



Review Article

Cell culture platform fabrication methods and applications

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ABSTRACT

Cell culture technologies are fundamental tool in biological research. Traditional two-dimensional (2D) cell culture methods, despite their widespread use and simplicity, fail to accurately replicate the physiological conditions of native tissues, leading to altered cellular behavior. Recent advancements in 3D culture techniques, combined with innovative fabrication methods such as photolithography, paper-based, and 3D printing, have substantially improved the fidelity of cell culture models. In parallel, numerical simulations have become indispensable for optimizing the design and performance of these systems, offering precise control microenvironmental factors such as fluid dynamics, nutrient and oxygen gradients, and shear stress within microfluidic platforms. These approaches for integration facilitate accurate modeling of cell-to-cell and cell-to-matrix interactions essential for physiological relation. Concurrently, the integration of multimaterial fabrication techniques provides scalable and customizable solutions for developing sophisticated microfluidic and cell culture systems. This review discusses recent developments in these fabrication methods and highlights their integration with numerical simulation for optimization design, explores their collective potential to advance biomedical research and applications.

KEYWORDS: *Cell culture, Lithography, Numerical simulation, Paper-based, Three-dimensional printing*

INTRODUCTION

Cell culture technologies are essential for drug discovery, tissue engineering, and disease modeling. Traditional two-dimensional (2D) cultures, performed in petri dishes, flasks, or multiwell plates, involve adherent cells growing on flat surfaces and are favored for their simplicity, low cost, and high throughput compatibility [1]. However, 2D models lack native tissue features such as spatial organization, cell-cell interactions, and extracellular matrix (ECM) cues, leading to altered morphology, polarity, and gene expression that reduce their predictive accuracy *in vivo*. In contrast, 3D culture methods, combined with advanced fabrication techniques, enable tissue-like architectures that better replicate physiological conditions, supporting applications in tissue engineering, organ-on-a-chip (OoC), and disease modeling [2-5].

Numerical simulations enable design optimization by predicting complex cell culture behaviors under varying conditions. Key factors such as device geometry, fluid dynamics, and mass transfer, including nutrient gradients, flow velocity, shear stress, temperature, and cellular responses, can be systematically evaluated [6,7]. This

supports efficient and cost-effective optimization of culture geometry and performance [8]. Photolithography combined with polydimethylsiloxane (PDMS) microfabrication has been instrumental in producing high-precision microscale culture devices. PDMS offers excellent gas permeability, supporting oxygen and carbon dioxide exchange critical for maintaining appropriate cell culture microenvironments [9-11].

Paper-based methods have emerged as cost-effective and portable microfluidic platforms, offering advantages such as flexibility, porosity, and easy stacking, making them ideal for 3D cell culture and biological assays [12,13]. 3D printing enables reproducible fabrication of microdevices, perfusion bioreactors, and organ-level constructs, with scalable resolution and material versatility. Integration of paper with biocompatible resins in 3D printing leverages both material and optical properties, promoting the development of hybrid platforms as sustainable, affordable alternatives. These combined fabrication strategies, enhanced by numerical simulation, have advanced point-of-care diagnostics and

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are increasingly applied in cell culture and tissue modeling, offering scalable and biocompatible environments for physiological relevance [14-16].

This review examines key manufacturing techniques for developing 3D cell culture systems in microfluidic and OoC platforms. It highlights the role of numerical simulations in optimizing culture conditions and device performance. Fabrication methods such as photolithography-based PDMS microfabrication, 3D printing, and paper-based platforms are also surveyed for their applications in advanced cell culture models.

CELL CULTURE PLATFORMS DESIGN AND FABRICATION METHODS

Numerical simulation and design optimization for cell culture platform

Microfluidic systems are powerful tools in biomedical engineering, supporting applications from diagnostics [17,18] and drug delivery to cell culture [17,19,20] and OoC models [21,22]. These platforms rely on precise control of fluid flow, chemical gradients, and microenvironments. Numerical simulation is essential for analyzing microfluidic behavior, offering insights into parameters such as shear stress and mass transport that are difficult to measure experimentally [23]. By enabling performance prediction and design optimization before fabrication, simulations reduce trial-and-error testing and improve development efficiency [24,25]. Various computational methods are used to simulate microscale fluid dynamics in microfluidic devices [7,8,26]. Computational fluid dynamics (CFD), implemented through tools like ANSYS Fluent, COMSOL Multiphysics, or OpenFOAM enable researchers to model, predict, and optimize microfluidic system performance with high accuracy. It typically employs finite volume or finite difference methods. The finite element method (FEM), widely used via COMSOL, offers flexibility for complex geometries and enables multiphysics coupling, such as fluid-structure interaction and mass transport [27]. Recent studies increasingly adopt ANSYS Fluent to model flow behavior in diverse cell culture systems [28-30]. Figure 1 summarizes the key technologies contributing to the advancement of 3D cell culture systems.

Fluid flow is a key factor in cell culture system design, directly affecting cell growth through parameters such as velocity and shear stress. Guzzi *et al.* [31] used numerical simulation to optimize and fabricate a passive microfluidic device capable of delivering media and drugs without external pressure sources, by adjusting fluid column heights and well apertures. CFD simulations via COMSOL evaluated streamlines, pressure drops, and inlet/outlet velocities, showing strong agreement with theoretical expectations. Geometry also plays a critical role; features such as channel width, height, and micropillar layout influence shear stress and pressure distribution, impacting cell morphology, function, and viability. Pisapia *et al.* [32] simulated four 3D microchannel designs, including circular chambers, longitudinal channels, parallel channels with micropillars, and closed micropillar channels evaluated the velocity fields, wall shear stress, and pressure profiles to inform design.

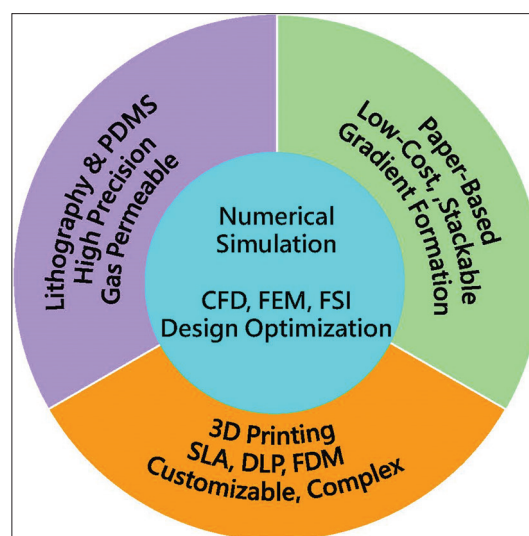


Figure 1: Key development technologies of three-dimensional cell culture systems. CFD: Computational fluid dynamics, FEM: Finite element method, FSI: Fluid-structure interaction, SLA: Stereolithography, DLP: Digital light processing, FDM: Fused deposition modeling

In cell culture microenvironments, limited spatial dimensions can cause rapid oxygen depletion, leading to hypoxia if delivery is insufficient. Numerical simulations are critical for analyzing oxygen and nutrient diffusion, mapping concentration profiles, and informing design strategies to improve supply. Gao *et al.* [33] proposed a culture device combining oxygen-permeable and impermeable materials to locally control oxygen tension. Using COMSOL Multiphysics, they simulated oxygen transport in PDMS and culture medium, and validated the results with PtTFPP-based fluorescence oxygen sensors, confirming simulation accuracy. In the tumor microenvironment (TME), spatial and temporal fluctuations in oxygen tension arise from uncontrolled proliferation and immature vascularization, affecting cancer cell behavior and promoting metastasis. To study cellular responses to oxygen gradients, Koens *et al.* [34] fabricated a microfluidic device with tunable oxygen gradients. Oxygen distribution was simulated using COMSOL Multiphysics by varying design parameters such as media/gas flow rates and channel configurations, solving Navier-Stokes and convection-diffusion equations. The resulting double-layer structure generated hypoxia more efficiently than single-layer devices. Building on this, the group [35] applied finite element analysis to model oxygen transport, accounting for solubility, diffusion coefficients, and cellular consumption in both PDMS and media. Aratake *et al.* [36] further developed microfluidic systems capable of precise oxygen control in collagen gels, simulating TME-like hypoxia. Simulations considered diffusivity and solubility in all materials, and were validated by oxygen-sensing films embedded at the gel bottom.

Cells are highly sensitive to shear stress, and excessive levels can cause detachment or damage. Numerical modeling enables precise mapping of shear stress across cell surfaces based on device geometry and flow conditions [37,38]. Sang *et al.* [38] proposed a zero-flow design using FEM to predict transport properties with minimal flow disturbance.

Compared with conventional structures, this design created a stable, low-shear microenvironment ideal for long-term culture and sensitive detection. Simulations and experiments confirmed that circular microchambers rely on diffusion rather than convection, promoting cell viability. Tomecka *et al.* [39] developed a micropillar-based system for both 2D and 3D cardiac cultures. In the 3D setup, hydrogel formed upon contact with the medium, centralizing the cell matrix in the channel. Simulations evaluated nutrient diffusion, pressure profiles, and wall shear stress, revealing slightly higher pressure and $\sim 1.5 \times$ shear stress in 3D cultures. Viability tests confirmed the 200 μm -deep microsystem supported stable cardiac cell growth. Temperature is another critical parameter in cell culture. Elevated ambient temperatures can impair cell adhesion and induce cell death [40]. In cryopreservation, cytotoxic effects of cryoprotective agents require their application at low temperatures to minimize cellular damage. Peng *et al.* [41] developed a three-layer microfluidic device with integrated cooling and heating systems for rapid, localized temperature control. Design parameters were validated through simulations, incorporating 3D laminar flow, conjugate heat transfer, and Joule heating to model thermal behavior and achieve precise on-chip temperature regulation.

Human tissues are heterogeneous, with function shaped by cellular interactions, structural organization, and molecular gradients. Canadas *et al.* [42] used CFD and discrete phase modeling (DPM) to simulate cell seeding, medium flow, and rotational dynamics in a dual-chamber bioreactor to replicate native tissue behavior. Simulations were performed using ANSYS Fluent, with the 3D device geometry designed in SolidWorks, a computer-aided design software that enables accurate modeling of complex bioreactor structures, and subsequently validated experimentally. The culture medium was modeled as an incompressible Newtonian fluid governed by the Navier–Stokes equations, which describe conservation of momentum in fluid flow, together with the continuity equation for mass conservation. Cells were represented as non-rotating spheres tracked via Lagrangian particle modeling. A four-regime adhesion model, including stick, rebound, spread, and splash was applied to capture cell-scaffold interactions. Nutrient transport between chambers was modeled using convection-diffusion equations with two distinct media, incorporating scaffold porosity, gravity-driven flow, and diffusion during rotation. While CFD models have been used to assess hydrodynamic parameters, biomechanical stress and strain within tissue-engineered blood vessel (TEBV) walls remain understudied. To address this, Wang *et al.* [27] integrated OoC technology with 3D fluid-structure interaction (FSI) modeling to examine TEBV biomechanics under perfusion. A cylindrical TEBV was fabricated and embedded in a PDMS-based microfluidic device, forming a vessel-on-a-chip system perfused by a peristaltic pump. Material properties, including TEBV stiffness and medium viscosity, were experimentally determined. A 3D FSI model simulated vessel deformation and pulsatile flow. Validation showed good agreement with experiments, with only 10.0% and 10.5% errors in diameter expansion and velocity, respectively. The simulation results demonstrated the wall

shear stress, internal stress and strain distributions, and flow patterns within both the culture medium and the vessel wall.

Lithography-based cell culture platform

Lithography-based microfabrication has been pivotal in developing advanced cell culture systems [43,44]. Photolithography is widely employed to create micropatterns on silicon or glass using photoresists such as SU-8 [45,46], which serve as master molds for PDMS casting. The resulting PDMS microfluidic devices are flexible, optically transparent, and ideal for biological applications [47,48]. PDMS-based microfluidics are broadly used to culture cells under well-defined microenvironments [49,50]. For example, Nashimoto *et al.* [51] constructed a 3D spheroid culture platform with a perfusable vascular network. A 100 μm -thick SU-8 mold was used to create five parallel microchannels, the central one for fibroblast spheroids, side channels for human umbilical vein endothelial cells (HUVECs) seeding and sprout induction, and outer channels for medium supply. Microposts enabled endothelial migration into the central zone.

Direct contact co-culture, which mimics tissue interfaces, can be achieved through shared chambers or layered channel designs. Gao *et al.* [52] fabricated a double-layer PDMS platform integrating pneumatic valves for dynamic 2D/3D co-culture control. By modulating valve designs, the system allowed either direct cell contact or soluble-factor interaction. The platform supported dynamic synapse imaging in fluorescently labeled hippocampal neurons. Shi *et al.* [53] developed two PDMS microfluidic devices, including vertically layered and four-chambered, for controlled CNS neuron and glia cells co-culture. Fabricated by SU-8 soft lithography and multilayer PDMS casting, the system used pressure-actuated valve barriers for reversible chamber isolation. Neurons adhered to PLL-coated glass; glia to the PDMS roof. The vertical proximity (50–100 μm) without contact promoted synaptogenesis, yielding a $\sim 2.5 \times$ increase in synaptic contacts compared to indirect co-culture. PDMS's gas permeability supports oxygen gradient generation. Gao *et al.* [33] developed composite microfluidic devices using gas-permeable PDMS and impermeable NOA81. Using two-layer SU-8 lithography, molds were created to fabricate chambers with embedded oxygen control zones. Integration of oxygen scavengers such as pyrogallol enabled spatial hypoxia control, verified by PtTFPP-doped PDMS oxygen sensors. In tumor models, oxygen gradients affect cancer progression [34–36]. Koens *et al.* [34] created a double-layer microfluidic device with a central gel channel flanked by media and gas channels. Oxygen gradients were established by flowing custom gas mixtures and blocking ambient exchange using polycarbonate films. Aratake *et al.* [35] used the same device to co-culture breast cancer and stromal cells under 21%, 5%, and 1% O_2 . After 7 days, cancer cell migration toward vasculature increased significantly under hypoxia, especially at 1% O_2 .

Lithographic techniques also support single-cell studies. Dura *et al.* [54] developed a high-throughput device to pair T cells with antigen-presenting cells under controlled microenvironments. Using two-layer SU-8

photolithography (2.2-3 μm pillars and 7–9 μm traps), the device optimized trap geometry for pairing efficiency and minimal clogging, enabling early immune activation analysis. Thick SU-8 films enable high-aspect-ratio structures for 3D culture. Liu *et al.* [55] used triple-layer spin-coated SU-8 2075 to form a 700 μm mold for a PDMS tumor vasculature model. After plasma bonding to glass, a 600 μm inner diameter polyurethane fiber embedded in TG–gelatin hydrogel was removed post-gelation to form a lumen. PEG 4000 enhanced hydrogel mechanics and nutrient diffusion, allowing long-term U87 and HUVEC co-culture in a 3D environment.

Hybrid techniques combine 3D printing with PDMS casting to fabricate complex microchannel structures. Hwang *et al.* [56] used four types of 3D-printed molds to create cylindrical PDMS microchannels (200–1000 μm). Despite comparable surface roughness, contact angles varied (68°–84°), serving as a predictor of capillary flow. Villegas *et al.* [57] improved PDMS channel smoothness using low-resolution 3D-printed molds coated with an omniphobic lubricant-infused layer, reducing surface roughness from 2 μm to 0.2 μm comparable to photolithography while eliminating cleanroom dependency. Resulting devices exhibited improved optical clarity, uniform shear stress, and consistent flow rates. Most conventional microfluidics rely on irreversible bonding, hindering post-culture retrieval. Feng *et al.* [58] addressed this by developing a reversibly bonded PDMS–polystyrene (PS) platform using low-concentration ($\leq 0.5\%$) APTES. This method allows device disassembly without structural damage. With 0.1% APTES, the system supported long-term cultures across multiple cell types. Compared to irreversible bonding, it improved cell viability (70% vs. 19%) and retrieval rates in 2D (72%) and 3D (82%) cultures.

Paper-based cell culture platform

Paper-based platforms are fabricated from commercially available or modified papers [12,59]. Their fibrous, porous structure supports cell adhesion, proliferation, and migration, enabling 3D cultures that better mimic tissue environments [13,60,61]. Capillary-driven flow allows the formation of nutrient and oxygen gradients essential for physiological relevance [62]. Common scaffold materials include chromatography paper, Whatman filter paper, photo paper, graphene-based paper, and lens cleaning paper, which are patterned with PDMS, SU-8, or wax to define culture zones. Surface coatings with Matrigel or peptides further enhance cell adhesion and promote differentiation.

Paper-based constructs can be vertically stacked to form layered architectures, enabling co-cultures and the formation of tissue-like microenvironments. These systems simulate physiological gradients and support 3D cell behavior studies with easy assembly, disassembly, and compatibility across cell types [13,60]. Tissue stacks using paper scaffolds replicate tumor heterogeneity by generating spatially distinct oxygen, pH, and nutrient zones. Derda *et al.* [62] introduced a scalable method using eight-layer, 200 μm -thick chromatography paper sheets loaded with ECM and cells to construct multilayered 3D cultures. Oxygen gradients were regulated using PDMS slices and cellulose acetate films. Breast cancer cells in these stacks exhibited proliferation, hypoxia, and apoptosis, mimicking

in vivo tumor behavior. The platform enables controlled gradients, precise cell distribution, and layer-by-layer analysis of gene expression, metabolism, and viability. Later, Derda *et al.* [63] developed the cells-in-gels-in-paper (CiGiP) method, patterning hydrophilic zones on Whatman filter paper via wax printing to localize cell-laden ECM hydrogels. Stacked sheets formed structured 3D cultures, allowing spatially resolved, high-throughput analysis with 200 μm resolution – overcoming limitations of bulk 3D cultures that yield only averaged responses.

Cellulose-based paper scaffolds provide a rigid, porous structure for 3D cell culture and are often combined with wax patterning or inkjet printing to form microfluidic networks. Commercial printers are typically used to deposit hydrophobic barriers that define fluidic channels and culture zones within the paper. Pupinyo *et al.* [64] developed a wax-printed, *in situ* paper-based 3D culture platform for rapid, low-cost screening of anti-melanogenic compounds. Whatman No. 1 paper was used as the scaffold, with Matrigel-encapsulated B16F10 melanoma cells cultured in three test zones defined by wax-printed hydrophobic boundaries. After compound incubation, the dried paper was scanned, and melanin levels were quantified using ImageJ. The platform enabled visible assessment of melanin production without the need for centrifugation or solubilization, reducing analysis time approximately from 3 h to 20 min. Fluorescent staining confirmed cell viability over 120 h, and SEM imaging with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) staining validated 3D cell structures. Sapp *et al.* [65] developed a multilayer 3D culture system using wax-patterned Whatman filter paper to support *in vitro* growth and analysis of aortic valvular interstitial cells (VICs). The system consists of stacked paper layers embedded with VICs in collagen gel, simulating native valve tissue architecture with adjustable thickness and cell density. The paper layers, modeled after a 96-well plate, were printed with a wax template and seeded with VIC in collagen mixtures that rapidly gelled. Layer stacking enabled precise customization of culture dimensions and cellular organization, effectively modeling the full thickness of valvular tissue. Tao *et al.* [66] developed a paper-based microfluidic system for 3D cell culture, offering a low-cost, disposable, and user-friendly alternative to glass or PDMS devices. The platform was fabricated by wax-printing hydrophobic barriers onto cellulose filter paper, followed by heat treatment to allow wax penetration and formation of microchannels and culture wells. Hydrophilic channels enabled passive liquid transport via capillary wicking. The system demonstrated cyto-compatibility and supported cell proliferation. Chemosensitivity testing with Huh-7 liver cancer cells exposed to doxorubicin (DOX) was conducted, and drug response was quantified using the CytoScan™ WST-1 Cell Cytotoxicity Assay. The WST-1 assay results demonstrated a marked reduction in cell viability in DOX treated regions, whereas control regions without drug exposure maintained normal cell growth.

In addition to wax printing, inkjet and PDMS ink printing offer low-cost alternatives for defining cell culture zones on paper substrates. Agarwal *et al.* [67] developed a paper-based device for 3D liver cell culture and bioassays

using double-sided LaserJet printing to create hydrophilic and hydrophobic regions. The platform supported the growth of various cell types, including HepG2, HUVECs, fibroblasts, and MSCs. Notably, paper grade and culture zone dimensions significantly influenced the functional phenotype of HepG2 cells. Juvonen *et al.* [68] developed patterned arrays for 2D cell culture by printing hydrophobic PDMS ink onto four types of coated paper substrates, using ARPE-19 retinal epithelial cells. PDMS-free regions served as culture zones, while PDMS-coated areas restricted cell adhesion. Atomic force microscopy was used to characterize nanoscale surface properties, including bearing area, roughness, contact angle, and surface energy. Higher roughness was associated with lower bearing area due to sharper surface features. Compared to paper substrates, PS dishes and printed PDMS films had smoother surfaces with 100% bearing area. The printed arrays effectively confined cell growth to designated regions. Surface characteristics influencing cell compatibility were identified. Substrates with high bearing area, low roughness, high total surface energy, and intermediate electron donor components supported cell growth, whereas opposite values led to poor adherence and proliferation.

Photolithography has been adapted for paper substrates by coating them with SU-8 photoresist and using ultraviolet (UV) exposure to define hydrophilic and hydrophobic regions. Hong *et al.* [69] developed a paper-based microfluidic device for generating drug concentration gradients to enable high-throughput drug screening. The device features a five-layer architecture built from Whatman chromatography paper, with hydrophobic SU-8 patterns forming hydrophilic channels, and PDMS used to form a sandwich structure. HeLa cells were cultured in collagen-filled microwells as 3D scaffolds. Capillary-driven flow autonomously established a DOX gradient across the culture zones. Cell viability analysis revealed a clear correlation between DOX concentration and cytotoxicity. To better replicate cardiac cell morphology and promote tissue maturation, Guo *et al.* [70] developed an optically active, paper-based scaffold for 3D cardiac tissue formation and stimulation. The scaffold incorporates gold nanorods (GNRs) and CdSe/CdS quantum dots (QDs) onto a fibrous paper matrix, enabling light-triggered thermal stimulation and *in situ* temperature sensing. Fabricated from APTES-treated Thorlabs lens cleaning paper, citrate-stabilized GNRs were electrostatically adsorbed, followed by EDC/NHS coupling of silica-encapsulated QDs. The scaffold was coated with ECM proteins and embedded in collagen gel for culture. HL-1 cardiac cells seeded on the scaffold exhibited high viability, alignment, and synchronous activity under optical stimulation.

Hybrid fabrication methods integrate diverse materials and techniques to combine mechanical stability, fluid control, and diffusion functionality. For example, layering polyvinyl chloride (PVC) or polymethyl methacrylate (PMMA) with paper enhances structural integrity and performance in paper-based cell culture platforms. Simon *et al.* [71] employed the CiGiP 3D system to study lung cancer cell responses to ionizing radiation under simulated TME conditions. The platform was constructed by stacking Whatman lens paper and

perforated PVC sheets containing hydrogels embedded with A549 cells, creating oxygen and nutrient gradients. Metabolic activity, radiation sensitivity, and gene expression varied by layer depth, with deeper layers exhibiting increased hypoxia, reduced proliferation, and greater radiation resistance. This system enables spatial analysis of radiation responses under physiologically relevant gradient conditions. Lei *et al.* [72] developed a paper/PMMA hybrid microfluidic platform for investigating cellular crosstalk in a 3D culture model. The device integrates wax-patterned microreactors on Whatman filter paper with PMMA diffusion channels fabricated via computer numerical control (CNC) engraving and aligned with the paper substrate. Hydrogel-filled channels in the PMMA base enable biomolecule diffusion to stimulate surrounding cells. In the experiment, Huh7, HepG2, BM-1, and control cells were seeded in side microreactors, while four conditions were applied to the central microreactor, including transfected cells, non-transfected cells, 0.5 µg/mL EGF treatment, and blank control. Cell proliferation was assessed using a colorimetric bioassay, and signal intensities from microreactors were quantified to evaluate cell responses under each condition. These findings validate the platform as a simple and functional tool for investigating cellular crosstalk.

Matrigel coatings are widely used to promote cell adhesion and differentiation. Supjaroen *et al.* [73] developed a paper-based co-culture model integrating intestinal epithelial and neutrophil cells within a customizable 3D-printed PLA transwell system. Wax-patterned Whatman paper membranes coated with Matrigel simulated the ECM and were stacked to form the culture platform. The system enabled cell-based permeability assays and modeled immune-mediated intestinal inflammation. Caco-2 cells cultured on the paper membrane exhibited time-dependent increases in transepithelial electrical resistance (TEER) signals, correlating with tight junction protein expression and microvilli localization, as confirmed by immunofluorescence. Differentiation of HL-60 cells with phorbol-12-myristate-13-acetate (PMA) induced neutrophil extracellular trap (NET) formation on the 3D scaffold, visualized via Hoechst 33342 staining, which revealed characteristic nuclear morphology and NET structures. The developed co-culture system provides a low-cost, customizable platform suitable for drug screening, nutrient transport studies, and biosensor applications. A major limitation of paper-based cell culture systems is the lack of continuous medium supply. To address this, Fu *et al.* [74] developed a wick-driven paper-based microfluidic device for 3D cell culture and anticancer drug screening, eliminating the need for external pumps. The device comprises two wax-patterned filter paper layers functioning as a self-wicking conduit. The folded hydrophilic strip extends into an external reservoir, enabling continuous medium delivery via capillary action. This design eliminates the need for hydrogels, as cells are seeded directly on hydrophilic regions, supporting hydrogel-free 3D culture.

Three-dimensional printing cell culture platform

3D printing has transformed the fabrication of 3D cell culture systems, enabling complex, customizable, and functional platforms for applications including cell culture and OoC. Key techniques such as stereolithography (SLA), digital

light processing (DLP), fused deposition modeling, selective laser sintering, and two-photon polymerization are critically evaluated for their roles in constructing structural, mechanical, and microfluidic components. Among them, SLA and DLP are widely adopted energy-based bioprinting strategies due to their high resolution and material versatility [15,75,76].

SLA enables precise control and smooth surface finishes, critical for reducing shear stress in interface-sensitive microfluidic cell culture systems. Sphabmixay *et al.* [77] developed a mesoscale physiological system (MePS) for long-term perfused 3D culture of primary human hepatocytes (PHH), fabricated using a custom high-resolution projection micro-stereolithography (PuSLA) technique. This method employed a novel photopolymerizable resin printed directly onto a porous membrane. The PuSLA system achieved micron-scale precision (2.3 μm z-axis, 6.8 μm lateral resolution), allowing the construction of structures replicating liver microenvironments. The MePS scaffold incorporated nanochannels and microporous channels to mimic physiological oxygen and shear stress levels, supporting long-term culture of over 10^6 PHH per device.

Paper-based and lithographically patterned PDMS platforms utilize capillary action for fluid transport without external pumps. Similarly, 3D SLA printing enables high-resolution fabrication of capillary-driven microfluidic devices. Esparza *et al.* [78] applied Young–Laplace and Navier–Stokes principles to design SLA-printed cardiovascular tissue models with or without micropost grids (DWPG, DWoP), using a Form3B printer and UV-curable Ostemer 322 polymer. The hydrophilic surfaces enabled self-driven fluid flow in closed circuits. Over five days, both device types supported elongation and organization of cardiovascular cells, particularly around microposts. Sitte *et al.* [79] proposed a continuous flow delivery system (CFDS) to address hypoxia and nutrient limitations in static 3D cultures. The modular CFDS integrates 3D printing and laser-cut acrylic components, supporting perfusion of cell-laden paper and gel scaffolds. Medium circulates via a peristaltic pump in a closed loop, and chambers can be configured for single or dual-compartment culture. The system maintains optimal flow rates to support the viability of M231 cells and formation of functional HUVEC monolayers replicating endothelial barrier behavior under flow.

Ding *et al.* [80] introduced a fully 3D-printed modular microfluidic platform for continuous harvesting of mesenchymal stem cells (MSCs) from microcarriers (MCs) in large-scale bioprocessing. Fabricated using high-resolution DLP 3D printing, the system integrates three modules, helical micromixers for cell detachment via Dean flow, a spiral microseparator for MC removal, and a zig-zag microconcentrator achieving up to $4.5 \times$ cell enrichment. Harvested MSCs maintained morphology, viability, and differentiation potential. The modular, multiplexable setup supports high-throughput, clog-free operation with reduced shear stress. To further advance ready-to-use microfluidics, Karamzadeh *et al.* [81] developed intrinsically hydrophilic capillary circuits using digital manufacturing completed in under 30 min. By formulating a PEGDA-250/acrylic acid-based hydrophilic ink (CCInk), monolithic capillary

devices with embedded channels, stop valves, and gyroid capillary pumps were directly printed via DLP, eliminating post-curing and external pumps. The system features a robust capillary valve design and circular conduits to prevent bubble trapping. This platform was demonstrated in a multilayer immunoassay chip for SARS-CoV-2 antibody detection, highlighting the potential for low-cost, rapid digital fabrication of functional, pump-free microfluidic systems.

While 3D printing enables high-resolution, multimaterial fabrication, it remains limited by residual resin toxicity and a narrow range of biocompatible materials – factors that must be critically assessed for their impact on cell culture and tissue engineering. Huh *et al.* [82] investigated combinations of 11 photoinitiators (PIs) and 5 UV absorbers (UAs) in DLP bioprinting, evaluating printability, resolution, and cytotoxicity. Using hydrogel crosslinking, they achieved high-resolution (25-50 μm) prints, including a perfusable, anatomically accurate heart-shaped construct with internal channels. C2C12 cells cultured with varying PI/UA concentrations revealed that 0.2% LAP and 0.5% R1800 offered optimal print fidelity and low cytotoxicity, maintaining over 90% viability after 14 days. Similarly, Fritschen *et al.* [83] compared three resin systems PlasCLEAR, PEG-1, and PEG-2, against PS and PDMS in terms of optical clarity and cytocompatibility in DLP printing. Spectroscopic analysis showed PEG-DA-based resins had higher UV/VIS transmission than PlasCLEAR. Post-processing (UV curing and solvent extraction) significantly improved polymer conversion, transparency, and reduced residual toxicity, leading to a 10-fold increase in cell viability. PEG-1 and PEG-2 also demonstrated superior compatibility with fluorescence imaging of HUVECs and L929 cells, confirming their suitability for microscopy-based applications in 3D printed microenvironments.

DLP is emerging as a promising technique for fabricating orthopedic implants due to its ability to construct complex geometries using polymers, metals, and ceramics. Martinez *et al.* [84] demonstrated the use of high-resolution DLP SLA to fabricate hydroxyapatite (HA) structures for bone tissue engineering. Using LithaBone HA400 slurry, HA coupon structures were printed and characterized for their physicochemical and biological properties. The constructs exhibited suitable hydrophilicity, porosity, and surface roughness for cell adhesion, with no significant extractables or leachables detected. Over a 35-day culture period, the HA coupons supported cell viability, proliferation, and osteogenic differentiation of both human osteosarcoma cells and MSCs. MSCs cultured in osteogenic medium for 14–35 days showed comparable morphology and differentiation markers on HA coupons and conventional tissue culture polystyrene, exhibiting both osteogenic and adipogenic traits.

Multimaterial DLP printing enables the integration of dissimilar materials within a single device, greatly expanding the functionality of microfluidic systems. Kim *et al.* [85] introduced a practical stereolithographic protocol using a “Print-Pause-Print” method to fabricate transparent, multimaterial microfluidic devices. The approach allows seamless resin exchange during printing, integrating

impermeable PEG-DA-250 for microchannel walls and permeable PEG-DA-575 or PEG-DA-700 for porous diffusion barriers. Channels are printed in three steps: bottom layer with PEG-DA-250, paused resin swap for porous barrier, and resumed printing to complete the top layer. Sliced image files ensure vertical alignment between layers. Demonstrated devices included serpentine and cross-channel diffusion chips that selectively permit hydrogen ion and fluorescein diffusion. Cytocompatibility assays using CHO cells and mouse hippocampal neurons showed viability comparable to commercial polystyrene dishes. Similarly, Jun *et al.* [86] developed a 3D printed perfusion bioreactor (3D PBR) for co-culturing human MSCs and vascular cells. The bioreactor incorporates a 0.4 μm porous PET membrane separating mesenchymal and vascular compartments while enabling media exchange. Printed via low-force SLA with surgical-grade methacrylate resin and assembled with precision bonding, the system supports dynamic perfusion via an integrated peristaltic pump and reservoir. Micro-computed tomography imaging validated the internal structure, and modeled shear stress confirmed appropriate flow conditions. The 3D PBR sustained >91% cell viability for MSCs and HUVECs across multiple perfusion days, demonstrating its suitability for tissue engineering applications.

OoC systems have transformed *in vitro* modeling by enabling dynamic, physiologically relevant cell-cell interactions. However, their complexity, high cost, and limited accessibility hinder broader adoption. Leveraging 3D printing, OoC platforms can now replicate organ-level functions with greater scalability and modularity. Rauti *et al.* [87] developed a reusable, modular insert chip fabricated via stereolithographic 3D printing. Designed to interface with standard well plates and multi-electrode arrays (MEAs), the chip features a porous membrane for co-culturing up to three cell types under controlled flow, and supports high-resolution

imaging and reuse. Functionality was validated by culturing HUVEC and Caco-2 cells under varied flow conditions, enabling straightforward integration of multiple measurement modalities. Shafique *et al.* [88] employed LCD-based 3D printing with a custom PEGDA-250-based photocurable resin to fabricate OoC devices with lateral features as small as 75 μm and vertical resolution down to 20 μm . Circular channels of 110 μm radius were achieved, and up to 3420 chips were printed simultaneously in an 8-h run, demonstrating scalable, low-cost manufacturing. Co-culture of spheroids and endothelial cells within the devices revealed endothelial sprouting, migration, and reorganization, highlighting the system's capacity for complex tissue modeling.

CONCLUSIONS AND FUTURE PERSPECTIVES

The integration of numerical simulation, photolithography, paper-based methods, and multimaterial 3D printing has markedly advanced the development of 3D cell culture systems. Numerical simulations enable precise optimization of microfluidic designs and culture conditions before fabrication. Paper-based techniques provide low-cost, flexible platforms well-suited for high-throughput biological assays. Multimaterial 3D printing facilitates the fabrication of high-resolution, structurally complex systems that replicate physiological microenvironments. Table 1 provides a comparative summary of the major fabrication methods used in 3D cell culture systems, outlining their respective advantages and disadvantages. Collectively, these technologies have established versatile and robust platforms for tissue engineering, drug screening, and disease modeling, driving innovation in *in vitro* cell culture research. Table 2 provides a comprehensive summary of the fabrication methods used for cell culture platforms, detailing the associated studies, the specific cell types used, and their intended applications. Future advancements in 3D cell culture systems are expected to emphasize scalability and

Table 1: Comparison of fabrication methods for cell cultures

Fabrication method	Advantages	Drawbacks
Photolithography and PDMS molding	High resolution (nanometer to micrometer scale)	Time-consuming and multiple-step process
	Excellent optical transparency	Requires high-cost cleanroom facilities
	High gas permeability, ideal for cell respiration	PDMS can absorb small hydrophobic molecules, skewing drug assays
	Well-established, robust protocols	Low mechanical stiffness; can deform under flow
Paper-based platforms	Biocompatible and flexible	Low structural integrity, can be fragile when wet
	Extremely low cost and disposable	Limited resolution compared to lithography
	Capillary-driven flow, no external pumps needed	Difficult to achieve dynamic, high flow rate perfusion
	Inherent 3D porosity supports 3D culture	Paper fibers can interfere with high-resolution imaging
3D printing (SLA/DLP)	Easily stacked for layered co-cultures and gradient generation	Potential for nonspecific binding of biomolecules
	Readily modified with wax printing, no cleanroom needed	Limited resolution compared to lithography
	Rapid prototyping and high design freedom	Resin cytotoxicity requires careful postprocessing
	Monolithic fabrication, fewer assembly steps	Stiffness, permeability material properties are resin-dependent
	Ability to create complex 3D architectures and internal channels	Can require support structures that are difficult to remove from microchannels
	Growing range of biocompatible resins	Surface roughness can be an issue for fluid flow
	Scalability for mass production	

PDMS: Photolithography combined with polydimethylsiloxane, DLP: Digital light processing, SLA: Stereolithography, 2D: Two-dimensional, 3D: Three-dimensional

Table 2: Summary of fabrication methods for cell culture platforms, associated studies, cell types, and applications

Fabrication method	Platform description/fabrication details	Purpose/application	Cell types	Reference
Lithography and PDMS	Double-layer device with gel/media channels and gas channels	To generate spatiotemporal oxygen tension heterogeneity in a 3D culture	MDA-MB-231	[34]
	Same platform as Koens <i>et al.</i> Cells cultured in adjacent gel channels	To investigate cancer cell migration and angiogenesis under controlled hypoxic conditions	MDA-MB-231 endothelial cells	[35]
	5-channel PDMS device from SU-8 mold, microposts allow cell migration	To create a 3D cellular spheroid with a perfusable vascular network	HLFs, HUVECs	[51]
	PDMS device with pneumatic valves to control a membrane barrier	Dynamic control of cell-cell interactions for studying synapse formation	Hippocampal neurons	[52]
	Vertically layered PDMS device with pressure-actuated valves	To study glia-neuron interactions and impact on synaptic number	CNS neurons, Glial cells	[53]
	Two-layer SU-8 photolithography to create master for cell trap arrays	Deterministic pairing of immune cells for single-cell analysis of immune activation	Lymphocytes T cells, APCs	[54]
	Paper-based	Stacked layers of chromatography paper impregnated with ECM gel	To create scalable 3D cultures that mimic tumor heterogeneity for analysis	Breast cancer cells
Wax-printed hydrophobic barriers on Whatman paper		Rapid screening of anti-melanogenic activity of natural compounds	B16F10 melanoma cells	[64]
Wax-printed channels and wells on cellulose filter paper		To create platform for 3D cell culture and chemosensitivity drug testing	Huh-7 human liver cancer cells	[66]
5-layer structure with SU-8 patterned paper and PDMS		To generate autonomous drug concentration gradients for high-throughput drug screening	HeLa cervical cancer cells	[69]
CiGiP stacks with perforated PVC sheets		To study metabolic response and radiation sensitivity under oxygen/nutrient gradients	A549 lung cancer cells	[71]
Wick-like device with two paper layers wicks medium via capillary action		To enable continuous medium supply for long-term 3D culture without external pumps	MCF-7, HepG2	[74]
3D printing		PμSLA printed on a porous membrane	To create a perfusable system for long-term liver culture	PHH
	DLP-printed modular system, micromixer, separator, and concentrator	Continuous harvesting of MSCs from microcarriers	MSCs	[80]
	DLP bioprinting with photoinitiator/UV absorber combo for low cytotoxicity	To fabricate cell-laden constructs	C2C12 myoblasts	[82]
	DLP stereolithography of hydroxyapatite slurry	To fabricate osteogenic bone tissue engineering scaffolds	Osteosarcoma cells, MSCs	[84]
	Multimaterial SLA "Print-Pause-Print" method to create permeable barriers	To create monolithic devices with integrated diffusion membranes	CHO	[85]
	SLA-printed modular insert chip with porous membrane for well plates	To create a reusable, modular Organ-on-a-Chip platform	HUVECs, Caco-2 cells	[87]

CiGiP: Cells-in-Gels-in-Paper, PDMS: Photolithography combined with polydimethylsiloxane, SLA: Stereolithography, MSCs: Mesenchymal stem cells, PHH: Primary human hepatocytes, DLP: Digital light processing, 2D: Two-dimensional, 3D: Three-dimensional, UV: Ultraviolet, ECM: Extracellular matrix, CNS: Central nervous system

standardization, enabling the translation of laboratory-based prototypes into reproducible and clinically relevant platforms. Equally critical is the development of biocompatible and sustainable materials, such as eco-friendly 3D printing substrates and paper-based scaffolds, to minimize cytotoxicity and reduce environmental impact. In addition, the integration of hybrid systems that combine biosensors, real-time imaging, and FSI simulations will be crucial for dynamic monitoring of cellular responses, paving the way for more sophisticated and responsive culture platforms.

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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