 2.6. Human induced pluripotent stem cell (iPSC)-derived liver spheroids fabrication

HiPSCs were maintained in NuwacellTM nTarget hPSC Medium (Nuwacell Biotechnologies, RP01020, Hefei, China), passaged via StemPro \(^\oplus\) Accutase\(^\circledast\) (Gibco, A1110501, CA, USA) detachment, and reseeded in the Vitronectin (Gibco, A31804, CA, USA)-coated 6-well plate filled with NuwacellTM nTarget hPSC Medium containing 10 \( \mu M \) Y-27632 (STEMCELL Technologies, 72304, BC, USA).

The hiPSCs were differentiated into foregut using a previously described method with minor modifications. The foregut cells were generated on day 6 and then detached into single cells by Accutase. The foregut cells were seeded in the agarose-based hexagonal-closely-packed microcavity arrays with each microcavity containing 200 cells. The seeded foregut cells were cultured in the liver spheroid formation medium supplemented with 10 \( \mu M \) Y27632 for the first 2 d and refreshed with the spheroid formation media for other 2 d. After the spheroid formation, the culture media was switched to the liver specification medium for 4 d. After the liver specification, the culture media was changed to the liver maturation medium for 14 d. Spheroid induction and culture were maintained at 37 \(^\circ\)C with 5% \( \text{CO}_2\).

### 2.7. Construction of hepatic lobule-like liver model

HiPSC-derived liver spheroids fabrication (\(~\sim1300\)) and HUVEC-encapsulating microgels (\(~\sim 50\)) were resuspended in 10 mg ml\(^{-1}\) fibrinogen (Sigma, MO, USA). The cell containing building blocks were loaded on cover glass (24 \( \times \) 24 mm) fixed at the
bottom of the assembly chamber by using DSA. After the sedimentation of cell containing building blocks, Faraday wave was applied with a driving frequency of 56 Hz and an amplitude of 120 mVpp, resulting in petal-shaped pattern formation similar in shape to the hepatic lobule. The immobilization of the assembled cytoarchitecture was accomplished by adding thrombin (12.5 UN ml\(^{-1}\)) for the crosslinking of fibrinogen.

### 2.8. Tissue culture

Assembled hepatic lobule-like liver models were cultured at 5% CO\(_2\) and 37 °C in a humified incubator in a 1:1 mixture of hepatocyte growth medium (HCM) and ECM. The six-well microtiter plate was placed on an orbital shaker at the speed of 75 rpm for dynamic culture of hepatic lobule-like liver models.

### 2.9. Cell viability assay

The cell viability was examined by using Hoechst 33342/propidium iodide (PI) staining. Tissue constructs were washed with 1X phosphate buffered saline (PBS) 3 times and incubated for 30 min at 37 °C in 1 ml staining solution containing 0.1% (v/v) Hoechst 33342 solution (Cell Signaling Technology, MA, USA) and 0.1% (v/v) PI solution (Beyotime Biotechnology, Shanghai, China). After that, tissue constructs were imaged under an inverted fluorescence microscope (IX83, Olympus, Japan) with a 10× objective. The percentage of area positively stained for PI was determined using ImageJ software (NIH, Bethesda, MD, USA) and was relative to the total area positively stained for Hoechst 33342.

### 2.10. Immunostaining and imaging

For in-situ immunostaining, assembled hepatic lobule-like liver models were fixed with 4% paraformaldehyde (Sigma, MO, USA) overnight at 4 °C and washed with 1X PBS supplemented with 0.5% Triton X-100 (Sinopharm, Beijing, China) three times. The models were blocked with 1% bovine serum albumin (BSA) (Sigma, MO, USA) supplemented with 0.1% saponin (Sigma, MO, USA) overnight at 4 °C. The models were stained overnight at 4 °C for 1:250 primary antibody, mouse anti-human hepatocyte nuclear factor 4 alpha (HNF-4α) (Santa Cruz Biotechnology, TX, USA). The next day, hepatic lobule-like liver models were washed with 1X PBS supplemented with 0.5% Triton X-100 3 times on a rocker and then incubated for 2 h at room temperature in the dark with 1:200 secondary antibody. The secondary antibody was Alexa Fluor 568 donkey anti-goat (Invitrogen, CA, USA), Alexa Fluor 568 goat anti-mouse (Invitrogen, CA, USA). After that, the sections were washed with 1X PBS three times on a rocker, and nuclei were stained with 1:1000 Dapi solution. The sections were imaged under an inverted fluorescence microscope with a 40× objective.

### 2.11. Period acid-Schiff (PAS) and Nile red staining

To access glycogen storage, we used PAS (Leagene, Beijing, China) staining. Nile red (Sigma, MO, USA) staining was performed to access lipid accumulation. PAS- and Nile red-stained images were captured under a light microscope (ICX41, Shunyu, China) or a laser scanning confocal microscopy (SP8 STED, Leica, Germany).

### 2.12. Human albumin enzyme-linked immunosorbent assay (ELISA)

To determine the secretion of albumin, supernatants of tissue culture were collected after 2, 4, and 6 d of culture. Levels of human albumin were measured by the human albumin ELISA quantitation set (Bethyl Laboratory, TX, USA) according to the manufacturer’s instructions.

### 2.13. Urea production assay

Supernatants of tissue culture were collected after 2, 4, and 6 d of culture and used to determine the urea production of hepatic lobule models. The concentration of urea was measured by the urea assay kit (BioAssay Systems, CA, USA) according to the manufacturer’s instructions.

### 2.14. Data analysis

The data were plotted by using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) software. All data are presented as mean ± SD values and were analyzed by SPSS Statistics 21 software. Differences were considered significant at *P < 0.05, **P < 0.01, and ***P < 0.001.
Figure 1. Differential bioassembly of different PS microspheres. (A) Scheme of acoustic differential bioassembly of three types of PS microspheres with different sizes and densities. (B) Acoustic differential bioassembly process of three types of PS microspheres to form a petal-shaped pattern. (C) Time evolution of the aggregation index during the bioassembly process. Vibration is applied at time zero. (D) Simulation of the gas–liquid interface deformation driven by Faraday wave. The color bar indicates the relative amplitude of the deformation. (E) The nodal and antinode regions of the Faraday wave. Scale bars, 2 mm.

3. Results

3.1. Differential bioassembly of three types of microspheres

The experimental setup majorly includes an arbitrary function generator, a power amplifier, a vertical vibration exciter, and an assembly chamber (figure S1). By increasing the driving frequency from 15 Hz to 100 Hz, we obtained a series of nodal patterns determined by the mode number pair \((m, n)\) of Faraday wave, where \(m\) presents the number of nodal diameters and \(n\) presents the number of nodal circles (figure S2(A)) \[40, 41\]. Using the mathematical physical model, we numerically simulated the force potential distribution as well as the streamlines of the flow field, which can be used to accurately predict the aggregation regions of PS microspheres (figures S2(B) and (C)). Furthermore, we selected an assembly chamber with 1.5 mm in height and 30 mg ml\(^{-1}\) PS microspheres for Faraday wave-based differential bioassembly, under which conditions the closely-packed and boundary clearly-separated assembly patterns were obtained (figures S3 and S4).

We simultaneously assembled three types of PS microspheres with different sizes and densities. Faraday wave was applied after the PS microspheres gravitationally settled down and randomly distributed at the bottom of the assembly chamber. The PS microspheres were driven to the regions with the lowest force potential which was determined by both the size and the density of the microspheres. Finally, the PS microspheres were assembled to form the predefined petal-shaped pattern (figure 1(A)). The dynamic process of the bioassembly could be divided into three different stages, namely pre-assembly stage, assembly stage, and stabilization stage. Correspondingly, the aggregation index of PS microspheres rapidly increased in the assembly stage from the
baseline to the plateau, and then reached the stabilization stage. A typical example of the acoustic bioassembly process took around 4 s (figures 1(B) and (C)) and was shown in video S1. The formed petal-shaped pattern was consistent with the deformation of the gas–liquid interface (figure 1(D)). We found that PS microspheres with blue and green colors were assembled beneath the nodal regions of the Faraday waves, while PS microspheres with red color were assembled beneath the antinode regions, thus resulting in the formation of a spatially-defined complementary pattern. Further, we found that the PS microspheres with blue color were surrounded by the PS microspheres with green color, resulting in the formation of a sandwich pattern (figures 1(E) and S5).

### 3.2. Differential bioassembly based on size effect

To elucidate the size effect on the acoustic bioassembly, we used five kinds of PS microspheres with a diameter ranging from 50 µm to 1000 µm (figure 2(A)). For PS microspheres with a diameter less than 500 µm (50, 100, and 200 µm), the numerical simulation of the force potential distribution...
indicated that the lowest force potential existed beneath the nodal regions (figure 2(B)), resulting in the formation of nodal assembly patterns (figures 2(C) and S6(A)). For PS microspheres with a diameter equal to or larger than 500 \( \mu m \) (500, 1000 \( \mu m \)), the antinode regions had the lowest force potential (figure 2(D)), resulting in the formation of antinode assembly patterns (figures 2(E) and S6(B)). The coverage rate for both nodal patterns and antinode patterns are nearly 50% and 20%, indicating PS microspheres with different diameters were accurately aggregated at the corresponding positions with the lowest force potential (figure 2(F)). By introducing two kinds of PS microspheres with complementary distributions of the lowest force potential, complementary assembly patterns were obtained (figures 2(G) and S6(C)). Specifically, 100 \( \mu m \) PS microspheres and 500 \( \mu m \) PS microspheres were differentially located beneath the nodal regions and the antinode regions respectively, resulting in the formation of the complementary petal-shaped pattern (figure 2(H)). In addition, we simultaneously assembled other two kinds of PS microspheres (500 \( \mu m \) PS microspheres and 1000 \( \mu m \) PS microspheres). Although both PS microspheres were located beneath the antinode regions, the microspheres with smaller sizes arranged around the microspheres with larger sizes, resulting in the sandwich patterns (figures 2(I) and S6(D)). In these sandwich patterns, 1000 \( \mu m \) PS microspheres and 500 \( \mu m \) PS microspheres were positioned at the center and the edge area, respectively (figure 2(J)). According to equation (4), PS microspheres with larger sizes will be preferentially arranged at the center area beneath the nodal or antinode regions due to larger force potential difference (figure S6(E)). The phenomenon that building blocks are differentially assembled to form complementary and/or sandwich patterns based on their sizes is defined as the ‘size effect’.

### 3.3. Differential bioassembly based on density effect

Furthermore, we investigated the density effect on the acoustic bioassembly using density-varied microspheres with diameters of 100 \( \mu m \) (figure 3(A)). We found that the microspheres with varied densities were all assembled beneath the nodal regions (figures 3(B) and S7(A)). We simultaneously assembled two types of PS microspheres with different densities (1.03 g cm\(^{-3}\) and 1.13 g cm\(^{-3}\), 1.13 g cm\(^{-3}\) and 1.20 g cm\(^{-3}\)). We found that PS microspheres with higher densities were assembled at the center area of the nodal regions, and the microspheres with lower densities were arranged at the edges of the nodal regions, resulting in the generation of sandwich patterns (figures 3(C) and S7(B)). The quantitative analysis of PS microsphere distribution also indicated that microspheres with lower densities were arranged around the microspheres with higher densities (figure 3(C)). Compared with PS microspheres with lower densities, PS microspheres with higher densities will be preferentially arranged at the center area beneath the nodal regions due to larger force potential difference (figure 3(D)). The phenomenon that building blocks are differentially assembled to form sandwich patterns based on their densities is defined as the ‘density effect’.

### 3.4. Construction of hepatic lobule-like liver model using acoustic differential bioassembly

In the liver, the hepatic lobule is the basic functional unit that consists of radially arranged hepatic cords and sinusoids around a central vein to form a hexagon-shaped heterocellular architecture (figure 4(A)). To construct a hepatic lobule-like liver model, we differentially assembled liver spheroids and HUVEC-encapsulating microgels into petal-shaped heterocellular architectures and cultured them in vitro (figure 4(B)). In the fabrication of cell-containing building blocks, the liver spheroids were generated from hiPSCs using an ultra-low adhesion agarose microwater array (figure S8(A)). Besides, GFP-transfected HUVECs were uniformly dispersed inside homogeneous GelMA microspheres (figure S8(B)). The average size of the liver spheroids and HUVEC-encapsulating microgels were 283 \( \mu m \) and 1340 \( \mu m \), respectively (figures S8(C)–(E)). We evaluated the biocompatibility of the Faraday wave-based differential bioassembly technique by cell viability assays. Dead cells were stained with PI in red fluorescence, and the total nucleus was stained with Hoechst 33342 in blue fluorescence (figure S9(A)). The cell viability of liver spheroid and HUVEC was larger than 97% and 81%, respectively. Cell viability before and after the bioassembly did not show significant differences, indicating that the cell viability was not affected by the bioassembly process and during the subsequent culture (figure S9(B)). We first separately assembled liver spheroids or HUVEC-encapsulating microgels into petal-shaped cytoarchitectures, in which the liver spheroids were distributed beneath the nodal regions (figure 4(C)), and the HUVEC-encapsulating microgels were assembled beneath the antinode regions (figure 4(D)). We further differentially assembled liver spheroids and HUVEC-encapsulating microgels into complementary petal-shaped cytoarchitecture, which not only mimicked native hepatic lobule structure but also potentially enabled direct contacts between hepatic parenchymal cells and supporting cells (figures 4(E) and S10(A)). The bioassembly process was shown in video S2. As soon as the bioassembly was accomplished, cells individually located in their respective regions without cell-cell interactions. During in vitro culture, the liver spheroids gradually fused over time based on the interplays among the spheroids, and between spheroids and their extracellular matrix [42]. The whole heterocellular architecture
became an integrated construct without losing the intrinsic patterns designed for different cell types (figure S10(B)).

Furthermore, we investigated liver-specific functions of the assembled hepatic lobule-like liver models (figure 5(A)). PAS staining and Nile red staining assays indicated that models developed liver-specific functions, including glycogen storage and lipid accumulation (figures 5(B) and (C)). In addition, we performed an immunofluorescence analysis of the hepatic lobule-like liver models on day 7 to investigate the protein expression of liver-specific markers. The results indicated that the models were positive for both albumin and HNF-4α (figures 5(D) and (E)). In non-assembled models in which the two types of cell-containing building blocks were randomly arranged, some of the cells were positive for albumin and HNF-4α while more non-parenchymal cells generated and existed outside the models expressing vimentin (VIM, a mesenchymal marker). The results revealed that these non-assembled models may gradually lose liver functions (figure S11). To further quantitatively assess the liver metabolic and synthetic functions of the hepatic lobule-like liver models, we examined albumin secretion and urea production over an extended period. The albumin and urea levels in the assembled hepatic lobule-like liver models were relatively higher than in the non-assembled models. Although the albumin level of the hepatic lobule-like liver models slightly decreased on day 4, both albumin secretion and urea production reached the highest levels on day 6 at the concentration of 130.9 ng ml\(^{-1}\) albumin and 4.8 mg dl\(^{-1}\) urea in the supernatant, which indicated that the

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**Figure 3.** Acoustic differential bioassembly based on density effect. (A) Scheme of acoustic differential bioassembly of PS microspheres based on density effect. (B) Bioassembly of a single type of PS microspheres with five different densities beneath the nodal regions of the Faraday wave. (C) Bioassembly of two kinds of PS microspheres with different densities and quantitative comparison of their distributions (Group 1: 1.03 g cm\(^{-3}\) and 1.13 g cm\(^{-3}\), Group 2: 1.13 g cm\(^{-3}\) and 1.20 g cm\(^{-3}\)). (D) The relationship between the difference in force potential and building block density. All scale bars indicate 2 mm.
Figure 4. Construction of a hepatic lobule-like liver model using acoustic differential bioassembly. (A) The cytoarchitecture of a hepatic lobule. (B) Scheme of construction of a hepatic lobule model by using acoustic differential bioassembly. (C) Liver spheroid nodal pattern corresponding to hepatic plate. (D) HUVEC-encapsulating microgel antinode pattern corresponding to hepatic sinusoid. (E) Fluorescent images of hepatic lobule models on day 0 and day 7. Scale bars: (C) and (D) 1, 2 mm, (E) 2 mm.
hepatic lobule-like liver models could reconstitute some critical liver-specific functions (figures 5(E) and (F)).

4. Discussion and conclusion

One major task for biofabrication is to fabricate tissue- and organ-specific functional units that are composed of heterocellular architectures. Acoustic bioassembly, as a direct cell manipulation technique that employs particle-wave interactions, has been regarded as an emerging biofabrication technique for bioengineering tissues with native cell proximity [43]. Specifically, Faraday wave-based bioassembly has been reported to efficiently assemble cells or cell spheroids into diverse cytoarchitectures in a reconfigurable way [30]. Although co-assembly of fibroblast and HUVEC spheroids has been demonstrated at the nodal regions of the acoustic field, differential assembly of different cell types in a cell-specific spatially-controlled manner has never been demonstrated [44]. In this study, we present an acoustic differential bioassembly technique to address this long-lasting challenge. Specifically, our differential bioassembly employed size and/or buoyant density dependent interactions between Faraday wave and cell-containing building blocks to spatially define cellular position. Based on this principle, we developed two types of differential assembly strategies: (a) differential assembly of cellular blocks beneath the nodal and antinode of Faraday waves to form complementary cytoarchitectures; (b) differential assembly of cellular blocks at the center and edge beneath the nodal or the antinode of Faraday waves to form sandwich cytoarchitectures. Our Faraday wave-based differential bioassembly technique demonstrates...
unique advantages over other bioassembly techniques. Although bioprinting techniques, such as extrusion and digital light processing (DLP)-based bioprinting [45], enable to generate heterocellular architecture with spatially-defined cell types, the printing process needs to alternate printheads or cell cartridges. Recent developed coaxial extrusion bioprinters overcome this limit and enable to simultaneously print two cell types in a spatially-defined way [46–48]. Compared to these bioprinting techniques, our acoustic bioassembly technique allows simultaneous assembly of different cell types into a heterocellular construct with spatially-defined cell types and human physiologically-relevant cell density. The assembly time only takes a few seconds and is irrelevant to the complexity of the formed structure, as it is majorly determined only by the intrinsic property of the physical process. Conventional bulk acoustic or surface acoustic based bioassembly also permit a fast assembly time and closely-packed cellular structure. Nevertheless, our acoustic bioassembly technique uniquely permits high-throughput parallelization without adding extra vibrational exciters/transducers [28].

As a proof-of-concept work, we differentially assemble hiPSC-derived liver spheroids and endothelial cells to form complementary heterocellular architecture, which emulates the native structure of the hepatic lobule. This hepatic lobule-like liver model presents several liver-specific functions, including lipid storage, glycogen accumulation, albumin secretion, and urea production. However, this model has some limitations in faithfully mimicking native hepatic lobules. First, the size of this hepatic lobule-like liver model is much larger than native hepatic lobules. Second, this model lacks perfusible vascularization. As the characteristic length of the assembly is determined by the half-wavelength of the Faraday wave, we expect the size of this model can be shrunk by employing a high-frequency vibrational exciter. Additionally, we believe perfusible central and portal veins of hepatic lobule can be obtained by introducing microfluidic technology [49].

In summary, we expect this novel Faraday wave-based differential bioassembly technique will become an important alternative tool to bioprinting for constructing human relevant in vitro organotypic models with spatially-defined heterocellular architectures, which can potentially facilitate broad applications in basic medical research, drug screening, and regenerative medicine.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest

P C is a cofounder of, and has an equity interest in: (a) Shenzhen Convergence Bio-Manufacturing Co., Ltd, a company that is developing convergence biomanufacturing technologies to enable artificial meat and regenerative medicine. U D is a founder of, and has an equity interest in: (a) DxNow Inc. a company that is developing microfluidic and imaging technologies for point-of-care diagnostic solutions, and (b) Koek Biotech, a company that is developing microfluidic and IVF technologies for clinical solutions. P C’s and U D’s interests were viewed and managed in accordance with the conflict of interest policies.

CRediT authorship contribution statement

Longjun Gu: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing—original draft, Writing—review and editing. Xiaodong Xu: Formal analysis, Investigation, Methodology, Visualization. Shanqing Jiang: Investigation, Methodology, Writing—original draft. Jibo Wang: Software, Writing—original draft. Fang Xu: Software. Han Fan: Investigation, Visualization. Jia Shang: Investigation. Kan Liu: Software. Utkan Demirci: Supervision, Validation, Writing—review and editing. Pu Chen: Conceptualization, Data curation, Formal analysis, Investigation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing—original draft, Writing—review and editing.

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