Sonolithography: In-Air Ultrasonic Particulate and Droplet Manipulation for Multiscale Surface Patterning

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Acoustic fields are increasingly being used in material handling applications for gentle, noncontact manipulation of particles in fluids. Sonolithography is based on the application of acoustic radiation forces arising from the interference of ultrasonic standing waves to direct airborne particle/droplet accumulation in defined spatial regions. This approach enables reliable and repeatable patterning of materials onto a substrate to provide spatially localized topographical or biochemical cues, structural features, or other functionalities that are relevant to biofabrication and tissue engineering applications. The technique capitalizes on inexpensive, commercially available transducers and electronics. Sonolithography is capable of rapidly patterning micrometer to millimeter scale materials onto a wide variety of substrates over a macroscale (cm²) surface area and can be used for both indirect and direct cell patterning.

Acoustophoretic techniques are increasingly recognized for their capabilities in noncontact particle manipulation. Standing acoustic pressure fields generated by one or more transducers contain regular distributions of high-pressure regions (anti-nodes) and zero-pressure regions (nodes). Relative properties of the particulate material and surrounding medium dictate the localization of the particles into defined and predictable regions within the acoustic field. Reliable and precise manipulation of populations of micrometer-to-millimeter scale particles by acoustic forces has been demonstrated for in-liquid applications including separation and filtration of contaminating species within a heterogeneous solution, concentration of cells in suspension, sorting in microfluidic devices, and spatial organization or patterning of particles in one, two, and three dimensions. In particular, acoustic patterning of materials within liquid media has been applied to alignment of conductive nanowires and reinforcement phases within a composite material, as well as directed polymerization to fabricate materials with defined microstructures.

Acoustic patterning techniques are particularly appealing for biotechnological applications due to the noncontact handling, relatively low power requirements as compared to optical tweezers, and no need for chemical or physical modifications to cells, which may be necessary in the case of magnetic manipulation techniques. Acoustic systems have been used to directly pattern cells, including aligning myoblasts to form the basis of muscle fibers, stenciling of Schwann cells to provide guides for neurite elongation, and distributing vascular cells within a construct to study microvascular formation. Alignment of cells within a bioprinted construct has also been demonstrated. These systems rely on particulates being held in a liquid medium; particle settling onto a substrate or a controlled phase transition from solid to gel maintains patterning once the field has been removed. Megahertz frequencies are required for these in-liquid systems as they lead to a wavelength in the tens to hundreds of micrometers range, and therefore in the size range of mammalian cells.

Acoustic technologies for handling particles in air typically use frequencies in the low ultrasonic range due to the rapidly increasing attenuation with increasing frequency. In-air ultrasonic standing wave devices have mainly been used for containerless processing or analysis. Acoustic aerosol concentrators have been developed, but thus far, applications of these technologies to the concentration and manipulation of aerosolized materials and their subsequent deposition onto substrates to generate functional, patterned surfaces have been limited. Patterning of solid particulates on a vibrating Chladni plate has long been recognized; this has been extended to generating patterned surfaces in liquids but relies on an accurately shaped and flexible surface.

Here, we introduce basic principles and techniques of sonolithography, an air-based acoustophoretic method for creating patterned surfaces from both aerosolized droplets and solid particulates. Prospective applications include production of patterned surfaces that can give rise to localized material properties, or convey additional functionality to a substrate, as well as generate hierarchical structuring or form the basis of composites or metamaterials. In biofabrication, precisely
positioned proteins and bioactive molecules can mimic the instructional cues of the native extracellular matrix (ECM), which provides both physical support and spatiotemporal regulation of various biophysical signals, thus influencing cellular behaviors.\textsuperscript{[24]} We apply sonolithography to biofabrication, showing its efficacy in providing defined regions for cell adhesion on a substrate, and indicating its potential use as a method for directly positioning mammalian cells. Sonolithography is also shown to pattern a variety of materials and substrates.

Sonolithography uses ultrasonic standing waves, in air, to pattern populations of liquid droplets or solid particles onto a substrate as they migrate through the acoustic field (Figure 1a). An array of standing waves was generated by arranging opposed pairs of 40 kHz ultrasonic transducers, yielding a half-wavelength distance in air, at room temperature, of 4.3 mm. An octagonal array (Figure S1, Supporting Information) was created, with four transducer pairs equally spaced around the perimeter, separated by $5\lambda$ (43 mm). Each transducer pair creates a pseudo-1D standing wave field. Increasing the number of transducer pairs increases the complexity of the resultant pattern and enables multiple patterns to be generated within a single array via activation of individual transducer pairs (Figure S2, Supporting Information).

The interference of the standing waves gives rise to time-averaged spatially consistent acoustic pressure fields with characteristic patterns. The spatial distribution of nodes and antinodes enables the manipulation of particles and droplets and their subsequent patterning. Typically, particles in the Rayleigh regime (i.e., small when compared to the wavelength) and of materials that are relatively dense with respect to the host fluid (e.g., water droplets in air) are governed by the potential theory of Gor’kov and migrate toward the nodes of acoustic pressure.\textsuperscript{[25]} The acoustic pressure field was simulated (Supporting Information)\textsuperscript{[26]} based upon the arrangement of the ultrasonic transducers (Figure 1b), which indicates the locations of the acoustic nodes and antinodes, and therefore the predicted distribution of patterned material.

Figure 1. Working principles of sonolithography. a) Schematic representation of the sonolithography process. In the case of a liquid material, droplets are generated, pass through an acoustic pressure field generated by ultrasonic standing waves, and are deposited into a pattern onto a substrate. Nodal localization is shown for larger blue particles, at the minimum amplitude points. b) The pattern can be predicted through simulation (details in the Supporting Information) of the acoustic radiation forces. Here, the simulated pressures are shown for four transducer pairs, arranged in an octagon with $5\lambda$ (43 mm) spacing between the transducers. A 25 mm $\times$ 25 mm square region of interest in the center is outlined in green, corresponding to experimental images. Zero acoustic pressure areas (nodes) are black and maximum acoustic pressure areas (antinodes) are white. c) A still from a video (Video S1, Supporting Information) of nebulized water being patterned onto water sensitive paper using the octagonal array, taken at $t = 15$ s, where the patterning has become clear. Contrast has been enhanced for ease of visualization. d) Nebulized water ($\Omega$ 1–5 $\mu$m), water dispensed from a droplet-on-demand (DOD) generator ($\Omega$ 25 $\mu$m), and colored sand ($\Omega$ 0.5–1 mm) have been patterned with the same octagonal array. Nebulized water localizes to antinodes, whereas DOD water and sand localize to nodes. For the combined water image (bottom left), both nebulized water and DOD generated water (here, $\Omega$ 80 $\mu$m) have been patterned consecutively onto the same piece of water sensitive paper. The photographs of sand (recolored to red) and nebulized water (in greyscale) have been contrast enhanced and overlaid (bottom right) to demonstrate the different physical arrangements of these particles. e) Image analysis was performed to compare the deposition patterns of the water and sand with the simulated pressures in (b). The radial average pixel intensity of greyscaled photos of the nebulized (green) and DOD water (blue) and sand (red) were plotted against the distance from the center. Pixel intensity has been normalized to the maximum intensity at the darkest regions, such that peaks correspond to the areas with greatest density of patterned material. Also shown is the simulated acoustic pressures (black dashed), where the peaks shown correspond to the antinodes, and zero values to the nodes.
Rapid patterning (<10 s) induced by the acoustic field was visualized using water in combination with water sensitive paper, which undergoes a yellow-to-blue color change when exposed to aqueous solutions (Figure 1c,d left; Figure S1 right, Supporting Information), and using colored sand (Figure 1d right; Figure S1 left, Supporting Information). A population of water droplets (\(D = 1–5 \mu m\)) was generated using the vibrating piezoelectric droplet-on-demand (DOD) generator, with nozzles of both 25 and 80 \(\mu m\) in internal diameter, was used to precisely control production of larger water droplets. Both systems were used to generate a range of droplet sizes. Droplets produced by the DOD device were patterned into concentric circles, which corresponded with the nodal regions predicted by the simulation. Colored sand particles (between \(\approx 0.5\) and 1 mm in diameter) localized to the same areas as the DOD droplets. Patterning also occurred rapidly upon introduction of smaller nebulized water droplets into the field (Video S1, Supporting Information). A color change was first observed after \(\approx 10\) s of exposure to the field, with the pattern fully visible by \(15\) s (Figure 1c). These time periods are dependent upon ambient conditions, e.g., any air flow in the room can cause droplets to move away from the field. In addition, there was a slight delay from the time the droplets landed on the water sensitive paper to when they were absorbed, giving rise to the color change. When compared to the simulation, these droplets were found to have patterned to the antinodes. A size dependence has been theoretically predicted, where smaller particles migrate to high acoustic pressure antinode regions, and larger (but still small relative to the wavelength) toward the nodes (Supporting Information).

The antinode localization of the droplets produced by the medical nebulizer, which were an order of magnitude smaller than those produced by the DOD generator, is clearly observed when both droplet generation methods were used sequentially on the same piece of water sensitive paper (Figure 1d). An overlay of images of the patterned nebulized water and sand yields a similar result. Plotting the normalized radial average pixel intensity versus the distance from the center of the patterned region confirms the antinode and node localizations (Figure 1e).

The observed node/antinode droplet discrimination indicates that the localization is a function of particle size, as predicted, rather than a material property, as water was used in both methods. While an experimental threshold for this cross-over behavior has not yet been precisely determined, theoretical analysis predicts a particle diameter of 26.6 \(\mu m\) (Equations (S2) and (S3), Supporting Information). This agrees with the order of magnitude indicated by our experimental studies. We observed that the threshold is between 5 and 25 \(\mu m\), which is of a similar scale to some mammalian cells. This also demonstrates the ability of sonolithography to pattern both solid and liquid materials over a size range of three orders of magnitude.

The gentle, noncontact manipulation of populations of particles and droplets is especially relevant for biofabrication techniques, where harsh conditions (e.g., high laser light intensity) can negatively impact the viability of cells, or even denature proteins. Sonolithography was used to pattern type I collagen onto a petri dish to generate spatially defined regions for cellular adhesion. This approach was inspired by the pioneering biofabrication strategy “cytoscritping,” and reflects a common application of soft lithography. Using the octagonal array shown in Figure S1 in the Supporting Information and medical nebulizer, a 0.2 mg mL\(^{-1}\) collagen I solution was applied to a 10 cm petri dish, made of tissue culture polystyrene (TCP). Without surface treatment, cells will adhere to TCP. To prevent nonspecific adhesion, the plates were treated with 2% bovine serum albumin prior to cell seeding, which binds to the TCP and blocks cell adhesion. Human umbilical vein endothelial cells (HUVECs), modified to stably express green fluorescent protein (GFP) for ease of visualization, were used as a model cell line. Cells were visualized using the tile-scan function of a widefield microscope to enable full imaging of the patterned area, which was \(\approx 18\) cm\(^2\). Localization of the cells to the patterned regions was maintained over the course of one week, with cells remaining viable. No cellular migration out of the patterned regions was observed (Figure 2a,b). Collagen I was nebulized without an acoustic field as a control system; cells were distributed across the surface of the dish (Figure S3, Supporting Information).

This demonstrates an ability to rapidly (<30 s) and repeatedly pattern cell-instructive molecules over cm\(^2\)-scale surface areas, while retaining regions that contain a few cells (center antinode) up to hundreds of cells (Figure 2b). Furthermore, this type of patterning can be applied to additional substrates in order to present spatially defined cell-interactive regions. By harnessing the size-based localization properties, multiple moieties could be patterned using the same array. By activating different transducer pairs in sequence, more complex patterns with multiple cell-interactive materials can be generated, providing an opportunity to potentially emulate the myriad proteins and cell-active compounds present in the native ECM.

Direct cell patterning via sonolithography was also explored. The medical nebulizer used to dispense the collagen is advantageous for the rapid patterning of sonolithography because of its ability to generate droplets covering a large surface area; however, nebulizing cells using this method would likely severely impact their viability. Accordingly, the DOD generator with the 80 \(\mu m\) nozzle was used to dispense viable cells (Figure 2c). The DOD generators used here are designed to precisely dispense single droplets, with positioning controlled by either moving the nozzle or the substrate. The combination of the droplet localization from the DOD device and the acoustic patterning resulted in multiple droplets coalescing in positions corresponding to areas on the nodal rings. This is in contrast to nebulizer’s more uniform dispersion of droplets across a larger surface area. Therefore, patterns generated with larger droplets had reduced resolution when compared to the patterns produced with nebulized droplets.

Since cells are being patterned in air with a small liquid volume (<0.3 nL per drop), dehydration and subsequent cell death is a key concern. Cells must adhere to the substrate prior to the introduction of culture media to prevent the loss of patterning. In order to facilitate accelerated cell adhesion, petri dishes were coated with type I collagen, which produced a hydrophilic surface. This caused droplets to spread on deposition, wetting into a liquid layer on the surface of the dish. It was then observed that the acoustic forces were able to deform...
this liquid layer.[31] Cells were then shown to adhere in regions corresponding to the deformed liquid layer, indicating a secondary mode of indirect patterning activity for sonolithography (Figure 2d).

Sonolithography is widely applicable to many patterned materials and substrates. Figure 3 illustrates patterning of carbon-based conductive ink, fluid from a highlighter pen, and a sucrose solution, which were all nebulized, as well as solid particulates and powders (expanded polystyrene, sand). Interestingly, a size-based separation was observed between the larger grains of sand and powdered fragments (Figure 3f). Patterning was also demonstrated on various surfaces, including plastics (tissue culture polystyrene, polymethylmethacrylate, and polycarbonate), glass, paper, Parafilm, and a calcium alginate film. Taken together, these results indicate that sonolithography has potential for use in a wide array of applications for rapid patterning of deposited material onto substrates.

Sonolithography is advantageous because of its speed, reliability, flexibility, and ease of use. Patterning takes place in less than 30 s, and because this is a field-based approach, hundreds to thousands of individual particles can be manipulated simultaneously. The patterning is repeatable, both over multiple uses with the same array, and between different arrays in the same physical arrangement (Figure S4, Supporting Information). The resultant pattern is flexible, based upon the physical positioning of the transducers, their frequency, and properties of the waves (e.g., amplitude and phase). Multiple patterns can also be made with the same system by activating different transducer pairs. An octagonal array with four transducer pairs operating in-phase can generate 15 different acoustic field arrangements, with at least one active pair (Figure S2, Supporting Information). When considering the ability to spatially segregate by size, the number of potential patterns increases to 45, when including individual patterns for both small and large particles, and patterns containing materials of both sizes (e.g., Figure 1d). For example, by activating pairs, lines can be generated at different orientations and by activating all elements in-phase, a central circular feature can be generated (Figure 1). In the horizontal plane, we produce repeating linear structures that are regularly featured.

Figure 2. Sonolithography as a tool for indirect and direct cell patterning. a) Tile-scan of GFP-HUVECs, one week after seeding onto a type I collagen-patterned substrate. b) A higher magnification of the region marked by a white box in (a) to show individual cells in the center antinode and the first antinode ring. c) A magnified image of the center node of an untreated petri dish substrate immediately after patterning GFP-HUVECs using the 80 µm DOD dispenser. Individual cells can be observed as dark spheres within the droplets; examples are indicated by red arrows. d) A tile-scan taken of cells deposited via DOD generator onto a type I collagen-coated dish, 1 d after patterning. Cell-containing droplets spread when deposited on a type I collagen-coated dish. The acoustic field deforms the surface of the liquid layer that is formed. An increase in cell density corresponds to regions in this dish where the liquid layer was deformed.
transducers enables further flexibility and user-defined con-
ments in dynamic and phased array systems, where each
transducer is controlled individually to generate real-time
changes in the acoustic field, [26b,33] could easily be adapted to
sonolithography. This could form the basis of more complex
patterns via integration with a phased array or using
acoustophoresis, there must be a difference in the
acoustic field is generated above the substrate—particle
transportable, as well as inexpensive and potentially disposable
in the case of use of contaminating materials.

Other technologies involving stereolithography and dynamic light projec-
tion modalities, such as photolithography. For example, by
print offers the potential for combination with other fabrica-
stances have been made to introduce real-time control over
these patterns via integration with a phased array or using
microbubbles in liquid,[34c,d] an air-based system based on
these ideas does not yet exist.

The frequency used in this work was 40 kHz. This yielded
feature sizes on the order of ≈100 μm over a large surface
area, enabling multiscale fabrication. Furthermore, by using
different frequency transducers, it is possible to further tune
the wavelength and feature size. For example, the wavelength
of a 25 kHz frequency transducer is ≈13.7 mm. Increasing the
frequency to 400 kHz generates wavelengths on the order of
hundreds of micrometers, approaching the scales of the MHz
water-based systems. In its current form, the system itself is
straightforward to fabricate and to use. The 40 kHz transducers
are commercially available and inexpensive, array supports can
be user-designed and 3D printed, and the wave generator is run
from a basic microcontroller board with low power (<10 W) and
voltage requirements (<20 V). Separate function generators and
amplifiers are not necessary, as with some of the liquid-based
systems. The device and control board are small and easily
transportable, as well as inexpensive and potentially disposable
in the case of use of contaminating materials.

Sonolithography is also applicable to a variety of materials,
both patterned and substrate, as the patterning is largely inde-
pendent of the materials’ physical and chemical properties.
The acoustic field is generated above the substrate—particle
localization occurs in air, prior to deposition, and not as a
result of the vibration of the substrate. In order for particles
to undergo acoustophoresis, there must be a difference in the
particle’s compressibility and/or density as compared to that
of the medium.[23b] Practically, this limits the ability to manip-
ulate liquid materials in a liquid system; sonolithography is
able to overcome this, as comparatively, density and compress-
bility of all liquid and solid particles will be significantly dif-
ter to the air medium. This also enables the patterning
of viscous liquids, though droplet formation and dispersion
may provide an additional challenge.[15] For solid materials,
if electrostatic or other forces between the patterned material
and the substrate are greater than that of the acoustic radia-
tion force, the ability to move the materials may be hindered.
While the acoustic radiation force dominates, as evidenced
by the correlation between the simulated first-order pressure
landscape and the experimental results, acoustic streaming[16]
is also observed, i.e., radial movement of droplets around the
array, which ceases when the droplets deposit on the surface.
Although high amplitudes were used to generate usable forces
and the effect is inherently nonlinear, no evidence of effects
of any harmonics of the 40 kHz drive signal on the patterning
were seen.[37]

Sonolithography’s flexibility in materials and small foot-
print offers the potential for combination with other fabrica-
tion modalities, such as photolithography. For example, by
patterning a photosensitive polymer and exposing the sub-
strate to the appropriate wavelengths of light for crosslinking,
a similar effect to photolithography could be achieved without
the use of a separate photomask. Combinations of method-
ologies involving stereolithography and dynamic light projec-
tion and devices employing standing surface acoustic waves
have already been developed.[80,7c] These hybrid systems have

in biological systems, and could be used to engineer muscle
fibers or nerves.[11,12] Points and circles are cross-sections of
lines and cylinders, respectively, which by extrusion or multi-
layer processing, may then form the basis of a 3D construction
of nerve fibers or blood vessels with arbitrary paths.

Modulation of the transducer phases and the addition of
transducers enables further flexibility and user-defined con-
trol of patterns. Reversing the phases of transducer pairs
offers a simple way to extend the number of patterns formed,
as well as provides a method of translating the pattern in
X and Y without physically moving the array.[12,32] Develop-
ments in dynamic and phased array systems, where each
transducer is controlled individually to generate real-time
changes in the acoustic field,[36b,31] could easily be adapted to
sonolithography. This could form the basis of more complex
sequential patterns; programmed alterations in phases may
be used to generate layers of specific particle distributions,
much like a slicing function of a 3D printer. Advances in
acoustic holography have improved the level of detail of user-
defined patterns achievable with acoustic systems.[34] While

Figure 3. Examples of various materials and substrates patterned
using sonolithography. a) Nebulized carbon-based conductive ink and
expanded polystyrene beads (Ø =1.5 mm) on paper. b) Nebulized high-
lighter fluid on paper, illuminated by a handheld blacklight. c) Nebulized
aqueous sucrose solution on glass. d) Nebulized water on a dehydrated
calcium alginate film. A black arrow indicates the second antinode from
the center. e) Nebulized water on Parafilm. f) A size segregation effect
observed with sand. Smaller dust fragments have been patterned to the
center antinode. Scale bars in all images are 1 cm.
shown utility in generating 3D materials with complex internal structures.

The rapid, noncontact manipulation will have specific applicability to biofabrication techniques where time to form a construct is a critical parameter in maintaining cellular viability. Sonolithography also introduces the potential for size-based spatial segregation within the same array pattern. This has application in creating both surfaces with multiplexed bioactive molecules, as well as region-specific localization for cocultures of cells. Further development is in progress to enable real-time manipulation of the acoustic field to generate more complex patterns, as well as integration into additional additive manufacturing and biofabrication modalities, such as extrusion-based bioprinting.

In conclusion, we have introduced sonolithography, an in-air patterning technique for populations of particles and droplets. The approach extends the capabilities of existing acoustic patterning and fabrication systems by removing some of the material requirements, resulting in a technique that is largely independent of both the materials being patterned and of the substrate. This opens sonolithography to a wide array of applications. In particular, we demonstrated that this is an effective method of functionalizing surfaces for spatially localized cellular adhesion and has potential for directly manipulating and focusing cells. Furthermore, the patterning is rapid, replicable, and straightforward, requiring no specialist acoustic knowledge or equipment. We envision sonolithography as a method of rapidly generating 2D patterns for a variety of materials, as well as a complimentary technique to additive manufacturing where surface patterning combined with layer-by-layer fabrication can facilitate generation of structures with more internal complexity.

**Experimental Section**

Transducer Arrangement and Force Modeling: Array patterns and resultant acoustic pressure fields were modeled using an in-house MATLAB (MathWorks, Natick, MA, USA) script, following the derivations outlined in ref. [26b]. Further mathematical detail is provided in the Supporting Information.

Device Construction: Using the dimensions generated from the modeling of the acoustic pressure field, a transducer support was designed using the online tool Tinkercad (AutoDesk, San Rafael, CA, USA). Ultimaker (Gelderlnsen, The Netherlands) Cura software was used for slicing and G-Code generation. The support was subsequently printed using polyactic acid filament on an Ultimaker 2+ 3D printer. Ultrasonic transducers (MA40S4S, Murata, Kyoto, Japan) with a diameter of 1 cm, operating at a frequency of 40 kHz, were affixed to the transducer support using hot glue, such that the faces of the transducers were flush with the inner face of the support. A separation of 1 mm in the support was used for signal generation (40 kHz square wave) with amplification (+86.3 dB) provided by an L298n H-bridge dual motor driver. The device was powered using a 12 V adapter to the mains.

Direct Patterning: A piezoelectrically driven droplet-on-demand dispenser (MicroFab, plano, TX, USA) was used to generate monodisperse populations of droplets of either 25 or 80 μm, depending upon nozzle inner diameter. Waveforms were generated using the JetDrive V signal box (MicroFab), controlled by the JetServer4 software (MicroFab). For water, unipolar pulse waveforms were used. For the 25 μm system, this consisted of a trapezoidal waveform with rise and fall times of 5 μs, a dwell time of 15 μs, and dwell voltage of 17 V, with an idle voltage of 4.5 V. For the 80 μm system, rise and fall times were 3 μs, with a dwell time of 17 μs, dwell voltage of 35 V, and idle voltage of 4.5 V.

HUVEC Culture and Maintenance: Immortalized HUVECs (Lonza, Basel, Switzerland), which had been stably transfected via lentivirus to express GFP, were received as a gift. Cells were cultured according to standard culture protocols using EGM-2 Media, supplemented with the associated BulletKit (Lonza). Media was refreshed three times per week. Cells were kept in a humidified incubator (95% RH, 5% CO2, 37 °C), and were subcultured or used for experiments at 80% confluence.

Direct Patterning: A 0.2 mg mL−1 collagen I solution was prepared by diluting 3 mg mL−1 Rat Tail Collagen I (Gibco, Thermo Fisher) with phosphate buffered saline (PBS, without Mg2+ and Ca2+, Sigma-Aldrich, St. Louis, MO, USA). This solution was added to the chamber of a medical nebulizer. Ultrasonic arrays were prepared as described previously. Collagen was nebulized into a 10 cm petri dish containing the octagonal array for 30 s with the ultrasound on, or off as a control. To prevent dispersion of the nebulized material, dishes were placed in an acrylic containment chamber during the patterning process. Following patterning, dishes were exposed to UV light for at least 30 min for sterilization within a biological safety cabinet. Following overnight blocking of the exposed tissue culture plastic with 2 wt% bovine serum albumin (BSA, Sigma-Aldrich) in PBS, HUVECs were seeded normally and allowed to settle at room temperature for 12 min to encourage initial cell adhesion to the collagen. The cell solution was then removed, and the dish was rinsed gently with PBS twice to remove nonadherent cells. Fresh cell media was added to each dish, and the cultures were incubated for at least 24 h prior to imaging.

Microscopy: Low magnification (5x) tile scans (300–500 individual images) were taken over the area of the dish after 1, 2, and 7 d of culture using a Leica (Wetzlar, Germany) DMi6000 inverted epifluorescence widefield microscope. Micrographs were contrast enhanced using the Leica LAS X software and Fiji.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.
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Conflict of Interest

The authors declare no conflict of interest.

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acoustic patterning, acoustophoresis, biofabrication, surface patterning, ultrasound

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