



Advances in micropillar arrays in cellular biomechanics detection and tissue engineering

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Abstract: Cellular biomechanical features contributed to the occurrence and development of various physiological and pathological phenomena. Micropillar arrays have emerged as an important tool for both the assessment and manipulation of cellular biomechanical characteristics. This comprehensive review provides an in-depth understanding of the fabrication methodologies of micropillar arrays and their applications in deciphering and fine-tuning cellular biomechanical properties and the innovative experimental platforms including organ-on-a-chip and organoids-on-a-chip. This review provides novel insights into the potential of micropillar technology, poised to update the landscape of stem cell research and tissue engineering.

Introduction

Cellular biomechanics, a fascinating field of study, exerts influences on a myriad of cellular behaviors and is involved in the occurrence and development of human diseases [1,2]. From the control of cell migration [3,4] to the orchestration of differentiation biological processes [5] and even shaping intracellular responses to external stimuli [6,7], the regulatory role of cellular biomechanics in cell function is indisputable. In tissue engineering, the ability to finely tune and modulate these behaviors represents a potential goldmine for regenerative therapies. While the classical approach has long relied on chemical cues to direct stem cell fate [8], recent research has thrown the spotlight on the role of mechanical cues, such as micropatterns, in shaping and guiding cellular behavior.

Mechanical cues, stemming from micropatterned surfaces, trigger a cascade of events within cells. They guide cell contact, induce reorganization of the cell's internal cytoskeleton, and even the deformation of the nucleus including orientation, morphology, Lamin A/C levels, and chromatin condensation degree [9,10]. What makes this even more intriguing is the diverse cellular responses observed depending on the specific geometric configuration of the micropatterns.

Micropillar arrays, consisting of precisely engineered cylindrical or pillar-like structures on substrates, present a controlled platform for exploring cellular responses to mechanical cues [11]. When mesenchymal stem cells (MSCs) were grown on triangular prism micropatterns, the well-structured F-actin fibers span the entire cell, efficiently transmitting compressive forces to the nucleus and resulting in the flattening of the nucleus and a stretching of nuclear pores, enhancing the nuclear import of YES-associated protein (YAP). Conversely, the story takes a different turn when cells grow on cylinder micropatterns, which exert a comparatively less compressive force on.

The nucleus, leading to a reduced translocation of YAP into the nucleus and a corresponding decrease in the secretion of angiogenic growth factors. The enhancement of the paracrine activity of MSCs facilitated tissue regeneration [9]. Micropillar arrays have become an exciting avenue for investigating and understanding how cells respond to mechanical cues, arrays represent a cutting-edge tool in the modulation of cellular behavior through biomechanical stimuli, opening up exciting new horizons in the field of tissue engineering and regenerative medicine (Fig. 1). Utilizing multilayer microfluidic chips integrated with polydimethylsiloxane (PDMS) micropillar arrays has been harnessed for the cultivation of specific brain region organoids derived from human induced pluripotent stem cells (hiPSCs) [12].

This review aimed to introduce the applications, fabrication techniques, and underlying mechanisms of micropillar arrays in cellular biomechanics detection and

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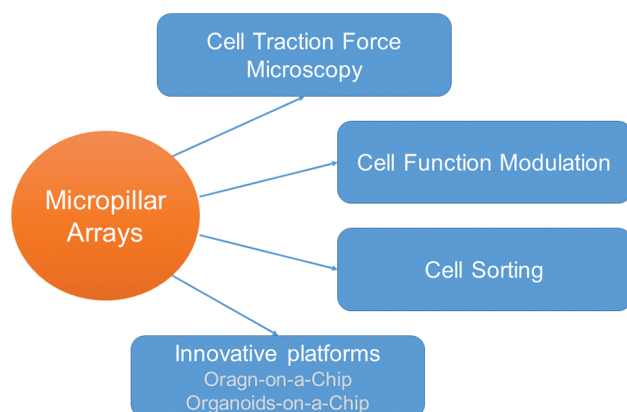


FIGURE 1. Application of micropillar arrays in cellular biomechanics detection and tissue engineering.

tissue engineering. We analyzed their utility in quantifying cell traction forces, assessing cellular mechanical properties, and guiding cell separation and stem cell differentiation. Additionally, we reviewed the innovative platforms enabled by micropillar arrays, including organ-on-a-chip and organoids-on-a-chip systems.

Fabrication of Micropillar Arrays

Soft lithography and micromolding

Soft lithography [13,14] and micromolding [15,16] are two well-established techniques for fabricating micropillar arrays. Micromolding involves casting a polymer material into a mold to create micropillar structures, offering high reproducibility and scalability for large-scale production [17].

Soft lithography, which emerged in the late 1990s, utilizes elastomeric materials like PDMS to create micro-holes as molds for micropillar arrays, which provide high resolution and precise control of micropillar dimensions [18].

Soft lithography replaces traditional lithography with elastic molds, mainly PDMS, for producing microstructures or micromolds. This technology offers flexibility and advantages such as the ability to create complex multi-layer or three-dimensional structures, even on irregular surfaces. Currently, soft lithography is applied in biology, particularly for controlling molecular composition on surfaces for cell fate control [19,20].

3D printing and nanofabrication

3D printing enables layer-by-layer deposition of materials to create micropillar arrays with complex architectures [21–23], offering versatility in terms of materials used and allowing for the incorporation of gradient properties within the micropillars.

Nanofabrication techniques, such as electron beam lithography and nanoimprint lithography, provide sub-micron resolution, enabling the creation of nanoscale micropillar arrays [24,25]. These techniques are particularly valuable for investigating cellular responses at the nanoscale.

Microfluidics-based methods

Microfluidic devices can generate monodisperse emulsions containing precursor materials that solidify into micropillars

upon exposure to Ultraviolet (UV) light or thermal curing [26–29]. This controlled assembly and scalability approach allows for the precise control of micropillar size, spacing, and composition. Furthermore, microfluidic platforms can facilitate the integration of biochemical cues or multiple cell types to construct complex micropillar arrays [30,31]. The fabrication techniques mentioned above are illustrated in Fig. 2.

Design parameters

A comprehensive analysis of how micropillar array design parameters affect cellular behavior and outcomes in tissue engineering has been reviewed [9,10]. For instance, the formation of adhesion patches is significantly affected by the dimensions of nanopillars. When the side length of square nanopillars on a substrate was less than 333 nm, both the expression of integrins and the formation of adhesion patches were dramatically reduced [32]. Shorter micropillars exhibit higher basal stiffness while increasing the height of micropillars reduces their basal stiffness. These variations in stiffness affect the area of adhesion plaques formed by MSCs, with smaller adhesion areas correlating with an increased propensity for adipogenic differentiation. MSCs on nanopillars with spacing less than 140 nm had faster skeleton formation and greater ability to differentiate towards osteogenic lineages. The spacing and height of the micropillars affected the cell migration speed [32]. When the spacing of the micropillars was 3 μm , the cells hardly moved. When the spacing increased from 3 to 5 μm , the cells became elongated, with a migration speed of 0.18 $\mu\text{m}/\text{min}$. When the height decreased from 20 to 10 μm , the cell migration speed was decreased from 0.23 to 0.84 $\mu\text{m}/\text{min}$ [33].

Cell Traction Force Microscopy Using Micropillar Arrays

Cell traction force microscopy (TFM) relies on measuring the deformation of the substrate caused by cell-generated forces and subsequently inferring the traction forces based on the mechanical properties of the substrate and its deformation [34–36]. This method enables the quantification of forces exerted by cells on their surrounding substrate, which is particularly valuable for studying cell-matrix interactions, mechanotransduction, and cellular responses to physical cues. There are two TFM approaches, including fluorescent magnetic bead-based TFM and micropillar-based TFM.

Fluorescent magnetic bead-based TFM

Cells grown on the substrate in which fluorescent magnetic beads are embedded. The interaction of the cells with the beads causes deformation of the substrate in response to the forces exerted by the cells. The deformation of the substrate is tracked by imaging the displacement of the beads, allowing the cell-generated forces to be calculated. This method offers high spatial resolution and is suitable for studying single-cell forces and their distribution [37].

Micropillar-based TFM

PDMS micropillar arrays provide another platform for cell traction force measurement (Fig. 3). The pillars within the array deform in response to traction force, and the extent of

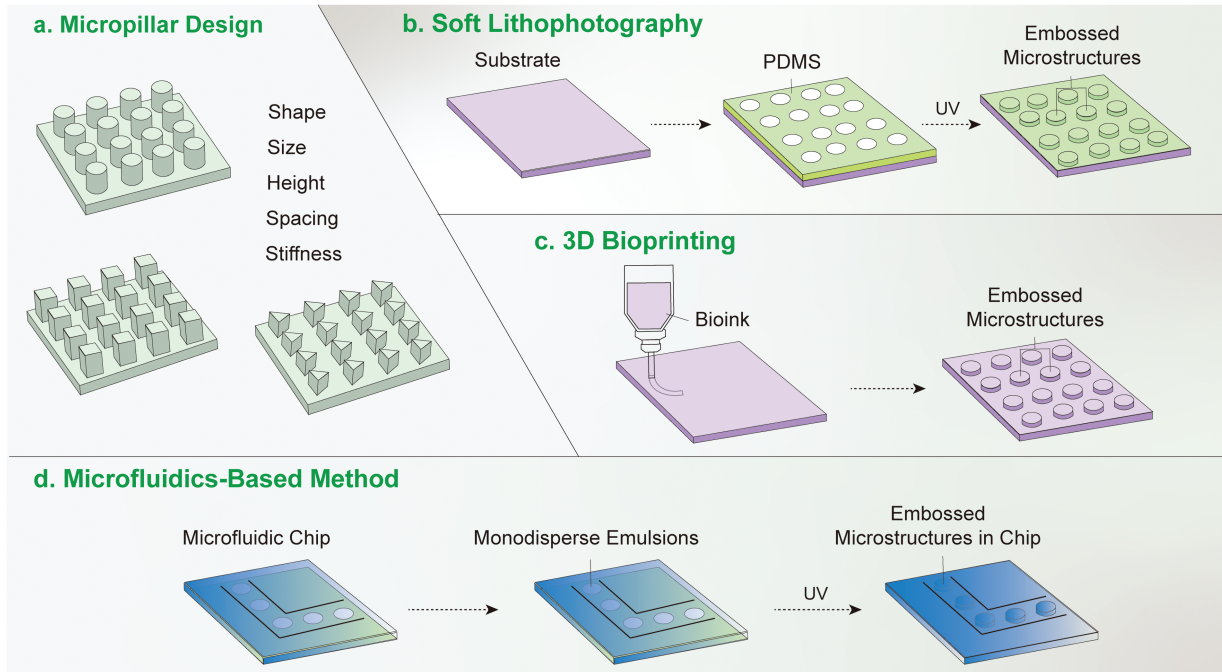


FIGURE 2. Methods of micropillar fabrication. (a) Considerations for micropillar preparation. Various parameters must be considered when preparing micropillars, encompassing their shape, size, height, spacing, and mechanical stiffness. (b) Soft lithography method. In the soft lithography approach, a substrate is coated with a photoresist. Ultraviolet (UV) light is then directed through a predefined irradiation mask, leading to the formation of an ordered micropillar array on the substrate. (c) 3D bioprinting technique. The 3D bioprinting method involves extruding material from a designated container onto a substrate, ultimately resulting in the creation of a micropillar array on the substrate. (d) Microfluidic-based method. The microfluidic-based approach follows a sequence of steps, commencing with the coating of a silicon plate with SU-8 photoresist. Subsequent UV irradiation creates a template, which is then employed as a mold for applying a polydimethylsiloxane (PDMS) precursor. The template is subsequently removed through thermoplastic molding, yielding the desired micropillar array.

pillar deformation is correlated with the magnitude of forces. Micropillar arrays can be fabricated with micron or nanometer scale sizes, which leverages the flexibility of PDMS to accurately capture the traction force over a large area [38,39].

However, the arrangement and composition of micropillar arrays modulate the cell traction force. Cells exhibit distinct force distributions on regions of different pillar densities. Specifically, cells tend to exert lower forces on regions of higher pillar density and higher forces on regions of lower density, with cells showing a preference to migrate towards regions of increasing pillar density [40,41]. Magnetic micropillars containing magnetic particles can alter pillar spacing and induce changes in cell attachment area and migration [10].

In other works, micropillar was thought to have a limited effect on cells. Within the heart-on-a-chip platform, photolithographically patterned Au microelectrodes are combined with printed 3D conductive polymer micropillars (200 μm in size). The low Young's modulus micropillars effectively prevent any deformation of the tissue during compression. Tissue itself, slightly displaces the pillars during the contraction [42]. The micropillar arrays were also used in external forces loading such as stretch [10].

Although the deformation of the micropillar arrays provides a method for cell traction force quantification, the micropillars may also influence the generation of cellular forces, and this complex interplay among micropillar,

extracellular matrix (ECM) and cell and how to accurately quantify the cell traction forces remains to be investigated.

Cell Properties and Micropillar Arrays

Cellular biomechanical properties

Micropillar arrays offer the means to assess cell spreading and cell membrane fluidity [43]. Also, micropillar arrays influence the viscoelastic properties of cells and their mechanical behavior. Primary human MSCs (hMSCs) were observed to be softer compared to human fibroblasts on smooth surfaces, while MSCs on micropillar arrays tended to exhibit linear growth, transitioning from a viscoelastic fluid property to a viscoelastic solid, indicating an increase in cell stiffness [44].

Cell function modulation

Micropillar arrays offer a 3D microenvironment that supports cell growth. Micropillar arrays have shown the potential to elongate and align cells. MSCs are adept at sensing matrix stiffness, pillar length, and the concavity/convexity of pillar surfaces, leading to specific cellular responses. PDMS micropillar arrays with varying stiffness and geometry can be tailored to induce elongation and alignment in hMSCs and cardiomyocytes. These anisotropic micropillars can accelerate elongation rates compared to traditional 2D microenvironments [45]. Coating PDMS surfaces with proteins, such as collagen or fibronectin, modulate the cell

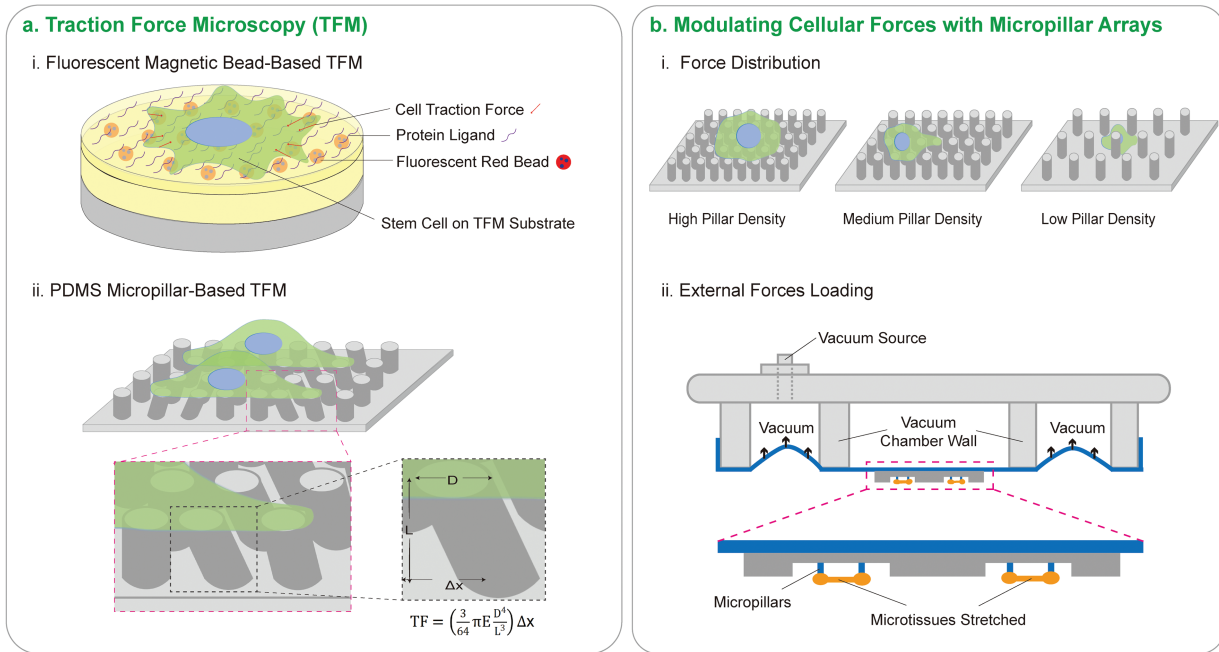


FIGURE 3. Cellular traction force detection and regulation. (a) Traction force microscopy (TFM) employs two primary methods: (i) Fluorescent magnetic bead-based TFM. Cells are cultured on a substrate with a known elastic modulus and are pre-labeled with fluorescent tracer particles. As cells contract, they exert traction forces that deform the substrate. These mechanical forces adhere to the principles of elastic mechanics. Utilizing a laser confocal microscope to monitor the displacement of fluorescent tracer particles, the degree of elastic deformation in the substrate can be quantified. Ultimately, employing an inverse algorithm, data pertaining to the traction forces exerted by the cells can be extracted. (ii) PDMS Micropillar-based TFM. Cell contraction causes deformation in PDMS micropillars. The extent of deformation in these micropillars can be observed and measured using an optical microscope. An appropriate equation can then be applied to calculate the force exerted by the cell on the micropillars. (b) Modulating cell forces with micropillar arrays. (i) Impact of micropillar array density on force distribution. At high densities, cells form focal adhesions predominantly on the micropillar surfaces. At medium densities, focal adhesions are observed on both the upper surfaces and sides of the micropillars. Conversely, at low densities, cells tend to occupy the inter-pillar spaces. (ii) External forces loading. Cells can be stretched by creating negative pressure that stretches the micropillar arrays on the membrane. Alteration in cells can be monitored using an inverted microscope, facilitating the observation of cellular responses to the mechanical application.

spreading area and cellular sensitivity via control of gene expression levels [46,47].

Focal adhesions (FAs), transmembrane protein complexes on the cell surface, detect the micropillars' topographical and mechanical properties, relaying these signals to the cell's interior. The cytoskeleton, tethered to both the intracellular FAs and the nucleus, acts as a conduit for mechanical signals, inducing alterations in nuclear and chromatin structure, and regulating ECM-related gene expression and protein secretion [48,49]. This feedback mechanism between the cell and the ECM sustains mechanical homeostasis and governs cell fates [50,51].

Moreover, the broader microenvironment shapes stem cell responses to micropillar arrays. The interplay between soluble factors, neighboring cells, and extracellular matrix composition all contribute to the signaling networks involved in determining stem cell fate.

Cell sorting using micropillar arrays

Micropillar arrays are combined with fluid dynamics to separate cells using deterministic laminar flow and asymmetrically distributed arrays of micropillars, where cells smaller than a critical size flow through the micropillar gaps while larger cells move laterally and undergo a lateral offset [52]. When nickel micropillars are integrated into microfluidic devices, programmatically controlled pneumatic

microvalves manipulate fluid flow, achieving separation [53,54].

By adjusting the size and shape of the micropillars, the cell capture rate can be improved. For example, changing the orientation of the triangular micropillar arrays creates a smoother hydrodynamic gradient and prolongs the contact time between the cells and the immune-modified micropillars, facilitating the capture of circulating tumor cells (CTCs) from blood [54,55]. The antibody-modified micropillars had been developed to enrich and recognize circulating nucleated red blood cells from maternal blood [56], which is expected to become a next-generation non-invasive prenatal diagnostic technology.

Innovative Platforms: Organ-on-a-Chip and Organoids-on-a-Chip

Organ-on-a-chip systems

The advancement of organ-on-a-chip systems benefits from a deeper understanding of cellular heterogeneity in complex and dynamic *in vivo* microenvironments [57]. The Chip-on-a-Transwell platform was used to investigate the microphysiological systems *in vitro* [58].

Organ-on-a-chip technology employs microfluidic devices to replicate the tissue-tissue interfaces, spatiotemporal chemical gradients, and dynamic mechanical

microenvironments *in vivo*, enabling specific physiological studies and the development of dedicated *in vitro* disease models (Fig. 4). As documented, organ-on-a-chip systems have been utilized to study endothelial differentiation of hiPSCs, incorporating quadrilateral micropillars to provide shear protection and enhance the physiological relevance [59,60]. Organ-on-a-chip systems can mimic the structure and function of alveolar-capillary interfaces and simulate conditions such as pulmonary oedema [61], providing alternatives to traditional cell culture models and animal testing, as well as applications in drug development and toxicology. Moreover, microfluidic chips with PDMS micropillar arrays have been used to develop *in vitro* blood-brain barrier (BBB) models incorporating the human brain microvascular endothelial cells (hBMECs), human pericytes (hPCs), and human astrocytes (hACs) [62], offering a tool to study drug permeability and neurological disorders.

Manufacturing limitations are a major obstacle to current advances in organ-on-a-chip. While laser-induced methods offer higher precision engineering capabilities, costs remain prohibitive. New methods such as 3D printing and injection molding show substantial potential for organ-on-a-chip manufacturing [57].

Organoids-on-a-chip

As an emerging field, organoid microarrays aim to facilitate the manipulation and control of organoids, enabling a comprehensive reflection of the complex internal environment *in vivo*. Organoid microarrays are characterized by their high throughput and high sensitivity microstructures, capable of integrating a series of experimental processes such as organoid sorting, cultivation, observation, stimulus induction, detection, and analysis. For instance, a micropillar concentrator (for capturing volatile

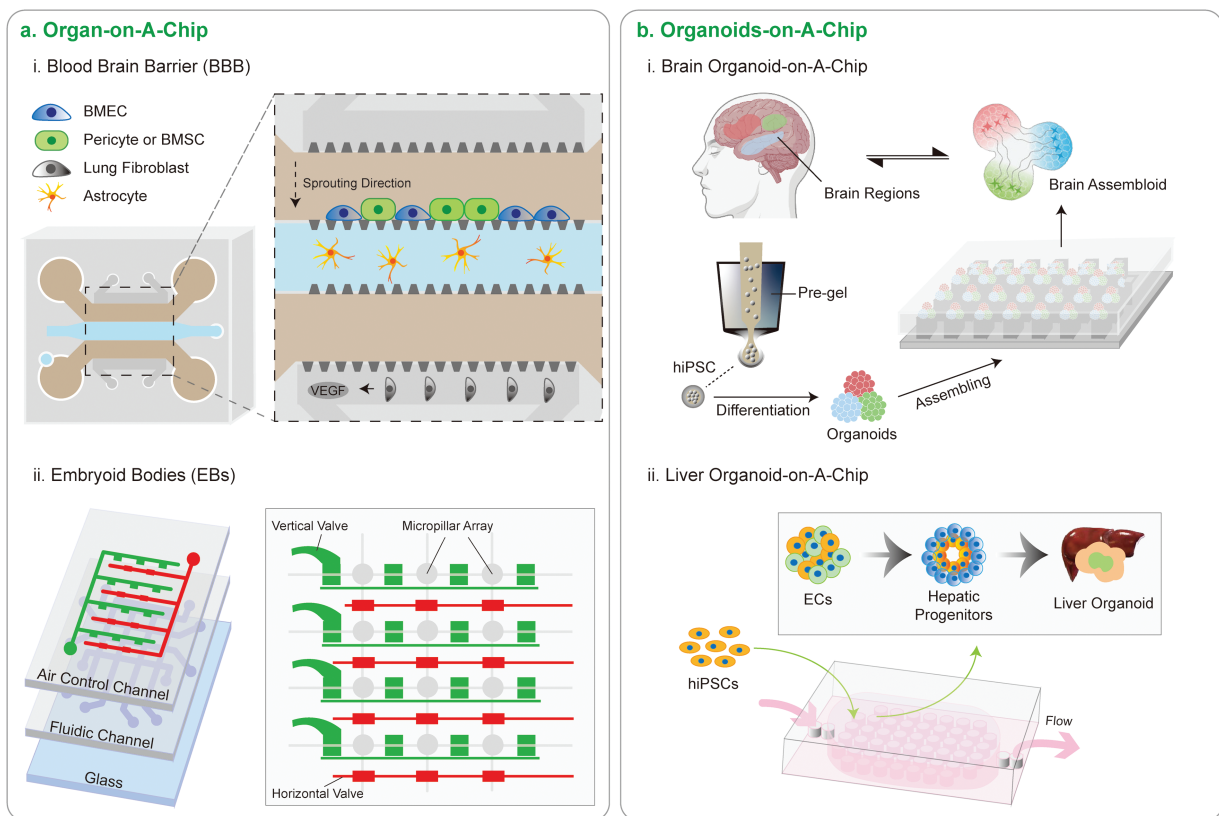


FIGURE 4. Micropillars advancing cutting-edge chip design. (a) Two organ-on-a-chips. (i) Directed sprouting of brain microvascular endothelial cells (BMECs). Organ-on-a-chip technology, exemplified by a vertically integrated microfluidic chip, mimics physiological organ behavior. In this context, the chip enables the directed sprouting of BMECs by localized delivery of vascular endothelial growth factor (VEGF) from lung fibroblasts. Notably, bone marrow mesenchymal stem cells (BMSCs) function as perivascular pericytes, displaying superior vessel-constrictive capabilities compared to human pericytes, thereby aiding the formation of a robust blood-brain barrier. Additionally, astrocytes play a pivotal role in maturing the capillary network. (ii) Regulating embryonic stem (ES) Cell fate. An integrated microfluidic culture device, featuring a 4×4 microfluidic arrangements with air control channels and fluidic channels, incorporates micropillar arrays ($300 \mu\text{m}$ in diameter). These micropillars, spaced $12 \mu\text{m}$ apart, facilitate ES cell docking. This microfluidic culture device, equipped with shear-protected micropillar arrays, offers substantial potential for uniform cell docking and forming of embryoid bodies (EBs). (b) Two organoids-on-a-chips. (i) Brain organoids-on-a-chip. Human induced pluripotent stem cells (hiPSCs), encapsulated in hydrogels, undergo controlled differentiation to form organoids representing distinct brain regions. These organoids can be precisely assembled using micropillar arrays, enabling the creation of fused organoids that closely mimic the complexity of the brain, providing a robust platform for studying intricate brain structures and functions within a controlled *in vitro* environment. (ii) Liver organoids-on-a-chip. The organoids-on-a-chip facilitates the formation of human liver organoids through hiPSC-based EBs cultured on perfusable micropillar arrays. This approach enables *in situ* hepatic differentiation, long-term 3D culture, and the generation of liver-like organs.

carbonyl metabolites with high efficiency) was integrated with a lung organ chip for the identification of cellular carbonyl metabolites [63]. HiPSC-derived multi-organoids-on-chip system (co-cultured liver and heart organoids on chip) can reflect the drug metabolism and responses at multi-organ levels [64]. These advanced platforms are applied across various biological fields, including the construction of developmental or disease models, drug discovery and development, immune response therapy, and microbial infections. This integration enhances the predictability of clinical treatment plans and significantly improves experimental efficiency.

The synergy between micropillar arrays and microfluidic organoid microarrays presents a powerful tool for both clinical and basic research (Fig. 4). Using multilayer microfluidic chips with PDMS micropillar arrays, brain-like organoids derived from hiPSCs were constructed and represent multiregional brain regions such as the cortex, hippocampus, and thalamus [12]. Multiregional brain organoids-on-a-chip offer a unique opportunity to investigate the interactions between different brain regions and their contribution to neurological disorders [12].

The integration of micropillar arrays with microfluidic chips allows precise control of the spatial arrangement and mechanical properties of the organoids and mimics mechanical cues such as compression and shear stress. By harnessing the power of micropillar arrays, researchers can create more accurate and physiologically relevant models of organs, paving the way for new discoveries in life.

Conclusion and Prospective

Micropillar arrays integrated with stem cell research hold great promise for breakthroughs in regenerative medicine. The interaction between stem cells and micropillars initiates complex signaling cascades that intricately shape cellular responses. Simultaneously, the stem cell microenvironment plays a pivotal role in governing their responses to micropillars. While our current understanding of how micropillars influence stem cell behavior is substantial, unveiling the mechanotransduction pathways and dissecting cellular responses to multidimensional cues, and the nuanced interplay among soluble factors, extracellular matrix composition, and neighboring cells continue to intrigue researchers deeply.

Unlocking the molecular machinery responsible for translating mechanical cues into specific cellular behaviors remains a hard pursuit. Scrutinizing mechanosensitive ion channels, cytoskeletal elements, and mechanotransduction molecules can shed light on the genetic and epigenetic changes triggered by micropillar arrays. Employing advanced techniques like single-cell sequencing and chromatin accessibility profiling holds promise for comprehensively understanding these alterations. Progress hinges on deciphering mechanotransduction mechanisms, probing complex cell-cell interactions, understanding microenvironmental influences, and dissecting the interplay of multidimensional cues.

Exploring how microenvironmental factors intersect with micropillar array effects could unveil complex signaling

networks orchestrating cellular behaviors. The synergy between physical cues from micropillars and bioactive molecules presents an enticing frontier. Functionalizing micropillar surfaces with such molecules could lead to synergistic effects, finely tuning stem cell responses.

Advancing micropillar technology stands to benefit greatly from integrating cutting-edge methodologies. Harnessing microfluidics for precise biochemical delivery, live-cell imaging for real-time observation, and dynamic modulation of mechanical forces for time-resolved studies open new vistas. High-throughput screening platforms that streamline the discovery of materials and treatments bridge the gap between research and practice. Investigating co-culture scenarios, such as combining mesenchymal stem cells (MSCs) with immune or endothelial cells in the presence of micropillar arrays, offers a promising avenue for insights into determinants of stem cell fate, immune modulation, and angiogenesis. Moreover, the integration of micropillar arrays with 3D bioprinting and organ-on-a-chip models presents novel avenues for research and application.

Despite great advancements in micropillar arrays, there remain other challenges. Future research should focus on refining *in vitro* models to better match *in vivo* physiological conditions, integrating more complex cell types, and recreating the cell-cell interactions within microenvironments. The development of novel materials that minimize drug and nutrient uptake while maintaining flexibility and biocompatibility is also crucial. The biocompatibility of materials used in micropillar arrays is critical for their application. PDMS, commonly used for its optical clarity and flexibility, has limitations such as non-specific absorption of small molecules [65]. To address these issues, alternative materials such as polyurethanes and cyclic olefin polymers could be explored.

Furthermore, ethical considerations, particularly in stem cell research, need to be addressed to ensure the responsible development and application of micropillar technologies. This includes adherence to ethical guidelines and maintaining transparency in research practices, including consent, privacy, and the long-term impact of regenerative therapies on patients.

Due to the reproducibility and precision of organoid microarrays, they are poised to revolutionize medical research, potentially replacing animal and 2D cellular experiments. These chips could become essential tools for preclinical research, offering more functional, integrated, automated, and personalized solutions. The future development of organoid chips is likely to focus on creating human organoid biomimetic chips composed of multiple organoids connected by pipelines [64]. Achieving this will require extensive research on the population ratios, differentiation conditions, and spatial distributions among various cell types, as well as the determination of amplification factors to simulate the complex physiological environment in the body.

In summary, micropillar arrays are a useful platform for cellular biomechanics and tissue engineering research. The development of this platform will unlock novel possibilities for understanding and manipulating cellular behavior,

driving innovative strategies in regenerative medicine, disease modeling, and personalized therapies.

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