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A review of stimuli-responsive materials in 4D bioprinting for biomedical applications

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Four-dimensional (4D) bioprinting has been increasingly explored owing to its potential to create dynamic, tunable structures that can respond to external stimuli. The fusion of dynamic stimuli-responsive materials with conventional three-dimensional printing technologies forms the foundation of 4D bioprinting and has the potential to revolutionize tissue engineering. 4D bioprinting involves the printing of structures whose shape, function, or properties can be altered as a function of time in response to environmental stimuli. Critical to 4D bioprinting is the development of smart biomaterials that respond to different stimuli, such as temperature, pH, light, and biochemical signals. This review explores various stimuli-responsive materials used in 4D bioprinting for biomedical applications. Furthermore, it provides an overview of material properties and categorizes them according to their responsiveness to external stimuli. Additionally, the current trends in stimuli-responsive materials for 4D bioprinting, and their applications and critical challenges in tissue regeneration, drug delivery, and personalized medicine are identified.

1. Introduction

Bioprinting is a form of advanced additive manufacturing with bioinks made up of living cells and biocompatible polymers to design complex, three-dimensional constructs to reproduce native tissue form and function. 4D bioprinting is an advance in the field of bioprinting that adds temporal elements to conventional 3D bioprinting.[1–3] This new strategy enables dynamic structures that are capable of undergoing shape or functional changes in response to an environmental stimulus such as temperature, pH, or light.[4] 3D bioprinting has significantly contributed to tissue engineering through the fabrication of complex tissue constructs using biocompatible materials and cells. The bioink used in conventional bioprinting does not respond to external stimuli. 4D bioprinting involves engineering materials that can sense their surroundings and respond to external stimuli by changing their properties.[5]

The history of 4D bioprinting traces back to the early 21st century, when researchers began investigating the capabilities of stimuli-responsive materials or 'smart materials'. These materials can undergo reversible changes in response to specific external stimuli. 4D printing was first postulated by Skylar Tibbits in 2013 when he suggested the use of self-assembled materials to make adaptive structures that change over a period of time.[6] With the development of biocompatible responsive materials, 4D bioprinting technologies can be applied in areas such as tissue engineering, drug delivery, and regenerative medicine.

Stimuli-responsive materials can be classified on the basis of their response to applied external stimuli, such as temperature-sensitive hydrogels, which swell or shrink with changes in temperature; pH-sensitive materials, which change their properties on the basis of the basicity/acidity of the environment; or light-sensitive polymers, which change their properties upon illumination by a specific wavelength of light. Furthermore, responsive materials have also been engineered to respond to other stimuli, such as humidity, electricity, or magnetic fields. The integration of such stimuli-responsive materials into bioprinting has revealed new paths for designing responsive and functional tissue constructs for biomedical applications.[7–9]

Using SRMs, tissue-engineered scaffolds that can dynamically modify their properties in response to biological stimuli can be developed by integrating stimuli-responsive materials into printed scaffolds. These scaffolds can be designed to degrade at a rate comparable with that of new tissue generation.[10–12] In addition, 4D bioprinting using SRMs enables the fabrication of shape-morphing constructs that can respond to multiple external or internal stimuli. This ability of SRMs opens avenues for engineering advanced tissue grafts that can adapt their mechanical or biochemical microenvironment *in situ* to better mimic native tissue dynamics. Furthermore, smart

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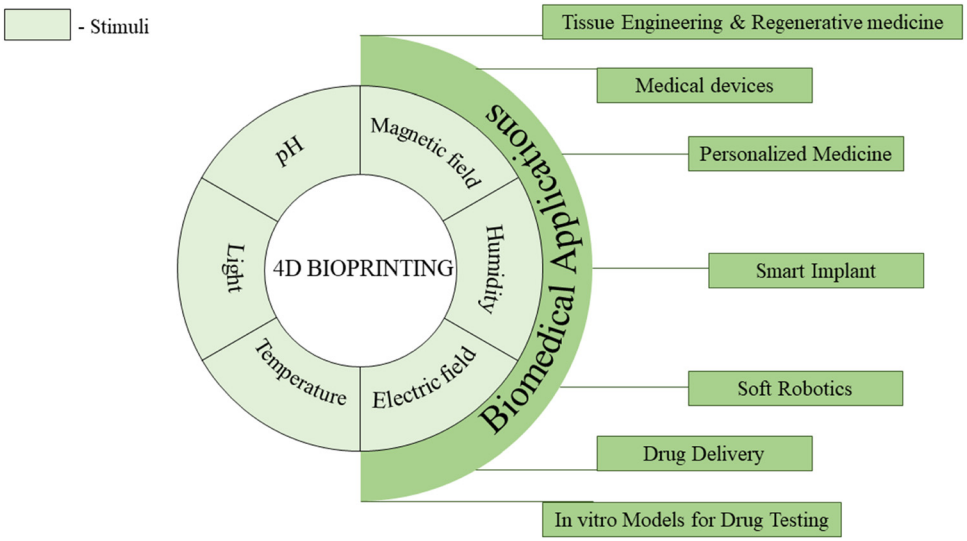

Fig. 1 Diagrammatic overview of various stimuli for 4D bioprinting and their biomedical applications.

hydrogels and shape-memory polymers incorporated in 4D bioprinted structures can facilitate controlled cell behavior, enhance vascularization, and promote tissue regeneration by dynamically interacting with the surrounding environment. These multifunctional constructs have the potential to be used to develop bioactuators, biosensors, and personalized implants that actively participate in healing processes and respond to changing physiological conditions (Fig. 1).[13–15]

Although many advances have been made in the field of 4D bioprinting, there are still challenges that need to be addressed. These include the development of more advanced bioinks, the optimization of printing conditions, and clinical translation of 4D bioprinted materials. Many recent review articles have provided in-depth analysis of individual classes of stimuli-responsive or 'smart' materials such as magnetic, temperature-responsive, pH-sensitive, and light-responsive polymers, primarily focusing on the detailed mechanisms, synthesis strategies, and specific biomedical applications of each material type.[16–18] This review aims to provide a broader perspective on 4D bioprinting, integrating information on the various classes of stimuli-responsive materials while highlighting their overall potential, challenges, and biomedical applications. By offering a comprehensive overview rather than an in-depth examination of individual material classes, this review aims to explain how these smart materials collectively contribute to the advancement of dynamic tissue engineering, regenerative medicine, and functional biofabrication.

2. Stimuli-responsive materials for bioprinting

Stimuli-responsive materials (SRMs) are distinct from other biomaterials because they can transform shape or properties in response to external stimuli.[19] These stimuli can trigger the shape memory or shape-morphing ability of the printed constructs. Upon exposure to an external stimulus, they create a memory effect that allows a transition from the original shape to a programmed temporary shape. SRMs can be engineered to revert back from their programmed temporary shape to their original shape upon the removal of stimuli. The incorporation of these stimuli-responsive materials in 4D bioprinting increases the functionality and adaptability of the resulting constructs for biomedical applications.[4,7,20]

2.1. Temperature-responsive materials

Temperature-sensitive materials, or temperature-sensitive polymers, are a group of smart materials whose physical properties significantly change in response to changes in temperature. Temperature-responsive materials can be classified on the basis of their critical solution temperature into lower critical solution temperature (LCST) or upper critical solution temperature (UCST) types. In materials that exhibit LCST behavior, a coil-to-globule transition is observed above the critical temperature. Below the LCST, the polymer chains have an expanded coil conformation with a homogeneous solution. Nevertheless, above the LCST, the polymer chains shrink into compact globules, resulting in phase separation and increased turbidity. This is due to entropy changes, especially the hydrophobic effect, which modify the ordering of water molecules around the polymer with increasing temperature (Fig. 2).[21,22]

Thermoresponsive polymers have been widely studied in 4D bioprinting because they enable constructs to respond dynamically to physiological conditions. One of the first natural polymers investigated was gelatin, which undergoes a sol–gel transition below 30 °C. Gelatin is mainly printed *via* extrusion-based bioprinting, as its viscosity can be controlled by cooling during deposition. Fibroblast- and chondrocyte-laden scaffolds were fabricated successfully; however, one challenge was that gelatin filaments lost shape fidelity and collapsed once they were warmed to 37 °C. This meant that gelatin alone could not form lasting printed constructs. Currently, gelatin is used as a

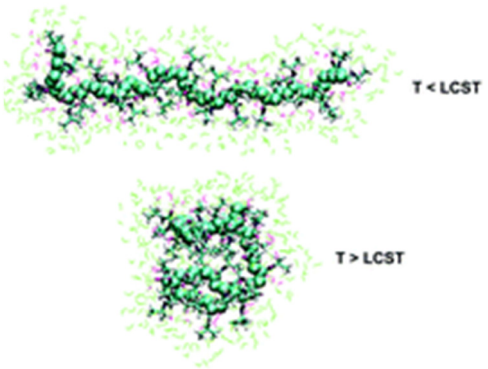

Fig. 2 Molecular representation of the coil-to-globule transition of poly(*N*-isopropylacrylamide) (PNIPAM) chains in water across the lower critical solution temperature (LCST). At temperatures below the LCST (T < LCST), PNIPAM chains adopt an expanded, hydrated coil conformation stabilized by hydrogen bonding with water. When the temperature exceeds the LCST (T > LCST), dehydration and enhanced hydrophobic interactions lead to a compact globular conformation. Reproduced from ref. 23 with permission from Creative Commons CC BY-NC 3.0.

sacrificial or supportive matrix for 3D or 4D bioprinting applications.[24,25] Various other temperature-responsive polymers that have been recently explored for use as SRMs include poly(*N*-isopropyl acrylamide) (PNIPAm), poly(ethylene glycol) (PEG) and poly(*N*-vinyl caprolactam) (PNVCL).[26,27] Recently, these polymers have gained attention and are used along with cells for printing tissue engineering constructs. To print tissue-engineered constructs using temperature-responsive polymers, a solution of the polymer along with the cells is maintained below the LCST of the polymer and extruded on the print bed *via* a bioprinter. The bed of the bioprinter is maintained above the critical temperature, resulting in gelation of the polymer solution with cells to form the tissue construct.[28]

Chemically crosslinked methylcellulose (MC) hydrogels are of particular interest as thermoresponsive biomaterials for advanced tissue engineering applications, especially for cell sheet engineering. In a recent study by Bonetti *et al.* (2021),[29] MC was chemically crosslinked with citric acid (CA), resulting in a tough hydrogel with greatly enhanced mechanical properties.[29] The extent of crosslinking can be controlled by varying the CA concentration, allowing for an 11-fold enhancement in the mechanical properties compared with those of noncrosslinked MC. Remarkably, even with chemical crosslinking, MC maintained its native thermoresponsive character. This property is due to the reversible hydrophilic–hydrophobic transition of MC chains at physiological temperatures ($\approx$ 37 °C). The hydrogel is hydrophobic, allowing cell adhesion and proliferation above the LCST; when the hydrogel is cooled below the LCST, the material becomes hydrophilic, inducing spontaneous cell sheet detachment without enzymatic digestion. This mild, temperature-mediated release maintains the integrity of the extracellular matrix and cell–cell junctions, which is an important requirement for building functional tissue sheets. From the perspective of 4D bioprinting, these thermoresponsive MC hydrogels offer the dynamic functionality necessary to develop smart, stimuli-responsive tissue constructs. The MC-CA hydrogels provided conditions for stepwise tissue maturation, on-demand release of sheets, and layer-by-layer assembly of the sheets. The tunable mechanical properties and cytocompatibility of these materials make them suitable for fabricating dynamic tissue constructs for 4D bioprinting applications for regenerative medicine and *in vitro* tissue modeling (Fig. 3).

Nie *et al.* (2022)[30] aimed to develop a novel hydrogel system that combines the benefits of temperature responsiveness and mechanical reinforcement for effective cell encapsulation. The hydrogel was synthesized by incorporating poly(*N*-isopropylacrylamide) (PNIPAM), a thermoresponsive polymer known for its lower critical solution temperature (LCST) behavior near physiological temperature, with hydroxyethyl-chitosan (HECS), a biocompatible polysaccharide. Graphene oxide (GO) nanosheets were integrated into the network to enhance the mechanical properties and stability of the hydrogel. This composite hydrogel demonstrated a reversible sol–gel transition in response to temperature changes, allowing for the encapsulation of cells at lower temperatures and gelation at body temperature. *In vitro* studies indicated that the hydrogel supported the viability and proliferation of encapsulated cells, suggesting

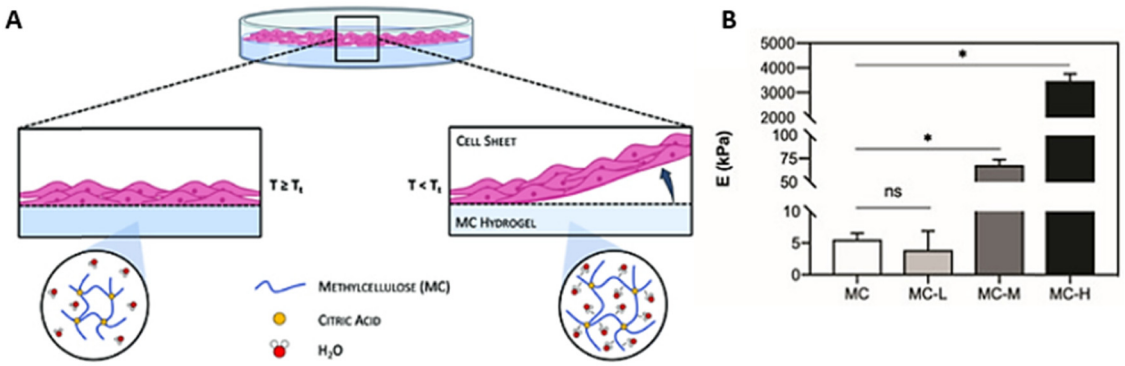

Fig. 3 (A) Thermoresponsive methylcellulose (MC) hydrogels crosslinked with citric acid (CA) at $T < T_t$ are in the sol (hydrophilic) state, and those at $T \geq T_t$ are in the gel (hydrophobic) state. (B) Young's modulus (E) of CA-crosslinked MC hydrogels. * = $p < 0.05$. MC-L, MC-M, and MC-H indicate low, medium, and high degrees of crosslinking, respectively. Reproduced from ref. 29 licensed under Creative Commons Attribution (CC BY).

its potential for cell-based therapies. However, the study did not report on *in vivo* evaluations. The developed temperature-responsive hydrogel offers a promising platform for cell encapsulation applications, combining the advantages of thermoresponsive behavior and enhanced mechanical properties through graphene oxide reinforcement.[30] Tan *et al.* (2009)[31] synthesized a new thermosensitive hydrogel from a copolymer of *N*-isopropylacrylamide (NIPAAm) and *N*-hydroxymethylacrylamide (HMAAm) for the encapsulation of chondrocytes.[31,32] The hydrogel was synthesized *via* free radical copolymerization of NIPAAm and HMAAm monomers in the presence of *N*,*N*′-methylenebisacrylamide as the crosslinker. The insertion of HMAAm units into the PNIPAAm backbone increased the hydrophilicity and biocompatibility of the hydrogel. Porcine articular chondrocytes were encapsulated in the PNIPAAm-*co*-HMAAm hydrogel. The hydrogel showed an LCST at 32 °C and a reversible sol–gel transition with a change in physiological temperature. At temperatures below the LCST, the hydrogel was in the form of a liquid, facilitating cell encapsulation. Upon reaching body temperature (37 °C), the hydrogel formed a solid gel that encapsulated the chondrocytes within its three-dimensional network. The encapsulated chondrocytes also showed good cell viability and an elliptical cell shape, which is the typical form of the native cartilage phenotype. The thermoresponsiveness of the PNIPAm-*co*-HMAAm hydrogel provided a minimally invasive route for delivering the cell-laden construct into cartilage defects. The PNIPAm-*co*-HMAAm bioink can be printed in liquid form and allowed to solidify where applied, thus creating a cartilage-regenerating matrix. This work demonstrated the feasibility of using a heat-sensitive hydrogel system for application in cartilage tissue engineering.[33]

In another study, Choudhury *et al.* (2024)[34] developed a 4D-printed vascular graft scaffold using a shape-memory thermoplastic polymer, PLMC (poly(lactide-*co*-trimethylene carbonate)), printed *via* melt-extrusion 3D printing technology with anisotropic infill patterns to encode programmable deformation. The constructs exploit thermal responsiveness at ∼80 °C, and the flat printed sheets spontaneously rolled into tubular structures, whereas the polymer's shape-memory property enabled them to be temporarily flattened and then recover back into the tube form at near-physiological temperature (∼37 °C). To demonstrate biofunctionality, the scaffolds were seeded with endothelial cells on a flat geometry, and subsequent recovery into a tubular form yielded a cell-lined lumen, as confirmed by live/dead staining, cytoskeletal (F-actin/DAPI) imaging, and proliferation assays, which revealed good viability and spreading. This dual shape-morphing and shape-memory mechanism illustrates a promising strategy for creating cellularized vascular grafts, where minimally invasive delivery of flat constructs followed by *in situ* morphing into tubular lumens could be harnessed for regenerative vascular applications.[34]

The development of biodegradable thermosensitive shape-memory polymers (SMPs), such as PLA-, PCL-, and polyurethane-based systems, represents a significant advance in the field of 3D printing. These materials are usually processed *via* the melt extrusion printing method (fused deposition modeling, FDM), which enables the fabrication of implants, self-expanding stents and bone scaffolds. However, the integration of SMPs with bioprinting causes thermal stress on cells, making it difficult to encapsulate living cells in SMP inks.[35] To overcome this, most studies have used SMPs to fabricate structural frameworks on which cells are subsequently seeded. Despite this limitation, SMPs outperformed earlier thermoresponsive systems in terms of structural robustness and translational potential. Combining thermoresponsive materials with other class of SRMs has greater potential in biomedical applications. Magneto-thermoresponsive composites were printed mainly *via* extrusion-based bioprinting of gelatin, alginate, or silk fibroin matrices containing magnetic nanoparticles.[36] This allowed the fabrication of cell-laden scaffolds capable of bending or contracting under localized heating by alternating magnetic fields. The printing fidelity was high, but challenges included nanoparticle sedimentation during extrusion, which caused inconsistent distributions within the printed constructs. In addition, nozzle clogging is occasionally reported to occur due to particle aggregation. Despite these issues, *in vitro* studies confirmed the high viability of encapsulated fibroblasts and stem cells, and *in vivo* rodent models demonstrated safe actuation.[37]

These studies demonstrate that temperature-sensitive materials can be used for 4D bioprinting applications. These materials support the growth of more than one cell type, thus supporting the development of functional tissue. Temperature-sensitive materials face a few challenges that may impact their effectiveness in biomedical applications. One among them is the inability to maintain the phase transition temperature with a high degree of precision during printing. Another challenge is that some thermoresponsive materials exhibit hysteresis or irreversible aggregation, limiting their real-world application. Further concentrations of other substances in media (*i.e.*, salts and proteins) can significantly affect the transition characteristics of such polymers, leading to variability in their responsiveness. Variability in polymer synthesis and batch-to-batch variations also contribute to differences in transition temperatures and rheological properties, making standardized printing difficult. Furthermore, the lack of commercially available thermoresponsive bioinks makes it difficult to standardize printing and validation across various laboratories. Although temperature-responsive polymers are highly promising, these problems need to be addressed before their application in drug delivery, tissue engineering, and regenerative medicine can be engineered.[38]

2.2. pH-responsive materials

pH-responsive polymers are an interesting class of smart materials whose physical and chemical properties change in response to environmental pH. This unique responsiveness is primarily due to the ionizing functional groups in their molecular chains. Polyacids and polybases both embody such materials. Polyacids containing acidic groups, such as carboxylic acids (–COOH) or sulfonic acids (–SO_3H), become protonated at low pH values, or negatively charged at high pH values. These acidic groups become deprotonated, resulting in the

accumulation of negative charges under relatively high pH conditions. Charge reversal results in electrostatic repulsion between the polymer chains, causing the material to swell and alter its physical properties. Conversely, polybases contain basic groups such as amines ($-NH_2$) that can accept protons at low pH and become positively charged. These materials tend to swell at pH values below their pKa, allowing dynamic structural changes in response to shifts in the environmental pH.[39–41]

The ability to respond to pH variations makes pH-responsive materials beneficial in various applications, most notably in drug delivery applications. They can be designed to release drugs in a controlled manner, initiated by the acidic conditions that are generally found in the microenvironments of cancers or inflamed tissues. This pH-sensitive drug release system enhances the therapeutic efficacy of drugs and minimizes potential side effects by preventing their premature release in normal tissue. pH-sensitive materials also offer flexibility in designing complex delivery systems that can be developed to deliver more than a single therapeutic agent, leading to improved efficacy in therapy for diseases. With continuous research in this field, there are numerous pH-sensitive materials that are generating new drug delivery platforms for tissue engineering applications.[39,40,42–44]

Research has been recently concentrated on enhancing the functionality of pH-sensitive polymers *via* novel synthesis methods. A study by Parimita *et al.* (2025)[45] introduced a new method for the preparation of pH-responsive bilayer hydrogel actuators with 4D printing technology. Researchers have created a bilayer system based on chitosan (CS) and carboxymethyl cellulose (CMC) hydrogels cross-linked with citric acid that achieves high interfacial adhesion without undergoing chemical surface modification. This high adhesion ensures antidelamination during actuation, which is typically a problem with bilayer hydrogels. With direct ink writing (DIW), an extrusion-based 3D printing technique, they printed intricate bilayer and patterned structures with controlled geometry and layer thickness. The ink rheology and printing parameters were optimized for the printability and mechanical stability of the hydrogel layers. The actuator exhibited programmable, reversible shape-shifting under different pH conditions. It displays bidirectional bending: the structure bends in one direction under acidic conditions and reverses under basic conditions, with neutral pH being a nonmorphing point. This is a result of differential swelling of the two hydrogel layers as a consequence of ionization of their functional groups, resulting in strain mismatch that triggers bending. Effective shape transformation without layer separation is ensured by strong interfacial adhesion. This pH-responsive programmability enables accurate control of the direction and magnitude of actuation, allowing for intricate shape transformations beyond mere bending. This research pushes the frontiers of 4D printing by overcoming the limitations of earlier pH-responsive actuators, which were primarily restricted to simple 2D films with poor interlayer adhesion. Through the combination of biopolymer hydrogels with DIW 3D printing, the authors present a scalable means to fabricate customizable, stimuli-responsive actuators with robust mechanical strength and intricate architectures. This research reveals future prospects in biomedical devices and soft robots, including smart valves and biomimetic structures, and the potential of 4D-printed hydrogels for programmable, multifunctional soft actuators.[45]

Research has investigated the use of alginate-based hydrogels as bioinks for 3D bioprinting applications aimed at articular cartilage tissue engineering. This study emphasizes the importance of alginate's molecular weight and the mannuronic (M) to guluronic (G) acid ratio in determining the viscosity and cross-linking behavior of hydrogels, which are crucial for achieving the desired printability and mechanical properties. The pH-dependent gelation process, facilitated by calcium ions, was explored to understand its impact on the structural integrity and resolution of the printed scaffolds. While the primary focus is on the material properties and printability of alginate-based bioinks, the findings suggest potential for cell encapsulation, offering insights into their application in tissue engineering. This study identified challenges such as nozzle clogging and inconsistent layer deposition during the printing process, which are attributed to the shear-thinning behavior of alginate and the need for precise control over cross-linking. Research has proposed blending alginate with other biomaterials to increase mechanical stability and printability, along with incorporating growth factors to improve the functionality and longevity of printed cartilage constructs.[46]

Jongprasitkul *et al.* (2023)[47] addressed the limitations of poor printability and low structural fidelity in hyaluronic acid (HA)-based bioinks for extrusion-based 3D bioprinting by developing a pH-responsive, gallol-functionalized HA hydrogel suitable for injection and bioprinting applications. The bioink was formulated by combining gallic acid-functionalized HA (HAGA), which provides pH-dependent viscosity for improved injectability and printability, with hyaluronic acid methacrylate (HAMA), which enables photocrosslinking post-printing to form a stable hydrogel network. The resulting HAGA–HAMA hydrogel demonstrated enhanced printing precision, viscoelastic properties, dimensional stability, tissue adhesiveness, and antioxidant activity. *In vitro* evaluations confirmed high biocompatibility, highlighting the hydrogel's potential for direct printing onto wound sites and broader applications in tissue engineering and regenerative medicine.[47] Compared with collagen-only systems, HAGA-containing composite hydrogels exhibit superior shape fidelity, with significantly reduced shrinkage ($\sim$20% *vs.* $>$90%) when loaded with fibroblasts, while maintaining biocompatibility with various cell types, including cardiomyocytes.[48] Gallic acid functionalization imparts potent antioxidant properties, with studies showing $>$90% antioxidant activity and effective scavenging of reactive oxygen species.[49,50] These hydrogels also demonstrate antimicrobial activity against wound-associated bacterial strains and inhibit matrix metalloproteinases and myeloperoxidase enzymes that impair chronic wound healing.[49] Injectable formulations promoted cell proliferation, migration, and angiogenesis and accelerated wound healing *in vivo* (Fig. 5).[50]

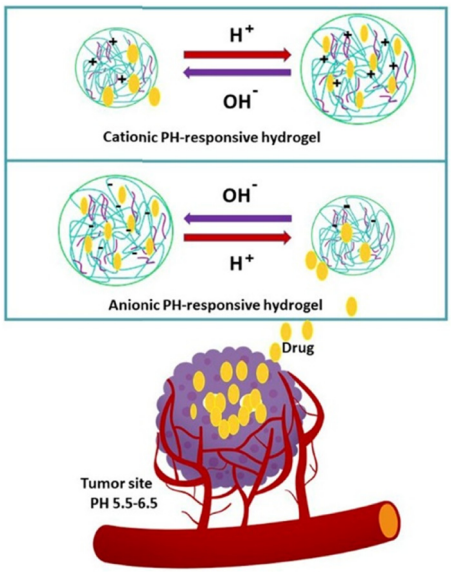

Fig. 4 Schematic representation of the pH-dependent behavior of cationic and anionic polymer hydrogels. Cationic hydrogels swell at acidic pH values because of protonation and electrostatic repulsion between polymer chains. This swelling facilitates the release of any encapsulated drug. Conversely, anionic hydrogels, with acidic groups, exhibit the opposite response: they swell at basic pH and collapse at acidic pH, enabling pH-triggered release of encapsulated drugs. Reproduced from ref. 51 licensed under Creative Commons Attribution (CC BY 4.0).

Overall, pH-responsive systems excel in their ability to respond to pathological environments such as acidic tumor tissue or inflammatory regions, giving them a clear niche advantage in smart drug delivery and disease-specific biomedical devices. However, they remain largely at the proof-of-concept or *in vitro* stage, with limited *in vivo* testing and no clinical applications. Compared with thermoresponsive systems (*e.g.*, Pluronics and PNIPAM), which have already advanced toward tissue engineering and regenerative medicine applications, pH-responsive materials lag in terms of mechanical robustness and translational maturity. pH-responsive systems, especially when combined with synthetic polymers or integrated into hybrid multistimuli designs, could eventually surpass thermoresponsive systems in targeted therapeutic delivery and adaptive implants, but for tissue engineering and structural constructs, thermoresponsive materials are currently the more reliable and clinically closer option. The ability of these materials to respond to pH variations makes them extremely useful in a variety of applications and, most notably, in biomedical applications. They can be designed to release drugs in a controlled manner, initiated by the acidic conditions that are generally found in the microenvironments of cancers or inflamed tissues. This pH-sensitive drug release system enhances the therapeutic efficacy of drugs and minimizes potential side effects by preventing their premature release in normal tissue. pH-sensitive materials also offer flexibility in designing complex delivery systems that can be developed to deliver more than a single therapeutic agent, leading to improved efficacy in disease therapy. With continuous research in this field, there are numerous pH-sensitive materials that are generating new drug delivery platforms for tissue engineering applications.

2.3. Photoresponsive materials

Photoresponsive materials represent a key area of interest in 4D bioprinting because they provide a way to dynamically control constructs. Photoresponsive materials undergo reversible physical and chemical changes upon exposure to specific wavelengths of light and thus can fine-tune their properties in response to external stimuli. The light responsiveness phenomenon typically involves photochemical reactions, where materials undergo structural changes as a result of light exposure, leading to changes in their solubility, swelling, or mechanical properties.[52,53] Common photoresponsive polymers include gelatin methacryloyl (GelMA), poly(ethylene glycol) diacrylate (PEGDA), and methacrylated hyaluronic acid (HAMA), all of which contain photocrosslinkable methacrylate groups that can be activated *via* photoinitiators such as irgacure or LAP

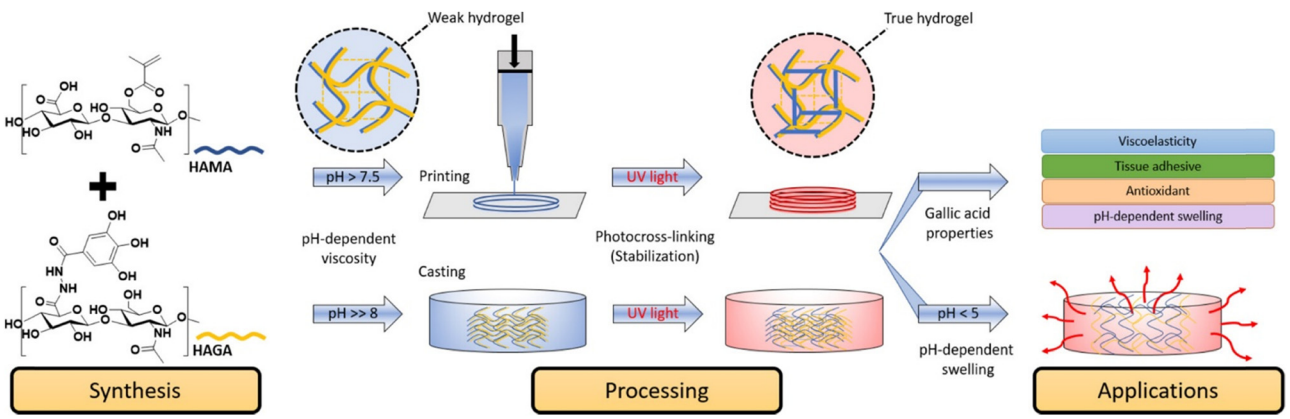

Fig. 5 Schematic representation of the HAGA–HAMA hydrogel, combining the viscosity modulation of pH-dependent precursors for casting and extrusion-based 3D bioprinting. 3D printing of the complementary network hydrogel is done in two steps: first, the viscosity of the precursor is enhanced *via* pH change, making it printable as an "ink", and next, photocrosslinking is used after printing. The GA-based hydrogels demonstrate viscoelasticity, tissue adhesion, and antioxidant and pH-dependent swelling behavior. Reproduced from ref. 47 licensed under Creative Commons Attribution (CC BY 4.0).

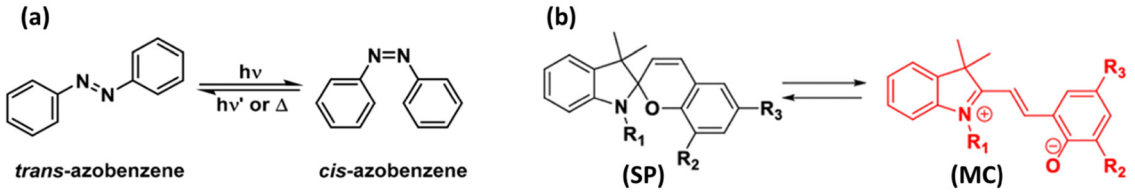

Fig. 6 Photoresponsive materials: (a) reversible photopolymerization of azobenzene in two states. Reproduced from ref. 55 licensed under Creative Commons Attribution (CC BY). (b) Reversible interconversion between ring-closed spiropyran (SP) and ring-opened merocyanine (MC). Reproduced from ref. 56 licensed under CC-BY-NC-ND 4.0.

(lithium phenyl-2,4,6-trimethylbenzoylphosphinate) under UV or blue light. Upon light exposure, these materials form covalent crosslinks that help retain the specific shapes of the printed structure. Additionally, azobenzene- or spiropyran-functionalized polymer systems are a class of photoresponsive materials that are being used for bioprinting applications (Fig. 6).[54]

Azobenzene derivatives are distinguished by their ability to undergo *trans–cis* isomerization when exposed to UV or visible light.[52,57,58] Reversible photoisomerization between the stable trans-form and metastable *cis*-form of azobenzene upon visible or UV light is depicted in Fig. 4. Substantial molecular geometry differences between the two isomers cause polymer conformation changes that lead to shrinking or swelling behavior.[59] Spiropyran is a heterocyclic compound that can reversibly switch between its closed (spiropyran) structure and its open (merocyanine) structure upon exposure to ultraviolet (UV) light.[60] Spiropyran and merocyanine are analogous chemical structures that involve photochromism, which is capable of reversibly controlling their structures *via* the input of a light stimulus. This is an important feature of spiropyran, where the open form (merocyanine) is more polar and possibly able to modulate its response to the environment compared to the closed form.[61] These molecules can be incorporated into films, hydrogels, and other systems used in bioprinting to induce controlled drug release for photothermal therapy or for tissue engineering applications.[62,63]

Caprioli *et al.* (2021)[64] developed a light-controlled 3D-printed hydrogel using azobenzene derivatives that were engineered for the controlled release of therapeutic agents upon illumination. Human epithelial cells were used for this study with the aim of developing a responsive system for treating inflammatory disease. The aim was to design a hydrogel that is dynamically responsive to an external stimulus to regulate drug delivery to modulate the effectiveness of the treatment of inflammatory diseases. Traditional drug delivery systems are generally incapable of regulating the spatiotemporal release of a drug, which results in less optimal therapeutic effects. Using light-sensitive materials, researchers have developed a system with more precise controlled drug delivery. The hydrogel was synthesized by mixing poly(ethylene glycol) (PEG) with azobenzene derivatives. The azobenzene moieties were incorporated into the network of the polymer so that the material could be triggered to undergo *trans–cis* isomerization upon exposure to UV light. The hydrogel was printed *via* a 3D bioprinting technique, and intricate structures could be achieved that would be capable of mimicking the extracellular matrix. Human epithelial cells were encapsulated in the hydrogel during the printing process. Upon UV light exposure, the azobenzene groups are isomerized, leading to swelling of the hydrogel and the release of entrapped anti-inflammatory drugs. The release pattern was controlled by changing the extent and duration of illumination. Azobenzene-containing hydrogels represent a highly promising method for controlling drug delivery systems for the treatment of inflammatory diseases. With the controlled delivery of anti-inflammatory drugs under light, the system avoids systemic side effects and enhances therapeutic efficacy. The system was capable of on-demand drug release, which is beneficial in clinical applications where treatment dosing must be controlled.[64]

A study by Jung *et al.* (2022)[65] explored the use of spiropyran-enveloped hydrogels in cancer treatment. Research has focused on cross-linking and printing the aforementioned hydrogels with human cancer cells, *i.e.*, human breast cancer cells (MCF-7), to establish a tumor microenvironment. The objective was to develop a photoresponsive material that would generate ROS for photodynamic therapy in a controlled and targeted manner. Traditional cancer therapies are likely to produce systemic side effects and lack cancer cell specificity. Using spiropyran, which is photoactivatable and capable of generating reactive oxygen species (ROS), researchers aimed to create a targeted therapy that would affect cancer cells with minimal damage to the surrounding healthy tissue. Spiropyran-loaded hydrogels were synthesized by mixing poly(lactic-*co*-glycolic acid) (PLGA) with spiropyran derivatives. Spiropyran units were introduced into the hydrogel network, where these moieties are photoactivated. High-precision bioprinting was utilized to generate a tumor-like microenvironment. When the printed constructs were exposed to UV light, spiropyran moieties were converted into an open merocyanine state, generating reactive oxygen species. The generation of ROS results in oxidative stress among surrounding cancer cells, resulting in cell death. The response was controlled by varying the intensity and duration of light exposure. The spiropyran hydrogel showed great potential for cancer treatment *via* photodynamic therapy. The photoresponsiveness of spiropyran to initiate therapeutic effects offers onsite treatment of cancers *via* a controlled mechanism, reducing the potential for off-target tissue response. This novel approach enhances cancer treatment efficacy by combining the advantages of light sensitivity and localized therapy. Scientists are engineering responsive

dynamic systems that respond to light activation with control over drug delivery and localized cancer therapy *via* azobenzene- or spiropyran-incorporated polymers along with living cells.[65] These advancements pave the way for more effective and personalized treatment strategies in the future.

GelMA-based bioprinting systems have evolved significantly through photoinitiator optimization. Early work with Irgacure 2959 and UV light showed promise but faced cytotoxicity challenges, with cell viability decreasing as the photoinitiator concentration and printing time increased.[66] LAP has emerged as a superior alternative, enabling visible-light curing with markedly improved photocuring kinetics and better cytocompatibility than Irgacure 2959.[67] However, oxygen inhibition remains a significant challenge affecting print fidelity in photopolymerized constructs. To address this, visible-light systems using ruthenium/sodium persulfate (Ru/SPS) were developed which demonstrated enhanced cell viability (>85% for 21 days), improved light penetration depth, enabling the fabrication of thick (10 mm) constructs, and better cellular functionality, including higher glycosaminoglycan content, than traditional UV systems.[68] These advances have established visible-light photoinitiating systems as preferred approaches for cell-laden GelMA bioprinting applications.

PEGDA is a synthetic polymer widely used in 3D bioprinting for tissue engineering applications because of its biocompatibility, tunable mechanical properties, and crosslinking capabilities.[69] Mazzoccoli *et al.* (2010)[70] demonstrated that blending different molecular weight PEGDA polymers (400 and 3400 Da) can optimize the mechanical properties, achieving compressive strengths of up to 1.7 MPa while maintaining approximately 80% cell viability at a 20 w/w% polymer concentration.[71] However, the lack of inherent cell-adhesive properties of PEGDA necessitates blending with bioactive polymers such as GelMA to support cell adhesion and proliferation. Compared with the PEGDA-only scaffolds, the GelMA/PEGDA/F127DA composite scaffolds resulted in superior bone regeneration, with 49.75% greater bone volume.[72] HAMA contributes to bioactivity and ECM mimicry in hybrid hydrogel systems,[73] presenting an innovative approach to minimally invasive diagnostics. Researchers developed a composite hydrogel microneedle (MN) patch *via* digital light processing (DLP) 3D bioprinting, which combines hyaluronic acid methacryloyl (HAMA) and gelatin methacryloyl (GelMA). By optimizing the HAMA-to-GelMA ratio, light intensity, and exposure time, they achieved well-defined MNs with robust mechanical properties and significant swelling capacity. The MN patches demonstrated effective skin penetration and efficient interstitial skin fluid (ISF) extraction; 8.5 mg of PBS was collected in just 1 minute. This advancement offers a promising platform for real-time health monitoring and point-of-care diagnostics.[74] Fowler *et al.* (2025)[75] utilized digital light processing (DLP) to 3D bioprint GelMA/PEGDA hydrogels with varying channel designs to increase tissue infiltration and vascularization in rodent models. The diameter of the channels significantly influenced vascularization, with 1 mm channels yielding the highest infiltration, whereas the channel length had minimal impact. These findings provide insights into optimizing the scaffold architecture for improved tissue integration and vascularization in tissue engineering applications.[75]

The evolution of photoresponsive biomaterials for 4D bioprinting reflects a balance between bioactivity, mechanical performance, and dynamic responsiveness, but critical challenges remain. GelMA-based systems have established themselves as benchmarks owing to their intrinsic bioactivity, cell-adhesive motifs, and proven vascularization *in vivo*, but issues of UV-induced cytotoxicity, oxygen inhibition, and curing depth highlight the need for continuous optimization of initiators and printing conditions. PEGDA offers structural precision and tunable mechanics but lacks biofunctionality, necessitating blending with ECM-mimetic polymers such as GelMA or HAMA. HAMA contributes valuable bioactivity for vascularization but requires reinforcement to overcome poor stiffness and slow kinetics. While photochromic and photothermal nanocomposite hydrogels demonstrate exciting possibilities for soft robotics, dynamic actuation, and localized therapy, their cytotoxicity, stability, and translational limitations prevent their immediate adoption as clinically viable, cell-laden constructs. Recent advances, such as hybrid GelMA/PEGDA systems for neural regeneration, CMCS/PEGDA hydrogels for dental applications, and DLP-printed architectures for vascularization, demonstrate the breadth of applicability but also underscore that each formulation requires context-specific optimization to balance viability, bioactivity, and mechanical demands. Overall, despite promising strides, a major translational bottleneck lies in achieving multifunctionality without compromising cytocompatibility, long-term stability, or regulatory safety, emphasizing the need for rational hybrid design and standardized evaluation across applications.

2.4. Magnetic-responsive materials

Magnetic responsive materials are a class of materials that play a vital role in 4D bioprinting and can introduce magnetic stimuli to enable post-fabrication changes in the function or shape of the printed construct upon exposure to an external magnetic field. Magnetic responsive materials, in broad terms, encompass magnetic nanoparticles, which are used to dynamically change the shape of the printed structure. The overall idea of magnetic responsive materials is the embedding of magnetic nanoparticles in a polymer matrix. The material is subjected to deformation or movement by an external magnetic field through the nanoparticles. This approach is applied in 4D bioprinting to design structures that can alter shape or properties over time when exposed to magnetic fields. The mixture of materials allows the printing of complex, multifunctional structures with the ability to carry out specific functions in tissue engineering.[36,76]

Betsch *et al.* (2018)[76] conducted a study involving the development of a new bioprinting process involving the use of magnetic fields to arrange collagen fibers within functional bioinks. This method attempts to print intricate, multilayered tissue that mimics native tissue. Bioinks composed of low-gelling temperature agarose and type I collagen were printed by the authors along with streptavidin-coated superparamagnetic

iron oxide nanoparticles (SPIONs). The inclusion of SPIONs made it possible for the collagen fibers to be controlled under magnetic field manipulation during the process of bioprinting. Human knee articular chondrocytes (hKACs) were used in the experiments to prepare the tissue constructs. The cells were embedded into magnetic nanoparticle-loaded hydrogels to form functional tissue architectures that are employed in cartilage tissue engineering. The printed structure was cultured at 37 °C in a humidified 5% CO_2 environment for 21 days. The culture medium was repeatedly changed to achieve the best conditions for differentiation and cell proliferation. A variety of analytical methods have been used by researchers to evaluate the efficiency of the bioprinting process as well as the properties of printed constructs. The findings demonstrated that exposure of the hydrogels to a magnetic field during bioprinting dramatically increased the orientation of the collagen fibers in the hydrogels. The hKAC-loaded aligned fiber constructs presented significantly greater expression of collagen II and other cartilage markers than did the randomly oriented fiber constructs after 21 days of culture. These findings indicate that the composition and structure of the bioink used in bioprinting play critical roles in cell differentiation and tissue formation (Fig. 7).[76]

Chakraborty *et al.* (2024)[36] developed a 4D bioprinted construct by integrating anisotropic magnetic nanoparticles (MNPs) into a silk fibroin-gelatin bioink with the addition of human bone marrow-derived mesenchymal stromal cells.[53] The thermal response and magnetic actuation of the magnetic field in the acellular construct were characterized and compared to those of the MNPs alone. The bioprinted scaffolds were subsequently exposed to magnetic actuation, and their effect on chondrogenesis was examined. Cyclic actuation was carried out every other day, with two different durations tested for actuation: 5 minutes and 30 minutes per day for 21 days. The protocol with a 30 min actuation period was previously shown to increase early (Sox9 and aggrecan) and late (collagen-II) chondrogenic marker expression and suppress hypertrophic marker expression (collagen-X and matrix metalloproteinase-13). In addition, the 30-minute actuation group presented greater matrix deposition, overall collagen, and glycosaminoglycan contents than the 5-minute actuation group and the construct with no MNPs.[36] This work effectively demonstrates the ability of magnetic fields to control collagen fiber orientation in 4D bioprinting, which paves the way for printing complex, functional tissue structures with a strong resemblance to native tissue architecture.

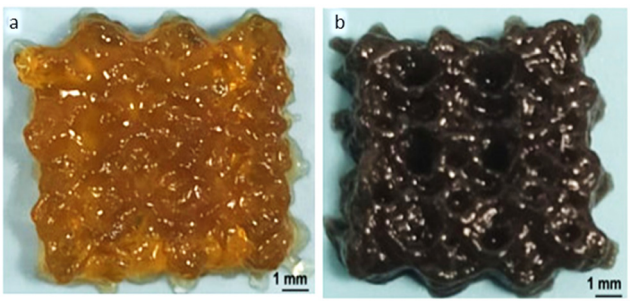

Fig. 7 Optical images of the 4D-bioprinted constructs (a) without magnetic nanoparticles (MNPs) as a control and (b) with MNPs (5 mg mL^{-1}). Reproduced from ref. 36 licensed under Creative Commons CC-BY.

A recent study by Li *et al.* (2025)[77] presented a novel approach to 4D printing by developing a bilayer hydrogel that exhibits magnetic responsiveness. This hydrogel is composed of a temperature-sensitive poly-*N*-isopropylacrylamide (PNIPAM) layer and an iron oxide (Fe_2O_3) magnetic layer. The magnetic layer is generated during the 3D printing process by introducing iron ions into the PNIPAM matrix, followed by treatment with NaOH to precipitate Fe_2O_3 nanoparticles within the polymer network. The bilayer structure exploits the differing swelling behaviors of the two layers: the PNIPAM layer responds to temperature changes, whereas the magnetic layer responds to external magnetic fields. This combination allows the hydrogel to undergo programmable shape transformations upon exposure to specific stimuli, demonstrating potential applications in soft robotics and responsive biomedical devices.[78]

While the integration of magnetic-responsive bilayer hydrogels in 4D printing represents a significant advancement, several challenges remain. The mechanical properties of hydrogels, such as their tensile strength and elasticity, are crucial for their practical application. Future research should focus on enhancing these properties without compromising the responsiveness of the hydrogel to external stimuli. Additionally, the scalability of the *in situ* nanoparticle formation process needs to be addressed to facilitate large-scale production. Moreover, the long-term stability and biocompatibility of these hydrogels under physiological conditions require thorough evaluation to ensure their safety and efficacy in medical applications. Addressing these challenges will be essential for translating this technology from the laboratory to real-world applications in tissue engineering, drug delivery systems, and adaptive soft robotics.

2.5. Electroresponsive materials

Electroresponsive materials are a class of materials that change their properties or shapes in response to electrical stimuli. Some of the electroresponsive materials used in 4D bioprinting applications include polythiophene, a conductive polymer known for its electroactive properties, making it suitable for applications in drug delivery and biosensing.[79] Another conductive polymer, polyaniline (PANI), is often utilized in bioinks because of its ability to respond to electrical stimuli, enhancing the functionality of bioprinted constructs.[80] Polypyrrole is characterized by its conductivity and is commonly incorporated into hydrogels for 4D bioprinting applications.[81] Poly(2-hydroxyethyl methacrylate) (PHEMA) is used in drug delivery systems and exhibits electroresponsive behavior, making it valuable for dynamic tissue engineering applications.[82] Owing to its excellent conductivity, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) is used in neural tissue engineering and microelectromechanical systems (MEMS)

because of its electroactive properties.[83] These types of electroresponsive materials can be used to create sophisticated bioprinted structures with an adaptive and responsive nature to external electrical stimulation, which opens new prospects in tissue engineering and regenerative medicine.

One such study by Ashtari *et al.* (2019)[84] aimed to create electroresponsive hydrogels based on poly(2-hydroxyethyl methacrylation) (PHEMA) with conductive polyaniline (PANI) dispersed within them for cardiac tissue engineering. The objective was to create a scaffold that mimics the electrical features of native heart tissue and permits the growth and function of cardiomyocytes. The hydrogel precursor solution was achieved by combining PHEMA, PANI, and the photoinitiator. Neonatal rat cardiomyocytes were dispersed in the bioink. The bioink was printed with a stereolithography-based bioprinter to create porous 3D structures. UV-crosslinked printed scaffolds were cultured in medium. PANI hydrogels are electric stimulus responsive and conductive, mimicking the electrical signals of the heart. The encapsulated cardiomyocytes also exhibited very high viability (>90%) after bioprinting and subsequent culture in electroresponsive hydrogels. Printed constructs supported the formation of cardiac tissue-like structures, and cardiomyocytes aligned and created gap junctions. The printed cardiac tissue exhibited coordinated contraction upon stimulation *via* an external electric field, indicating functional integration of the cardiomyocytes.[84]

A study by Doblado *et al.* (2021)[85] explored the application of electroactive conductive polymers as materials in neural tissue engineering. The electroactive polymers used were polypyrrole (PPy) and polyaniline (PANI), which are electrically conductive and biocompatible. This paper illustrates the process by which neural stem cells (NSCs) are loaded with a precursor solution of an electroactive hydrogel polymer and 3D printed by bioprinting methods. The constructs were cross-linked to stabilize the structure, and they were subsequently grown in supplemented growth factor medium. The findings showed that the electroactive hydrogels promoted NSC viability and induced neuron differentiation when the hydrogels were stimulated electrically. Research has shown that the use of electroactive materials in 3D-printed scaffolds can affect neural tissue regeneration by promoting neurite outgrowth and extension in differentiated neurons. The present research proves the viability of electroactive polymer application in neural tissue engineering as a novel promising field for the development of advanced, electric stimulus-responsive scaffolds to enhance therapeutic efficacy in neural regeneration.[85]

Alkahtani *et al.* (2024)[86] developed a 3D-printed electroresponsive drug delivery system designed for programmable, on-demand release of therapeutics. The system integrates the conductive polymer poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) with thermoplastic polyurethane (TPU) to achieve both electrical responsiveness and mechanical stability. Fabrication was performed *via* direct ink writing (DIW) *via* semisolid extrusion, enabling precise 3D architectures. The PEDOT:PSS component allowed the hydrogel-based constructs to respond to applied electrical stimuli (±1.0 V), modulating the release rate of methylene blue (MB) as a positively charged model drug. Experiments demonstrated that the electroresponsive constructs achieved significant, rapid changes in cumulative drug release over 180 minutes compared with passive diffusion, showing clear responsiveness to pulsatile voltage inputs. The system's programmability, combined with its compatibility with Internet of Things (IoT) integration, positions it as a promising platform for smart, real-time therapeutic interventions in personalized medicine (Fig. 8).[86]

Electroresponsive materials, including conductive polymers and hydrogels, offer significant potential in 4D bioprinting by enabling dynamic tissue modulation, cardiac pacing, neural differentiation, and programmable drug release. While studies have demonstrated high cell viability, functional integration, and precise responsiveness to electrical stimuli, challenges remain in terms of the long-term biocompatibility, degradation, mechanical stability, and scalability of 3D-printed constructs. Most research is limited to *in vitro* or small-animal models, and translating these systems to clinically relevant, vascularized tissues requires hybrid strategies that combine conductivity with bioactivity, degradability, and controlled fabrication methods. Addressing these limitations is critical

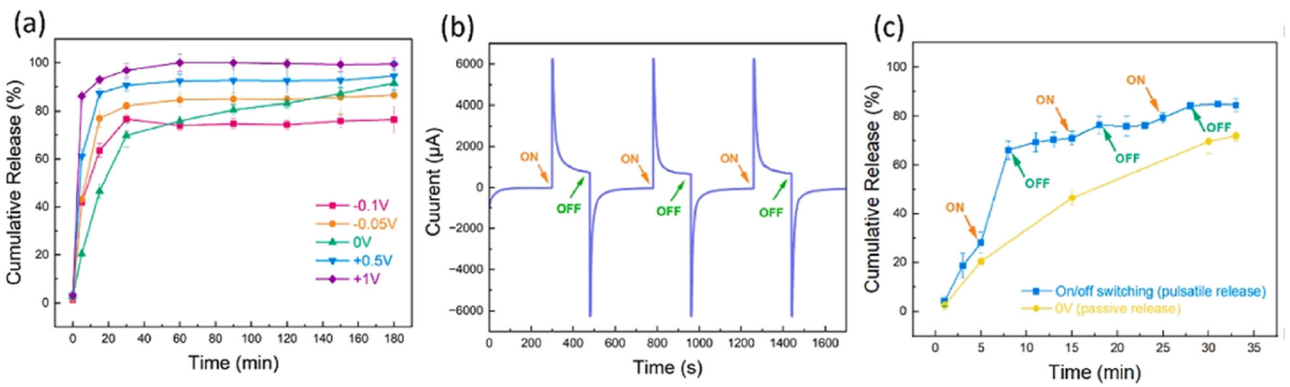

Fig. 8 (a) Cumulative release profiles of MB upon stimulation at −1.0, −0.5, +0.5, or +1.0 V and without stimulation (0.0 V). (b) Current–time response during the chronoamperometry experiment. (c) Pulsatile release profile of MB with on/off switching compared with its passive release (0.0 V). Reproduced from ref. 86 licensed under Creative Commons CC-BY.

to fully exploit electroresponsive materials for regenerative medicine and smart biomedical applications.

2.6. Humidity-responsive materials

The humidity responsiveness of 4D bioprinted materials is mostly obtained through the application of stimuli-responsive polymers, *i.e.*, hydrogels. Hydrogels can reversibly change their volume with changes in humidity and hence facilitate dynamic shape changes in the printed structure. Hydrogels are cross-linked polymer networks that can absorb and retain a certain amount of water. When subjected to water or high humidity, hydrogels absorb water and swell, expanding their volume. The hydrogels release water and recover their original volume when the humidity decreases. There are other polymers that show surface-tunable hygroscopicity such that they are able to selectively adsorb or repel water molecules depending on their chemistry. Inclusion of such polymers within the composite material allows scientists to regulate water absorption and desorption activity, thereby providing precise humidity-sensitive actuation.[87,88]

Shape memory polymers (SMPs) are capable of exhibiting the shape memory effect and recovering the same effect when induced by an external stimulus, such as humidity. SMPs turn soft under high humidity conditions and recover their original shape, but under low humidity conditions, they maintain the deformed shape. The composition, chemistry, type of additives, polymers, and cross-linkers of the material may influence the humidity responsivity. Balanced tuning and optimization of the components are crucial for achieving favorable actuation dynamics.[89] The printing conditions, such as extrusion pressure, nozzle size, and layer thickness, may affect the internal structure and porosity of the printed material, consequently influencing the desorption and water absorption kinetics. The temperature and relative humidity during and after printing can influence the material's responsiveness. The conditions need to be controlled for reliable and reproducible humidity-induced shape changes.[90]

Hydrogels such as poly(acrylic acid) (PAA) and poly(*N*-isopropylacrylamide) (PNIPAm) have been widely used because of their water-absorbing and water-releasing properties upon humidity change.[91–93] Cellulose-based materials such as cellulose nanofibers and cellulose derivatives have also shown potential humidity-sensitive properties due to their hydrophilic character and ability to form hydrogen bonds with water molecules.[94–96] Chitosan, a biopolymer of chitin, is pH- and humidity-sensitive and has potential uses in drug delivery and tissue engineering. Synthetic polymers such as poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG) have been functionalized with humidity-sensitive groups and employed as responsive materials for numerous applications. Furthermore, inorganic materials such as silica and metal–organic frameworks (MOFs) have been explored for humidity-sensitive applications since they have high surface areas and tunable pore structures.[97–99]

Several studies have been conducted on bioprinting with humidity-sensitive materials combined with living cells. De Souza *et al.* (2021)[100] explored the use of chitosan and hyaluronic acid hydrogels as possible bioinks for use in tissue engineering owing to their positive biocompatible characteristics. Research shows that the utilization of responsive materials along with bioprinting technologies to achieve optimal cell viability for tissue engineering applications is feasible. By employing these mechanisms and controlling the material composition and printing conditions, it is feasible to design 4D bioprinted structures with humidity sensitivity that have wide applications in tissue engineering, biomedical devices, and soft robotics.[100] Furthermore, Hull *et al.* (2021)[101] conducted experiments utilizing PEG-based formulations that were formulated to induce the proliferation of fibroblasts under humidity-sensitive conditions. Research has emphasized the ability of PEG formulations to facilitate a favorable microenvironment for fibroblast growth, an aspect important in tissue repair and wound healing. By optimizing the humidity sensitivity of such formulations, the study aimed at achieving the maximum cell survival and growth rates and, in the process, enhancing the efficacy of therapeutic usage. Research has shown that PEG hydrogels can be applied effectively where the retention of moisture is needed, thereby presenting new fields of application for biomaterials in regenerative medicine.[101]

Mondal *et al.* (2023)[102] developed a bidirectional shape-morphing 4D bioprinted hydrogel system capable of sequential deformation in opposite directions upon exposure to a single stimulus. This study aimed to overcome limitations in conventional 4D hydrogels, which typically deform in only one direction, by designing graded semi-interpenetrating network (IPN) hydrogels that respond dynamically to water immersion. The constructs were fabricated *via* extrusion-based 3D printing with a hydrogel precursor ink composed of methacrylated carboxymethyl cellulose (CMC-MA, 4% w/v), methylcellulose (MC, 6% w/v), chitosan methacrylate (CS-MA), polyethylene glycol dimethacrylate (PEGDMA), and the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, 0.1% w/v). The photocrosslinkable methacrylate groups within CMC-MA, activated by LAP under light exposure, enabled precise structural fixation during printing. This study also examined the sequential, bidirectional shape-morphing behavior of CS-MA/PEGDMA/MC hydrogel beams with different layer configurations. Three designs were tested: 1B-1T (one bottom layer, one top layer), 1B-2T (one bottom layer, two top layers), and 2B-1T (two bottom layers, one top layer). The results showed that the layer arrangement strongly influences the swelling kinetics, response time, maximum bending angle, and final curvature, with some designs exhibiting slower swelling and reduced bending than others. This demonstrates that layer design is a key parameter for programming precise bidirectional deformation in 4D-printed hydrogels. L929 mouse fibroblasts were encapsulated to evaluate their cytocompatibility, and Alamar blue assays along with 3D confocal microscopy confirmed high cell viability, normal morphology, and proliferation within the hydrogel matrices. This system provides significant advantages for soft deployable devices, minimally invasive therapeutic delivery, and dynamic tissue engineering scaffolds, offering

programmable deformation, biocompatibility, and potential for constructing complex, adaptive tissue-like architectures.[102]

From a critical perspective, achieving reproducible and predictable humidity-induced shape changes requires careful optimization of both the material formulation and printing parameters, including extrusion pressure, nozzle diameter, layer thickness, and postprint environmental conditions. Additionally, while synthetic polymers such as PEG and PVA offer tunable properties, their integration with natural polymers for cell-laden applications must balance mechanical stability with bioactivity, a trade-off that remains a central challenge. Future research should focus on enhancing the response speed, improving the structural durability under cyclic humidity changes, and integrating multistimuli responsiveness to expand functional applications. The combination of advanced polymer chemistry, controlled microstructure design, and standardized printing protocols will be crucial to fully exploit humidity-responsive 4D bioprinted materials for tissue engineering, biomedical devices, and soft robotics.

2.8. Multistimuli-responsive materials

Multiple stimuli-responsive materials can respond to two or more diverse external stimuli, such as temperature, pH, light, magnetic fields, or redox conditions. These materials have the ability to alter their properties, structure, or behavior in response to more than one environmental stimulus, allowing them to perform advanced functions such as controlled drug delivery, tissue engineering, and sensing. Their ability to respond to combined stimuli increases their versatility and simulates intricate biological processes, which makes them promising for accurate, on-demand therapeutic and diagnostic uses.

Liquid-crystal elastomers (LCEs) are novel multistimuli-responsive materials that are excellent candidates for 4D bioprinting and tissue engineering applications. The distinctive feature of LCEs is attributed to their molecular structure, which has elastomeric and liquid crystalline properties. Owing to this synergy, LCEs can respond to a wide range of environmental stimuli, including temperature, light, and electric fields, and experience drastic changes in shape and mechanical properties. The chemistry of LCEs is based on crosslinked polymer networks that exhibit liquid crystalline behavior. These materials are made up of liquid crystalline monomers that are polymerized into a three-dimensional network. The liquid crystalline phase allows the polymer chains to be oriented in a way that can be controlled by external stimuli. With increasing temperature, the phases of the LCEs change, resulting in reversible shape deformation. Through the use of liquid crystalline moieties and the ability to control the crosslinking density, scientists can tune their physical and mechanical properties. This approach is useful for modeling the mechanical properties of tissues in an attempt to support successful cell growth and differentiation. The LCE can be designed to be biocompatible for use in a broad variety of biomedical applications. Biocompatible LCEs consist of nontoxic monomers and cross-linkers that aid in cell adhesion and growth.[103–106] LCE scaffolds can stimulate the alignment and growth of a variety of cell types, such as neural stem cells, myoblasts, and chondrocytes. Having liquid crystal moieties within the scaffold will improve cell alignment, mimicking native tissue architecture, which is ideal for tissue engineering purposes.[107] Bera *et al.* (2015)[108] developed LCEs that respond to mechanical stimuli, temperature, and light and combined them with myoblasts for muscle tissue engineering. The LCE scaffolds guided myoblast proliferation and differentiation, forming muscle fibers in response to mechanical stimuli and photostimuli, indicating their potential application in muscle tissue regeneration.[108] Sharma *et al.* (2015) developed porous, biocompatible, and biodegradable liquid crystal elastomer scaffolds appropriate for spatial cell culture.[109]

McDougall *et al.* (2023) studied an innovative embedded 4D printing method that allows the construction of intricate free-standing 3D LCE structures.[110] This technique involves the extrusion of a specifically designed hydrophobic LCE ink into a water-based supporting thixotropic LAPONITE® gel matrix. The gel is used as a temporary scaffold to maintain the intricate geometries of the printed LCE during printing. Following printing, the structures are UV-cured to achieve full crosslinking, especially when facilitated by the inclusion of pentaerythritol triacrylate (PETA), which enhances the mechanical stability and shape retention. The gel matrix is subsequently washed, resulting in strong, self-standing LCE architectures that retain excellent actuation characteristics. This method surpasses the past limitations of LCE 4D printing, which is limited mainly to planar or simple geometries because of the necessity for an external support or post-processing alignment procedures. Direct printing of programmable, complex 3D LCE structures with aligned molecular structures (mesogens) is especially important in biomedical applications. LCEs produced through this technique can be designed to exhibit controlled, reversible motion, which makes them strong contenders for soft actuators in minimally invasive surgical devices, dynamic tissue-mimicking scaffolds, and adaptive drug delivery systems responsive to physiological stimuli. Owing to their biocompatibility, mechanically tunable nature, and sensitivity to biologically relevant stimuli, LCEs are attractive materials for future biomedical devices that need adaptive, gentle, and programmable actuation (Fig. 9).

Nain *et al.* (2024)[111] developed a novel 4D-printed, nanoengineered hydrogel scaffold designed for fabricating programmable and perfusable T-shaped vascular bifurcations. This system utilizes a dual-component ink composed of alginate and methylcellulose (Alg : MC) in two formulations (3 : 9 and 4 : 6 ratios), each with different swelling behaviors. The inks were nanoengineered with carbonized alginate (CAlg), obtained by mild pyrolysis, or pristine alginate, to introduce bioactive functionalities such as antioxidant, anti-inflammatory, and antithrombotic properties. Extrusion-based printing was performed, where alternating layers of two hydrogels were printed with designed infill angles. Upon immersion in a calcium chloride ($CaCl_2$) solution, the constructs underwent shape transformation from 2D flat sheets to complex 3D structures such as tubes and T-shaped vascular channels. The developed

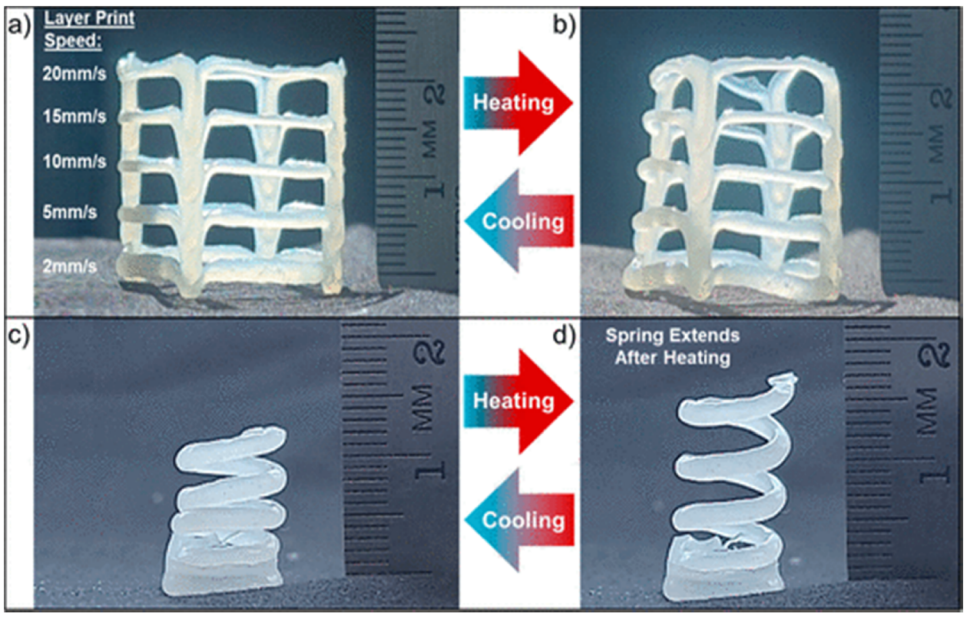

Fig. 9 Illustration of the embedded 4D printing of an LCE to achieve unique 3D shape transformations in response to temperature. (a) LCE ring structure with each layer printed at different speeds. (b) Actuation of the LCE ring structure shows that each ring actuated differently in response to heating. (c) LCE spring after printing and removal from the gel matrix. (d) LCE spring exhibiting linear expansion in response to heating. Reproduced from ref. 110 licensed under CC-BY-NC-ND 4.0.

hydrogel is a dual-stimuli responsive system that is specifically moisture responsive and ion responsive. The moisture-induced anisotropic swelling is imparted by differential expansion between the two hydrogel layers, which causes bending and folding. Ionic crosslinking *via* Ca^{2+} further stabilized the shape through coordination with alginate. For biological assessment, the constructs were seeded with human umbilical vein endothelial cells (HUVECs) and NIH3T3 fibroblasts, which exhibited excellent viability, morphology, and cytocompatibility. This study highlights a promising route toward self-actuating, bioactive vascular scaffolds with potential applications in cardiovascular tissue engineering, particularly for creating anatomically relevant and minimally invasive grafts for conditions such as coronary artery disease.[111]

Okihara *et al.* (2024)[112] developed dual stimuli-responsive hydrogels that can dynamically alter their physical and chemical properties in response to varying ultraviolet (UV) exposure times and temperatures.[112] The materials used in this research include 7-methacryloyloxycoumarin (MAC) and methoxyoligoethylene glycol methacrylate (OEGMA). The copolymerization of these components results in the –P(MAC-*co*-OEGMA) polymer, which results in lower critical solution temperatures (LCSTs) that depend on the composition. The elastic modulus of the hydrogels formed from this polymer increased with increasing UV exposure, indicating that the gelation process was triggered by light (Fig. 10).

The dual stimulus-responsiveness of the hydrogels allows them to respond to temperature as well as UV light. The crosslinking under UV light is caused by the photodimerizable groups (coumarin) present in MAC. The temperature responsiveness, however, is attributed to the OEGMA building block, which adjusts the hydrophilicity of the hydrogel on the basis of temperature, affecting the adhesion and spreading of cells. This paper employs L929 mouse fibroblasts to examine cell behavior on surfaces of such gels. It was discovered that cells are preferentially attached to hydrogels with superior elastic moduli and spread preferentially at temperatures above the LCST. This research highlights the potential applications of these dual stimuli-responsive hydrogels in tissue engineering, where they can be utilized to dynamically modulate cell behavior, providing a more biomimetic culture for cell growth and differentiation.

The graphene oxide (GO)-embedded extracellular matrix (ECM)-derived hydrogels developed by Rueda-Gensini *et al.* (2021)[113] represent a significant advancement in the field of 4D bioprinting, offering a multiresponsive platform that combines the structural and biological benefits of the ECM with the functional properties of graphene oxide. These hydrogels are typically synthesized by modifying decellularized ECM, such as small intestine submucosa (SIS), with methacryloyl groups to facilitate photocrosslinking and then incorporating GO nanosheets to increase the mechanical strength, electrical conductivity, and responsiveness to external stimuli. The inclusion of GO not only improves the printability and structural integrity of the hydrogel but also imparts the ability to respond to various stimuli, such as electrical fields, pH, and temperature, making them suitable for dynamic tissue engineering applications. Studies have demonstrated that these GO-embedded ECM hydrogels support high cell viability and promote cellular behaviors such as adhesion, proliferation, and differentiation, which are essential for the development of functional tissue constructs.[113]

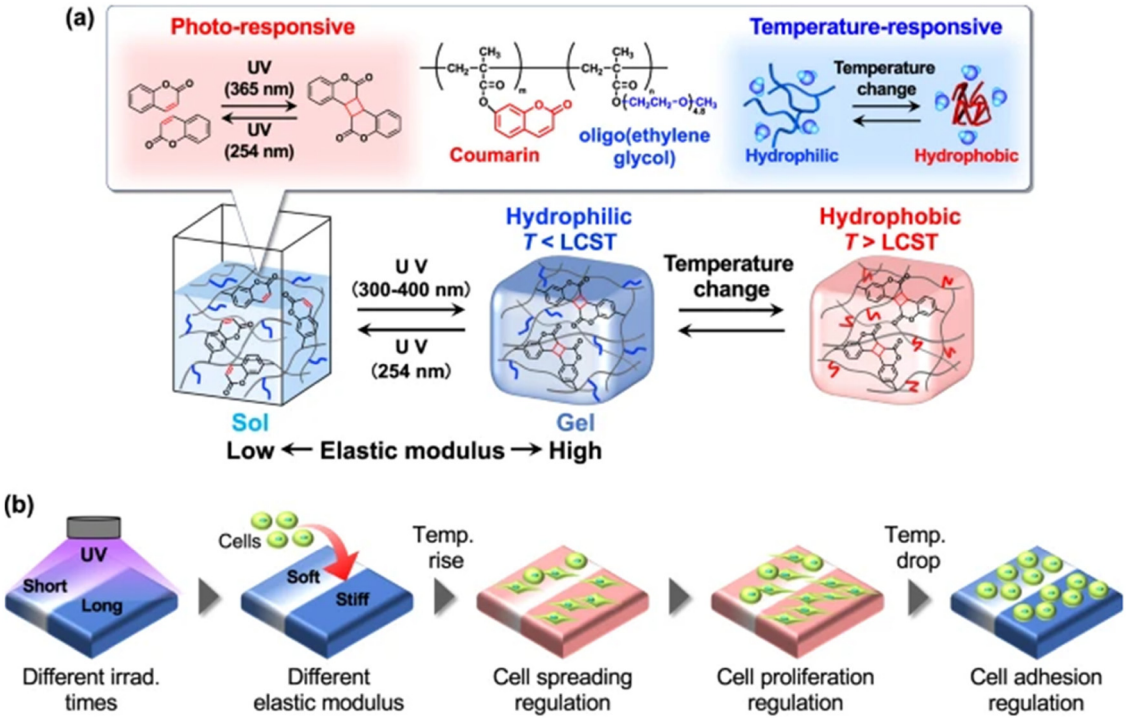

Fig. 10 (a) A strategic design of dual stimuli-responsive polymer gels exhibiting dynamic changes in their physical and chemical properties in response to variations in UV exposure time and temperature. (b) Schematic of cell regulation by tuning the elastic moduli and hydrophobicity/hydrophilicity of dual stimuli-responsive polymer gels. Reproduced from ref. 112 licensed under Creative Commons CC BY.

Multistimuli-responsive materials in 4D bioprinting, such as LCEs and dual-stimuli hydrogels (*e.g.*, Alg:MC and P(MAC-*co*-OEGMA)), enable dynamic shape changes, offer tunable mechanics, support high cell viability, and guide proliferation and differentiation. They allow complex 3D designs and controlled cell behavior, mimicking native tissues. However, challenges remain for clinical translation, including material complexity, scale-up limitations, long-term mechanical stability, uniform responsiveness in larger constructs, and limited *in vivo* validation. While promising *in vitro*, overcoming these challenges is essential for realizing their full potential in tissue engineering and regenerative medicine.

3. Recent advancements and challenges

In recent years, 4D bioprinting has emerged as a transformative approach that allows dynamic, stimuli-responsive constructs to be fabricated with high spatial control. Unlike static 3D bioprinted scaffolds, these constructs can sense, adapt, and remodel in response to environmental or biological cues. The continuous development of smart bioinks has accelerated this progress, enabling programmed shape changes, controlled biomolecule release, and improved tissue integration. By combining photopolymerizable matrices with conductive polymers, responsive nanoparticles, or enzyme-cleavable linkages, researchers are moving toward biofabricated systems that more closely recapitulate the dynamic nature of living tissues.

Owing to their precision and spatiotemporal control, light-responsive bioinks continue to dominate this field. Recent advances in volumetric bioprinting have enabled rapid fabrication of centimeter-scale tissue constructs with high fidelity. Bernal *et al.* (2019) demonstrated volumetric bioprinting *via* visible light projection to create cell-laden constructs exceeding 85% viability within seconds to tens of seconds, producing anatomically correct trabecular bone models and meniscal grafts.[114] Loterie *et al.* (2020) achieved high-resolution tomographic volumetric manufacturing, producing centimeter-scale parts in under 30 s with 80 μm positive features *via* controlled photopolymerization kinetics.[115] Wolfel *et al.* (2025) introduced bioxolography using diphenyliodonium chloride and *N*-vinylpyrrolidone as photoinitiator enhancers, enabling >1 cm^3 constructs at $\sim$20 μm resolution within minutes while maintaining excellent cell viability.[116] Kim *et al.* (2021) developed light-activated decellularized extracellular matrix bioinks with ruthenium/sodium persulfate systems, demonstrating rapid dityrosine-based crosslinking for centimeter-scale constructs with improved mechanical properties and shape fidelity.[117] Parallel advances have been made in multistimuli systems, such as gelatin-norbornene hydrogels that exhibit both photo- and enzyme-responsiveness and magneto-thermal composites that integrate nanoparticles for remote actuation. These dual-responsive approaches are particularly promising for generating dynamic tissue environments that adapt to both external and cell-mediated conditions.

Electro and mechanoresponsive systems have been leveraged to target tissues where electrical and mechanical signaling

Table 1 Comparative analysis of stimuli-responsive materials: advantages, challenges, applications and clinical translation

Stimuli	Materials	Advantages	Challenges	Applications	Clinical translation and scalability	Ref.
Temperature	Poly(*N*-isopropylacrylamide) (PNIPAm), pluronics, poly(*N*-vinylcaprolactam) (PNVCL), poly(ethylene glycol) (PEG), *N*-hydroxymethyl acrylamide (HMAAm), liquid crystal elastomer (LCE)	Sharp LCST response near body temperature; reversible swelling; potential for minimally invasive actuation	Residual monomer cytotoxicity; poor cell adhesion; slow response due to diffusion; limited mechanical strength	Drug delivery systems, tissue engineering, cell culture scaffolds	Limited by cytotoxicity; requires copolymer blends for safe biomedical use	21,26,27,38
Light	Azobenzene, spiropyaran, liquid crystal elastomer (LCE), gelatin methacryloyl (GelMA), methacrylated hyaluronic acid (HAMA), polyethylene glycol diacrylate (PEGDA)	High spatial/temporal control; rapid crosslinking; biocompatible with long-wavelength initiators	Limited light penetration; photoinitiator cytotoxicity; heterogeneous curing in thick constructs	Photothermal therapy, photodynamic therapy, regenerative medicine	Promising for vascular constructs and thick tissues with visible/red-light systems	52,53,57,59
pH	Chitosan, gelatin, polyacrylic acid, poly(lactic-*co*-glycolic acid) (PLGA), poly(*N*-isopropyl acrylamide)	Tunable swelling and deswelling; controlled drug release; targeted therapeutic delivery; compatibility with extrusion bioprinting; potential for precise shape-shifting	Mechanical instability with repeated cycles; difficulties in maintaining cell viability and biofunctionality during printing and actuation	Drug delivery systems, wound dressings, tissue scaffolds	Clinical potential in vascular and bone regeneration; control of ion gradients is key	39,42,139
Magnetic field	Superparamagnetic iron oxide nanoparticles (SPIONs), magnetic nanoparticles, along with suitable polymers	Remote actuation; noninvasive control; reprogrammable shapes	Aggregation/sedimentation; ROS generation; heterogeneity in actuation	Targeted drug delivery, tissue engineering scaffolds, alignment of cells	Potential for targeted therapy and soft robotics, but reproducibility issues hinder clinical scaling	76,82,140,141
Electric field	Polythiophene, polyaniline (PANI), polypyrrole, poly(2-hydroxyethyl methacrylate) (PHEMA), poly(3,4-ethylene dioxythiophene) polystyrene sulfonate (PEDOT:PSS)	High conductivity (1–40 S cm^{-1}); enables biosensing and stimulation; stable interfaces	Long-term stability; immune compatibility	Cardiac and neural tissue engineering applications	Strong potential for bioelectronics and neural/cardiac scaffolds; clinical adoption requires ISO biocompatibility validation	19,83,140,142
Humidity	Poly(*N*-isopropyl acrylamide) (PNIPAm), poly(ethylene glycol) (PEG), cellulose nanofibers, polysaccharide derived from chitin, poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG), hyaluronic acid hydrogels	Simple, low-energy actuation; reversible bending/swelling	Limited precision; weak mechanical robustness; environment-dependent	Antimicrobial membrane, dynamic drug delivery system, soft robotics for biomedical devices, responsive scaffolds for tissue engineering applications	More suited for soft robotic devices than implants; early stage for biomedical use	91,93,94,96,98,143

are critical. When blended into biocompatible hydrogels, conductive polymers such as PEDOT:PSS, polypyrrole, and polyaniline provide support for cardiomyocyte synchronization and neuronal activity under stimulation.[118–120] Liquid crystal elastomers and mechanophore-functionalized hydrogels, on the other hand, enable the release of constructs that undergo reversible deformation or controlled biomolecule release when subjected to stress or strain.[121] These features allow for the engineering of active tissue constructs such as cardiac patches and musculoskeletal constructs, but long-term stability and extrusion printability remain limited.

Enzyme-responsive materials are also gaining attention for their ability to create bioinks that degrade or remodel in response to cell-secreted proteases.[122] Matrix metalloproteinase (MMP)-cleavable linkages have been integrated into PEG derivatives, allowing encapsulated cells to dynamically remodel their environment, thereby increasing tissue maturation. In parallel, secondary photo- or click-based crosslinking is being introduced to improve long-term structural stability while still preserving responsiveness. Ion-responsive systems, particularly calcium-mediated alginate hydrogels, continue to be used for rapid bioprinting, but newer designs incorporate dynamic ion exchange or reversible ionic interactions to enable controlled swelling, drug release, and shape morphing. These features make them attractive for creating transient vascular networks or stimuli-driven soft actuators, although their relatively weak mechanics and ion diffusion instability limit their clinical potential.

A recent and emerging area is the use of immune-responsive hydrogels for 4D bioprinting. Immune-responsive hydrogels have emerged as a transformative class of materials for 4D bioprinting and are designed to sense and respond to host immune signals such as inflammatory cytokines or immune cell activity. Studies have demonstrated that chitosan-based hydrogels can provide antibacterial, antioxidant, and anti-inflammatory properties, making them ideal candidates for creating dynamically responsive scaffolds in tissue engineering. Hao *et al.* (2023) further highlighted that hydrogel matrices could act as immunomodulatory platforms, enabling the controlled delivery of bioactive agents and even entrapped vaccines while minimizing systemic immune activation.[123] These materials offer significant advantages for 4D bioprinting, including on-demand release of therapeutic molecules, mitigation of chronic inflammation, and promotion of tissue integration and vascularization. Their capacity to dynamically degrade or release payloads in response to local immune cues closely mimics native tissue healing processes. However, the field faces substantial limitations: achieving precise reproducibility in terms of responsiveness, fine-tuning sensitivity to diverse immune environments, and ensuring long-term biocompatibility remain critical challenges. Moreover, variations in patient-specific immune responses and the complexity of integrating these systems into

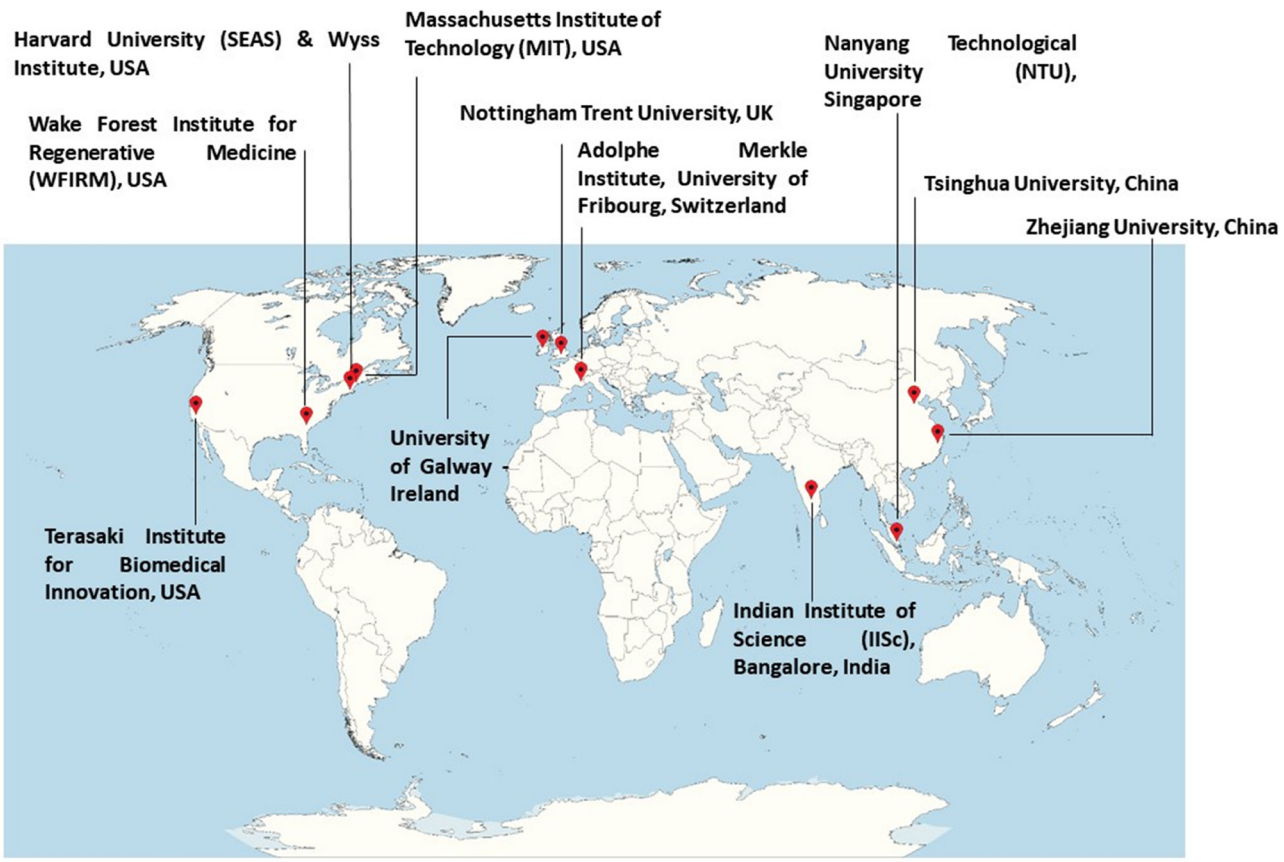

Fig. 11 Global distribution of leading research institutions actively engaged in 4D printing of stimuli responsive materials for biomedical applications.

larger, functional 4D constructs complicate clinical translation. Thus, immune-responsive hydrogels represent a promising frontier in the development of adaptive, self-regulating bioprinted tissues with potential applications in wound healing, organoid fabrication, and implantable tissue construction.

Despite these advancements, major challenges continue to restrict the widespread translation of 4D bioprinted materials. Light-based systems are limited by their penetration depth and oxygen inhibition; conductive and magnetic fillers face issues with aggregation, cytotoxicity, and regulatory concerns; enzyme- and ion-responsive systems suffer from variability and poor mechanical robustness; and immune-responsive approaches, although highly innovative, remain in their infancy with limited *in vivo* validation. Moreover, scalability, nozzle clogging, batch-to-batch reproducibility, and standardized safety testing all remain unsolved bottlenecks. A comparative summary of these stimuli-responsive systems, including their key advantages, limitations, applications, and current stage of clinical translation, is presented in Table 1. A critical analysis of the field reveals that the most successful systems are hybrid designs that combine multiple stimuli-responsive mechanisms with ECM-mimetic bioactivity, but these formulations are also the most complex to regulate and manufacture. To achieve clinical translation, future work must balance functionality with reproducibility, develop long-term preclinical models that capture immune interactions, and integrate intelligent design tools such as AI-guided optimization of bioink formulations.

The rapidly evolving field of 4D bioprinting is marked by significant global contributions from multiple leading research institutions, as illustrated by the geographical distribution in Fig. 11. The key centers of innovation span North America, Europe, and Asia, encompassing Harvard University and the Wyss Institute (USA);[124] Wake Forest Institute for Regenerative Medicine (USA);[125] the Massachusetts Institute of Technology (USA);[126,127] the Terasaki Institute for Biomedical Innovation (USA);[128,129] the Nottingham Trent University (UK);[130] the Adolphe Merkle Institute (Switzerland);[131,132] the University of Galway (Ireland);[133] the Indian Institute of Science (India);[34,102,111] Tsinghua University and Zhejiang University (China);[134,135] and Nanyang Technological University (Singapore).[77] These institutions have collectively propelled the development of smart bioinks, adaptive scaffolds, and stimuli-responsive constructs that offer control over dynamic tissue engineering processes. The diversity in research focus, from light-activated bioinks and volumetric bioprinting to multistimuli responsive systems employing electro, mechano-, enzyme-, ion-, and immune-responsive materials, reflects the multifaceted challenges and innovations within the field. Despite impressive strides in creating biomimetic, shape-memory constructs and immune-modulating hydrogels, major challenges related to material robustness, precise responsiveness, scalability, and regulatory approval persist. The global collaboration network underscored by this mapping highlights the interdisciplinary and international efforts driving 4D bioprinting toward clinical translation, emphasizing the importance of hybrid material systems and AI-driven optimization for future progress.[136–138]

4. Conclusion

Tissue engineering and regenerative medicine have progressed remarkably with the transition from conventional 3D bioprinting to the more dynamic paradigm of 4D bioprinting. The incorporation of stimuli-responsive materials (SRMs), including shape memory polymers, liquid crystal elastomers, and shape memory hydrogels, has opened new opportunities in tissue construct design by enabling printed scaffolds to sense, adapt, and transform in response to environmental cues. Such functionality has already shown promise in applications ranging from biomimetic blood vessel fabrication to smart drug delivery systems. However, despite these exciting advances, significant challenges remain before 4D bioprinting can be clinically translated. Limitations include the cytotoxicity and long-term safety of certain SRMs, variability in responsiveness under physiological conditions, limited scalability of complex bioinks, and the lack of standardized testing for regulatory approval. Furthermore, achieving reproducibility in patient-specific constructs while ensuring vascularization, immune compatibility, and predictable biodegradation remains a formidable task. This progress will require hybrid bioink designs that balance functionality with safety, the development of scalable bioprinting platforms, and systematic preclinical validation in large animal models. Thus, 4D bioprinting has the potential to move beyond experimental demonstrations and provide clinically viable, personalized, and functionally responsive tissue constructs for next-generation regenerative therapies.

Author contributions

Conceptualization: Mathew Peter, Pramod K. Namboothiri, Rudra Nath Ghosh and Akshatha; formal analysis: Akshatha and Mathew Peter; funding acquisition: Mathew Peter and Pramod K. Namboothiri; methodology: Mathew Peter and Akshatha; supervision: Dr Mathew Peter; writing – original draft: Akshatha; writing – review and editing: Akshatha and Mathew Peter; all the authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest in the publication of this article.

Data availability

All data for this article were created by Akshatha Bhandari, using the facilities in the Department of Biomedical Engineering, Manipal Institute of Technology, MAHE.

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