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Acoustic lithography: Field-directed cell patterning for bio-systems engineering

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Abstract

The precise spatial patterning of living cells represents a foundational capability in bio-systems engineering, enabling the systematic study of collective cellular behaviors and the fabrication of increasingly complex functional tissues. Conventional methods for achieving this control, while numerous, are often constrained by static pattern formation, the need for biochemical labels that can alter cell function, or requirements for non-physiological media. In this context, acoustic-field-based manipulation has emerged as a uniquely powerful and biocompatible alternative. This review synthesizes these advancements under the unifying concept of "acoustic lithography," a framework that captures the technology's capacity for rapid, parallel, and label-free cellular organization. The discussion covers the core physical principles of acoustic radiation force and acoustic streaming before surveying the diverse technological landscape, from bulk and surface acoustic waves to advanced acoustic holography. It further highlights the impact of these tools across a spectrum of applications, including high-throughput analysis, biomimetic co-culture engineering, advanced biofabrication, and clinical sorting. Collectively, these applications demonstrate the field's trajectory as it moves beyond static patterning to encompass the integrated control of structure, environment, and function. Viewing the technology through this broader engineering lens underscores its significance as a vital platform, charting a course for the next generation of dynamically engineered living systems.

Keywords: Acoustic lithography; Acoustofluidics; Biofabrication; Bio-systems engineering; Cell patterning

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1. Introduction

The ability to actively engineer the spatial distribution of cells is pivotal for advancing biological discovery and regenerative medicine.[1,2] While modern microscopy has unlocked unprecedented vistas of the microscopic realm, the capacity to move beyond

passive observation and actively pattern cells into defined structures is essential for understanding and directing collective biological functions.[3,4] This technique, known as cell patterning, is fundamental for decoding processes such as tissue morphogenesis, directed migration, and intercellular signaling.[5–7] Such processes underpin transformative applications in drug screening, disease modeling, and the engineering of functional tissues, laying the groundwork for advanced biofabrication and tissue engineering strategies.[8–11] Critically, conventional two-dimensional (2D) cell cultures fail to recapitulate the complex microenvironment and cell–cell interactions of native tissues, and animal models present challenges in throughput and species-specific differences, limiting their predictive power for human physiology.[12–14] Cell patterning emerges as an essential strategy to bridge this gap, providing geometrically controlled microenvironments that enable the systematic investigation of cellular physiology in a manner that reflects *in vivo* complexity.[15–18]

The drive to engineer increasingly complex cellular constructs has fueled the evolution of diverse patterning methodologies, each presenting distinct advantages and limitations.[4,5,19] Template-based techniques, such as those using elastomeric stencils, offer simplicity and high throughput by physically confining cells within predefined apertures.[20,21] However, this static approach affords limited flexibility for dynamic studies, and stencil removal requires careful consideration to maintain pattern integrity.[22] In contrast, field-directed strategies provide dynamic, reconfigurable control.[23,24] Magnetic manipulation, for instance, uses functionalized nanoparticles to guide cells with high specificity,[25,26] though this reliance on pre-labeling necessitates careful optimization to avoid influencing the cells' native state. For ultimate precision, optical tweezers employ focused laser beams to achieve subcellular resolution.[27] The highly focused energy, however, requires careful power management to mitigate potential photothermal effects, and scaling this high-precision method for high-throughput applications remains a challenge.[6,28] Another label-free alternative, dielectrophoresis (DEP), separates cells based on their intrinsic dielectric properties.[22,29] For optimal performance, DEP is typically conducted in low-conductivity buffers, which may require careful formulation to ensure long-term compatibility with cell health.[30] In contrast, acoustic field-directed patterning operates effectively in standard physiological buffers, as it relies on intrinsic mechanical properties rather than electrical polarizability.[31–36] This key advantage, combined with its tag-free and minimally invasive nature, positions acoustic manipulation as a uniquely versatile approach.[37–39]

To capture these collective advancements and emphasize their potential for creating user-defined, complex biological structures, this review frames the field under the concept of "acoustic lithography," by analogy with optical lithography in microfabrication. It is important to differentiate this concept from the broader, well-established field of acoustofluidics. Acoustofluidics encompasses a wide range of dynamic operations like separation, mixing, and transport. We propose acoustic lithography, much like optical lithography, to define its specific role in fabrication. The essence of this concept is the use of acoustic fields to construct preset and complex cellular structures.[40,41] The method's exceptional biocompatibility is well-documented, with studies showing preserved viability in erythrocytes and unaltered development in zebrafish embryos after prolonged exposure. This advantage stems from the fundamental nature of acoustic energy transfer: as mechanical waves, acoustic waves can propagate deep into optically opaque tissues with minimal absorption and negligible thermal loading, a significant benefit over light-based methods.[42,43] Furthermore, because acoustic forces are generated based on intrinsic mechanical properties (density and compressibility), the technology functions effectively in standard physiological buffers, whose high ionic strength would otherwise screen the electric fields required for dielectrophoretic. This reliance on inherent acoustic contrast obviates the need for the chemical labels required for magnetic approaches, thus preserving the native state of the cells.[44]

The rapid evolution of this field has created a need for an updated, cohesive synthesis of its principles and applications. While several excellent reviews cover the fundamentals of acoustofluidics and key applications in tissue engineering,[31,32,45–49] the technological landscape has expanded dramatically. Breakthroughs now extend beyond static patterning to include the integration of acoustics with smart, stimuli-responsive biomaterials for 4D biofabrication, pioneering *in vivo* applications such as targeted drug delivery and the remote assembly of tissue constructs within living organisms.[50–52] These emerging areas, alongside novel techniques like acoustical holography, sonogenetics, and single-molecule force spectroscopy,[53–59] are often dispersed across various disciplines. This fragmentation creates a pressing need for an updated synthesis to bridge the gap between fundamental acoustofluidic principles and their translational potential. This review synthesizes these advancements through the framework of acoustic lithography, providing a strong control-oriented perspective. This perspective is highlighted in our discussion on broader engineering principles, which illuminates the field's trajectory through a clear engineering logic arc—progressing from first,

controlling cellular structure, to second, engineering the dynamic microenvironment, and ultimately to third, regulating biological function. We believe this broader engineering perspective, which focuses on unifying design goals rather than disparate methodologies, provides a fresh vantage point that is critical for guiding future innovation in the field. Indeed, the expanding body of research reveals an increasingly sophisticated and diverse technological ecosystem, integrating novel materials, computational design, and multi-physics approaches to achieve unprecedented levels of control.[56–63]

This review is structured to build a comprehensive understanding of acoustic lithography from the ground up (Figure 1). We first elucidate the fundamental physics that form the toolkit for acoustic manipulation. Following this, we systematically survey the key methodologies, showcasing how these tools are engineered into increasingly sophisticated machines for cell patterning. We then explore the diverse applications of these machines in solving critical problems in bio-systems engineering. Finally, to fully appreciate the field's trajectory and potential, we step back from the technical details to introduce a cross-disciplinary perspective. This synthesis highlights the field's key advantages: its excellent biocompatibility in native media, its capacity for true three-dimensional (3D) precision, and its unique potential for dynamic, reconfigurable control, which together chart a course for future innovation.

2. Physical principles of acoustic cell manipulation

The ability of acoustic waves to manipulate cells and other microparticles in a fluid stems from nonlinear effects that generate time-averaged forces and bulk fluid motion.[39,64,65] Even at small scales relevant to cell patterning, these acoustic effects dominate, easily overcoming gravitational forces, which are negligible for individual cells in suspension. This manipulation is primarily achieved through two distinct yet interconnected physical phenomena: the acoustic radiation force (ARF) and acoustic streaming (a steady, time-averaged fluid motion generated by the acoustic field).[66] The ARF is directly exerted on a particle due to the scattering of the incident acoustic wave.[67] This force effectively creates a potential energy landscape, pushing or pulling the particle toward stable trapping positions. Simultaneously, acoustic streaming is an indirect effect,[68] in which the absorption of acoustic energy within the fluid generates steady, often vortical, flows that induce a gentle drag on suspended particles.[69] The interplay and relative dominance of these two forces—determined by factors

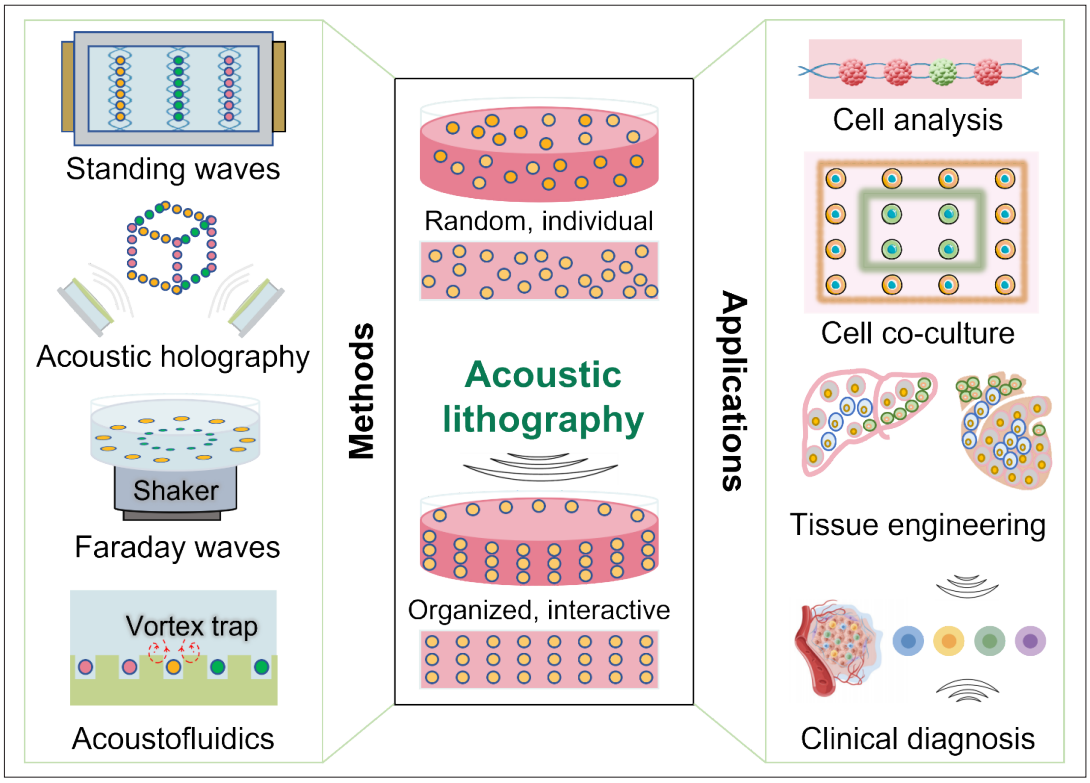

Figure 1. The overarching framework of this review.

such as the acoustic frequency, particle properties, and fluid environment—form the cornerstone of all acoustic lithography techniques.[70]

2.1. Acoustic radiation force

The ARF arises from the transfer of momentum from the acoustic wave to the particle. A complete description of this force requires considering the time-averaged momentum flux tensor, $\langle \prod \rangle$, integrated over a surface S enclosing the particle.[71,72] The brackets $\langle\ \rangle$ denote the time-average.

$$F = -\oint_s \langle \prod \rangle \cdot dS \tag{1}$$

This general formulation is comprehensive but complex. Gor'kov derived a simplified and powerful expression for a specific and highly relevant case: when particles are much smaller than the acoustic wavelength. This condition defines the Rayleigh regime, where the product of the wavenumber k and particle radius R is $kR \ll 1$ —a condition that most cells satisfy. He achieved this by treating the interaction as an effective potential energy field, U. The resulting force is then simply the negative gradient of this potential[73]:

$$F_{ARF} = -\nabla U \tag{2}$$

The Gor'kov potential U is a function of the time-averaged squares of the acoustic pressure $\langle p^2 \rangle$ and fluid velocit $\langle v^2 \rangle$ at the particle's location, and is given by:

$$U = V_p \left(\frac{1}{2} f_1 \kappa_f \langle p^2 \rangle - \frac{3}{4} f_2 \rho_f \langle v^2 \rangle \right) \tag{3}$$

Here, V_p is the particle volume, while ρ_f and κ_f are the fluid density and compressibility, respectively. The particle's response to the acoustic field is captured by two dimensionless acoustic contrast factors, f_1 and f_2, which depend on the particle's compressibility (κ_p) and density (ρ_p) relative to the surrounding fluid:

$$f_1 = 1 - \frac{\kappa_p}{\kappa_f} \tag{4}$$

$$f_2 = \frac{2\left(\rho_p - \rho_f\right)}{2\rho_p + \rho_f} \tag{5}$$

The first term in the potential, proportional to f_1, represents the force contribution from the particle's compressibility (a monopole scattering term), while the second term, proportional to f_2, represents the contribution from its density (a dipole scattering term). In a standing wave, which is the most common configuration for cell patterning, the pressure and velocity fields are spatially modulated, creating a periodic potential landscape. In a simple one-dimensional standing wave, this results in a force that can be expressed as:

$$F_{ARF} = -\left(\frac{\pi p_0^2 V_p \kappa_f}{2\lambda} \right) \Phi(\kappa, \rho) \sin(2kx) \tag{6}$$

where p_0 is the pressure amplitude, V_p is the particle volume, λ is the wavelength, x is the position, and Φ is the overall acoustic contrast factor,[74] which combines the effects of compressibility and density differences between the particle and the medium:

$$\Phi(\kappa, \rho) = \frac{5\rho_p - 2\rho_f}{2\rho_p + \rho_f} - \frac{\kappa_p}{\kappa_f} \tag{7}$$

The sign of this contrast factor determines the particle's destination.[39] For most mammalian cells in aqueous media, $\Phi > 0$, causing them to migrate to the pressure nodes (regions of minimum pressure amplitude). Conversely, for less dense and more compressible objects like lipid droplets or gas bubbles, $\Phi < 0$, driving them to the pressure antinodes.

When multiple particles are brought into close proximity by the primary radiation force, inter-particle forces, known as secondary radiation forces or Bjerknes forces, become significant. These forces arise from the acoustic field scattered by one particle acting upon its neighbors. For two pulsating bubbles in an acoustic field, for instance, this interaction force can be attractive or repulsive depending on their relative phase of oscillation, and it scales with the inverse square of the distance between them.[75,76] For cells, these secondary forces are generally attractive and are crucial for promoting the formation of tight, compact multicellular clusters and spheroids.[77] Thus, in most cell patterning applications, it is the dominant role of the ARF that drives cells toward designated positions, establishing it as the primary power source for acoustic actuation.

2.2. Acoustic streaming

In any real fluid, acoustic energy is dissipated through viscosity, leading to a transfer of momentum from the wave to the fluid.[78] This process generates the steady, time-averaged fluid motion known as acoustic streaming.[79] The force exerted by this flow on a particle is a drag force, which

can be harnessed for transport, mixing, and pumping. The general source term for acoustic streaming, F_S, is given by the negative divergence of the Reynolds stress tensor:

$$F_S = -\rho_0 \nabla \cdot \langle \tilde{v} \otimes \tilde{v} \rangle \quad (8)$$

where $\tilde{v}$ is the first-order acoustic velocity field and ρ_0 is the density of the fluid at rest. The specific nature and utility of the resulting flow depend critically on where the acoustic energy is dissipated. For clarity, it is useful to distinguish between different types of streaming phenomena arising from this principle. Theoretically, Eckart streaming arises from thermoviscous dissipation in the bulk of the fluid, far from any boundaries. This typically results in a large-scale, unidirectional flow.

While fundamental, this type of streaming is not the dominant effect in microfluidic devices, where the system size is small and boundary interactions are ubiquitous. In such micro-scale systems, dissipation at solid boundaries is the primary driver of streaming. This gives rise to Schlichting streaming, a flow confined within the thin oscillatory viscous boundary layer adjacent to any surface.[80] This layer has a thickness given by $\delta_v = \sqrt{2\nu / \omega}$, where ν is the kinematic viscosity and ω is the angular frequency. This boundary-layer flow acts as the fundamental engine for the most useful streaming patterns. The shear generated by this engine, in turn, drives a larger-scale, stable flow in the bulk fluid known as Rayleigh streaming.[81]

It is this Rayleigh streaming, often manifesting as stable, counter-rotating vortices, that is most important for practical applications in microfluidics. By precisely engineering the geometry of the channel walls, such as by incorporating sharp edges or trapping microbubbles, researchers can control the location and intensity of the underlying Schlichting streaming.[82] This allows the creation of highly localized and predictable Rayleigh streaming vortices, which serve as powerful micro-tools for trapping, concentrating, and manipulating cells. The drag force exerted by these engineered streaming flows on a small spherical particle is well-described by Stokes' law:

$$F_{drag} = 6\pi\rho_f \nu R v_s \quad (9)$$

where v_s is the relative velocity between the particle and the fluid. The linear dependence of this force on particle radius makes acoustic streaming particularly effective for manipulating larger objects or for size-based sorting applications. This clear distinction between the "engine" (Schlichting) and the "tool" (Rayleigh) provides the physical basis for designing sophisticated acoustofluidic devices. Excellent reviews on detailed mathematical models and acoustic streaming mechanisms are provided by Wiklund *et al.*[79] and Sadhal, respectively.[83]

2.3. Interplay and synergy of forces

ARF and acoustic streaming invariably coexist, and their interplay determines the ultimate trajectory and final position of the target cells. The relative importance of the two forces is strongly dependent on particle size. The ARF scales as R^3 because it is a volume-dependent effect, whereas the streaming-induced drag force scales linearly with the radius R. Consequently, there exists a critical particle size below which streaming effects dominate and above which the radiation force dictates the motion. For a typical 2 MHz system in water, this transition has been shown to occur at a diameter of approximately 2 μm. Most biological cells are larger than this, so the radiation force is often the dominant positioning mechanism, dictated by their intrinsic mechanical properties.[84,85]

However, this is not a universal rule, and the two forces can be synergistically combined to achieve manipulation capabilities that neither could accomplish alone. A prominent strategy involves using large-scale streaming flows to transport cells over long distances into a region of interest, where finely tuned radiation forces then perform precise, short-range patterning. Another powerful synergistic approach has been demonstrated for the assembly of larger biological structures. In these systems, acoustic streaming is engineered to create stable microvortices that gently capture and confine a cellular spheroid, while a carefully configured radiation force field acts as a soft potential well, preventing the spheroid from escaping. This dual-force mechanism enables complex, programmable 3D assembly.[86,87] A clear understanding and deliberate control of the balance between these forces is therefore crucial for designing effective and versatile acoustic lithography systems.

These physical principles map directly onto the application scenarios discussed later. For instance, high-throughput bulk acoustic wave (BAW) cell sorting typically employs the ARF at frequencies in the 1–10 MHz range.[88] High-resolution surface acoustic wave (SAW) patterning also relies on ARF, but at much higher frequencies, often in the sub-gigaHertz range, to achieve finer spatial control.[89] In contrast, localized streaming, which dominates precision trapping and rotation, is often generated at lower frequencies, from the kiloHertz range to a few megaHertz.[90] Volumetric assembly via holography also operates in the 0.5–5 MHz range to balance penetration and resolution,[91,92] while large-area Faraday-wave patterning operates at the very low hydrodynamic frequencies of 10–100 Hz.[93,94]

This section has outlined the fundamental physical principles governing the interaction between acoustic fields and microparticles. The primary mechanisms—ARF and acoustic streaming—provide the foundational toolkit for acoustic lithography. While the diverse patterning methodologies discussed in the subsequent sections may involve more complex, geometry-specific phenomena, their underlying actuation mechanisms are invariably rooted in the interplay of these two principal forces. A clear understanding of these core principles is therefore essential for the rational design and optimization of any acoustic cell manipulation system.

3. Acoustic patterning methodologies

A variety of techniques have been developed to harness acoustic forces for cell patterning, many of which rely on the generation of acoustic standing waves to create force potential landscapes. This section surveys key methodologies based on the complexity of this wave engineering. We begin with foundational techniques like BAW and SAW, which create simple, periodic standing waves. The discussion then progresses to acoustic holography as a powerful extension of this principle, capable of generating complex, arbitrary standing wave fields for user-defined 3D assembly. Finally, we cover two distinct approaches: Faraday waves, which leverage fluid–surface instabilities for large-area patterning, and the novel and flexible approach of localized acoustofluidics, which employs engineered microstreaming for precise, dynamic manipulation.

3.1. Bulk acoustic waves

In acoustofluidic technology, BAW represents a core method with tremendous potential for cell patterning. Its primary working principle involves generating a standing wave field within a fluid-filled cavity: a piezoelectric transducer converts an electrical signal into mechanical vibrations, launching acoustic waves into the fluid. These waves interfere with reflected waves or waves from an opposing transducer, forming a stable standing wave with stationary pressure nodes and antinodes.[95] Cells, driven by the ARF, aggregate at the pressure nodes.[96] The final pattern is dictated by experimental parameters such as wave frequency, device dimensions, and fluid properties, allowing for precise control over cell arrangement.[60]

With the rapid development of cell patterning in biomedical applications, research on BAW technology has produced a series of innovative methods that expand its scope and practicality. For example, a common design for 2D patterning employs two orthogonal pairs of piezoelectric transducers (Figure 2A).[41] By independently controlling the standing waves in each direction, this design allows flexible adjustment of both the orientation and spacing of cellular grid patterns. To achieve more complex arrangements, devices with more intricate transducer configurations have been developed. The heptagonal acoustic tweezer (Figure 2B)[97] is a notable example, using seven transducers whose frequencies and phases can be independently controlled. By activating different combinations, cells can be organized into complex grid-like patterns that meet the requirements for simulating sophisticated biological structures.

To better integrate BAW technology with biomaterial fabrication, methods combining BAW transducers with microfluidic conduits have also been developed. One such approach (Figure 2C)[98] uses a transducer coupled to a glass capillary. The acoustic standing wave pattern cells within a liquid hydrogel precursor, which is then fixed by photopolymerization. This enables the continuous extrusion of hydrogel fibers with embedded, organized cells, providing an effective means for constructing anisotropic tissues such as muscle. Similar research (Figure 2D)[99] has explored how capillary shape influences the acoustic pressure field, enabling the formation of unique radial cell patterns. These patterns can also be fixed within hydrogel fibers or tubules, further enriching the application of BAW technology in biomaterial preparation.

In principle, BAW-based devices are foundational and robust. Their core strength lies in generating acoustic waves that propagate through the entire volume of a fluid-filled chamber, enabling true 3D force fields.[56,100] The versatility of BAW systems has been significantly expanded through these innovative device configurations, making them invaluable tools for both fundamental patterning and applied biofabrication.[101] While BAW systems are robust for 3D patterning, SAW systems, which we discuss next, offer a pathway to higher-resolution, planar manipulation by confining energy to a substrate.

3.2. Surface acoustic waves

SAWs are generated by applying an alternating electrical signal to an interdigital transducer (IDT) fabricated on a piezoelectric substrate. This process creates mechanical waves confined to and propagating along the substrate's surface. The most common type of fluid manipulation is the Rayleigh waves, as its vertical displacement component efficiently leaks acoustic energy into an overlying fluid, whereas other types like shear-horizontal SAWs cannot effectively couple with liquids and are mainly used for sensing applications. When these surface waves leak into a microchannel, they create pressure fields capable of manipulating particles. By precisely adjusting the frequency, phase, and power, and by optimizing the geometry of the IDTs, whose finger width and spacing directly define the

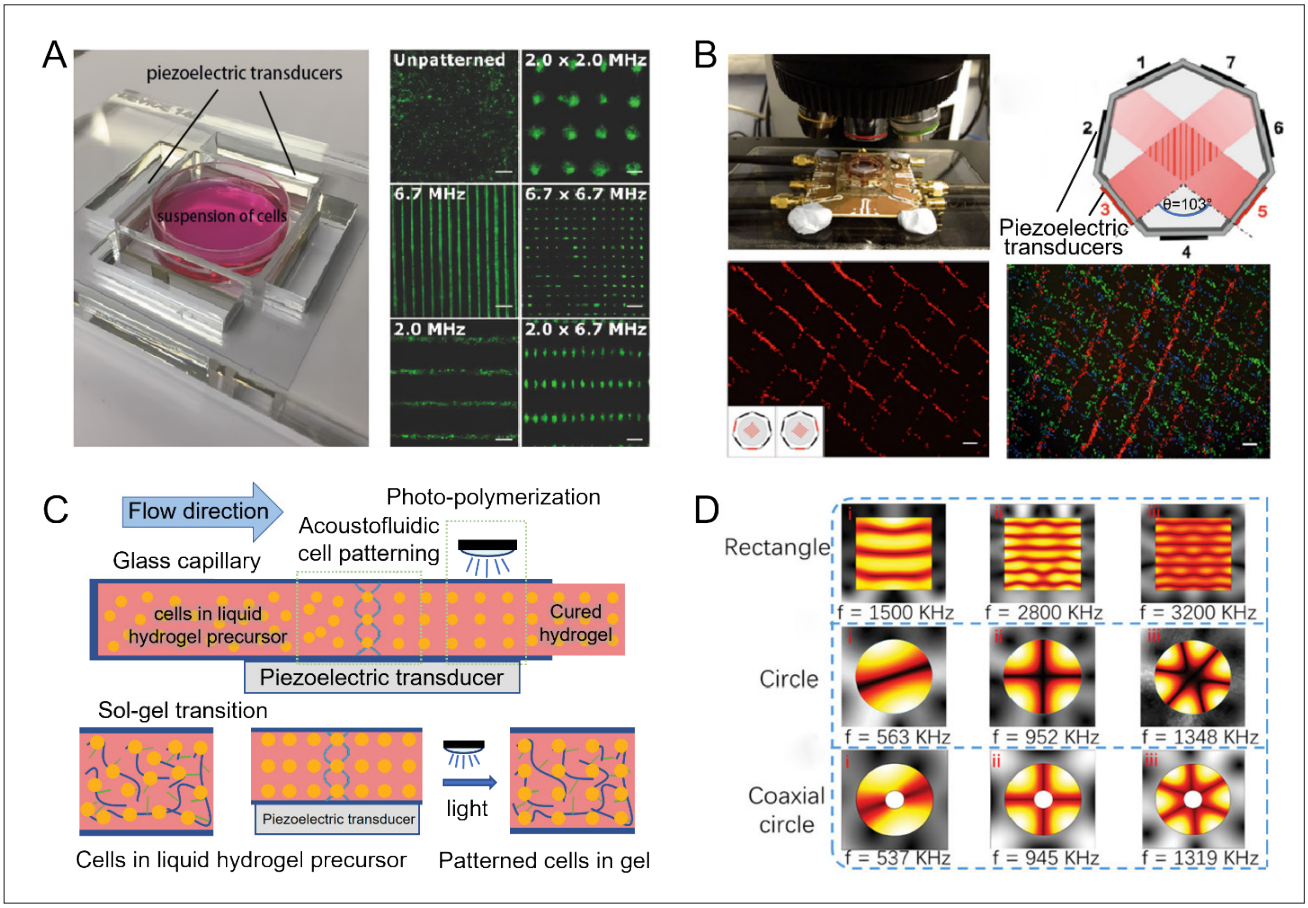

Figure 2. Methodologies for cell patterning using the BAW technology. (A) A 2D patterning device employing orthogonal pairs of piezoelectric transducers to dynamically control the orientation and spacing of cellular grids. Scale: 200 μm. Reprinted from Ref.[41] (B) A heptagonal acoustic tweezer that generates complex, reconfigurable patterns by selectively activating different transducer combinations. Scale: 100 μm. Reprinted from Ref.[97] (C) Integration of BAW with biomaterial fabrication, where a transducer coupled to a glass capillary enables the continuous production of hydrogel fibers with embedded cellular structures. Adapted from Ref.[98] (D) Influence of capillary geometry on the acoustic pressure field, demonstrated through simulations and experimental results of tunable radial cell patterning. Reprinted from Ref.[99] Abbreviations: f, frequency; 2D, two dimensional; BAW, bulk acoustic wave.

acoustic wavelength, acoustic energy can be focused to generate nodes and antinodes for non-invasive trapping, transport, and rotation of cells. A key breakthrough of SAW technology is the miniaturization of acoustic field control: the micron-scale period of lithographically defined IDTs allows for the formation of subwavelength-scale pressure nodes, supporting nanoscale positioning accuracy.[102,103] Furthermore, SAW devices can operate at much higher frequencies (up to ~1 GHz) than typical BAW systems, making them exceptionally well-suited for the high-resolution manipulation of sub-micrometer particles.

The versatility of SAW technology enables diverse device configurations and manipulation strategies. When interdigital IDTs are fabricated on the surface of a piezoelectric substrate and bonded with a microfluidic channel layer made of polydimethylsiloxane, the SAWs excited by a pair of IDTs propagate to the polydimethylsiloxane–piezoelectric interface, where acoustic energy leakage occurs, generating leaky waves. These waves couple upward into the liquid region within the channel, exciting longitudinal acoustic waves in the fluid. When two pairs of IDTs are positioned in the medium, they generate counter-propagating SAWs. The interference of these waves establishes a standing acoustic wave field in the fluid, enabling precise manipulation of microparticles. The ARF induced by this periodic pressure distribution serves as the key physical mechanism for separating particles or cells (Figure 3A).[103] For nanoscale bioparticle separation, a tilted-angle standing SAW system can be configured for continuous, two-stage processing to achieve complex separations. In a representative application for blood component isolation, the first stage removes components larger than 1 μm, such as red blood

cells, white blood cells, and platelets, while the second stage fractionates vesicle subsets. This system has demonstrated the capability to directly isolate exosomes from whole blood at a flow rate of 4 μL/min. A key advantage of this technology is its operational flexibility: the separation process can be actively controlled by switching the acoustic field on or off (Figure 3B).[104]

In cell patterning, when a standing SAW is generated through the interference of counter-propagating waves from opposing IDTs, a 2D grid-like acoustic field composed of multiple pressure nodes is established within the fluid. When the wavelength of the high-frequency SAW is on the same order of magnitude as the cell size (λ ~ D), the intersecting waves form a 2D array of potential wells. This configuration enables highly precise single-cell-per-well patterning (Figure 3C).[30] A significant advancement in SAW-based platforms is the wavenumber-spiral acoustic tweezers, designed for dynamic and reconfigurable cell manipulation. This system employs multiple pairs of centrosymmetrically arranged IDTs that are simultaneously and independently controlled by a single multi-frequency signal. By modulating the signal's frequency, phase, and amplitude, the interference of the resulting multidirectional waves reshapes the acoustic field in real time, overcoming the static limitations of conventional tweezers. This capability enables a suite of powerful functions, including transforming entire cell patterns from one lattice configuration to another and

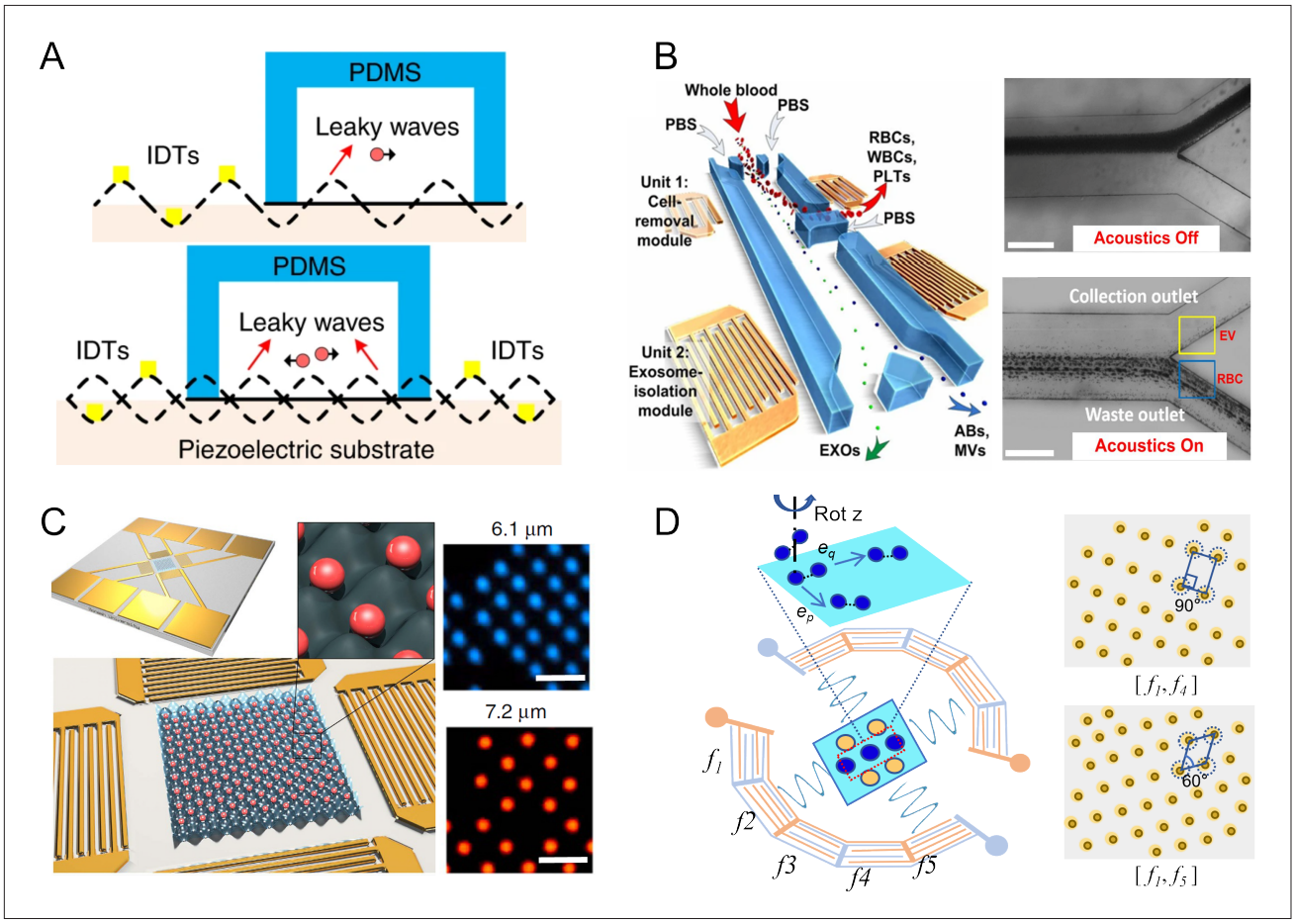

Figure 3. Key methodologies for cell and particle patterning using SAWs. (A) An integrated, two-module acoustofluidic chip for complex separations, where tilted-angle standing SAWs are applied sequentially to remove cells and then isolate nanoscale exosomes from whole blood. Scale: 500 μm. Reprinted from Ref. [103] (B) Massively multiplexed, high-density submicron particle patterning using an array of acoustically oscillating nanocavities as individual trapping sites. Reprinted from Ref. [104] (C) A single-cell patterning method using intersecting high-frequency SAWs with wavelengths comparable to cell diameters to form a 2D grid of single-cell traps. Scale: 30 μm. Reprinted from Ref. [30] (D) Dynamic, reconfigurable manipulation using wave-number spiral acoustic tweezers. Modulation of a multitone excitation signal enables real-time reshaping of acoustic fields to translate patterns or move particles along complex trajectories. Adapted from Ref. [105] Abbreviations: Abs, antibodies; EXOs, exosomes; IDTs, interdigital transducer; MVs, microvesicles; PBS, phosphate-buffered saline; PDMS, polydimethylsiloxane; PLTs, platelets; RBC, red blood cell; WBCs, white blood cells; 2D, two dimensional; SAWs, surface acoustic waves.

precisely guiding individual particles along complex, user-defined trajectories (Figure 3D).[105]

Collectively, these attributes position SAW as a high-precision toolkit within acoustic lithography.[104] Its defining characteristic is the confinement of acoustic energy to the substrate surface, which fundamentally distinguishes it from the volume-based actuation of BAW. This provides higher spatial resolution and facilitates seamless integration with planar microfluidic platforms, making SAW a powerful tool for both precise cell manipulation and integrated sensing.[106–108] While BAW and SAW methods excel at creating periodic or simple geometries, acoustic holography constitutes a significant leap forward, enabling arbitrarily complex, user-defined fields.

3.3 Acoustic holography

Acoustic holography represents a major advance in patterning technology, enabling the creation of arbitrarily complex 3D acoustic fields to engineer cell patterns that mimic natural biological structures. The core principle involves using 3D-printed acoustic holograms or phased-array transducers to precisely control the phase and amplitude of acoustic waves.[109,110] This produces a user-defined complex acoustic field in which cells are guided by radiation forces and acoustic streaming into a preset pattern.[111–113]

Implementation of this principle has evolved through several innovative strategies that progressively enhance spatial and temporal control. The foundational approach utilizes a static, 3D-printed acoustic hologram as a passive phase mask driven by a simple plane-wave transducer (Figure 4A).[54] The hologram's topography encodes phase information, which shapes the wavefront to assemble cells into a 2D pattern. To overcome the "one hologram, one pattern" limitation, a programmable method was developed that uses a single plate to achieve switchable patterning by altering the sound speed of the fluid medium, thus changing the effective wavelength and resulting diffraction pattern (Figure 4B).[114]

Building on these concepts, acoustic holography has been extended into the third dimension to achieve one-step volumetric 3D cell assembly (Figure 4C).[91] This is accomplished by using multiple holograms to collaboratively form a complex pressure field that matches a target 3D shape, enabling the simultaneous aggregation of cells throughout the entire volume and offering a significant advantage over slower, layer-by-layer fabrication methods. For the ultimate level of control, the passive hologram is replaced by a dynamic phased array transducer, which functions as a fully electronic, reconfigurable hologram (Figure 4D).[92] By electronically regulating each array element, complex acoustic fields such as focused vortex beams can be generated and morphed in real-time, enabling sophisticated tasks including the precise positioning and orientation of organoids.

Acoustic holography provides an unparalleled level of control for cell patterning. Its defining advantage is the ability to generate arbitrarily complex, user-defined acoustic energy landscapes,[91] moving far beyond the simple periodic patterns of BAW and SAW. The capacity for one-step volumetric assembly and the transition to fully dynamic phased arrays position acoustic holography as a key enabling technology for advanced applications in organoid construction, complex tissue engineering, and targeted *in vivo* therapies.[53,92] The methods discussed so far—BAW, SAW, and holograms—rely on engineered transducers to shape the sound field. In contrast, the following sections explore two alternative approaches: fluid–surface instabilities, known as Faraday waves, and localized microstreaming.

3.4. Faraday waves

Faraday waves offer a unique method for large-area patterning based on fluid-surface instabilities.[115] These are nonlinear standing waves that spontaneously form on the surface of a fluid subjected to vertical vibration when the driving intensity exceeds a critical threshold.[116] The initially flat surface transforms into a regular pattern of crests and troughs. The core principle for cell patterning lies in harnessing the hydrodynamic forces generated by these waves, such as acoustic streaming and pressure gradients, to drive the directional migration and aggregation of suspended cells into patterns that mirror the underlying wave structure.[117,118]

The practical application of this phenomenon has been demonstrated through several key strategies. A typical setup involves a chamber containing the cell suspension mounted on a vertical shaker (Figure 5A).[93] As the chamber vibrates, periodic standing waves form, and suspended particles migrate to the nodal regions, producing a large-area, periodic pattern. This platform has been used to investigate the differential assembly of cells based on their intrinsic physical properties (Figure 5B).[119] By leveraging subtle differences in size and density, various cell types can be spatially segregated, enabling structured co-culture arrangements. To extend this inherently 2D technique into the third dimension, a layer-by-layer assembly method has been explored (Figure 5C).[94] In this approach, a thin layer of cell-laden hydrogel is patterned and then rapidly polymerized, and by repeating this process, complex 3D cellular constructs with defined geometries can be fabricated.

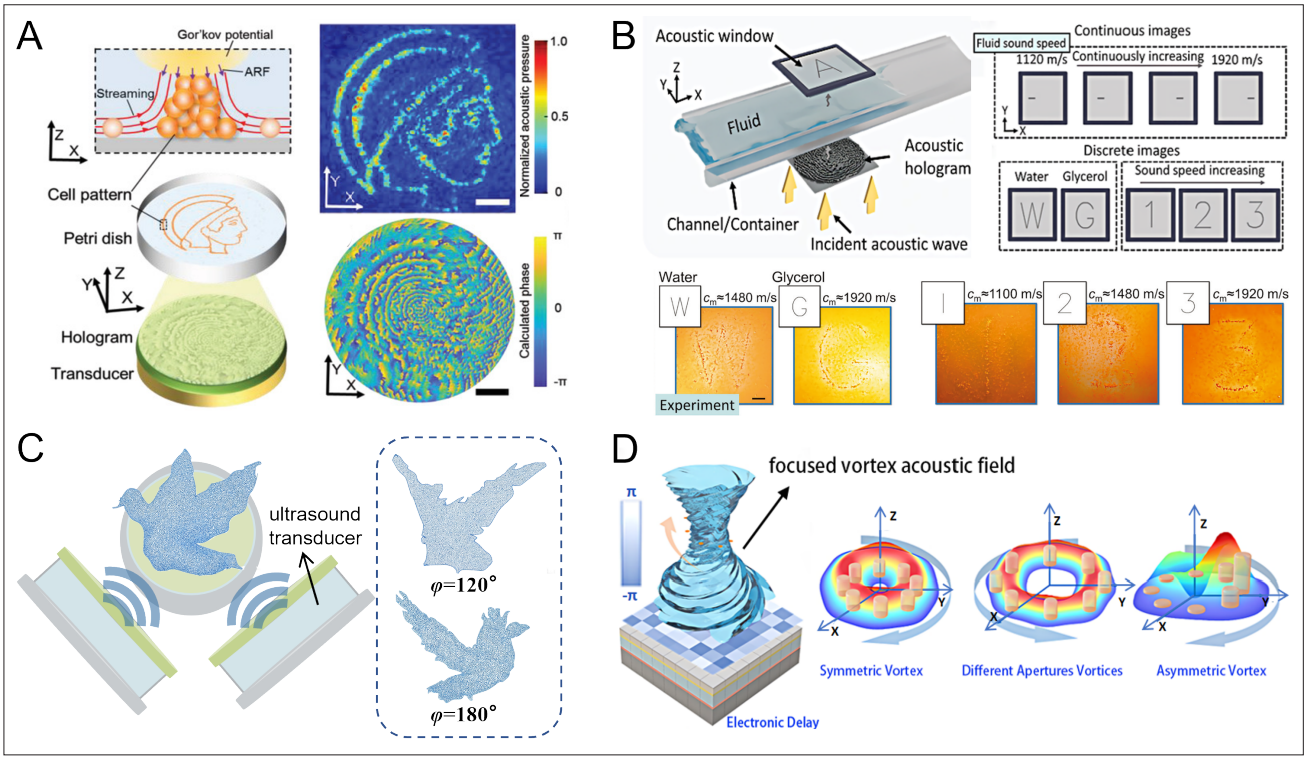

Figure 4. Strategies for cell patterning using acoustic holography. (A) Static 2D patterning using a 3D-printed hologram as a passive phase mask for planar wavefront shaping. Scale: 5 mm. Reprinted from Ref.[54] (B) Reconfigurable patterning from a single hologram achieved by modulating the sound speed of the intervening medium, thereby altering the resulting diffraction pattern. Reprinted from Ref.[114] (C) One-step volumetric assembly of 3D structures using multiple holograms to collaboratively shape a complex acoustic field. Adapted from Ref.[91] (D) Dynamic patterning and manipulation using an ultrasonic phased array acting as an electronic, reconfigurable hologram capable of generating complex acoustic fields in real time. Reprinted from Ref.[92] Abbreviation: ARF, acoustic radiation force.

Faraday-wave-driven patterning stands apart from other acoustic methods because it reliance on a macroscopic fluid instability rather than direct acoustic–particle interactions within the bulk fluid. Its primary advantages are the simplicity of the setup and its intrinsic capacity to generate highly ordered, large-area periodic patterns without complex transducers.[120] Although its resolution may not match that of high-frequency techniques, its scalability makes it a valuable tool for applications requiring large, uniform cellular arrays and for fundamental studies of pattern formation.[38]

3.5. Acoustofluidics using localized phenomena

While many methods rely on global, chamber-scale acoustic fields, a significant branch of acoustofluidics leverages highly localized phenomena for precision manipulation.[121] Instead of creating large-scale standing waves, these devices use micro-machined features such as sharp edges or oscillating microbubbles that act as active acoustic actuators. When a global acoustic field is applied, these microstructures become sites of intense energy dissipation, generating stable and highly localized microvortices via acoustic streaming.[39,45] These well-defined vortices serve as non-contact micro-tools for trapping, concentrating, and rotating individual cells or small clusters with exceptional precision.[5,122]

This approach is particularly powerful for single-cell analysis and precision fluid control. For instance, one method for single-cell extraction uses a spiral array of micropillars. When subjected to circular vibration, this array generates a global whirling flow that transports cells toward a central trapping mechanism made of a thermo-responsive gel, which can then capture and release a single cell through temperature modulation (Figure 6A).[123] Another powerful technique achieves highly localized manipulation using 3D-printed, subwavelength micro-resonators. These mass-spring structures are driven into resonance by a global BAW, generating intense, localized acoustic fields that trap nearby particles. Because these resonators can be arranged into arbitrary patterns, this method enables the coordinated assembly of particles into complex, user-defined shapes, such as letters and logos (Figure 6B).[124] A versatile platform for dynamic control is the reconfigurable acoustofluidic metasurface, a passive

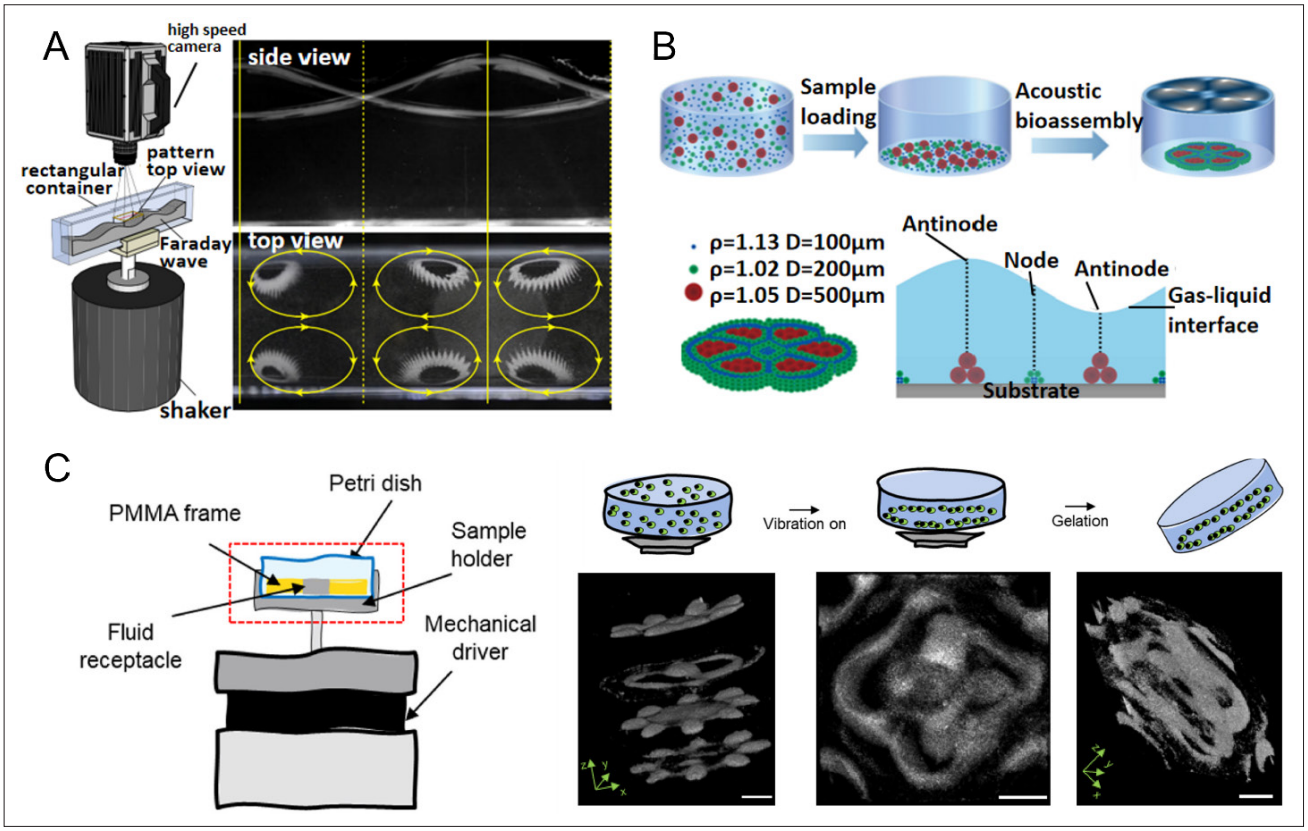

Figure 5. Principles and applications of cell patterning using Faraday waves. (A) Fundamental mechanism: vertical vibration of a fluid interface generates periodic standing waves. Reprinted from Ref. [93] The Faraday wave setup consists of a rectangular acrylic container (26.5 × 280 × 55 mm) connected to a vertical electromechanical shaker. (B) Differential bioassembly showing spatial segregation of heterogeneous cell populations based on intrinsic physical properties. Reprinted from Ref. [119] (C) Layer-by-layer fabrication strategy of 3D cellular constructs, shown with a computed tomography reconstruction of a multi-layered assembly. Scale: 3mm. Reprinted from Ref. [94] Abbreviation: PMMA, polymethyl methacrylate.

array of membrane resonators that generates localized, subwavelength standing waves. By tuning the frequency and phase of an external far-field acoustic source, the trapping field can be reshaped in real-time to enable not only collective patterning but also the precise translation and rotation of individual particles (Figure 6C).[125] Acoustic streaming can also be engineered for complex, multi-degree-of-freedom manipulations in 3D space, rooted in the precise control of 3D fluid phenomena.[126] By oscillating a single bubble trapped at a micropipette tip, a highly controllable 3D vortex is formed, which can serve as an acoustic "hand" for non-contact trapping, rotation, and cross-planar transport of objects ranging from single cells to spheroids (Figure 6D).[90]

Acoustofluidics based on localized phenomena provides a complementary toolkit to global-field techniques.[127] The primary advantage of this streaming-dominated approach is the unparalleled control it offers at the single-cell and small-volume scale. By engineering microfeatures to act as local actuators,[128–130] researchers can create dynamic, on-demand fluidic operations using simple external acoustic fields, making this an indispensable technology for single-cell genomics, rare-cell isolation, and point-of-care diagnostics.[131]

The diverse methodologies surveyed in this section—each leveraging acoustic phenomena in a unique manner—collectively form the versatile toolkit of acoustic lithography. To provide a clear comparative overview, the distinct characteristics and operational trade-offs of these principal techniques are summarized in Table 1. This synthesis highlights a clear progression in the field, moving from the generation of simple, periodic patterns using global resonance phenomena (BAW and Faraday waves) toward increasingly sophisticated and reconfigurable control over cellular organization. SAW technology represents a pivotal step in this evolution, offering higher resolution and seamless integration with microfluidic systems by confining energy to a substrate surface. This

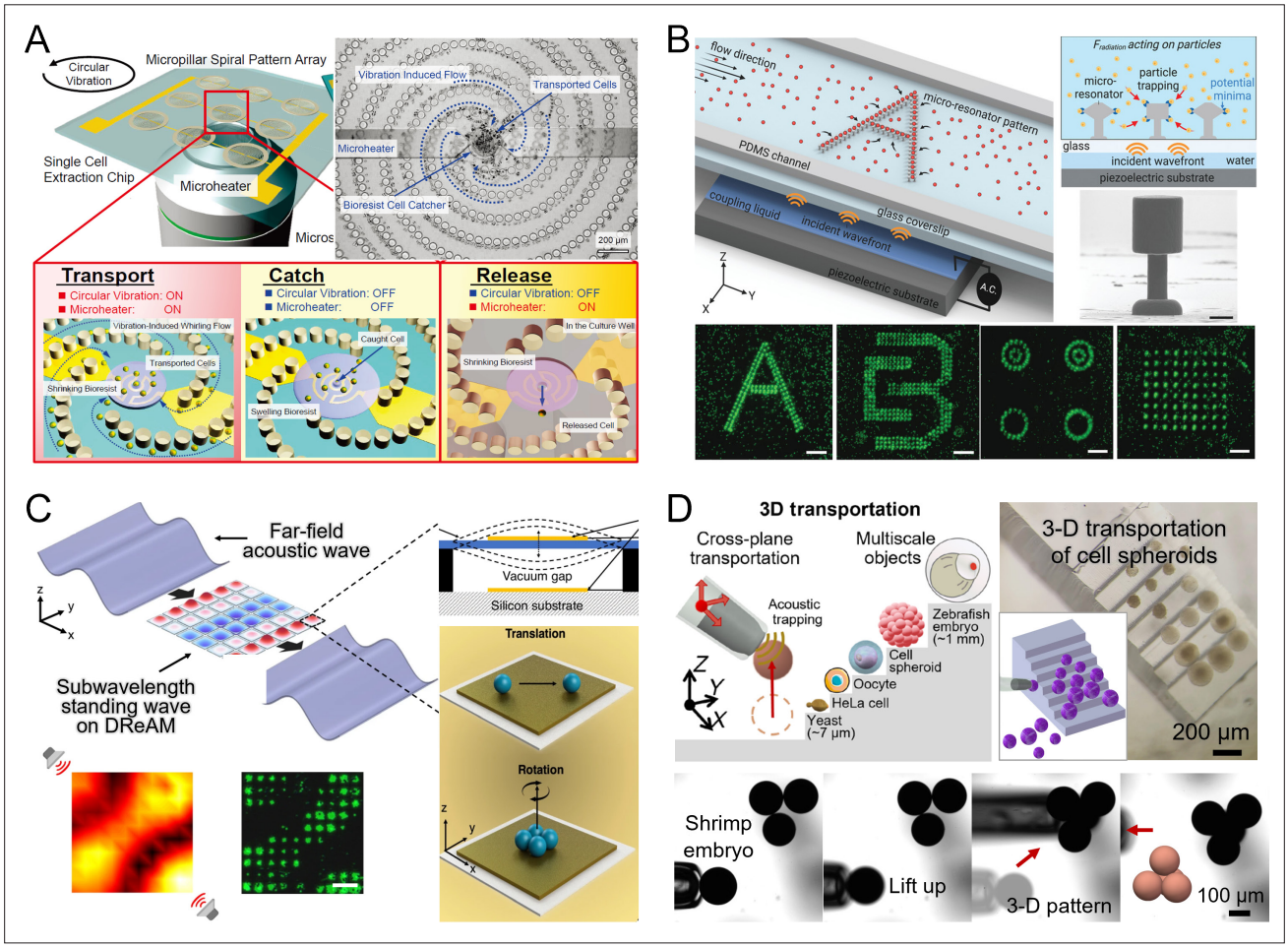

Figure 6. Precision microscale manipulation using localized acoustofluidic phenomena. (A) A single-cell extraction chip employing vibration-induced whirling flow from a spiral micropillar array for cell transport, combined with a thermo-responsive gel for capture and release. Reprinted from Ref. [123] (B) 3D acoustofluidics using sub-wavelength micro-resonators, where a global acoustic wave drives 3D-printed structures into resonance to create highly localized fields for trapping and assembling particles into arbitrary, user-defined patterns. Scale: 100 μm. Reprinted from Ref. [124] (C) A dynamically reconfigurable acoustofluidic metasurface that generates localized standing waves. Frequency and phase tuning reshape trapping patterns in real-time to enable particle translation and rotation. Scale: 200 μm. Reprinted from Ref. [125] (D) The μSonic-hand technique, in which an oscillating bubble at a pipette tip generates a controllable 3D vortex for complex manipulations including cross-planar cell transport. Reprinted with permission from Ref. [90] Copyright © 2025, American Association for the Advancement of Science. Abbreviations: DReAM, dynamically reconfigurable acoustofluidic metasurface; *F*, frequency; PDMS, polydimethylsiloxane.

enables more complex, yet still largely 2D, patterning capabilities. Acoustic holography offers a fundamentally new approach, moving beyond the constraints of device geometry to generate arbitrarily complex and, through phased arrays, fully dynamic 3D acoustic fields, providing unparalleled control over volumetric cell assembly.

Complementing these radiation-force-dominant methods, acoustofluidics based on localized streaming phenomena capitalizes on engineered microstructures to convert global acoustic fields into precise microscale fluidic operations. This approach excels in high-precision single-cell manipulation rather than large-scale patterning. The choice among these methods is therefore dictated by a balance of required resolution, desired pattern complexity, throughput, and the scale of the intended biological construct—reflecting a trade-off between the large-area patterning capabilities of simpler systems and the high-precision, dynamic control offered by more advanced technologies. Having established this diverse technological toolkit, researchers are now positioned to address key challenges across bio-systems engineering, as explored in the following section.

Table 1. Comparison of acoustic patterning methodologies

Methods	Characteristics	Resolution	Reconfigurability	Throughput	Typical applications
BAW	Generates strong volumetric force fields; pattern complexity limited by device resonance	Moderate (typically >100 μm)	Low: Produces simple, periodic 1D or 2D patterns (lines, grids); limited tuning via frequency changes	High	High-throughput cell alignment, patterned hydrogel fiber fabrication, cell washing
SAW	High resolution and microfluidic-compatible; energy confined near the surface	High (micron to sub-micron)	Moderate: Enables complex 2D patterns; dynamically reconfigurable with multiple IDTs	Scalable	Single-cell tweezers, dynamic pattern generation, biosensing
Acoustic holography	Allows arbitrary, user-defined 3D field shaping; complex dynamic control	High (wavelength-dependent)	High: Supports arbitrary 2D and 3D patterns; fully reconfigurable with phased arrays	Moderate to high	Complex tissue engineering, organoid assembly, targeted cell therapy delivery
Faraday waves	Simple, scalable, large-area patterning; limited to low-resolution periodic patterns	Low (mm to cm scale)	Low: produces large-area periodic patterns (squares, hexagons) with limited reconfigurability	High	Fundamental fluid physics studies, periodic cellular array creation, materials assembly
Acousto-fluidics	Enables high-precision single-cell manipulation; unsuited for large-scale patterning	Very high (single-cell level)	N/A	Low to moderate	Cell/particle sorting, focusing, single-cell analysis, rare-cell isolation

Abbreviations: BAW, bulk acoustic wave; IDTs, interdigital transducers; N/A, not available; SAW, surface acoustic wave.

4. Applications in bio-systems engineering

The fundamental physical principles of acoustic lithography, namely, its ability to exert gentle forces in a tag-free manner, make it an exceptionally versatile tool for bio-systems engineering. By preserving cell viability and native physiological states, this technology directly addresses the core limitations of many conventional manipulation techniques, propelling its adoption across a wide spectrum of applications. This section reviews the remarkable impact of acoustic lithography, tracing its evolution from foundational research tools to complex, multi-component systems with direct translational relevance. We survey its applications in foundational cell analysis, the engineering of biomimetic co-culture systems, advanced biofabrication, and its pivotal role in high-throughput sorting for clinical diagnostics, thereby illustrating how precise physical control can be translated into sophisticated biological function. The section concludes with an engineering perspective that synthesizes these applications, illustrating the conceptual progression from simple structural patterning to the sophisticated regulation of biological function.

4.1. Cell manipulation and analysis

As the fundamental units of life, the structure, function, and behavior of cells are directly linked to key biomedical questions concerning physiological regulation, disease mechanisms, and drug responses. Precise and efficient cellular analysis is therefore essential. It not only advances our understanding of fundamental life processes but is also critical to evaluating novel therapeutics, elucidating complex systemic interactions, and improving clinical prognostics in precision and regenerative medicine.[132–135] While traditional methods are often limited in precision, throughput, or biomimetic capabilities, acoustic techniques offer innovative solutions by leveraging their non-invasive, label-free, and dynamically controllable characteristics.[136] For instance, acoustic tweezers have become powerful tools for probing biophysical properties at a fundamental level through applications like force spectroscopy.[137] By trapping and applying piconewton-level forces to microbeads attached to a single molecule, these systems enable high-throughput measurements of molecular interactions.[138] Furthermore, localized acoustic streaming can precisely rotate single cells, facilitating multi-angle 3D imaging or detailed studies of cellular mechanics.

These foundational capabilities are increasingly integrated into sophisticated microfluidic platforms to achieve complex analytical tasks. A prominent application is sheathless particle separation, which addresses a key limitation of many flow-based sorting systems.[139] One such device employs a two-stage microfluidic design: standing SAWs first focus a mixed population of particles into a single stream without requiring sheath flow, thereby avoiding sample dilution and system complexity.[140,141]

In the second stage, the aligned particles are exposed to a different acoustic field that drives them toward off-center pressure nodes, enabling size-based separation into multiple collection outlets (Figure 7A).[142] This integrated approach provides a basis for high-throughput, contact-free sorting of cells and exosomes. Beyond sorting, acoustic energy has been incorporated into culture platforms for biofabrication.[143] Standing SAWs have been used to rapidly form cell spheroids for applications such as bioinks. This method has been shown to be highly biocompatible: cells patterned using both low-frequency (10.4 MHz) and high-frequency (23.8 MHz) excitation maintained viability above 90% for up to 7 days, with no statistical difference from the unmanipulated control groups, confirming the safety of the patterning process (Figure 7B).[140]

Furthermore, acoustic levitation has been developed into a robust, scaffold-free method for long-term 3D cell culture. Multi-trap acoustic levitation platforms can produce and maintain hundreds of uniform mesenchymal stem cell spheroids in parallel for over 24 hours (Figure 7C).[144] Cells in these levitated spheroids remain viable, maintain their characteristic surface markers, and exhibit a higher differentiation capacity compared to standard 2D cultures, highlighting the technology's potential for creating highly biomimetic models for drug screening and tissue engineering. To quantify this enhanced potential, Guo *et al.* applied programmable acoustic stimulation at varying power levels (300, 400, and 500 mW) to mesenchymal stem cell spheroids (Figure 7D).[145] Fluorescence images of the stimulated cells and analysis of molecular uptake, such as doxorubicin, showed a clear power-dependent effect. Their results confirm that precisely manipulating mechanical forces and inducing cell deformation via acoustic streaming enables controlled modulation of cell membrane permeability. This provides a promising new tool for drug delivery and cell therapy.

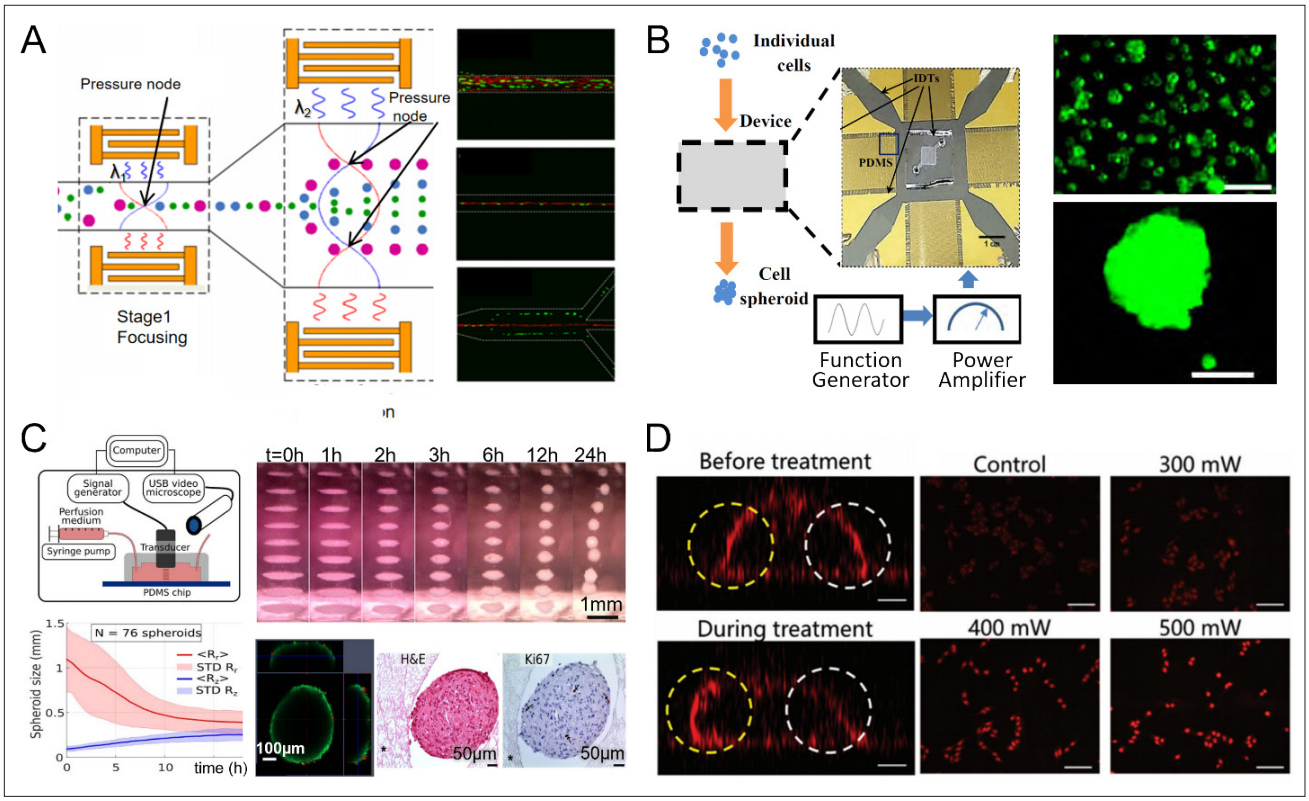

Figure 7. Acoustofluidic cell sorting and spheroid culture. (A) A two-stage surface acoustic wave (SAW) sorting strategy using wavelength switching for size-based separation. Fluorescent images show the distribution of 10 μm (green) and 3 μm (red) particles. Reprinted from Ref.[142] (B) Demonstration of high biocompatibility during spheroid formation with standing SAWs; viability remains above 90% at both low and high frequencies, comparable to controls. Scale: 20 μm. Reprinted from Ref.[140] (C) On-chip formation and functional characterization of viable, proliferative spheroids, including quantitative growth curves and histological analysis. Scale: 1 mm, 100 μm, and 50 μm, respectively. Reprinted from Ref.[144] (D) Programmable acoustic streaming applied to cells at varying power levels to modulate membrane permeability. Representative fluorescence images show power-dependent uptake of doxorubicin, demonstrating that acoustic stimulation can control intracellular transport. Scale: 5 μm. Reprinted from Ref.[145] Abbreviation: PDMS, polydimethylsiloxane.

4.2. Engineering co-culture systems

In vitro cell culture is essential for applications such as drug screening and the study of cell–cell interactions.[146] However, traditional monocultures fail to replicate the complex *in vivo* environment, where cells interact with neighboring cells, the extracellular matrix, and soluble biochemical factors. Co-culture systems, which incorporate two or more distinct cell types, partially address this limitation and are crucial for studying heterotypic interactions.[147]

Nevertheless, conventional co-culture methods often lack sufficient spatial control and microenvironmental regulation, making it difficult to replicate the dynamic interplay found in tissues such as the tumor microenvironment. Acoustic technology offers a novel solution to these challenges.[148] Its non-contact, biocompatible, and highly adjustable nature enables the precise spatial arrangement of multiple cell types and the dynamic regulation of the microenvironment,[149] providing a technical foundation for constructing highly biomimetic co-culture systems.[150] This engineering capability allows for the creation of specific 3D configurations, such as core–shell structures that mimic vascularized tissues, by sequentially patterning different cell types.[151,152]

Recent advancements have demonstrated the power of acoustics in constructing sophisticated co-culture models that more faithfully recapitulate physiological and pathological conditions.[153,154] In one approach to simulate tumor–immune interactions, tumor cells are first mixed with an acoustic virtual 3D scaffold hydrogel to promote self-assembly into tumor-like tissues, which are then co-cultured with T cells (Figure 8A).[155] Further, acoustic trapping technology can overcome the spatial limitations of traditional methods by precisely assembling tumor, immune, and stromal cells into 3D multicellular clusters (Figure 8B).[156] This system facilitates direct drug treatment within a biomimetic microenvironment and allows real-time monitoring of tumor cell survival, creating an efficient platform for rapidly screening personalized drug regimens.

Acoustic streaming effects can also be harnessed to dynamically regulate the culture environment by enhancing convective transport. For example, in a vascular co-culture model, the application of acoustic streaming improved barrier function—as evidenced by a 50% reduction in macromolecular leakage (Figure 8C)[157]—by promoting the diffusion of paracrine factors that strengthen cell junctions. Moreover, standing SAWs have been applied to fabricate functional therapeutic tissues that mimic the structure of natural vasculature. In this application, endothelial cells and adipose stem cells are co-aligned into 3D collateral cylindroids within a biocompatible hydrogel. This precise acoustophoretic patterning enhances cell–cell contacts and the secretion of angiogenic and anti-inflammatory factors, which, when tested in an *in vivo* ischemia model, lead to remarkable recovery of damaged tissue (Figure 8D).[89] This enhanced function arises because the acoustic field can act as a surrogate for the *in vivo* mechanical microenvironment: by physically condensing cells, the acoustic force promotes the critical cell–cell interactions and paracrine signaling essential for tissue maturation and function.

4.3. Biofabrication and tissue engineering

The construction of functional tissues and organs is a critical goal in biomedicine,[158] aimed at addressing organ transplant shortages and advancing disease modeling.[159] Tissue engineering combines biomaterials, cells, and growth factors to repair or replace damaged tissues. However, traditional methods often struggle to create complex structures, facing issues like uneven cell distribution and inefficient nutrient delivery.[160]

Acoustic technology, with its non-invasive and tunable properties, offers new methodologies to overcome these limitations. It enables the precise, contactless assembly of cells and biomaterials into organized structures, facilitating the fabrication of tissues that better mimic native complexity.[161,162] This has been demonstrated in the creation of functional tissues such as anisotropic muscle fibers and aligned cardiac tissue, where cellular organization is critical for function.[163] A key challenge in engineered tissues is vascularization; acoustic patterning has proven highly effective at organizing endothelial cells into predefined networks within scaffolds, significantly enhancing their integration with host vasculature upon implantation.[164]

The versatility of acoustic biofabrication is highlighted by its diverse applications across tissue types and fabrication strategies. A significant innovation is the integration of acoustics with volumetric 3D printing, a method termed SonoPrint. This technique first employs an acoustic standing wave field to organize reinforcement particles into complex, user-defined patterns—such as parallel lines, hexagons, or polygons—within a photosensitive liquid resin. A volumetric printer then solidifies the entire 3D structure simultaneously by projecting a series of patterned light images onto the rotating vial. This approach fabricates a complete composite object within minutes, embedding the preformed acoustic pattern throughout the volume and enhancing the final structure's mechanical properties (Figure 9A).[165]

Subsequent polymerization of the surrounding gel fixes the configuration, allowing the cells to proliferate and form hollow tubular structures that mimic blood vessels or nerve bundles. In another application, ultrasonic standing waves

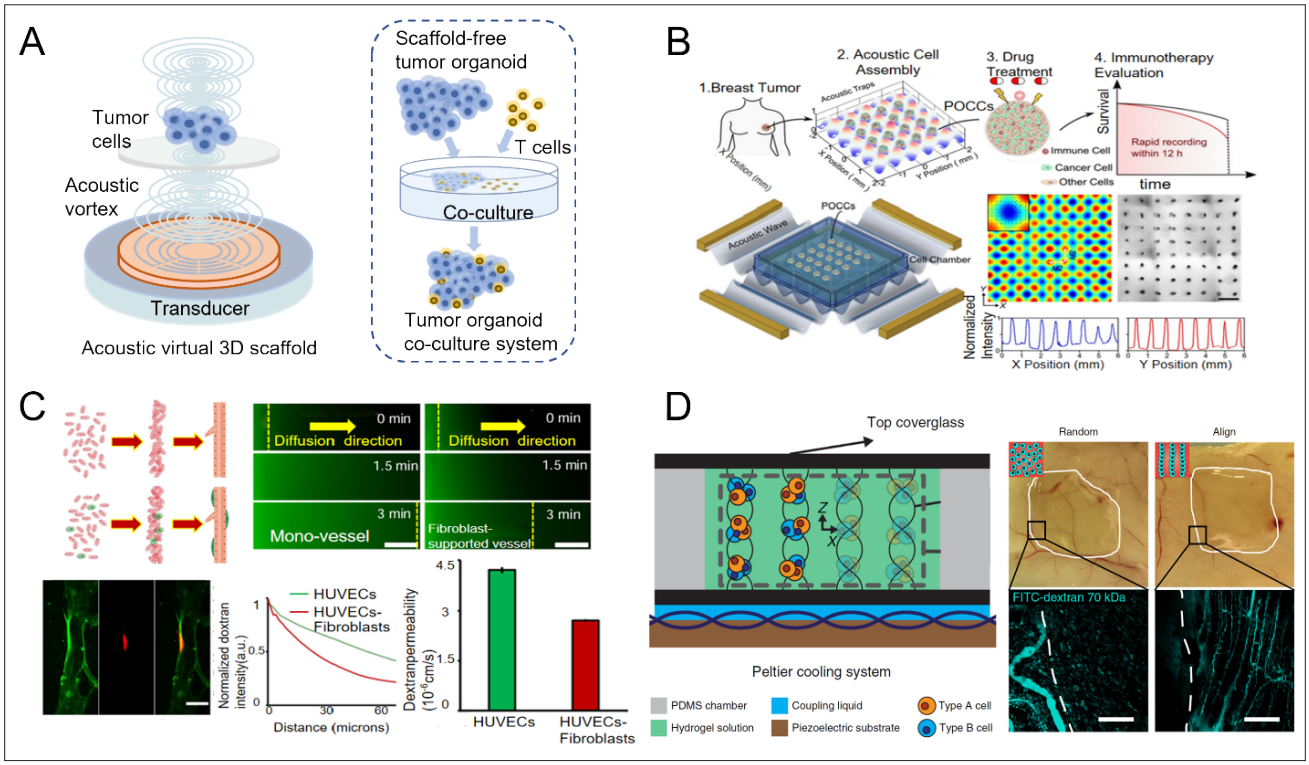

Figure 8. Acoustically engineered biomimetic co-culture models for advanced tissue microenvironment studies. (A) Tumor organoid formation via acoustic-assisted self-assembly in AV-Scaf hydrogel. Adapted from Ref. [155] (B) High-throughput tumor-immune droplet platform for real-time cytotoxicity screening and drug testing. Reprinted from Ref. [156] (C) Acoustic streaming enhances vascular barrier function in an endothelial–fibroblast co-culture model, demonstrated by reduced macromolecular leakage. Scale: 1 mm. Reprinted from Ref. [157] (D) High-resolution 3D cell patterning to construct functional vascular tissue: standing surface acoustic waves co-align endothelial and stem cells into therapeutic cylindroids within a hydrogel, restoring function in a mouse ischemia model. Scale: 200 μm. Reprinted from Ref. [89] Abbreviations: AV-Scaf, acoustic virtual 3D scaffold; FITC, fluorescein isothiocyanate; PDMS, polydimethylsiloxane; POCCs, primary tumor-derived organotypic cell clusters.

have been used to manipulate chondrocytes in an agarose solution, guiding them into a radially layered structure that recapitulates the zoned structure of natural cartilage (Figure 9B).[166] After thermal gelation and *in vitro* culture, this process yields engineered cartilage with a distinct, biomimetic organization. ARF also provides a flexible strategy for patterning cells suspended in a photosensitive hydrogel precursor into complex arrangements like stripes or arrays, which are subsequently fixed through UV-induced polymerization (Figure 9C).[98]

Significant advances in precision patterning are also being made through analytically driven holographic methods. A prime example is acoustography, which uses analytically designed IDTs to generate intricate, configurable 3D acoustic fields based on orbital angular momentum beams (Figure 9D).[167] This platform achieves enhanced resolution with a pixel size of ~25 μm and has been used to pattern microparticles into complex 3D structures, such as a triple helix, and to manipulate DNA carriers. This approach represents a move toward high-fidelity, configurable patterning for advanced biofabrication. Another distinct concept in acoustic additive manufacturing is holographic direct sound printing, in which acoustic holograms induce sonochemical polymerization for rapid, remote fabrication through barriers.[168]

4.4. Sorting for clinical applications

Cell separation and sorting are indispensable processes in biomedical applications, including diagnostics and therapeutics.[169–171] This is particularly crucial in oncology, where the isolation of rare circulating tumor cells (CTCs) from blood provides invaluable insight into a patient's disease status.[172–174] The extreme rarity of CTCs necessitates separation methods that are not only highly efficient and pure but also gentle enough to preserve cell viability for downstream analyses such as drug sensitivity testing and *in vitro* expansion. In this context, acoustic patterning has emerged as a robust, label-free strategy for CTC isolation that maintains high cell viability and supports high-throughput processing.

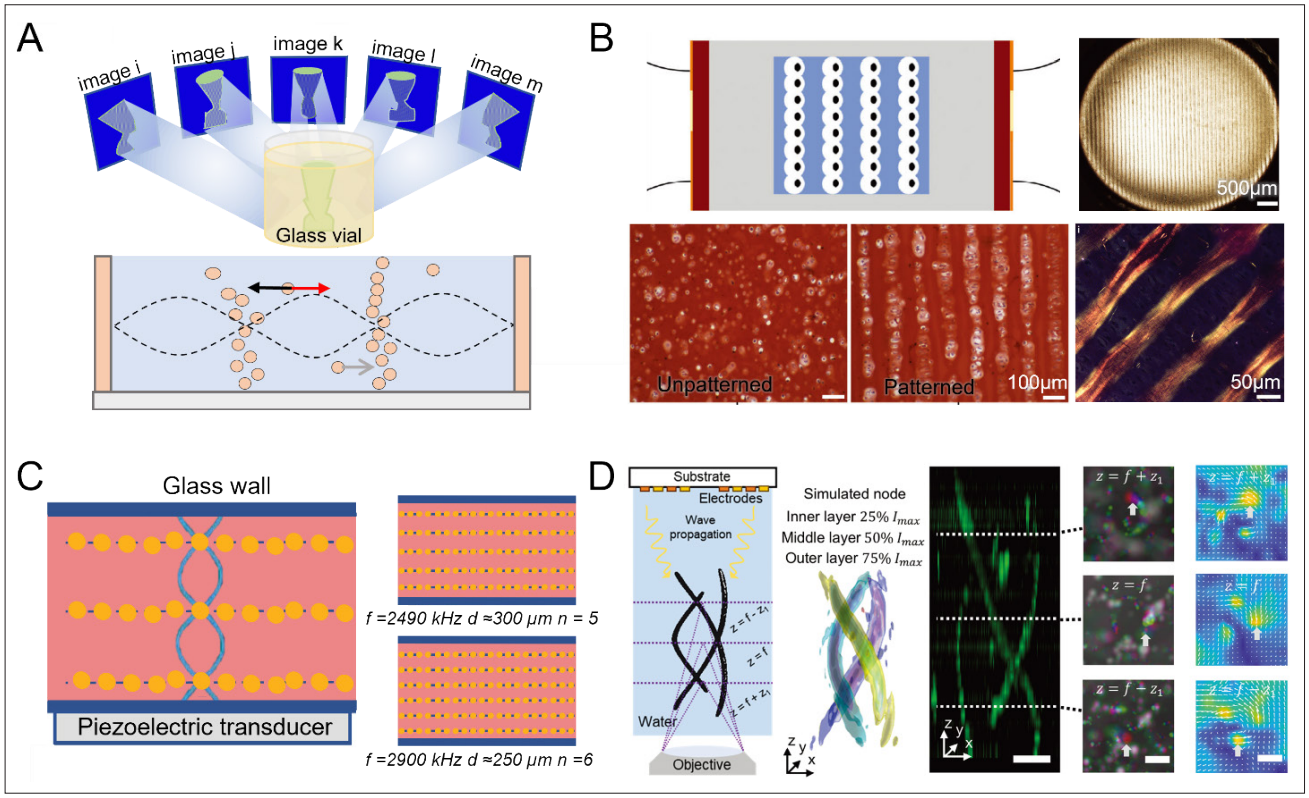

Figure 9. Advanced biofabrication strategies for tissue engineering using physical fields. (A) SonoPrint: an acoustically assisted volumetric 3D-printing method in which an acoustic field first patterns reinforcement particles within a photosensitive resin before simultaneous polymerization to produce mechanically enhanced composites. Adapted from Ref. [165] (B) Ultrasound standing-wave patterning of chondrocytes in agarose followed by thermal gelation to create structured cartilage. Reprinted from Ref. [166] (C) Patterned cell organization within a phase-changeable hydrogel for constructing 2D and 3D tissue models. Adapted from Ref. [98] (D) Advanced 3D acoustography using the BEACON platform, demonstrating correspondence between a simulated 3D triple-helix acoustic trap and experimentally patterned microparticles (captured using confocal microscopy). The technology uses an analytically designed interdigital transducer to generate complex, reconfigurable acoustic fields. Scale: 100 μm. Reprinted from Ref. [167] Abbreviations: BEACON, beam engineering and acoustic control node; *d*, diameter; *f*, frequency.

A variety of acoustic platforms have been developed to meet this clinical need, each leveraging a distinct strategy to isolate CTCs from the much more numerous blood cells. One prominent BAW-based approach utilizes a two-stage acoustic process within a microfluidic chip. First, a high-frequency pre-focusing wave aligns all cells into a single band. Subsequently, a lower-frequency separation field exploits intrinsic differences in cell size, density, and compressibility; the larger CTCs migrate more rapidly toward the central pressure node for collection, while smaller blood cells are carried away. This method has successfully enriched CTCs from whole blood with higher sensitivity than commercial platforms, providing a new liquid biopsy tool (Figure 10A).[175]

Another strategy employs a two-step acoustophoretic method designed for processing whole blood after red blood cell lysis. An initial acoustophoresis step enriches a population of CTCs based on their acoustic properties, followed by a secondary purification step that uses antibody-functionalized particles to deplete residual leukocytes via negative selection. This gentle, contact-free approach delivers viable cancer cells suitable for *in vitro* culture, paving the way for personalized therapeutic strategies (Figure 10B).[176]

Innovation has also come from hybridizing acoustic forces with other physical principles. One multistage platform combines inertial and acoustic forces: a helical microchannel first uses inertial forces to pre-focus the cells, after which the cells enter a straight segment where a SAW field generates multiple pressure nodal lines. Cells are then captured and aligned on these lines according to their diameter, achieving rapid separation of CTCs, leukocytes, and erythrocytes (Figure 10C).[88] To further enhance capture efficiency, other platforms have applied an alternating-frequency acoustic field to a piezoceramic microfluidic chip. This approach has successfully isolated cancer cells from various tumor origins with a capture efficiency exceeding 94% and has been validated on patient

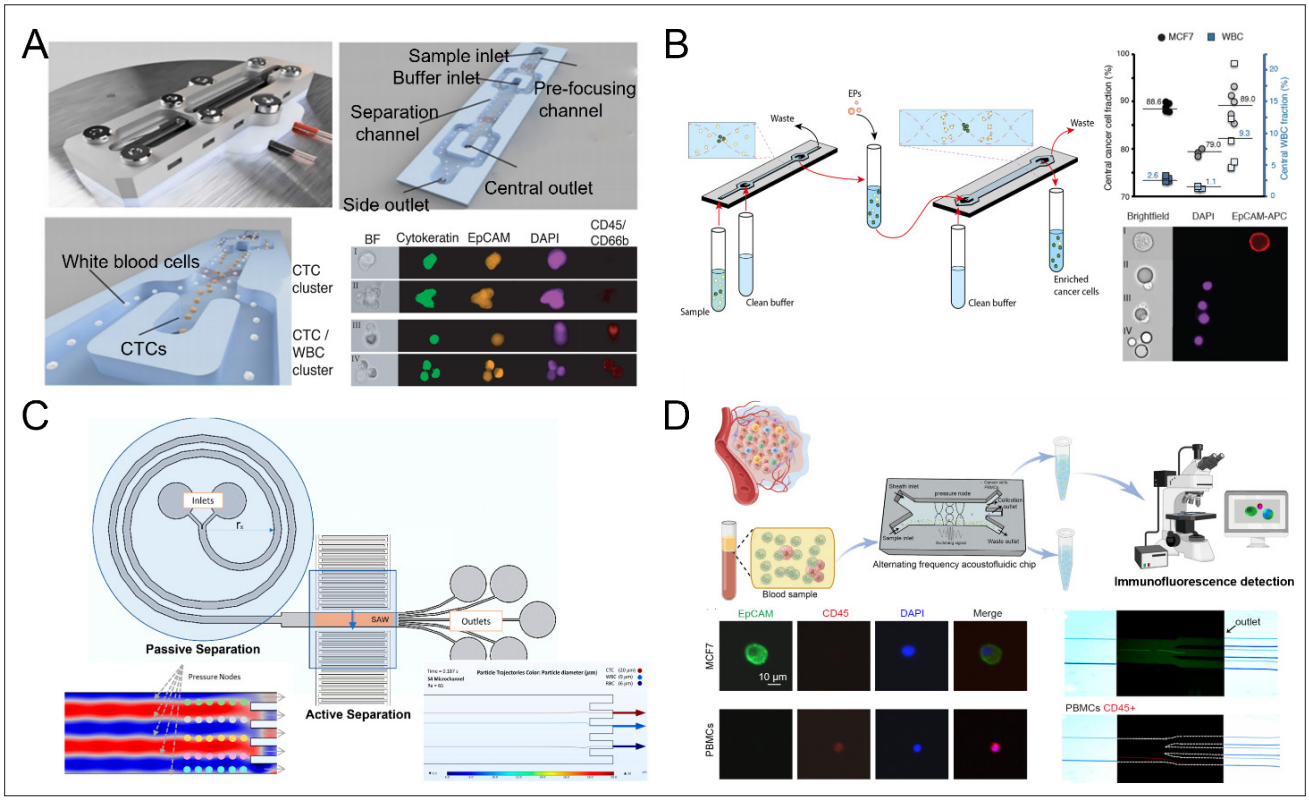

Figure 10. Acoustofluidic platforms for clinical cell sorting. (A) Illustration of the acoustofluidic microchip and the cell separation principle. The device features an initial prefocusing channel (20 mm × 300 μm × 150 μm) for cell aggregation. Reprinted from Ref. [175] (B) A two-step acoustophoresis method for separating live tumor cells from whole blood, utilizing a microchannel with dimensions of 30 mm × 380 μm × 150 μm. Reprinted from Ref. [176] (C) Schematic of a hybrid spiral microfluidic platform combining passive and active IDT components for cell focusing and separation. Reprinted from Ref. [88] (D) Schematic diagram of the separation process in an alternating frequency acoustofluidic chip. Scale: 10 μm. Reprinted from Ref. [177] Abbreviations: APC, allophycocyanin; BF, bright field; CD, cluster of differentiation; CTCs, circulating tumor cells; DAPI, 4',6-diamidino-2-phenylindole; EpCAM, epithelial cell adhesion molecule; PBMCs, peripheral blood mononuclear cells; WBC, white blood cell; n.s., no significance

samples spanning different cancer types. A key advantage of this method is that effective separation is achieved even when CTCs and other blood cells exhibit similar sizes. These examples collectively underscore the significant clinical potential of acoustic lithography as a robust and versatile liquid-biopsy tool for oncology and, more broadly, for applications such as non-invasive prenatal diagnostics and stem cell therapies (Figure 10D).[177] The high viability preserved in these sorting applications is a key advantage, stemming directly from the technology's mechanism: acoustic forces are inherently gentle, label-free, and operate in native physiological media, avoiding the high shear stresses or harsh chemical treatments of many other sorting methods.

4.5. Considerations for bio-systems engineering

A critical challenge for bio-systems engineering is translating these dynamic patterning methods into stable, functional constructs without compromising cell viability. Because acoustic forces merely hold cells in place, the transient pattern must be fixed via a rapid crosslinking step. This introduces the main engineering constraint: the fixation process itself must be both rapid and cytocompatible, whether through photopolymerization of materials such as gelatin methacrylate,[93] thermal gelation of hydrogels like agarose,[166] or chemical crosslinking of materials such as hyaluronic acid–catechol.[89] A successful workflow must precisely tune the acoustic patterning time, which may be only seconds, to match the material's gelation kinetics, ensuring both high pattern fidelity and high cell viability.

The preceding sections have detailed a wide range of applications, from high-throughput sorting to high-precision 3D assembly. To provide researchers with a clear, implementation-level quantitative reference, Table 2 summarizes the typical performance metrics, resolution, and compatible fixation methods for these applications. This quantitative summary leads into the following discussion, which steps back from these specific

applications to synthesize the unifying engineering logic underlying the field's trajectory.

4.6. Broader perspectives: from patterning to function

The applications detailed above showcase the current capabilities of acoustic lithography. To chart its future trajectory, however, it is instructive to step back and recognize that these specific successes are manifestations of a universal engineering principle: the precise command of acoustic and other physical fields to organize matter and orchestrate function.[57] By placing acoustic lithography within this broader context, we can identify shared principles with other advanced scientific domains and draw inspiration for future innovation.[51,56] This perspective reveals a common, multi-scale engineering logic for acoustic control that progresses from manipulating foundational structure to dynamically shaping the environment and, ultimately, regulating emergent biological function.

The principle of controlling structure to dictate function is fundamental across engineering disciplines. Its importance is well-established in materials science, where creating hierarchical structures from base materials is a core strategy for achieving desired macroscopic properties, as seen in the fabrication of advanced functional materials.[38,41,178–182] This same principle is evident in food science, where preserving the integrity of the cellular matrix is the primary determinant of food texture and quality.[183–185] The innovation of acoustic lithography lies in providing the acoustic tools needed to apply this established principle to living systems, building upon seminal works that demonstrated the ability to create arbitrary, complex structures using acoustic holography.[91,109,110] By consciously borrowing concepts of hierarchical assembly validated in materials science, future acoustic control strategies could be designed not only to position cells but also to guide their multi-scale self-organization, moving beyond simple patterning[186–191] toward more sophisticated forms of acoustic structural bio-control.

Beyond static structure, truly functional systems require the active engineering of their operational environment. To understand how to achieve this, we can draw valuable insights from parallel fields. In antimicrobial technology, for instance, the goal is to create microenvironments hostile to pathogens, using either natural compounds or targeted physical energy.[192–202] Similarly, advanced nano-

Table 2. Performance metrics for typical acoustic lithography applications

Application/ goal	Methods	Scale/ throughput	Patterning time	Post-patterning viability	Key outcome/ metric	Compatible fixation/ crosslinking	Ref.
Spheroid formation	BAW levitation	High (parallel, >100/run)	~15–30 min	High (no necrosis)	High uniformity in size & shape	Scaffold-free	[144]
Anisotropic tissue patterning	BAW adhesion/ SAW in gel	Medium (cm-scale)	~10 s–30 min	~90%	High cell alignment (>80% oriented)	Oxidative (HA–CA) or adhesion	[98,89]
Zoned cartilage engineering	Standing waves	Low-medium	~10 min	High (n.s. vs control)	Zoned matrix deposition	Thermal (agarose)	[166]
Complex volumetric 3D assembly	Acoustic holography	Low-medium (volumetric)	~1 min	High (n.s. vs control)	Arbitrary 3D complexity	Chemical (hydrogel) or culture	[91,92]
Large-area periodic patterning	Faraday waves	Very high (wafer-scale)	< 5 min	>95%	Large-scale order; simplicity	Photopolymerization	[93,94]
Complex co-culture assembly	Acoustic trapping	Low (cluster-by-cluster)	~10–20 min	N/A	Heterogeneous 3D clusters	Culture	[156]
Clinical sorting	Acoustophoresis	~75 µL/min	Continuous	N/A (fixed cells)	CTC & cluster detection	N/A	[88]
High-precision single-cell analysis	Localized streaming	Very low (single-cell)	Continuous/< 1 min	>90%	Precise 3D rotation & positioning	N/A	[90]

Abbreviations: BAW, bulk acoustic wave; CA, catechol; CTC, circulating tumor cell; HA, hyaluronic acid; n.s., no significance; N/A, not available; SAW, surface acoustic wave.

delivery systems are engineered to transport and release bioactive compounds in a controlled manner.[160] This vision of environmental engineering is directly achievable in acoustofluidics, a concept articulated in extensive reviews on the applications of acoustic streaming.[79] By integrating these ideas, we can envision the next generation of acoustic platforms in which the dynamic fluid control provided by acoustic streaming is combined with acoustically triggered release from nanocarriers. Such systems would transform acoustic devices from simple cell patterning tools into microenvironment regulators capable of creating truly biomimetic *in vitro* systems.

The pinnacle of this engineering logic is the direct regulation of function. This goal is exemplified in biotechnology, where microbial consortia in bioreactors are precisely controlled to optimize the production of therapeutics or chemicals, and physical fields such as ultrasound are used to enhance reaction efficiency. The key lesson here is the shift from merely providing an environment to using sound to actively direct a system's dynamic behavior and functional output.[92,157,203–210] This represents the ultimate frontier for acoustic lithography. The concept is rooted in the broader field of ultrasound-responsive systems, where acoustic energy is already used to trigger drug release or modulate biological barriers.[38] By learning from the control strategies used in bioreactors, acoustic fields could be programmed to guide stem cell differentiation *in situ* through targeted mechanotransduction or to enhance the efficacy of immunomodulatory drugs.[150,154,211–213] This approach would establish a new class of "acousto-ceuticals," in which acoustic energy becomes an active, programmable regulator of biological processes.

5. Bottlenecks and challenges

While the near-term outlook detailed in the following section is promising, the widespread adoption of acoustic lithography in clinical settings hinges on addressing several fundamental challenges. One of the most significant hurdles is the persistent trade-off between manipulation resolution and throughput. Although techniques like acoustic holography offer unprecedented pattern complexity, achieving true single-cell or even subcellular precision in dense, 3D environments remains a formidable challenge. Future research must focus on developing novel acoustic field-generation methods, potentially leveraging acoustic metamaterials or phononic crystals, to overcome the diffraction limit without sacrificing the ability to process biologically relevant sample volumes.

Another critical area requiring systematic investigation is the biological impact of acoustic exposure. Although acoustic methods are generally considered highly biocompatible, a comprehensive understanding of the dose–response relationship between specific acoustic parameters (frequency, intensity, duration) and long-term cellular fate is still lacking.[214] Standardized protocols and reporting metrics are urgently needed to enable cross-platform comparisons. Future studies must move beyond simple viability assays to explore how acoustic forces influence complex cellular processes such as mechanotransduction pathways, gene expression, and epigenetic modifications. Understanding these mechanisms is critical not only for ensuring the safety and efficacy of therapeutic applications but also for establishing the biosafety and ethical guidelines required for responsible *in vivo* translation.[215]

Furthermore, translating these technologies for *in vivo* applications presents a unique set of obstacles. These include the miniaturization of acoustic devices for implantation, integration with real-time imaging, and efficient transmission of acoustic energy through heterogeneous biological tissues. Moreover, the inherent system complexity and cost of advanced platforms, such as multi-transducer phased arrays or holographic setups, may limit their practical adoption outside specialized centers. The development of intelligent, biocompatible micro-robots powered and controlled by ultrasound represents an exciting frontier, but achieving this vision will require significant advances in materials science, robotics, and control theory before clinical deployment becomes feasible.[216]

6. Conclusion and outlook

Acoustic lithography has matured from a promising set of techniques into a versatile and powerful platform for bio-systems engineering, bridging the gap between high-throughput assembly and single-cell precision.[217] By leveraging the gentle, non-contact forces of acoustic radiation and streaming, it overcomes various limitations of conventional methods, offering unparalleled biocompatibility in native physiological media. This review has surveyed the fundamental physical principles, diverse methodologies—from BAW and SAW to advanced acoustic holography—and the broad applications of these tools in orchestrating cellular structures.[218] The preceding discussion on broader engineering principles places these achievements in context. It crystallizes the review's control-oriented perspective and highlights the field's clear engineering logic: a progression from controlling structure, to engineering the environment, and finally to regulating biological function. By drawing inspiration from established approaches in other engineering domains, the field can accelerate its transition from a manipulation tool to a functional control system.

Looking forward, the field is poised for transformative growth, likely to be driven by deeper integration with other cutting-edge technologies. Synergy with artificial intelligence and machine learning, for instance, could automate the design of highly complex, bespoke acoustic fields, accelerating research in developmental biology and regenerative medicine.[219–223] Combining acoustic patterning with 3D bioprinting and smart, acoustically responsive materials will unlock possibilities for creating four-dimensional constructs that change their shape or function over time in response to acoustic stimuli.[224,225] These near-term advancements will push the boundaries of what is possible in fabricating dynamic, life-like systems *in vitro*.

Ultimately, acoustic lithography is catalyzing a transformation in bio-systems engineering. As the technology matures and becomes more accessible, its translation from the research laboratory to clinical settings, for applications ranging from point-of-care diagnostics to *in situ* tissue repair, will become increasingly viable. By addressing the fundamental challenges of precision, control, and standardization, the field is well-positioned to unlock new therapeutic strategies and usher in a new era of engineered biology and regenerative medicine.

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Conflict of interest

The authors declare that they have no competing interests.

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