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4D printing of pH-responsive bilayer with programmable shape-shifting behaviour

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ABSTRACT

4D printing of smart materials has seen remarkable advancements in the domain of biomedical devices, with a particular focus on developing responsive and adaptive programmable structures. In this work, we report the 4D printing of two solvent-responsive hydrogels forming a bilayer that undergoes bidirectional actuation depending on the pH of the solvent. A strong interlayer adhesion between the hydrogels is formed without subjecting either of their surfaces to any chemical modification. These hydrogels have an interfacial toughness of 71.8 J/m^2 and undergoes no delamination during actuation inside a solvent. Conventionally, pH-responsive actuators are only limited to simple 2D films prepared by solvent casting. However, our work focuses on the design and fabrication of complex bilayer and patterned structures using Direct-Ink Writing (DIW) approach. These printed structures actuate upon immersion in a solvent medium, and the actuation is reversible in nature. The influence of programmable variables on the morphed structure was studied systematically by modifying the rheological properties (in the range of 10^2–10^5 Pa s) and printing parameters (optimized at a printing speed of 5 mm/sec, with the extrusion pressure of 5–6 bar and nozzle diameter of 0.5 mm) of the 3D printed bilayer structure. The physicochemical properties of the printable hydrogel ink were tuned such that the same structure can respond to both acidic and basic pH (with non-morphing point at pH 7), by altering the directionality of actuation. We have demonstrated the applications of these pH-responsive actuators in smart valves.

1. Introduction

4D printing is an advanced additive manufacturing process that integrates time as the fourth dimension [1]. It has attracted significant interest because of its capability to achieve memory adaptability in complex structures in the development of soft robots and actuators [2], biomedical applications for wound dressing, drug delivery systems, and tissue engineering [3–5], showing time-dependent variations in response to external environmental stimuli such as solvent [6], pH [7,8], light [9–12], heat [13–15], magnetism [16], and electric [16,17], among others. This responsiveness allows the 3D printed structures to alter, self-assemble, or change their shapes over time, usually in a predefined manner. The inclusion of these dynamic features enhances the functionality and adaptability of 3D printed objects. So far, numerous materials have been developed to achieve this objective, such as hydrogels, shape-memory materials, polymer fibres, and more. Interestingly, hydrogels have the capability to transform external stimulations into mechanical motions and are likewise engineered to behave smartly upon exposure to various stimuli.

Recently, 4D printing of solvent-responsive actuators have been drawing attention in biomedical applications due to the physiological environment of the body [6,18–20]. Moreover, the physiological pH of the body varies from organ to organ; for example, the stomach works in the 1–3.5 pH range, and the pancreas works in a slightly basic pH (8–8.3) range, and the pH level in the physiological environment varies depending on the conditions in the body. Therefore, there is a need for the development of a pH-controlled responsive actuator. The pH-responsive materials utilize the distinct swelling properties of the material in acidic or alkaline environments to control the driving force [7]. Shape-morphing pH-responsive bilayer hydrogel is the focus of our present work, wherein the shape or actuation of the structure is altered intelligently by varying the pH environment [21]. The actuation mechanism of pH-responsive hydrogels is primarily driven

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by the ionization of acidic and basic functional groups within the polymer network that makes up the hydrogel [22]. Several authors have reported the application of pH-responsive bilayer hydrogels as soft actuators for promising applications in drug delivery, microfluidic switches, artificial muscles, and flexible sensors [23–28]. Kumar et al. [7] fabricated a hydrogel-based pH-responsive bilayer actuator with multiple non-morphing points for regulated inhibition and release of drugs to a specific site within the body. Several authors have reported a pH-responsive actuator that operates either only in the range of acidic or basic pH [29,30]. Limited work has been explored on pH-responsive actuators in the complete spectrum of pH [31]. The developed pH-responsive bilayer by Kumar et al. [7] showed actuation-induced inhibition and release of model drugs to target specific site within the body. In this work, the non-morphing point of the prepared bilayer was tuned by modulating the concentration and crosslinking density of the bilayers involved. However, these hydrogels still remain unutilized due to their limitations in fabrication techniques into customized structures for the targeted system. For fabricating such stimuli-responsive systems, two main requirements are: (i) a compliant stimuli-responsive material and (ii) a process that enables controlled deposition of these materials to achieve a programmed response [32,33]. Chitosan and carboxymethyl cellulose have potential functionalities to be programmed as smart materials as demonstrated in one of the work [7]. The second requirement involves the identification of a suitable fabrication technique that enables programmability and fabrication of hydrogels. It has been demonstrated that 3D printing can overcome this limitation of geometric complexity [6,34]. The pH-responsive hydrogels (3D printing) can provide a suitable foundation for pH-responsive drug release, along with soft robotic actuators for minimal invasive surgery [28,35]. Research in 3D printing of pH-responsive hydrogels is still evolving, and ongoing efforts focus on improving the printing techniques, optimizing material formulations, and exploring new applications.

Multiple stimuli-response is of great importance in the field of 4D printing research for expanding the practical application of the printed structure in diverse environments [4,14,36]. A simple example is a bilayer hydrogel that can be actuated using two different stimuli on both sides. A strong bilayer adhesion is necessary to transmit the forces between the layers to achieve effective actuation. A strong adhesion ensures that the two layers of the hydrogel remain well-bonded during the shape change, preventing delamination or separation that could impede actuation. The choice of materials for the two layers is crucial. The hydrogels should have compatible chemistries to promote adhesion. Sometimes, surface modifications or cross-linking agents are used to enhance interlayer adhesion [37]. The strength of the bilayer adhesion directly influences the controllability and precision of the shape transformation in hydrogels. A strong bilayer adhesion in shape-morphing pH-responsive hydrogels plays a critical role in expanding the range of applications and improving reliability and effectiveness of these dynamic hydrogel systems. However, limited past work has been attempted in this regard because the conventional techniques are intensive in nature and are limited to only simple 2D films. Several authors have reported the application of bilayer hydrogels as biomedical devices in their studies [38], yet complex designs have not been attempted, which can be overcome by 4D printing [39,40]. Although 4D printing can overcome the limitation of design complexity, the printability of a multimaterial (more than one) system is a challenge. A major challenge in the 4D printing of bilayer hydrogel is control over the printability of the two-material system with strong interlayer adhesion during actuation. Rheological characterization, along with the optimization of the printing parameters, can be utilized to print bilayer structures. Applications of 4D printing of bilayer structures have rarely been reported owing to the loss of interfacial adhesion and structural integrity upon exposure to stimuli.

3D printing techniques such as fused deposition modelling(FDM), direct ink-writing (DIW), stereolithography (SLA), and digital light processing (DLP) are commonly used to print stimuli-responsive materials. DIW-based 3D printing is a versatile and cheaper technique where point-by-point printing is achieved by applying pressure to extrude the material through a nozzle [41]. This method imposes stringent requirements on the viscosity of the material. However, the interface bonding between printed layers is often compromised, resulting in reduced mechanical strength and delamination of layers along the cross-sectional direction. The properties and applications of structures generated by DIW largely depend on the properties of the ink components. Therefore, ink development is one of the main directions in the emerging field of DIW printing. The morphing characteristics of a pH-responsive actuator depend on factors such as concentration of polymer and crosslinking density, which, as a result, affect the thickness of structures and swelling characteristics. In this work, pH 7 was considered as the non-morphing point, and the directionality of actuation was altered about this point. 3D printing of pH-responsive hydrogels along the whole spectrum has not yet been attempted due to challenges in the formulation of pH-responsive ink to control both printability and its actuation characteristics. Two important criteria were taken into account for the design of a pH-responsive printable ink: control over composition to obtain correct rheological properties for printing and shape-morphing behaviour of 3D printed structures upon exposure to different ionic solvents. In our work, we have developed a 3D printable ink for the DIW process that could respond to pH variations in the solvent without any delamination and change its directionality of actuation at neutral pH.

In this study, 4D printing of solvent-responsive hydrogels consisting of chitosan (CS) and carboxy methyl cellulose (CMC) cross-linked with citric acid (CA) was designed and fabricated with strong interfacial adhesion. These structures underwent bidirectional actuation depending on the pH of the solvent immersed in without any delamination. The influence of programmable variables on the morphed structure was studied by modifying the rheological properties and optimizing the printing parameters of the 3D printed bilayer structure. The physicochemical properties of the printable hydrogel ink were tuned such that the structure can respond to both acidic and basic pH by altering the directionality of actuation. The CS and CMC ink was printed into the bilayer and patterned architectures to generate programmable designs to mimic human fingers and artificial valves. The actuation of these 3D printed pH-responsive structures was demonstrated as a potential application in smart valves.

2. Experimental section

The chemicals such as chitosan powder (degree of deacetylation $<$ 90%, viscosity (100–200) cps, medium molecular weight), carboxymethyl cellulose sodium salt (high viscosity 1100–1900) cps, acetic acid (extrapure AR $>$ 99.9%), citric acid monohydrate(extrapure AR 99.7%) were procured from SRL Chemicals Pvt. Ltd (India) for the preparation of chitosan and CMC hydrogel ink. For analysis, sodium hydroxide pellets (low chloride) were purchased from Merck Life Sciences, India, and hydrochloric acid (37%, ACS reagent) was purchased from Sigma-Aldrich (Bangalore, India).

2.1. Preparation of 3D printable ink

The present bilayer hydrogel consists of chitosan(CS) and carboxymethyl cellulose(CMC), which are prepared independently. The printability of these inks was analysed through rheological characterization.

Chitosan ink was prepared by dissolving chitosan powder at a concentration of 8% (w/v) in 50% v/v acetic acid solution. Around 20 wt% (out of the total weight of the chitosan powder) of citric acid was added to the chitosan solution and was kept undisturbed for 12 hours. The solution was then mechanically agitated at 200 RPM for 2 hours to solvate the chitosan and citric acid particles homogeneously and centrifuged at 3000 RPM for 1 hour to remove air bubbles.

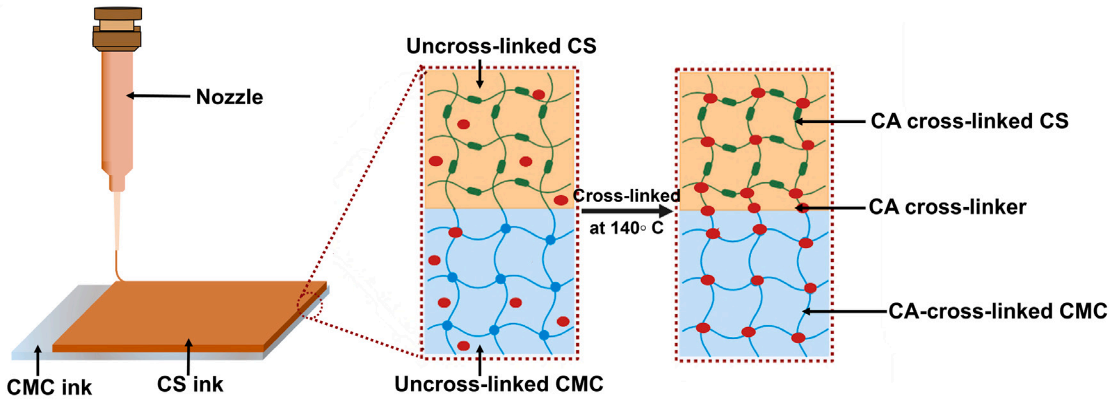


Fig. 1. The schematic diagram shows 3D printing of a two-material system composed of chitosan and carboxymethyl cellulose using direct-ink writing (DIW) technique and cross-linked with citric acid at 140 °C.

CMC ink was obtained by dissolving 15% (w/v) of CMC powder in DI water. Around 60 wt% (out of the total weight of the CMC powder) of citric acid was added to the CMC solution and was kept undisturbed for 12 hours. The solution was then mechanically stirred at 200 RPM for 2 – 4 hours to dissolve the CMC and citric acid particles homogeneously and centrifuged at 3000 RPM for 1 h to remove air bubbles.

2.1.1. 3D printing of CS/CMC bilayer structures

The bilayer hydrogel samples were manufactured by using our custom-built direct-ink writing (DIW) 3D printer. A stainless steel needle (21G) with an inner diameter of 0.5 mm was used to print all objects. In this study, all the printed structures were fabricated at a fixed layer thickness of 1 mm for CMC and 0.5 mm for chitosan. The samples were printed with 100% infill density and with a concentric infill pattern to avoid overlapping lines. During printing, the CMC layer was first printed on an acrylic substrate. After partial solidification at room temperature(<8 h), chitosan ink was printed on the printed CMC structure. The bilayer printed structures were dried in the oven at 50 °C for 24–48 h and cured at 140 °C for 10 min to cross-link the samples (as shown in Fig. 1) [6]. The dual-hydrogel ink was 3D printed into various designs that transformed into desired shapes upon exposure to pH solvent molecules.

2.2. PH-responsive actuation of bilayer 3D printed structures

The CS/CMC bilayer structures were printed into 7 mm × 2 mm sizes for all actuation experiments. The strips were held with CS to the left side layer. 0.1 M HCl and 0.1 M NaOH solutions were prepared for the pH-based actuation experiments. From 0.1 M HCl and 0.1 M NaOH, solvents of pH 4 and pH 10 were prepared, respectively. Distilled water was used as a pH 7 solvent. A pH metre was used for the accurate measurement of the pH of the solvent, and slight fluctuation in pH was adjusted. From all pH-responsive bilayer structures prepared, 15 samples were tested for actuation. The actuation videos of the experiments were recorded in real-time using a 64-megapixel camera with a resolution of 1440 * 1440 and at a rate of 30fps. The images were analysed using Matlab™software to determine the total bending time, bending angle of bilayer actuation, and degree of bending. A valve of dimension 30 mm × 30 mm was 3D printed with four leaf-like flaps for demonstration of the opening and closing of the smart valve. In order to mimic smart textile a structure of dimension 50 mm × 50 mm was printed with four flaps pointing outwards and a passive structure inside. To mimic a smart vent/window, a structure with multiple strips/flaps of 50 mm was printed. The flaps were printed in a fashion with a bilayer of CS/CMC placed in the same and opposite to each other on the centre axis.

3. Results and discussion

The findings provide a detailed study of the pH-responsive mechanism of the printed bilayer structures. The dynamics of the bending phenomenon were studied, and the related mechanisms were explained. The direction of actuation of 3D printed CS/CMC bilayer structures depends upon its exposure to pH solvents. These actuation characteristics provide insight for designing complicated structures with a combination of chitosan and carboxymethyl cellulose.

For the actuation experiments, the bilayer structures were 3D printed into rectangular strips of dimensions 7 mm × 2 mm. These rectangular strips were fixed at one end and dipped vertically in different pH solvent. The actuation videos were captured, and the curvature attained was calculated. The bilayer bends towards the CMC layer in an anticlockwise direction upon exposure to acidic pH. Similarly, the structure bends towards the chitosan side in a clockwise direction upon exposure to basic pH, as shown in Fig. 2. At pH 1, a maximum curvature of 93 ± 5° is attained after 900 s Whereas at pH 4, a maximum curvature of 54 ± 5° is attained after 600 s At pH 7, the bilayer structure did not show any actuation and remained flat with net-zero curvature. At pH 13, the maximum curvature is attained after 900 s with a curvature of 65 ± 5°, whereas at pH 10, the maximum curvature of 40 ± 5° is attained after 600 s The curvature achieved by the bilayer when dipped inside different pH solvents remains permanent or irreversible, i.e., the folded state is maintained.

3.1. Mechanism

Hydrogel actuators can be programmed to change their shapes and perform mechanical work when triggered by stimulants such as solvent, humidity, temperature, light, and so on due to the anisotropic swelling behaviour of these structures. A pristine 3D printed hydrogel structure undergoes out-of-plane bending when subjected to solvent. When a single side of the structure is exposed, the solvent molecules diffuse through the polymer matrix. However, when the above-printed structure is submerged in a solvent, it does not bend as there is uniform diffusion of solvent in all the faces of the structure, resulting in a net-zero concentration gradient. Therefore, the primary mechanism of bending of a single-layer hydrogel is the concentration gradient that develops throughout the thickness. A 3D printed bilayer structure with an active and passive layer bends towards the passive side when completely immersed in a solvent. The primary actuation mechanism of bending of a bilayer system is the differential swelling between the adjacent layers of a bilayer material system [6]. However, these 3D printed bilayer structures with a single active and passive layer have limited application due to uni-directional actuation behaviour. To bring out bi-directionality in the actuation behaviour of the structures, both layers are designed such that each respond to a particular stimulus.

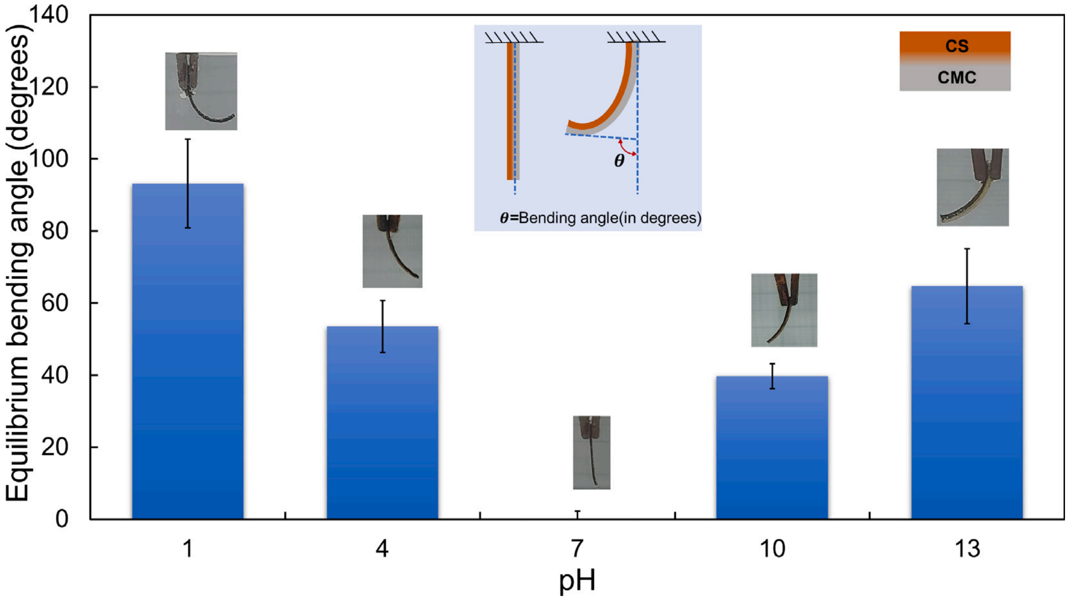


Fig. 2. The plot shows bending attained by the 3D printed bilayer structure at different pH values.

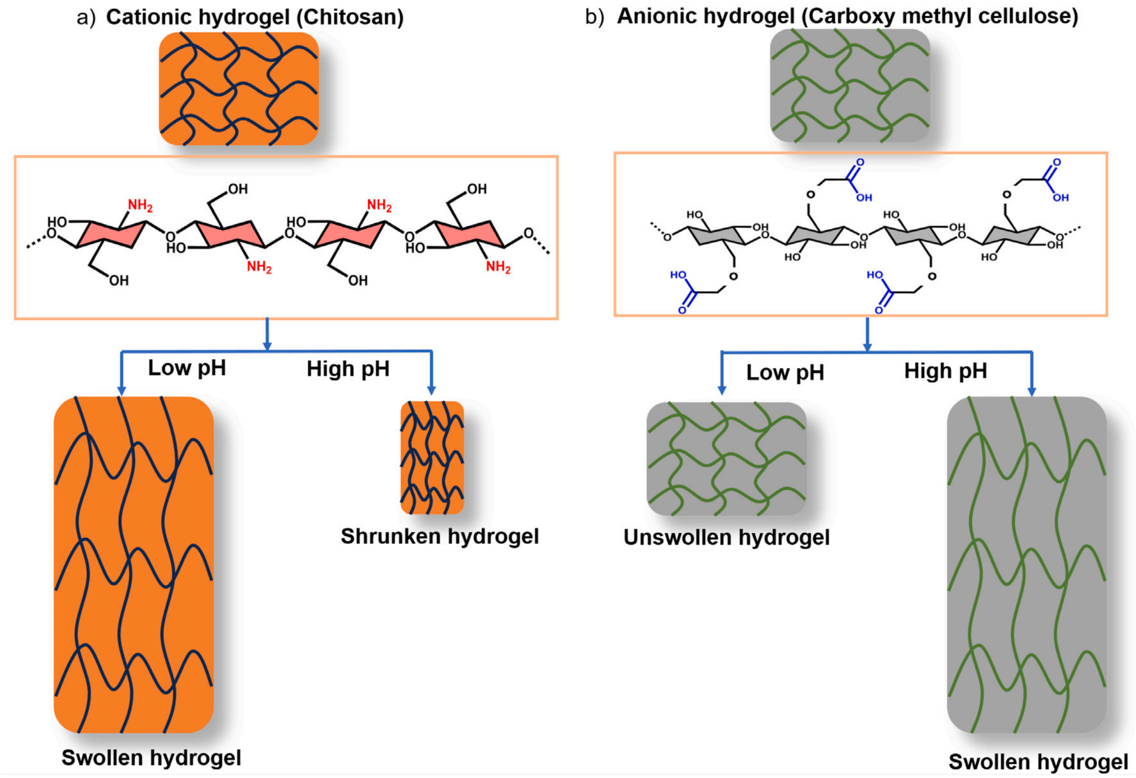


Fig. 3. Swelling mechanism of cation and anion hydrogel in low and high pH solvent in which (a) cationic hydrogels swell in low pH and shrink at high pH while (b) anionic hydrogel remains unswollen at low pH and swells at high pH.

In this work, both the layers of the bilayer structure are active; one responds to an acidic solvent, and the other to a basic solvent. The swelling behaviour of each printed layer was studied in pH solvents (explained in detail in supplementary section S3) to understand the actuation mechanism of these bilayer structures.

The overall curvature achieved by the CS/CMC bilayer when exposed to various pH solvents is influenced by a combination of factors (as explained in the work [7]):

- ionic repulsion;
- bending stiffness of layers involved;
- swelling of the materials in each layer.

The primary mechanism on which pH-responsive hydrogel-based systems function is ionic repulsion. The concept involved in this mechanism is illustrated using the standard CS/CMC bilayer schematic in fig.S4. Understanding the factors that influence the swelling ratio of a layer is also crucial. The cross-linking density and the presence of solvent-interacting chemical species play a significant role in determining this ratio. When the swelling ratios of the layers differ, the bilayer can achieve a greater curvature.

When a 3D printed chitosan hydrogel structure is immersed in an acidic solvent, a large swelling of the structure is observed (as shown in Fig. 3a). Chitosan hydrogels are cationic in nature, and the amino groups in chitosan have a pKa value of (6.2–7). The pendant groups in a cationic hydrogel get ionized below the pKa of the polymeric network [42]. When the chitosan is subjected to an acidic environment, the amino groups get ionized to NH_3^+ groups below the pKa of the polymeric network. The presence of the NH_3^+ ions generates an electrostatic repulsive force. The presence of these positive charge ions results in a large osmotic swelling force that makes the hydrogel swell at a lower pH.

A 3D printed carboxymethyl cellulose hydrogel structure swells less compared to chitosan upon exposure to an acidic medium (as shown in Fig. 3b). CMC hydrogels are anionic in nature, and carboxyl groups in CMC have a pKa value of 4.3. The pendant groups in an anionic hydrogel get ionized above the pKa of the polymeric network. The COO-groups in CMC remain un-ionized in lower pH leading to an unswollen hydrogel structure.

When a 3D printed bilayer CS/CMC hydrogel structure is immersed in an acidic medium, the structure actuates in an anti-clockwise direction, i.e., towards the CMC side. Upon exposure to acidic pH, larger swelling is observed in the CS layer due to the protonation of amine groups, as explained earlier, compared to the CMC layer, which gives rise to a differential swelling in the bilayer across the thickness of the hydrogel structure. This results in out-of-plane bending towards the CMC layer.

A larger curvature is attained in the 3D printed bilayer structure when immersed in pH 1 compared to pH 4. As we move from more acidic to neutral pH, the concentration of H^+ ions reduces, which leads to less protonation of amine groups in CS, resulting in less swelling of the structure. Due to the reduced swelling of chitosan in the bilayer structure at pH 4, the moment exerted by the CS layer in the bilayer induces lesser curvature compared to pH 1 (as shown in Figs. 2 and 4). The printed bilayer structure showed small actuation upon exposure to pH 7 because of the different hydration of the two hydrogels. It was found that the swelling degree of both the CS and CMC layer at pH 7 is approximately 200%. Due to a similar swelling ratio, a differential swelling was not developed in the bilayer structure.

The bending stiffness of the layers is crucial in regulating the curvature of actuation. It is evident that a layer with greater bending stiffness experiences more resistance to bending compared to a layer with lower stiffness. Alternatively, in order to get the same degree of bending, a stiffer layer requires a greater amount of generated moment. The bending stiffness can be expressed as Bending Stiffness $\propto$ EI, where E represents the modulus of the individual layer and I represents the moment of inertia of the section around the bending axis. Altering either the cross-linking density or the layer thickness can result in a modification of the bending modulus (E). For a rectangular cross-section, the moment of inertia (I) varies as Moment of Inertia $\propto h^3$ for a constant width. Therefore, even a small change in thickness can have a substantial impact on the flexibility of the material. Therefore, from our previous work [7], we have kept the layer thickness as constant, i.e., the thickness of CMC is twice that of CS. The cross-linking density is also kept unaltered for the preparation of printable ink.

Since no net moment of actuation was observed at pH 7 with a designed layer thickness of 0.5 mm and 1 mm in CS and CMC. Therefore, this was considered as the standard bilayer, taking into account all the above three factors.

The reverse phenomenon occurs when a cationic and anionic hydrogel is subjected to a basic environment. When the CMC hydrogel structure is exposed to a basic medium, the –COO- groups get deprotonated, resulting in an electrostatic repulsion among the negatively charged ions. This results in a larger osmotic force and swelling of the hydrogel structure. While the amine groups present in chitosan do not get ionized in the basic medium, and this results in an unswollen hydrogel structure. When a 3D printed bilayer CS/CMC hydrogel structure is immersed in a basic medium, the bilayer actuates in a clockwise direction, i.e., towards the CS side, due to the combined effect of more swelling ratio of the CMC compared to the CS layer and shrinkage of the CS layer in basic pH, which reduces the swelling ratio. The larger swelling ratio of CMC is due to deprotonation of the carboxyl group present, causing the repulsion of more like charges between the chains. Since the CS solution was prepared in 50% acetic acid solution, the prepared layer already has charged amine groups. When dipped in a basic medium, these protonated amine gets neutralized, and the chains come closer as there is no like charge repulsion. This causes some shrinkage of the CS layer. Due to the competitive swelling of CMC and shrinkage of CS in the bilayer system, a differential swelling was obtained across the thickness of the hydrogel structure. This induces a stress field, as explained above, and results in out-of-plane bending towards the CS layer.

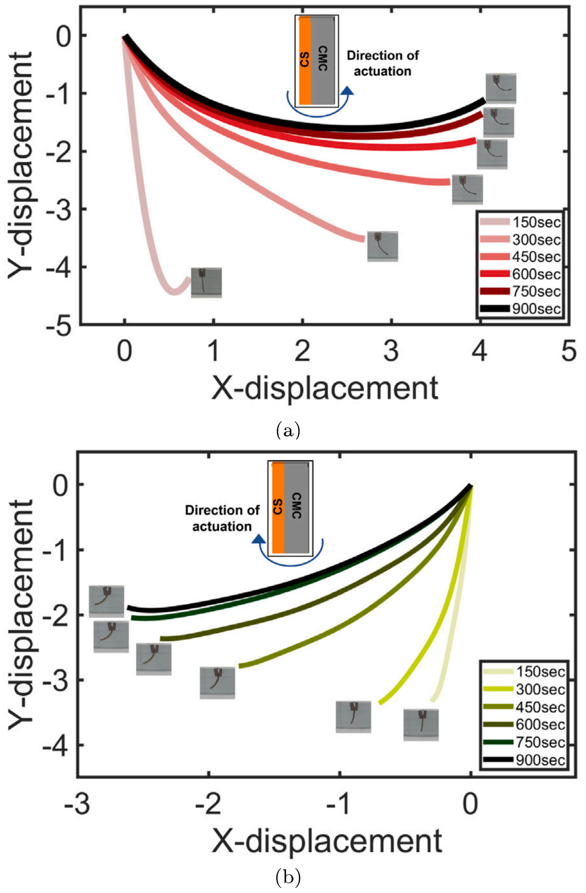


Fig. 4. Actuation plot showing X-Y displacement of 3D printed bilayer hydrogel structures (a) when exposed to acidic solvent of pH 1, (b) when exposed to basic solvent of pH 13.

A larger curvature is attained in the 3D printed bilayer structure when immersed in pH 13 in comparison to pH 10. This is because as one moves from a more basic pH to a neutral pH, the concentration of OH^- reduces; this leads to less deprotonation of carboxyl groups in the CMC layer, resulting in a lower swelling ratio. Due to the reduced swelling ratio at pH 10, the moment exerted by the CMC layer in the bilayer attains lesser curvature compared to pH 13.

All the actuated structures were irreversible in nature due to the permanent differential swelling in the bilayer structure. The original shape can, however, be retrieved by exposing it to suitable solvents.

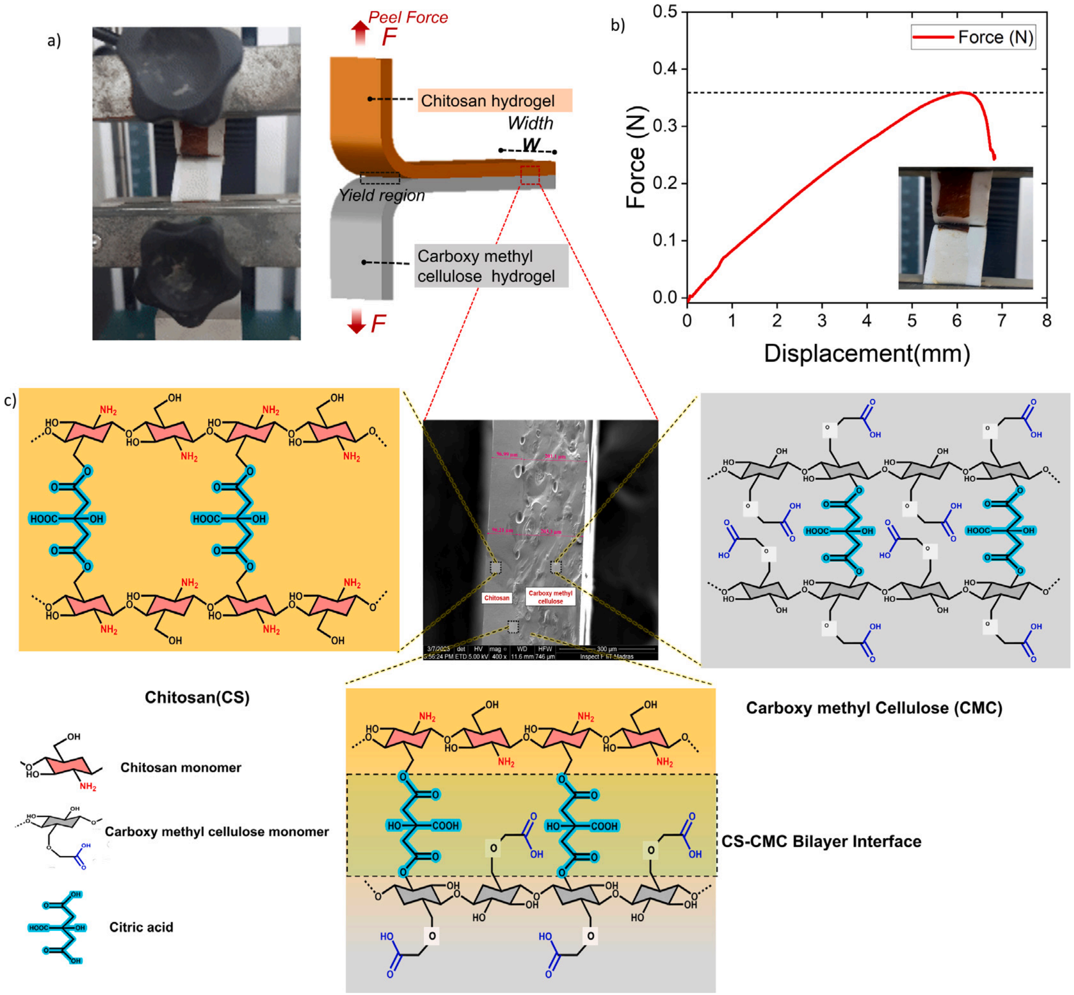


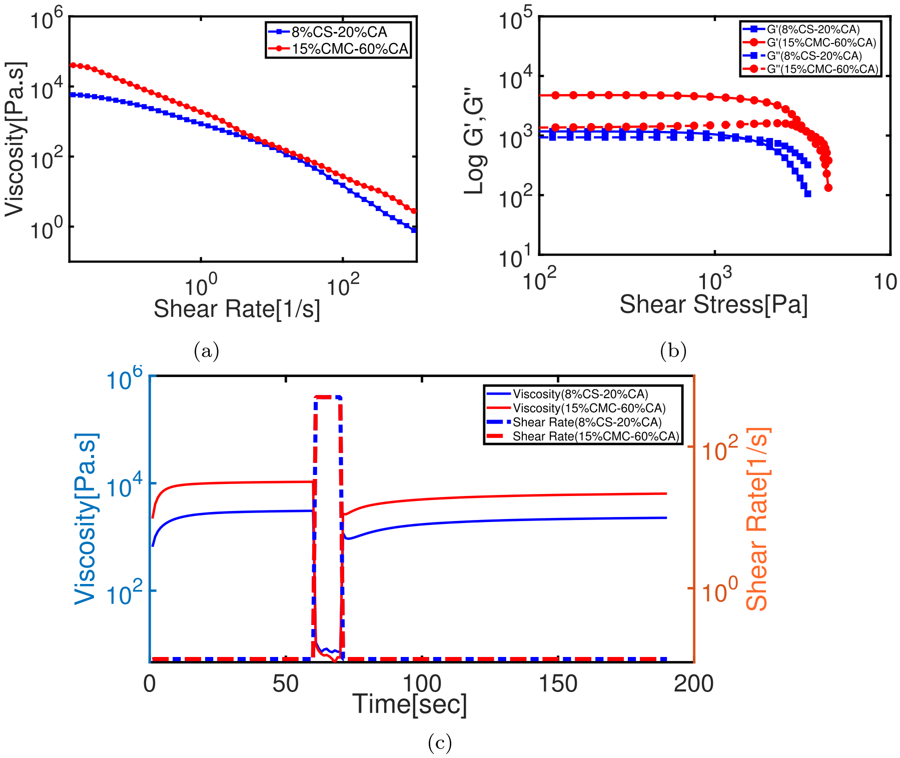
Fig. 5. Figure illustrates (a) set up for a T-peel test where there is a strong diffusive layer of chitosan and CMC, (b)Force–displacement curve showing the failure of the bilayer structure, (c) SEM analysis with a strong bonding of CS and CMC with interfacial bonding of –COOH- groups between 3D printed CS/CMC bilayer structure.

The permanent folded structures were reverted to a flat state using ethanol as solvent. Ethanol is an organic solvent highly miscible with water and is a poor solvent for CMC and chitosan. When the permanently folded structure is dipped in ethanol, the polymer chains in CMC and chitosan tend to precipitate as they try to avoid interacting with the organic solvent, while the water molecules inside the polymer matrix are taken up by ethanol. Due to these interactions, the concentration gradient diminishes, causing the folding structure to unfold to its initial state. Similarly, when an acid (pH 1) dipped structure that has folded in the anti-clockwise direction is dipped in a basic medium (pH 13), the increased swelling ratio of the CMC layer balances out the swelling ratio of the CS layer, causing the concentration gradient to diminish and thus the bilayer unfolds.

3.2. *Strong bilayer formation without chemical modification*

A hydrogel system with two distinct materials finds application in soft robotic actuators and drug delivery due to better control of bidirectional actuation with multi-responsive behaviour. Despite its multi-responsiveness, bilayer structures with different polymers suffer delamination during actuation. The stress caused by inhomogeneity in the swelling of the layers at the interface of the bilayer hydrogels leads to debonding of the layers. As a result, significant adhesion at the layer interface is required for sensitive deformation of bilayer hydrogels. A conventional bilayer structure usually consists of a three-layered structure where the top and both layers are the active layers, and the middle layer is the interface that strongly binds the top and bottom layers. Fabricating this three-layered structure usually needs some additional processing techniques, such as spin coating. However, the technique of fabricating conventional sandwich bilayer structures finds limitations due to complex designs and also the fact that it cannot be extended to multiple material systems.

In this work, a different approach was employed to prepare the bilayer structures using chitosan and CMC hydrogels with citric acid as a cross-linker using 3D printing. This method not only can be used for complex designs but also eliminates the need for a third interfacial layer between the two active layers. In our research, the CS and CMC layers were cross-linked by citric acid through covalent bonds, as shown in Fig. 5. A strong chemical cross-linked network between NH_3^+ groups in chitosan and COO- groups in CMC significantly contributed to the tight adhesion in the interface of the bilayer hydrogels, resulting in diverse complicated deformations without delamination. A T-peel test was used to quantitatively analyse the interfacial bonding between the two hydrogel layers (refer to supplementary section S4). The interfacial toughness was found to be 71.8 J/m^2, which shows that there is strong bonding between CS and CMC layers. Further, scanning electron microscopy (SEM) was used to investigate the hydrogel's bilayer structure, confirming that the bilayer system had strong interlayer adhesion. A strong electrostatic interaction between protonated amine and deprotonated carboxylic acid groups along with a covalent cross-linking of citric acid with hydroxyl groups of both polymers contribute to the stability of these bilayer structures (as shown in fig. S1). In addition,



(a)

(b)

(c)

Fig. 6. (a) A printable chitosan and carboxymethyl cellulose hydrogel ink showing shear thinning ability, (b) Linear viscoelastic behaviour of printable chitosan and carboxymethyl cellulose ink illustrating that the storage modulus dominates loss modulus after flow point, (c) Thixotropic property behaviour of printable chitosan and carboxymethyl cellulose ink showing shape fidelity of the ink after printing.

bilayer hydrogel actuators that have two active layers demonstrate bidirectional deformation behaviours, unlike most bilayer hydrogel actuators that only have one active layer and exhibit unidirectional deformation performance.

Several works have reported that creating bilayer hydrogel films through solvent casting is limited to simple 2D structures and poses challenges when creating complex 3D structures. The process of designing and fabricating a suitable mould for these complex structures is difficult, and incomplete polymerization and curing of the hydrogel can lead to improper actuation. However, 3D printing has the potential to overcome the limitations of fabricating hydrogels with greater shape-morphing flexibility and complicated rapid deformation requirements. The printability of a hydrogel depends on its rheological properties (viscosity, yield stress, shear thinning) and cross-linking mechanisms. Kumar et al. [7] used citric acid as a cross-linker to create CS/CMC bilayer films and engineered different functionalities and properties with varying concentrations of CMC. The concentration of chitosan used was 1.5%, and CMC concentration was varied from 2%–4% in order to engineer the non-morphing point of actuation for controlled drug release. However, pure chitosan and CMC inks in the form of low-concentration sols (<6 wt%) have a viscosity of less than 100 Pa s and tend to diffuse after 3D printing through the nozzles causing distortion of the 3D structure. To address this issue, hydrogel ink with high viscosity in the range of $10^2 - 10^4$ Pa s is needed to prevent oozing out of the nozzle as well as is easily extrudable without clogging the nozzle during printing [6]. Therefore, a hydrogel ink with good rheological properties is necessary to fabricate 3D hydrogel structures, which is discussed in detail in the next section.

3.3. *Analysis of printability of hydrogel ink*

Hydrogels are an excellent choice for direct ink writing for complicated 3D structures because their viscoelastic properties can be precisely optimized over several orders of magnitude to enable flow through nozzles and produce filaments that preserve their shape even as they span gaps in the underlying layers of the printed structure. In extrusion-based printing, the ink, initially at rest, transitions to a high shearing condition while flowing through the nozzle and changes viscosity, finally reaching a new resting state. In this work, two important criteria were taken into account when designing the hydrogel ink. They must demonstrate good control over the viscoelastic response so that they flow through the deposition nozzle and then set immediately to allow for shape retention of the deposited ink. Secondly, these printed structures should have shape-morphing behaviour upon exposure to different ionic solvents. These two aspects must be simultaneously taken into account for ink design.

3.3.1. *Rheological characterization of hydrogel ink*

Rheological measurements have been carried out to investigate the viscoelastic characteristics of hydrogel ink and relate their properties with a DIW 3D printing process. The tests were performed with a 25 mm parallel plate and a measurement gap of 0.55 mm using a rotational rheometer MCR301, Anton Paar, Austria. The shear thinning property, amplitude sweep, and thixotropic characterization of chitosan and carboxymethyl cellulose hydrogel ink were performed at room temperature (the working temperature of the 3D printer).

3D printing of hydrogel requires high viscosities in the range of 10^2–10^5 Pa s [6]. Generally, higher viscosities result in greater printing quality. However, high viscosity also increases shear stress, which can affect the polymer entanglements in the hydrogel ink. The molecular weights and concentrations are the most important factors that determine the viscosity of polymers in solution. In our study, keeping the molecular weight constant, i.e., medium MW (viscosity of 150–500 m Pa s) for chitosan and high MW (viscosity of 1100–1900 m Pa s) for CMC, the hydrogel concentration was varied to prepare printable ink. The composition of chitosan was varied from 7%–9% [6], CMC

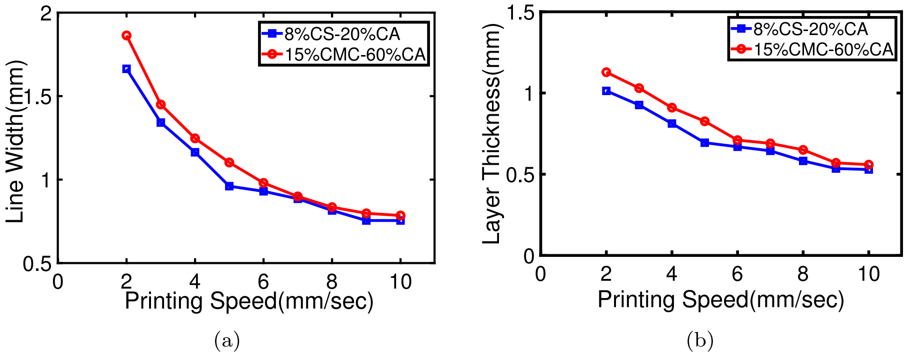


Fig. 7. Study of printing parameters at different printing speeds (1–10 mm/s) (a)variation of line width with printing speed, (b) variation of layer thickness with printing speed.

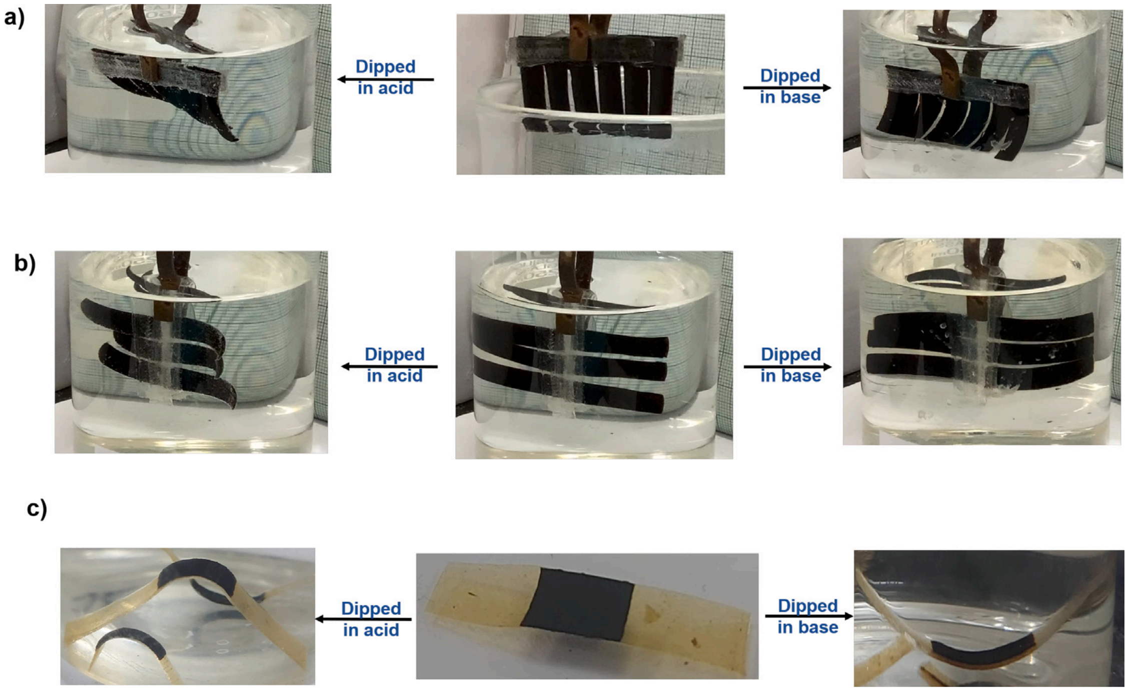


Fig. 8. Actuation of 3D printed structures with exposure to acidic and alkaline solvents, (a) with bilayer of CS/CMC, (b)with bilayer of CS/CMC placed opposite to each other of the centre axis and (c) with bilayer strips in different angles and spacings.

was varied from 10%–15% [43,44], and citric acid from 20%–60% [7] to alter the degree of cross-linking. It was observed that the viscosity of chitosan ink and CMC ink increased with an increase in the concentration of the solute particles in the solution. The viscosity of chitosan ink was 1.87×10^3, 5.82×10^3, 2.4×10^4 Pa s for 7%, 8%, and 9% respectively with 20% citric acid (as shown in Fig. 6(a)). The viscosity of CMC ink was 1.27×10^4, 4.26×10^4, 8.0×10^4 Pa s for 10%,15%, and 20%, respectively with 20% citric acid (as shown in Fig. 6(a)). The viscosity of the ink was in the range $10^2 - 10^5$ Pa s, and it could be concluded that the ink is stable inside the nozzle at rest [45]. The increased concentration of solute particles will increase the number of polymer chains in the solution, leading to a rise in its viscosity. It was observed that the viscosity slightly increased upon increasing the concentration of citric acid in both chitosan and CMC ink. This is because citric acid is only physically cross-linked. Therefore, with an increase in the degree of cross-linking, more polymer chains get entangled within the hydrogel ink, thus increasing the bonding energy and resulting in an increase in viscosity. The secondary cross-linking after printing gives a permanent shape to the printed structure.

Shear thinning ability of printable ink

The plot in Fig. 6(a) showed a non-linear behaviour which confirmed that the prepared chitosan and CMC hydrogel ink were non-newtonian fluids. The viscosity decreased as the rate of shear increased from 0.1 to 1000 s^{-1}, indicating that both hydrogel ink are shear thinning in nature. The partially cross-linked chitosan and CMC hydrogel inks exhibited shear-thinning properties due to changes in polymer configuration and macromolecular orientation along the shear flow. The entanglement of the polymers helps to give partial shape gain to the printed structure. The long term/permanent shape stability is achieved by secondary cross-linking of citric acid in ink after printing at 140 °C for 10 min.

Linear visco-elastic behaviour of printable ink

The ink in extrusion printing should display both flow and shape-retention properties. The ink should flow with minimal internal resistance while passing through the nozzle. In contrast, the properties should be opposite with immediate build-up of internal forces opposing

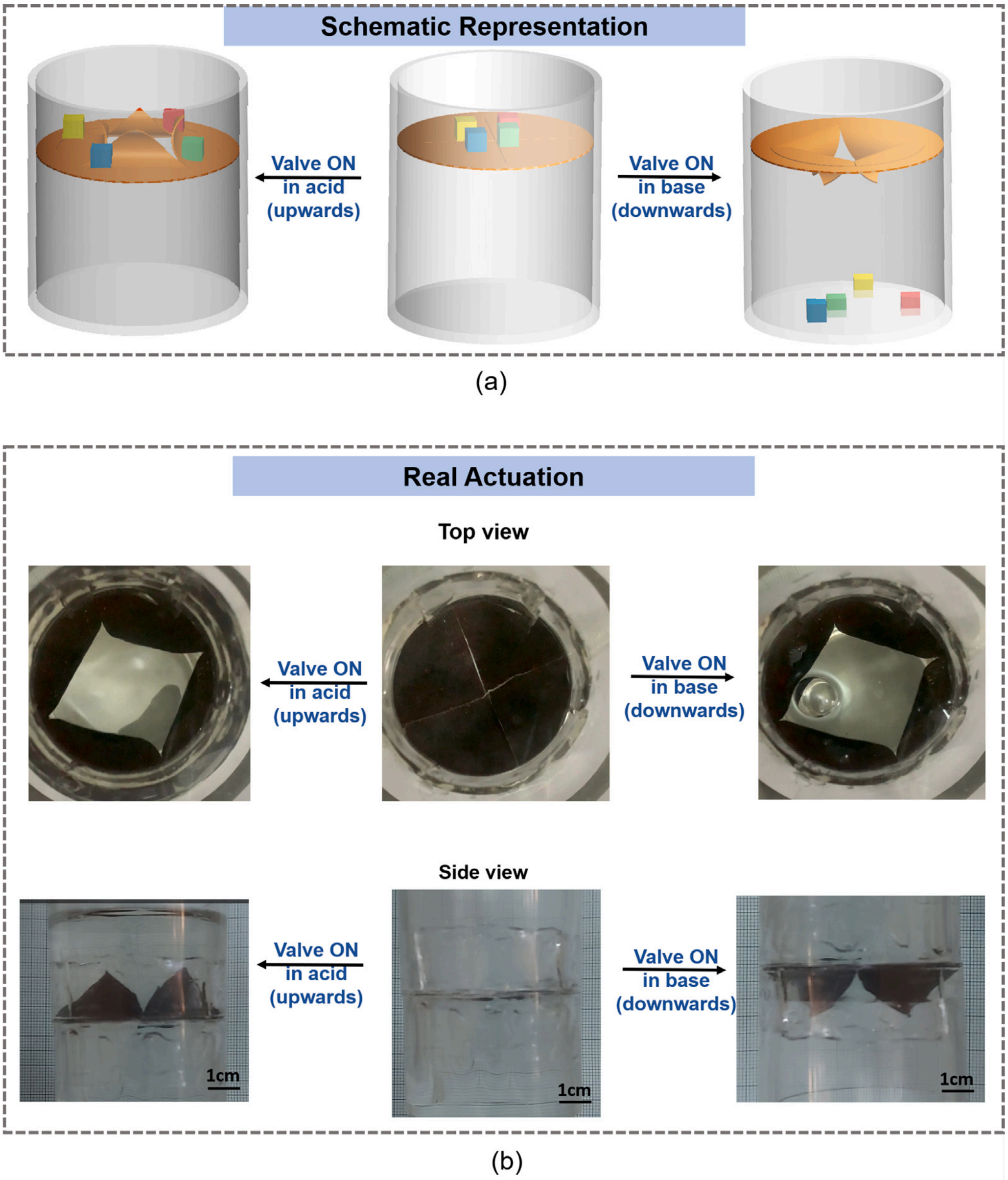


Fig. 9. An open and closed valve opens in one direction in acidic solvent and opens in the reverse direction in the alkaline condition and liquids/objects could pass through (a)schematic representation and (b)real-time experiment of a smart valve which allows and inhibits solid particles with opening and closing of valve upon exposure to solvents of different pH.

deformation upon flow discontinuation to retain elastic shape. This behaviour was described using two parameters: the storage (or elastic) modulus G' and the loss (or viscous) modulus G''. In Fig. 6(b), it was observed that the deformation occurred when the yield point (or yield stress) exceeded the flow point due to progressive breakage and disentanglement of molecular structure.

Thixotropy behaviour of hydrogel ink

The transition from deformation of fluid flow to elastic shape retention was quantified by studying the thixotropy behaviour of the hydrogel ink. The viscosity of the two inks was found to be significantly reduced at a high shear rate of 500 s^{-1}, indicating that the ink can be extruded into a continuous filament for printing. This is because the physical cross-links between the polymer chains broke due to the application of high stress during extrusion. The recovery in viscosity of hydrogels post-printing was observed when decreasing the shear rate to 0.1 s^{-1} and retaining it for 120 s (as shown in Fig. 6(c). This stage represents the ink's resting condition after being on the bed. The viscosity returned to their original values indicating that the prepared chitosan and CMC ink retained its shape after printing. This recovery was due to the ability of hydrogels to regenerate broken cross-links during rest, resulting in increased and recovered viscosity.

From the literature, it was found that 8% and 15% chitosan and CMC ink were the most suitable compositions for printing. Kumar et al. [7] used 20% and 60% cross-linking in chitosan and CMC hydrogel to obtain a non-morphing point at pH 7 to study the actuation in various pH solvents. Therefore, a composition of 8% CS- 20% CA and 15% CMC- 60% CA was chosen to study the printability and actuation behaviour for our further studies.

3.3.2. Optimization of printing parameters

The printability of any ink is influenced by pre-printing parameters (rheological and nozzle features), printing design, slicing, g-code parameters (e.g., pressure, temperature, and feed rate), non-g-code

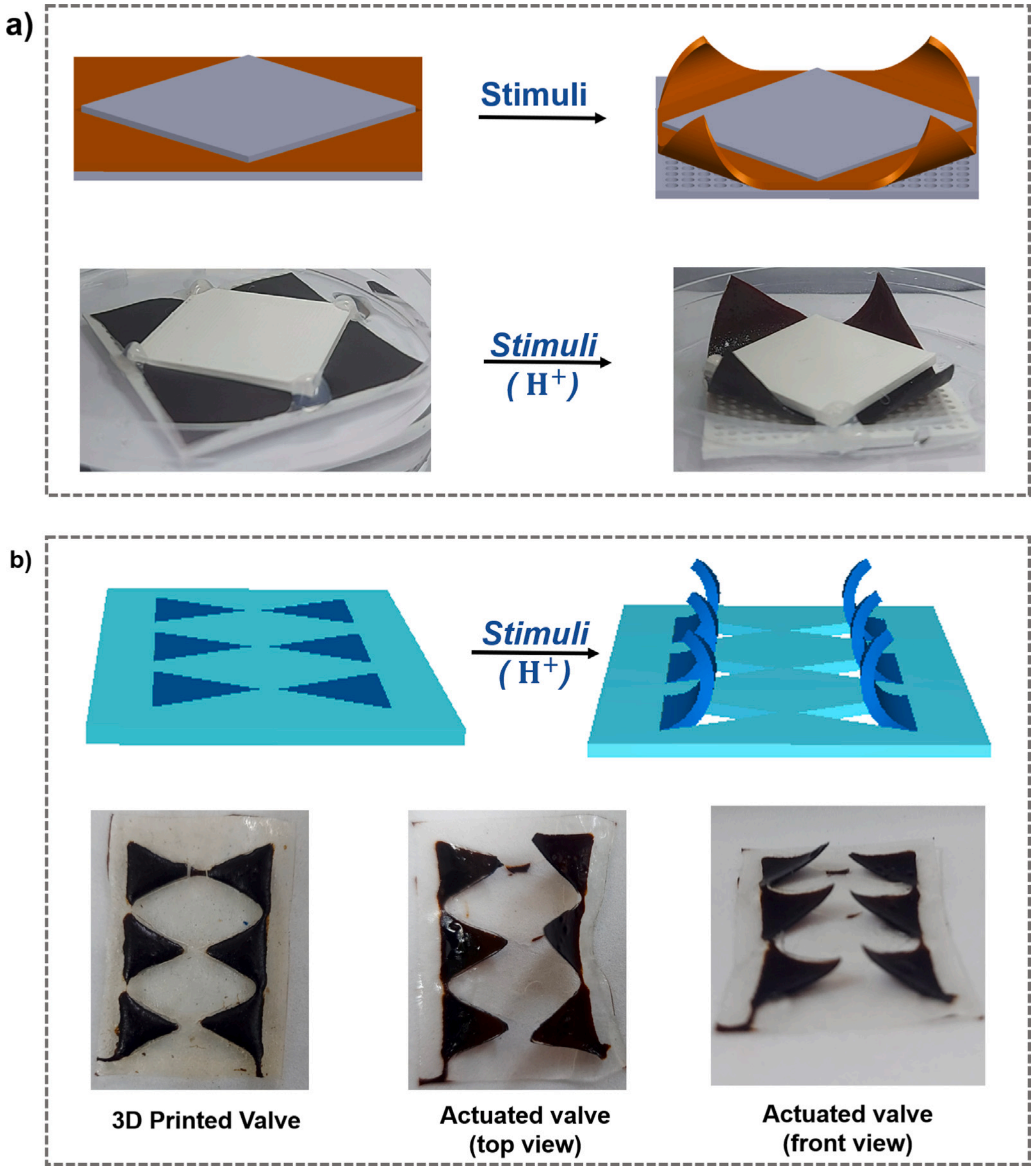


Fig. 10. Application demonstrations of the 3D printed bilayer hydrogel as smart/artificial valve in acidic environment.

parameters (e.g., environmental conditions), and post-printing parameters (e.g., cross-linking, coating, or drying techniques). Therefore, the printability of a hydrogel ink can not only be influenced by rheological parameters but also by considering geometrical and instrumental parameters [46].

During 3D printing of structures using a single hydrogel, good rheological properties of a single material and precise control of the printing parameters using a single micronozzle are necessary. But, for printing using a dual material system, there should be precise control of the printing parameters of the system.

The extrusion pressure directly affects the printed line width and layer thickness. The optimum conditions of print pressure to enable a continuous extrusion of structurally stable 3D structures on both the material system(CS and CMC) was chosen. A pressure of 5 bar for CS and 6 bar for CMC was selected based on the die swelling effect of the printable ink [6]. Correlating the printing pressure and printing speed with the layer thickness and line width were studied for both inks (as shown in Fig. 7). The optimal parameters were chosen based on multiple experiment trials, and the final structure was printed using those parameters (as listed in the table S1).

3.4. pH-responsive morphing and application of 3D printed structures

A printed structure with pH-responsive shape morphing abilities has broad applications in the biomedical field, like sensors, actuators, artificial valves, artificial muscles and tissue engineering, which require different shape-adaptive structures and folding patterns.

A simple to complex 3D deformation of printed structures was achieved using advanced programming of dual material systems in a DIW 3D printer. The deformation was used to design a hydrogel actuator that mimics the folding pattern of human fingers (Fig. 8(a)). This can be used as a potential application for drug delivery and capturing/transferring cells depending on the pH conditions of the environment. In addition, programmable complex deformations were also achieved by patterning the design of the pH-responsive layer in alternate positions (Fig. 8(b)). By adjusting the angle and placement of the patterned bilayer strips, the twist of the pH strip can also be controlled (Fig. 8(c)).

We demonstrated the smart valve actuation as an application, where different pH solvents can be used as stimuli. Recently, many researchers have studied smart/stimuli-responsive valves that are fabricated of stimuli-responsive materials and actuate in response to certain stimuli [47]. Stimuli-responsive hydrogels [47] and a few phase-changeable polymers [48] have been investigated as smart valves due to the complex physics and behaviour of the material. The hydrogel-based valve is of interest, as stimuli-responsive hydrogels have the ability to control the fluid flow in a system through different mechanisms. The smart valve design should aim to regulate the flow of fluid. Gargava et al. fabricated hydrogel-based valves inspired by the stomata in plants, which control the transport of water and gases in and out of leaves [49]. Yu et al. [50] demonstrated a biomimetic hydrogel valve with different pH sensitivities that becomes activated or deactivated reversibly as a result of volume and shape changes as a result of local pH. In comparison to traditional microfluidic valves, this hydrogel valve not only combines sensing and actuation capabilities but also requires fewer fabrication steps. The fabrication of these valves needs sophisticated technology due to the complexity of material fabrication and design aspects for the specific application to be considered.

A 3D printed bilayer pH-responsive hydrogel is proposed in the present work and can be used to fabricate a smart/artificial valve. Its actuation was demonstrated in extreme acidic and basic pH conditions, as shown in Fig. 9. A valve was designed and fabricated with a four-bilayer leaflet structure. This open and closed valve system was used to study the transfer of necessary objects (using dummy solid particles) into a fluid channel. When exposed to high pH, differential expansion in the bilayer of hydrogel causes the valve to bend during swelling, opening in a downward direction, thus allowing the particles to flow into the channel. At low pH, the valve actuates in the opposite direction, thus inhibiting the particles from flowing to the targeted channel.

Another valve was also designed with multiple parallel gating systems, which opened in either direction (as shown in Fig. 10) upon exposure to pH solvents. Smart hydrogel responds to many other stimuli, such as temperature [51], electric fields, ionic strength [7], light, carbohydrates, and protein. Soft material bi-strip valve designs can be difficult, but with the right technology, such as 4D printing, they can be made into useful components for chemical sensing and fluid regulation in microfluidic systems.

4. Conclusions

The development of chitosan/carboxymethyl cellulose bilayer structures through 4D printing presents an innovative pathway in the field of smart materials. We demonstrated a 4D printing strategy with bilayer structures from chitosan (CS)/carboxymethylcellulose (CMC) containing citric acid (CA) as a cross-linker with strong adhesion between both layers. These dual stimuli-responsive structures responded to both extreme conditions of pH (i.e., acidic and basic medium) and remained flat at neutral conditions. The present work aimed to systematically evaluate the rheological characterization and printing parameters of a bilayer structure. Specifically, the study examined the impact of programmable factors on the deformation shape. The printing structure, characterized by its diversity, underwent significant structural modifications in response to one or both pH conditions. By enabling programmable and reversible bidirectional bending, these structures hold significant potential for wide-ranging applications in smart valves, soft grippers, artificial muscles, and tissue engineering. As the domain of smart materials and manufacturing continues to evolve, further investigation into these kinds of structures could lead to even more exciting discoveries and advancements with multi-response systems. The current study is only limited to film-like structures (2D form) due to the low mechanical stability of the printed structures. However, advanced printing techniques can be utilized using this material system to fabricate 3D structures with more complex geometry, resulting in shape-adaptable 3D shapes, which is our next recent work. The cutting-edge field of smart hydrogels continues to promise a future where dynamic, adaptable, and responsive technologies become an integral part of our lives.

CRediT authorship contribution statement

Smruti Parimita: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Amit Kumar:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hariharan Krishnaswamy:** Writing – review & editing, Validation, Supervision. **Pijush Ghosh:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors 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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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eurpolymj.2024.113581.“Experimental procedures and characterization data for FTIR spectra, swelling tests, mechanical tests, and interfacial adhesion tests are available in the supplementary file”.

Data availability

Data will be made available on request.

References

[1] S. Tibbits, 4D printing: multi-material shape change, Architect. Des. 84 (1) (2014) 116–121.

[2] L. Chen, Y. Zhang, K. Zhang, F. Li, G. Duan, Y. Sun, X. Wei, X. Yang, F. Wang, C. Zhang, et al., Multi-stimuli responsive, shape deformation, and synergetic biomimetic actuator, Chem. Eng. J. 480 (2024) 148205.

[3] Y. Cheng, K. Ren, D. Yang, J. Wei, Bilayer-type fluorescence hydrogels with intelligent response serve as temperature/ph driven soft actuators, Sensors Actuators B 255 (2018) 3117–3126.

[4] L. Ren, Y. He, L. Ren, Z. Wang, X. Zhou, Q. Wu, K. Wang, B. Li, Q. Liu, Multi-parameter-encoded 4d printing of liquid crystal elastomers for programmable shape morphing behaviors, Addit. Manuf. 61 (2023) 103376.

[5] Y. Lin, J. Yu, Y. Zhang, U. Hayat, C. Liu, X. Huang, H. Lin, J.-Y. Wang, 4D printed tri-segment nerve conduit using zein gel as the ink for repair of rat sciatic nerve large defect, Biomater. Adv. 151 (2023) 213473.

[6] S. Parimita, A. Kumar, H. Krishnaswamy, P. Ghosh, Solvent triggered shape morphism of 4d printed hydrogels, J. Manuf. Process. 85 (2023) 875–884.

[7] A. Kumar, R. Rajamanickam, J. Hazra, N.R. Mahapatra, P. Ghosh, Engineering the nonmorphing point of actuation for controlled drug release by hydrogel bilayer across the ph spectrum, ACS Appl. Mater. Interfaces 14 (2022) 56321–56330.

[8] J. Kang, X. Yang, X. Yang, J. Sun, Y. Liu, S. Wang, W. Song, Carbon dots-enhanced ph-responsive lubricating hydrogel based on reversible dynamic covalent bondings, Chin. Chem. Lett. 35 (5) (2024) 109297.

[9] G. Liman, E. Mutluturk, G. Demirel, Light-and solvent-responsive bilayer hydrogel actuators with reversible bending behaviors, ACS Mater. Au (2024) http://dx.doi.org/10.1021/acsmaterialsau.4c00005.

[10] D. Park, J.W. Kim, C.O. Osuji, Programmable thermo-and light-responsive hydrogel actuator reinforced with bacterial cellulose, ACS Appl. Eng. Mater. 2 (3) (2024) 772–780.

[11] A. Saifi, C. Negi, K. Kumar, Visible light responsive soft actuator based on functional anthracene dye, Eur. Polym. J. 171 (2022) 111176.

[12] A. Saifi, C. Negi, A.P. Singh, K. Kumar, Visible light responsive soft actuator based on pva-dr1, ChemistrySelect 8 (39) (2023) e202301628.

[13] B. Liu, H. Li, F. Meng, Z. Xu, L. Hao, Y. Yao, H. Zhu, C. Wang, J. Wu, S. Bian, et al., 4D printed hydrogel scaffold with swelling-stiffening properties and programmable deformation for minimally invasive implantation, Nature Commun. 15 (1) (2024) 1587.

[14] L. Ren, B. Li, Q. Liu, L. Ren, Z. Song, X. Zhou, P. Gao, 4D printing dual stimuli-responsive bilayer structure toward multiple shape-shifting, Front. Mater. 8 (2021) 655160.

[15] G. Bond, A. Mahjoubnia, W. Zhao, S.D. King, S. y. Chen, J. Lin, 4D printing of biocompatible, hierarchically porous shape memory polymeric structures, Biomater. Adv. 153 (2023) 213575.

[16] J. Simińska-Stanny, M. Nizioł, P. Szymczyk-Ziółkowska, M. Brozyna, A. Junka, A. Shavandi, D. Podstawczyk, 4D printing of patterned multimaterial magnetic hydrogel actuators, Addit. Manuf. 49 (2022) 102506.

[17] T.Y. Koh, A. Sutradhar, Untethered selectively actuated microwave 4d printing through ferromagnetic pla, Addit. Manuf. 56 (2022) 102866.

[18] J.-K. Chen, C.-J. Chang, Fabrications and applications of stimulus-responsive polymer films and patterns on surfaces: A review, Materials 7 (2) (2014) 805–875.

[19] S. Mandal, A. Vignesh, S. Debnath, U. Ojha, Mechanically robust anisotropic hydrogel–organogel conjugates for soft actuators with fast response time and diverse bi-axial programmable folding ability, Chem. Mater. (2022).

[20] D. Cao, S. Malakooti, V.N. Kulkarni, Y. Ren, H. Lu, Nanoindentation measurement of core–skin interphase viscoelastic properties in a sandwich glass composite, Mech. Time-Dependent Mater. 25 (3) (2021) 353–363.

[21] M. Shojaeifard, S. Niroumandi, M. Baghani, pH-Responsive Hydrogel Bilayer With Reversible, Bidirectional Bending Behavior, Front. Mater. 9 (2022) 865652, http://dx.doi.org/10.3389/fmats.2022.865652.

[22] M. Nan, K. Guo, T. Jia, G. Wang, S. Liu, Novel acid-driven bioinspired self-resettable bilayer hydrogel actuator mimicking natural muscles, ACS Appl. Mater. Interfaces 16 (2024) 9224–9230.

[23] S. Jiang, L. Xia, Bioinspired high-performance bilayer, ph-responsive hydrogel with superior adhesive property, Polymers 14 (20) (2022) 4425.

[24] M. Shojaeifard, S. Niroumandi, M. Baghani, Ph-responsive hydrogel bilayer with reversible, bidirectional bending behavior, Front. Mater. 9 (2022) 865652.

[25] P. Gupta, K. Vermani, S. Garg, Hydrogels: from controlled release to ph-responsive drug delivery, Drug Discover. Today 7 (10) (2002) 569–579.

[26] M. Krogsgaard, M.A. Behrens, J.S. Pedersen, H. Birkedal, Self-healing mussel-inspired multi-ph-responsive hydrogels, Biomacromolecules 14 (2) (2013) 297–301.

[27] P. Techawanitchai, M. Ebara, N. Idota, T.-A. Asoh, A. Kikuchi, T. Aoyagi, Photo-switchable control of ph-responsive actuators via ph jump reaction, Soft Matter 8 (10) (2012) 2844–2851.

[28] J. Liu, Y.-S. Huang, Y. Liu, D. Zhang, K. Koynov, H.-J. Butt, S. Wu, Reconfiguring hydrogel assemblies using a photocontrolled metallopolymer adhesive for multiple customized functions, Nature Chem. (2024) 1–10.

[29] X. Li, X. Cai, Y. Gao, M.J. Serpe, Reversible bidirectional bending of hydrogel-based bilayer actuators, J. Mater. Chem. B 5 (15) (2017) 2804–2812.

[30] J. Shang, P. Theato, Smart composite hydrogel with ph-, ionic strength-and temperature-induced actuation, Soft Matter 14 (41) (2018) 8401–8407.

[31] J. Duan, X. Liang, K. Zhu, J. Guo, L. Zhang, Bilayer hydrogel actuators with tight interfacial adhesion fully constructed from natural polysaccharides, Soft Matter 13 (2) (2017) 345–354.

[32] J. Zhang, D. Ji, X. Yang, X. Zhou, Z. Yin, 4D printing of bilayer structures with programmable shape-shifting behavior, J. Mater. Sci. (2022) 1–15.

[33] M.C. Mulakkal, R.S. Trask, V.P. Ting, A.M. Seddon, Responsive cellulose-hydrogel composite ink for 4d printing, Mater. Des. 160 (2018) 108–118.

[34] T. Gu, H. Bi, H. Sun, J. Tang, Z. Ren, X. Zhou, M. Xu, Design and development of 4d-printed cellulose nanofibers reinforced shape memory polymer composites: Application for self-deforming plant bionic soft grippers, Addit. Manuf. 70 (2023) 103544.

[35] W. Zhao, C. Yue, L. Liu, Y. Liu, J. Leng, Research progress of shape memory polymer and 4d printing in biomedical application, Adv. Healthc. Mater. 12 (16) (2023) 2201975.

[36] X. Wang, H. Huang, H. Liu, F. Rehfeldt, X. Wang, K. Zhang, Multi-responsive bilayer hydrogel actuators with programmable and precisely tunable motions, Macromol. Chem. Phys. 220 (6) (2019) 1800562.

[37] R.K. Meena, S.D. Rapaka, R. Pratoori, R.K. Annabattula, P. Ghosh, An embedded interface regulates the underwater actuation of solvent-responsive soft grippers, Soft Matter 18 (2) (2022) 372–381.

[38] X. He, S. Wang, J. Zhou, D. Zhang, Y. Xue, X. Yang, L. Che, D. Li, S. Xiao, S. Liu, et al., Versatile and simple strategy for preparing bilayer hydrogels with janus characteristics, ACS Appl. Mater. Interfaces 14 (3) (2022) 4579–4587.

[39] J. Tiren, L. Tenerz, B. Hök, A batch-fabricated non-reverse valve with cantilever beam manufactured by micromachining of silicon, Sensors Actuators 18 (3–4) (1989) 389–396.

[40] W.S. Trimmer, Microrobots and micromechanical systems, Sensors Actuators 19 (3) (1989) 267–287.

[41] R. Goyal, S. Sahu, S. Mitra, R. Niranjan, R. Priyadarshini, R. Yadav, B. Lochab, Nanocellulose-reinforced 4d printed hydrogels: Thermoresponsive shape morphing and drug release, ACS Appl. Polymer Mater. 6 (2) (2024) 1348–1361.

[42] M. Rizwan, R. Yahya, A. Hassan, M. Yar, A.D. Azzahari, V. Selvanathan, F. Sonsudin, C.N. Abouloula, Ph sensitive hydrogels in drug delivery: Brief history, properties, swelling, and release mechanism, material selection and applications, Polymers 9 (4) (2017) 137.

[43] L. Diaz-Gomez, I. Gonzalez-Prada, R. Millan, A. Da Silva-Candal, A. Bugallo-Casal, F. Campos, A. Concheiro, C. Alvarez-Lorenzo, 3D printed carboxymethyl cellulose scaffolds for autologous growth factors delivery in wound healing, Carbohydr. Polymers 278 (2022) 118924.

[44] A. Brandley, R. Hollfelder, S. Nesaei, B. VanWie, N. Abu-Lail, B.A. Gozen, Direct-ink-writing of degradable carboxymethylcellulose, Procedia Manuf. 26 (2018) 993–1002.

[45] A. Schwab, R. Levato, M. D'Este, S. Piluso, D. Eglin, J. Malda, Printability and shape fidelity of bioinks in 3d bioprinting, Chem. Rev. 120 (19) (2020) 11028–11055.

[46] S. Bom, R. Ribeiro, H.M. Ribeiro, C. Santos, J. Marto, On the progress of hydrogel-based 3d printing: Correlating rheological properties with printing behaviour, Int. J. Pharm. (2022) 121506.

[47] A. Beck, F. Obst, D. Gruner, A. Voigt, P.J. Mehner, S. Gruenzner, R. Koerbitz, M.H. Shahadha, A. Kutscher, G. Paschew, et al., Fundamentals of hydrogel-based valves and chemofluidic transistors for lab-on-a-chip technology: A tutorial review, Adv. Mater. Technol. 8 (3) (2023) 2200417.

[48] R. Greiner, M. Allerdissen, A. Voigt, A. Richter, Fluidic microchemomechanical integrated circuits processing chemical information, Lab Chip 12 (23) (2012) 5034–5044.

[49] A. Gargava, C. Arya, S.R. Raghavan, Smart hydrogel-based valves inspired by the stomata in plants, ACS Appl. Mater. Interfaces 8 (28) (2016) 18430–18438.

[50] Q. Yu, J.M. Bauer, J.S. Moore, D.J. Beebe, Responsive biomimetic hydrogel valve for microfluidics, Appl. Phys. Lett. 78 (17) (2001) 2589–2591.

[51] L. Nie, J. Li, G. Lu, X. Wei, Y. Deng, S. Liu, S. Zhong, Q. Shi, R. Hou, Y. Sun, et al., Temperature responsive hydrogel for cells encapsulation based on graphene oxide reinforced poly (n-isopropylacrylamide) /hydroxyethyl-chitosan, Mater. Today Commun. 31 (2022) 103697.