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# 3D fabrication of artificial cell microenvironments for mechanobiology

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Christine Selhuber-Unkel<sup>1</sup> and Barbara Schamberger<sup>1</sup>

Artificial scaffolds are indispensable tools in unraveling the complexity of mechanobiology under controlled conditions. Recent breakthroughs in microfabrication techniques for biological applications have revolutionized the field, enabling well-defined features that span from the subcellular to the multicellular scale. These methods particularly allow for unprecedented control of cell stimulation. This review will showcase research that combines such scaffolds with various stimulation techniques: mechanical stimulation, actuation by magnetic or electric fields, chemical stimulation, or manipulation by light. Additionally, it will introduce passive scaffolds that are actuated by the cells themselves. These systems help to understand forces applied by the cells to their environment and pave the way toward dynamic biohybrid, cell-based systems.

## Addresses

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This review comes from a themed issue on **Scaffold-based and scaffold-free approaches for mechanobiology, in-vitro disease modelling and treatment**

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## Keywords

Artificial cell environments, Mechanobiology, Multi-scale stimulations, Controlled micro-environments, Silica fume.

## Scaffold-driven actuation

The actuation of cells or tissues by their scaffold is an important tool to mimic the *in vivo* conditions of cells and to stimulate cell reactions, such as differentiation, migration, reorientation, or contraction [1,2]. The

cellular processes involved in the mechanical stimulation of cells are called mechanosensing, mechano-transduction, and mechanoresponse. Their role in the regulation of cell functions was reviewed in Ref. [3]. Indeed, tissue shape and mechanical properties of cell environments evolve over time. At a short timescale, they change due to organ activity (e.g. breathing, digestion, muscle contraction) in a reversible manner. Whereas at a long timescale, they are often modified in an irreversible way (e.g. during embryo development, tissue aging, disease progression such as cancer). These aspects are not only important for cells involved in active motion, like muscle cells, but also for all other cells as they are able to sense and react to changes in the mechanical environment. For example, cells of the cardiovascular system are exposed to cyclic stress and adjust to it by strain-stiffening. This can in turn have pathological consequences in, e.g. atherosclerosis lesion formation [4]. In tumor growth, the mechanical properties of the extracellular matrix have an influence on the progression of cancer and invasiveness. Furthermore, the mechanical properties of tumor and stroma are changing during tumor growth, leading to a positive feedback loop of cancer progression [5]. The investigation of the mechanical environment of the cells and their cellular responses is challenging *in vivo*. Being able to precisely mimic this environment *in vitro* would allow a precise control of the physical parameters that cells encounter. This should lead to a deeper understanding of their impact on cell behavior and functions. A direct application of that would be the achievement of complex but minimal models that reproduce cell behaviors *in vitro*, allowing them to model physiopathological conditions and limit animal use. Also, a better control of cell differentiation, such as of osteogenic cells, would improve tissue regeneration and more personalized treatments. Finally, flexible and precise methods to manipulate cells open new perspectives for diagnostic devices. Therefore, simplification of the cell–matrix system in the form of *in vitro* experiments is commonly employed. There are several methods available to change the mechanical environment of cells *in vitro* statically or dynamically. Methods like indentation, stimuli-responsive gels, constriction devices, and deformable membranes have been recently reviewed in Refs. [6,7]. In contrast to these methods, this review will highlight the advances made in 3D cell scaffold designs for mechano-transduction studies in recent years and will give an overview of different actuation mechanisms. The focus

will be, but not exclusively, on submillimeter scaffolds, whose size corresponds to the cell and tissue scale, and their novel fabrication techniques. Two-photon-polymerization (2PP) will be highlighted throughout this review, as it offers a powerful tool to create adaptive cell scaffolds [8,9]. This high-resolution 3D printing method allows for the fabrication of features down to the submicrometer length scale. In this technique, the polymerization of the resin is locally initiated by the nonlinear absorption of two photons inside voxels [8,9]. Additionally, several scaffold stimulation mechanisms have shown great promise in recent years, including external mechanical stimulation, magnetic and electrical fields, chemically induced material changes through swelling or shrinkage, and light-based activation.

### Physical actuation

#### Direct mechanical stimulation by indentation

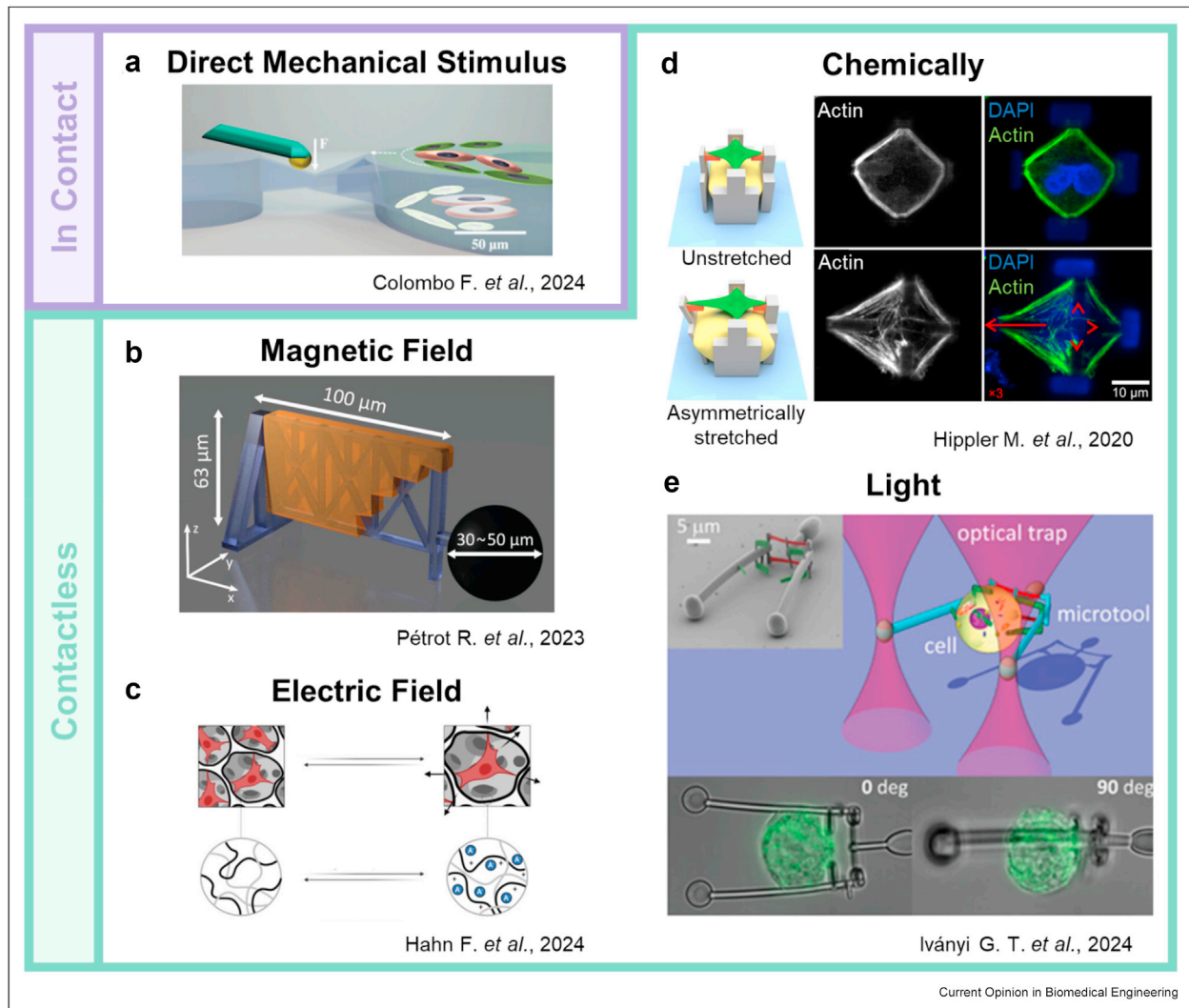
A simple method to change the mechanical environment of cells dynamically is introducing an external stimulus. One widely recognized approach involves platforms to stretch elastic substrates, thereby stimulating the cells mechanically [10–13]. This method primarily targets single cells or monolayers of cells that adhere to a substrate. As reviewed by Constantinou et al., imaging cells during stretching poses a challenge, for instance, due to drifts of the membrane relative to the objective, necessitating refocusing. Additionally, optical transparency of the substrate is required [10]. By using a more advanced actuation of the scaffold, e.g. through atomic force microscopy (AFM) or other microindentation approaches, recent studies have shown the possibility of mechanical stimulation in three-dimensional (3D) systems. For example, cells encapsulated in a 3D-printed hydrogel by 2PP and enclosed by an elastomer ring can be stimulated in a highly controlled manner. A nanoindentation device was used to deform the ring and consequently the hydrogel with defined frequencies (Figure 1a). There, it was shown that even short stimulation (30 min) of a 3D organotypic cell culture can lead to significant changes in the cellular morphology and its actin organization [14]. This method allows for the precise stimulation of cells in mono- and co-culture or even organoids, embedded in a 3D gel that mimics the physiological environment of the extracellular matrix [14]. Compared to classical methods where cells are cultured in extracellular matrix (ECM)-like matrix gels actuated by, for example, light, this method generates a force gradient across the sample. This gradient enables comparison between regions with different levels of applied force, or even no force, within a single sample. Another study focused on cell–cell adhesion using a 3D-printed platform actuated by an AFM tip [15]. This single-cell adhesion microtensile tester, manufactured by 2PP, enables to apply high strains while simultaneously observing cytoskeletal changes and stress increase in the cell–cell junctions. Remarkably, it also allows for the visualization of junction ruptures, as shown with keratinocyte cells [15,16]. The mentioned studies

require direct contact in the form of a cantilever and therefore are only suitable for manipulation from the surface of the scaffold. In the following sections, contactless methods are highlighted, which allow for manipulation in the inner core of a 3D scaffold. Such methods have many advantages compared to the ones mentioned so far. Therefore, a strong emphasis of this review is on different stimuli responsive materials and how they can be used in 3D printing for mechano-stimulation.

#### Magnetic field induced stimulation

A contactless method to apply a mechanical stimulus to a scaffold and the cells it is hosting can be achieved by magnetic fields. This can be realized by applying magnetic fields to a substrate made of magnetic components, i.e. magnetic particles [17,18]. Another approach is to attach a nonmagnetic scaffold to a magnetic particle. There, the scaffold can be bent by altering the position of the magnetic particle by an external magnetic field [19]. An advantage of using magnetic fields is that they can be applied without direct contact, allowing for actuation in areas that are not at the surface of a scaffold. Furthermore, it can be used as a very localized approach when applied to a multimaterial scaffold that has only some magnetic parts. The disadvantage of controlling scaffolds by magnetic fields is that typically only small forces in the range of pN can be applied [20,21]. A specialized magnetic tweezers system, commonly used for controlling the active motion of nanorobots, could be employed to exert higher forces. However, this is more challenging to handle compared to conventional magnets [22]. Another challenge in using magnetic particles is their limited biocompatibility, which is derived from multiple factors such as surface chemistry, charge, or size. Therefore, their toxicity must be carefully assessed for each particle type and its intended application. One approach to overcome this challenge is coating the particles with polymers or proteins, e.g. polyethylene glycol (PEG) or albumin [23,24]. There are several cases where a bulk material was successfully altered with magnetic nanoparticles, leading to cell stimulation by the deformation of the scaffold [17,18,21,25]. A vivid example shows the use of cyclic strain applied by a magnetic field to an implant material *in vivo* to modulate the host-immune response [17]. Another study showed how to arrange pillar-shaped magnetic particles in a biocompatible elastomer using a magnetic template. Having the particles in a defined pattern allows for more precise deformation of the cell culture substrate by the magnetic field [26]. Such actuatable setups can indeed be used to study the mechanotransduction of cells by combining them with traction force microscopy (TFM) [26,27]. A more localized actuation of a defined 3D environment has been realized by attaching scaffolds onto magnetic particles using 2PP (Figure 1b) [19]. A compliant silica-based hybrid polymer and a gelatin-based hydrogel were combined and linked to a magnetic bead. The combination of these two materials ensures structural integrity

Figure 1



Actuation of cells by scaffold: (a) by direct mechanical stimulus by indentation [14] (b) by magnetic field [19], (c) by electric field [31], (d) chemically [35], (e) with light [45]. (a) Reproduced under the terms of the CC by Creative Commons Attribution 4.0 International license (<https://creativecommons.org/licenses/by/4.0>) [14]. Copyright 2024 The Authors. Advanced Functional Materials published by Wiley-VCH GmbH. (b) Reproduced under the terms of the CC by Creative Commons Attribution 4.0 International license (<https://creativecommons.org/licenses/by/4.0>). Copyright 2023 The Authors. Advanced Functional Materials published by Wiley-VCH GmbH [19]. (c) Reproduced under the terms of the CC by Creative Commons Attribution 4.0 International license (<https://creativecommons.org/licenses/by/4.0>). Copyright 2024 The Authors. Published by the American Chemical Society [31]. (d) Reprinted with permission from AAAS [35]. Copyright 2020, AAAS. (e) Reprinted with permission from Wiley-VCH GmbH [45]. Copyright 2024 The Author(s). Advanced Materials published by Wiley-VCH GmbH.

as well as mimicking the physical properties of the extracellular matrix by the latter. Displacing the magnetic bead causes the bending of the scaffold, thus applying a well-controlled curvature to the cells [19]. It is also important to mention that not only scaffolds can be manipulated by magnetic fields, but also magnetically labeled cells themselves. This was applied, for example, to stimulate cardiomyogenesis in embryoid bodies of magnetically labeled cells by applying cyclic magnetic forces [28]. In this case the magnetic particles were

utilized to first form the embryonic bodies in a high throughput manner (up to 900 on one substrate) and then to stimulate the cells. Achieving such a high sample number would require substantial effort using classical methods.

#### Electrical field induced stimulation

Modifying the electrical field has proven to be a highly effective, contactless approach for altering the mechanical properties of electroconductive hydrogels [29].

Similarly to magnetic stimulation, the stimulation by electrical fields takes place not only on the surface but throughout the scaffold, enabling three-dimensional actuation. However, some limitations include the processability of electroresponsive materials and the hard predictability of their behavior [29,30]. Several polymer materials are known to be electrically responsive and are promising materials in cell culture and tissue engineering applications [29,30]. For example, poly(3,4-ethylenedioxythiophene) (PEDOT) is a conductive polymer that is biocompatible. Hydrogels with PEDOT as their electroactive component need only low voltages to elicit volume changes, which is beneficial for their application in cell culture [29,31]. An interesting example demonstrated the fabrication of polymerized high internal phase emulsion (polyHIPE)/PEDOT scaffolds with controllable porosity for the electromechanical stimulation of cells *in vitro* (Figure 1c) [31]. The polyHIPE material is composed of trimethylolpropane tris(3-mercaptopropionate) (TMPTMP) cross-linked poly(ethylene glycol) diacrylate (PEGDA). The inner channel surfaces are lined with a PEDOT coating. When a positive voltage is applied, PEDOT is oxidized, resulting in anion migration into the scaffold, which leads to its expansion. This process is fully reversible by applying a negative voltage. The responsive scaffold can adsorb ECM proteins without compromising its performance, allowing it to host and stimulate fibroblast cells [31]. Additionally, it is important to highlight that significant advances have been made in the field of printed electronics for recording and stimulating cells [32]. This technology holds great potential for applications in dynamic 3D microenvironments for studying various (multi)cellular processes.

#### Scaffolds responsive to chemical stimulation utilizing host-guest-chemistry

Additionally to the aforementioned stimulation methods, there are materials that alter their properties upon chemical changes in their environment. However, this strategy is limited for biomaterials, as chemical changes in the extracellular environment might significantly affect the cells as well. An interesting method to build materials responsive to chemical stimulation is to introduce host-guest interactions into the scaffold [33]. Host-guest chemistry belongs to the field of molecular recognition and describes the noncovalent bonding between supramolecular host molecules and complementary guests, i.e. ions or small molecules. These dynamic interactions are responsive to various external stimuli, which have been reviewed by Sinawang et al. [34]. In particular, the  $\beta$ -cyclodextrin/adamantane system is intriguing to fabricate hydrogels responsive to chemical stimulation [35,36].  $\beta$ -Cyclodextrin is a toroidally shaped macrocyclic molecule consisting of seven glucose units with a hydrophobic center, which can host adamantanes, forming the reversible host-guest

complexes [37]. When copolymerizing the  $\beta$ -cyclodextrin hosts and adamantane guests with e.g. acrylamide, the number of crosslinks in the hydrogel corresponds to the number of formed host-guest complexes. Since these complexes are in a dynamic, chemical equilibrium, the number of crosslinks can be decreased by the addition of a competitive adamantane-based guest to the media. Consequently, the Young's modulus of the hydrogel is decreased when the competitor is added and the material swells [35]. Hippler et al. combined this responsive hydrogel with other nonresponsive materials in a remarkable way at the subcellular level using 2PP. This enables entirely different dynamic stimulation compared to conventional 3D-printed scaffolds [35]. The resulting scaffolds take advantage of the host-guest interaction-based swelling to apply symmetrical and asymmetrical stretches to single cells (Figure 1d). The scaffold consists of four beams surrounding the responsive hydrogel, which facilitates a stretch of 1.5  $\mu\text{m}$  per beam for the symmetrical stretching after the addition of the adamantane competitor. For the asymmetrical setup, stretches around 5  $\mu\text{m}$  were achieved [35]. The studied cells showed remodeling in their cytoskeleton upon stretching and hence an increase in their traction forces [35]. A few years later, the same hydrogel material was also used on the macroscale to characterize the mechanosensing of human mesenchymal stem cells (hMSCs) [36]. Traction forces, YAP/TAZ signaling, and morphology were monitored, to reveal that dynamic changes of the Young's modulus first lead to change in traction force. Then, alteration of the YAP/TAZ localization as well morphological changes were observed [36]. An advantage of this method is its scalability, as multiple structures with cells can be arranged on a single substrate. Additionally, the cells can directly be imaged during stretching, which is applied perpendicular to the imaging direction. Finally, the cells can also be fixed and stained while in the stretched condition [35,36].

#### Manipulation of scaffolds by light

##### Light responsive materials

Light responsive materials are highly interesting for dynamic cell scaffolds, since they can be stimulated with high spatial and temporal control by adjusting the light intensity, illumination duration, and localization. Furthermore, they offer a significant advantage by eliminating the need for advanced manipulation setups such as optical tweezers, nanoindenters, and magnetic or electrical field generators. A crucial parameter in applying light to materials and cells is the power and wavelength of the illumination. Longer wavelengths in the visible or near-infrared (NIR) range are more feasible for *in vitro* applications than UV light [38]. Next to light-driven bond formations and breakages [39], isomerizations are found in e.g. azobenzenes [40] or molecular motors [41], may be employed into materials to drive scaffold actuations. For example, the

photosensitive allyl sulfide bond rearrangement was used to alter the viscoelasticity of a PEG hydrogel crosslinked by these bonds. Upon irradiation, the stress of the bulk material decreases up to 75% depending on the illumination time [42]. The hydrogel was employed to alter the epithelial curvature of embedded intestinal organoids, generating the characteristic structure of intestinal epithelia [42]. This was achieved by applying light patterns of 30–70  $\mu\text{m}$  width to the material adjacent to the organoids. In the irradiated regions, the allyl sulfide bond rearranges and softens the material. The organoids directly react to this mechanical change in the microenvironment, which was observed by the change in their curvature in the irradiated regions [42]. It was shown that the formation of pockets of the epithelial layer (known as crypts), which are randomly distributed on unpatterned intestinal organoids, could be directed by the irradiation patterns applied [42]. In a study employing a different class of light-responsive building blocks, motor amphiphiles were used to create light-responsive, nontoxic supramolecular artificial muscles [41]. There, the scaffolds were manufactured based on the self-assembly of molecular motor units, which were equipped with hydrophilic and hydrophobic side chains. Upon irradiation, this synthetic molecular motor isomerizes, causing the scaffold to bend. Due to the addition of hydrophilic end groups commonly found in extracellular matrix (ECM), mesenchymal stem cells could readily be cultured up to 5 days on these scaffolds. Then, the scaffolds were actuated by light illumination at 365 nm for 30 s [41]. So far, this approach is still in its early stages, but the development of synthetic printable motor amphiphiles have strong potential to provide more complex scaffold designs for advanced applications in the future. In particular the short wavelength needed to actuate the scaffold is a limitation for *in vitro* applications. Another method to change material properties by light is an indirect approach based on the photothermal effect [43]. This effect can be used by introducing gold nanoparticles to a transparent, thermoresponsive material. For example, near-infrared irradiation was used to excite gold nanoparticles embedded in a thermoresponsive poly-*N*-isopropylacrylamide (pNIPAM) hydrogel to locally actuate stem cells [43]. The gold nanoparticles transform the NIR irradiation locally into heat followed by the hydrogel shrinkage. By this method, hMSCs were stimulated with different irradiation patterns leading to osteogenic differentiation [43]. Using the photothermal effect by localized NIR irradiation, the global heating of the system is not required, thereby preventing potential cell damage. However, there are some examples, e.g. cell sorting devices, where pNIPAM can be applied successfully by short global heating processes. This was shown for a microfluidic chip equipped with 2PP printed pNIPAM grippers, which sort and catch cells guided through the chip. After the catching, the cells were released upon heating the system shortly, in the range of

a few minutes, to 42 °C [44]. This proof-of-concept work shows that the combination of microfluidics, 2PP and responsive materials has great potential for the fabrication of adaptive lab- or organ-on-chip devices to manipulate cells.

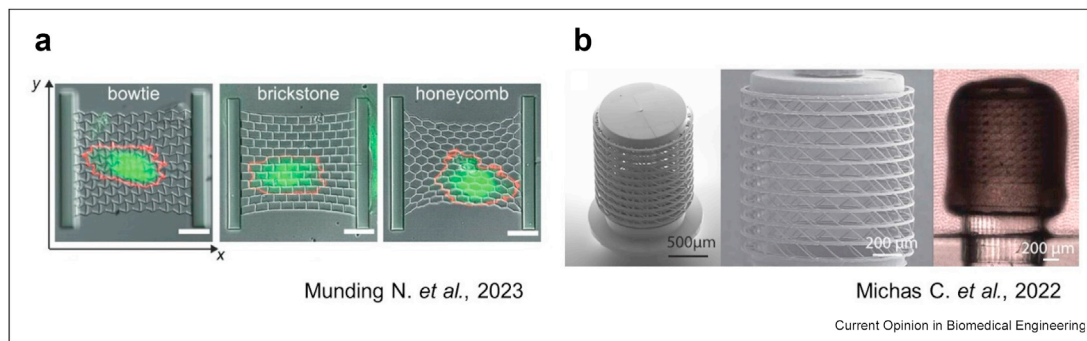
#### Actuation using optical tweezer

Additionally to light-responsive materials, optical tweezers can manipulate either cells directly or the scaffold, e.g. microrobots, and in turn indirectly stimulate cells mechanically. The latter strategy has the advantage of avoiding phototoxicity affecting the cells. Using this so-called indirect optical manipulation, Iványi *et al.* have remarkably shown that light-driven microrobots can be actuated to capture cells and study, e.g. cell–cell interactions (Figure 1e) [45]. Such microrobots, based on a multimaterial design, were fabricated by 2PP with elastic building blocks. These elastic parts are the key elements of the system, enabling its optical actuation. It was demonstrated that cells can not only be trapped but also positioned and released into microcontainers (200  $\mu\text{m}$  diameter, 50  $\mu\text{m}$  height) with speeds of up to 300  $\mu\text{m s}^{-1}$  in the timeframe of a few minutes [45]. The cell trapping solely relies on the actuation by the optical tweezers and the elasticity of the microrobot arms, so the cell can exit the robot after the manipulation [45]. This is an important advance, as other cell trapping approaches are often irreversible because they rely on biochemical bonding between the scaffold and cells [46]. Therefore, the described systems have the potential to revolutionize single cell manipulations in the future.

#### Actuation of materials by cells

So far, we have mainly discussed the actuation of cells by materials responsive to external stimuli. However, cells themselves can also actuate materials and thus play a role as active actuation units, like small motors, in a material. To understand and quantify the forces cells exert on their environment, several methods have been established, including AFM, TFM, or shear flow assays [47,48]. Despite such well-established methods, novel techniques in microfabrication allow for more complex cell scaffolds to mimic the extracellular environment of the cells. Since recent years, the most commonly used technique for scaffold manufacturing has been 2PP due to its high resolution (subcellular) and the expanding list of biocompatible materials that can be printed with this technique [49]. An example using 2PP to study cell–scaffold interactions is utilizing metamaterials, adding functional properties beyond the ones from the bulk material [50]. For example, a study used 2D metamaterials printed by 2PP to investigate the effect of the elastic properties of the scaffold towards cell behavior (Figure 2a) [51]. There the authors printed metamaterials with different unit cells, which is the simplest pattern that is periodically repeated in the

Figure 2



Cells on a metamaterial to (a) investigate cellular behavior [51] or (b) to actuate the material to create a pump [53] (a) scale bars: 30  $\mu\text{m}$ . (a) Reprinted with permission from Wiley-VCH GmbH [51]. Copyright 2023, The Authors. *Advanced Functional Materials* published by Wiley-VCH GmbH. (b) Reprinted with permission from AAAS [53]. Copyright 2022, the American Association for the Advancement of Science.

scaffold. The difference in the unit cell design led to changes in the elastic properties of the metamaterial, i.e. the effective Poisson's ratio being negative, close to zero, or positive. In turn, these different elastic properties had an effect on the cellular morphology [51]. By utilizing the concept of metamaterials for the design of 3D scaffolds, it is possible to fabricate microenvironments for multicellular systems with high elasticity. An example of such a system is a structure with auxetic design. These structures have a negative Poisson's ratio, a property commonly found in certain biological tissues, making them excellent models for studying cellular behavior [52]. The auxetic 3D scaffold made of a stiff material allowed cultured mouse fibroblasts to deform the structure and furthermore to direct cell migration on the scaffold [52]. The increase in complexity going from 2D sheets toward a 3D scaffold design as well as from a single cell to a multicellular tissue raises challenges in guiding and analyzing the cellular behavior. However, it holds promises to provide new insights into cell control by mechanical properties in the future. Employing the concept of growing tissue on a 3D metamaterial has already been used to create a cell-actuated micropump [53]. This was achieved by cultivating human induced pluripotent stem cells (iPSCs) derived cardiomyocytes on a cylindrically shaped metamaterial (Figure 2b). The 2PP printed metamaterial guided the cardiac tissue formation, and bidirectional flows were produced by cyclic contraction of the tissue. In a second step, the cardiac pump was connected to supply and output wells *via* microvalves on a microfluidic chip, enabling the direction of the flow within the device [53]. Designs like these can also be used to model and measure forces applied collectively by cells in the presence of an extracellular matrix, unlike most setups that focus on individual cells and do not mimic physiological

conditions. Thus, this presents a captivating example of how cells can be employed to actuate a scaffold, effectively creating a fully functional micromachine.

### Conclusion and outlook

To conclude, physical forces are powerful regulators of biological systems. Consequently, forces exerted on cells by the scaffold lead to significant responses within cellular systems. Conversely, forces applied by cells, acting as small motors on the scaffold, can influence a scaffold's structure and functionality. This feedback loop holds great promise for investigating and utilizing mechanobiological processes in cells and should be carefully considered when selecting methods for such investigations. In this review, we described 2PP as an important method for producing cell scaffolds for mechanobiological investigations. Its high 3D resolution, which is smaller than the length scale of a cell, allows precise control of the cell environment, both structurally (resolution  $<1 \mu\text{m}$ ) and mechanically. The latter being reflected in the variety of materials already existing for 2PP printing with stiffnesses similar to soft tissue (several hundred Pa) up to stiff tissue (several GPa). The transition from 2D to 3D scaffolds and their increase in complexity leads to challenges in fabrication, imaging, and analysis [54]. Especially, long printing times for extended structures with features on the microscale limit their application for systematic cell studies or biomedical research. Another challenge in the field is the development of responsive materials that are biocompatible. Often either the material itself is toxic to the cells or the stimulation method (light, pH, temperature). This review shows studies overcoming this challenge by e.g. utilizing host-guest chemistry [35], localized heating using gold nanoparticles in pNIPAM [43], or indirect optical trapping [45]. Most of the

presented materials can only be used after their fabrication, due to cytotoxic steps involved in the fabrication process. This limits the way cells can be positioned on and actuated by the systems. One example shown successfully constrained and altered epithelial organoids within a biocompatible light-responsive material *in situ* [42]. Additionally, 2PP in the presence of cells with a subsequent direct mechanical stimulation on the surface of the scaffold was presented [14]. However, a remaining challenge is the development of materials for 2PP, which can be printed in the presence of cells and actuated not only through the surface but throughout the scaffold. Despite these drawbacks, the reviewed methods and materials show potential to study the mechanically induced interplay between cells and extracellular structures and pave the way for further advances within the field.

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Chat GPT-4 and deepL Write in order to improve the readability and language of the manuscript. After using this tool/service, the author(s) manually reviewed all content to ensure technical accuracy and edited the content as needed. The author(s) take(s) full responsibility for the content of the published article.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christine Selhuber-Unkel, but also other authors reports financial support and travel were provided by Funding agencies, as listed in the acknowledgements and funding list. Christine Selhuber-Unkel reports a relationship with Other universities, societies, foundations etc. that includes: board membership, consulting or advisory, funding grants, speaking and lecture fees, and travel reimbursement. Barbara Schamberger reports a relationship with Other universities, societies, foundations etc. (Paris Lodron University, EuroCurvoBioNet etc.) that includes: board membership, employment, funding grants, and travel reimbursement. Caterina Tomba reports a relationship with Other universities, societies, foundations etc. (ANR, CNRS, EuroCurvoBioNet etc.) that includes: employment, funding grants, speaking and lecture fees, and travel reimbursement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Data availability

No data were used for the research described in the article.

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Papers of particular interest, published within the period of review, have been highlighted as:

- \* of special interest
- \*\* of outstanding interest

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