Supporting Information

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

Smruti Parimita[a], Amit Kumar[b,c], Hariharan Krishnaswamy[a,e,*], Pijush Ghosh[c,d,*]

[a]*Manufacturing Engineering Section, Department of Mechanical Engineering, IIT Madras, Chennai-600036, India*
[b]*Department of Biotechnology, Indian Institute of Technology Madras, Chennai-600036, India*
[c]*Department of Applied Mechanics and Biomedical Engineering, Indian Institute of Technology Madras, Chennai-600036, India*
[d]*Centre for Soft and Biological Matter, Indian Institute of Technology Madras, Chennai-600036, India*
[e]*Additive Manufacturing Research Group, Indian Institute of Technology Madras, Chennai-600036, India*

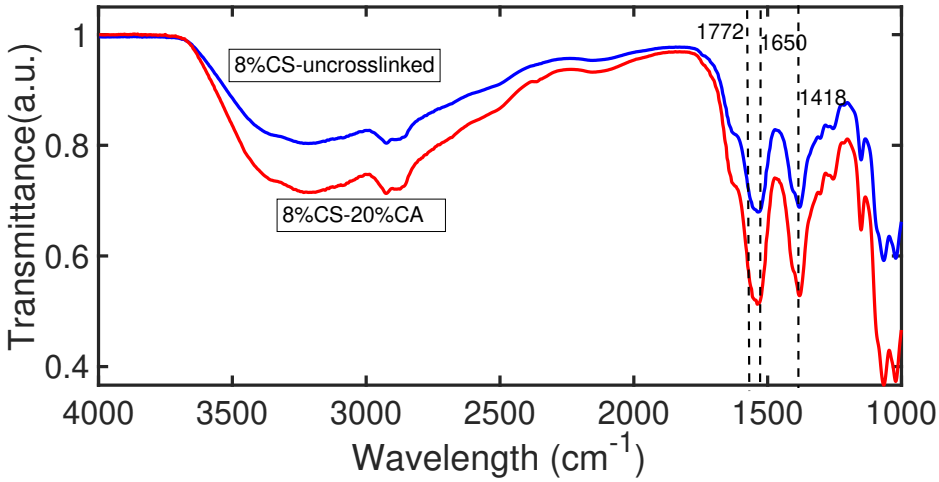
---

*Corresponding author
Email addresses: hariharan@iitm.ac.in (Hariharan Krishnaswamy), pijush@iitm.ac.in (Pijush Ghosh)

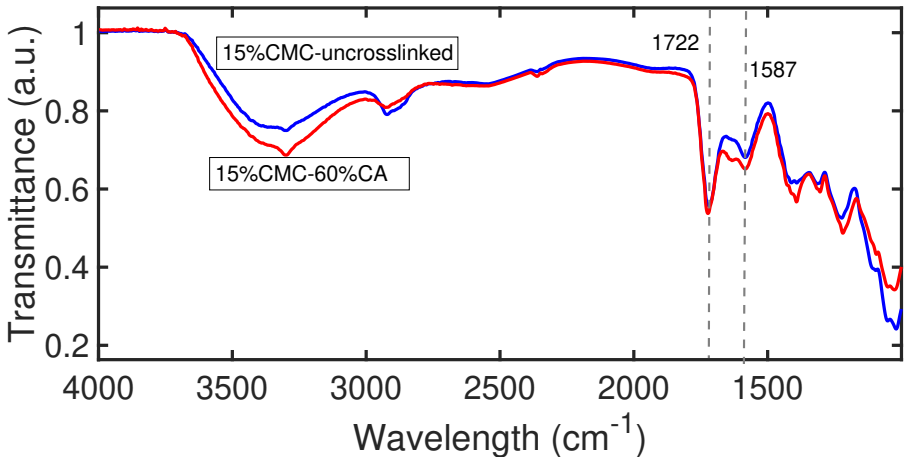
S1. Fourier Transform Infrared Spectrophotometer

The FTIR spectra were obtained using Bruker's Fourier transform infrared spectrophotometer for chemical structure elucidation before and after CA crosslinking of CS and CMC. 64 scans were collected in the range 800-4000 cm^{-1} with a resolution of 8 cm^{-1}. The IR spectra were analysed using lab solutions series software.

Fig.S1 shows the ATR-FTIR spectrum, which confirms the cross-linking between carboxyl groups of CA with the hydroxyl groups of both CS and CMC, thereby forming ester linkages. On the IR spectrum, peaks at 1722 cm^{-1} and 1650 cm^{-1} correspond to C=O and C-O-C stretching, respectively, confirming the ester bond formation, and a peak at 1587 cm^{-1} corresponds to -COO- was observed. The presence of a peak with negligible transmittance intensity corresponding to the amide group was observed at 1418 cm $^{-1}$. This might be due to the presence of some of the reacted primary amine groups with the carboxyl groups of CA to form these amide linkages. However, this does not affect the pH-responsive behaviour of the CS layer, which happens mainly due to the protonation of amine groups. The ATR-FTIR result implies that CA has been cross-linked with the CS and CMC molecules at 140°C for 10 min successfully.



(a)



(b)

Figure S1: FTIR spectra of 3D printed samples indicating the presence of ester linkages of (a)-OH of CS and and -COOH of CA, (b)-OH of CMC and and -COOH of CA

S2. Rheological characterisation

The amount of cross-linker was chosen based on the combined effect of all three factors, as discussed in section 3.1. The cross-linker will surely affect the swelling properties of the printed structures; however, it doesn't have a significant effect on the rheological properties of the ink (as shown in Fig. S2). Therefore, changing the ink composition will certainly not affect the 3D printability much as the rheological properties remain unaltered but will surely affect the actuation properties.

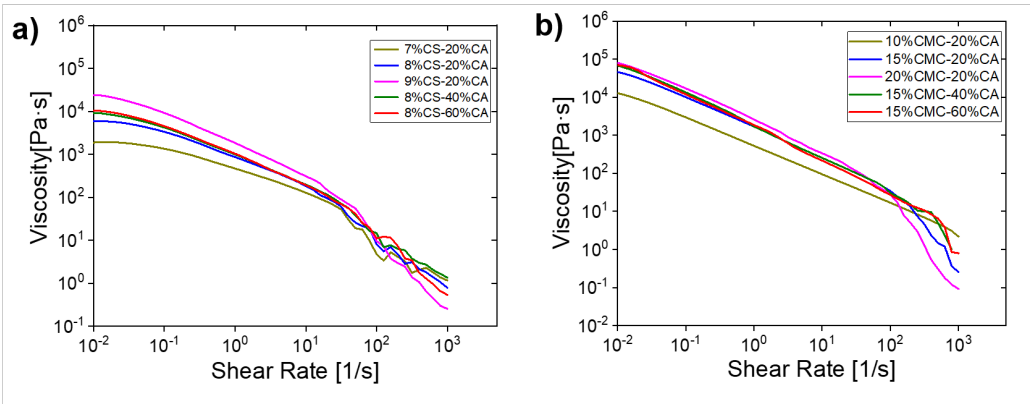


Figure S2: A printable chitosan and carboxymethyl cellulose hydrogel ink showing shear thinning ability a)different composition of CS ink with varying cross-linker content, b)different composition of CMC ink with varying cross-linker content

S3. pH-sensitive swelling behaviour of individual layers

The gravimetric method was utilised to measure the pH-dependent swelling behaviours of CS and CMC separate layers. Samples of CS and CMC of diameter 20 mm were 3D printed for the swelling measurements. Samples were immersed in specific pH-adjusted deionized water at room temperature. pH was adjusted using 0.1M NaOH or 0.1M HCl solutions. The weight of each sample was measured before (W_1) and after immersion (W_2) for 60 min at room temperature. The swelling degree was calculated by the following equation:

$$S\% = (W_2 - W_1)/W_1 * 100 \tag{1}$$

Measurements were taken in triplicate for each system.
Fig. S3 shows the swelling ratio of CS and CMC 3D printed films. The swelling ratios were checked after 60 min of swelling at different pHs. The swelling time could not be extended further as the film loses its properties and get completely soluble for some pH values.

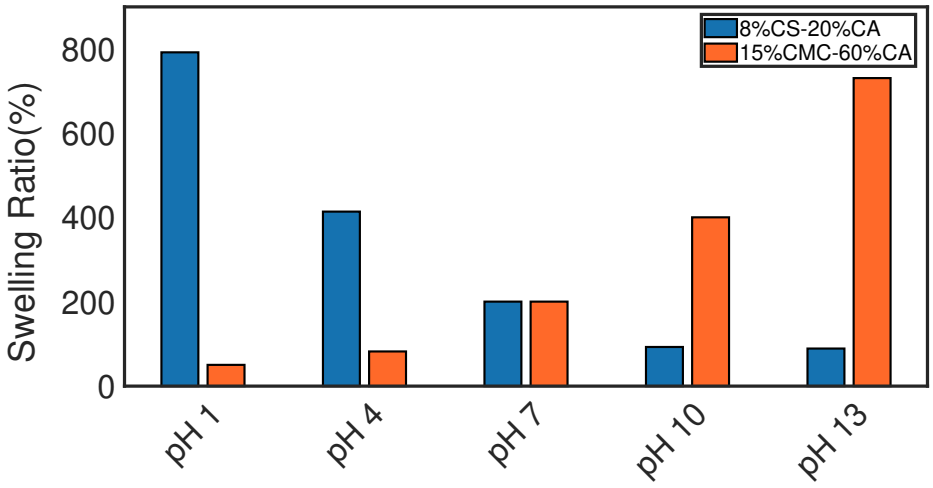


Figure S3: Dependence of the swelling ratio of CS and CMC hydrogel on the pH value

S4. Actuation Mechanism

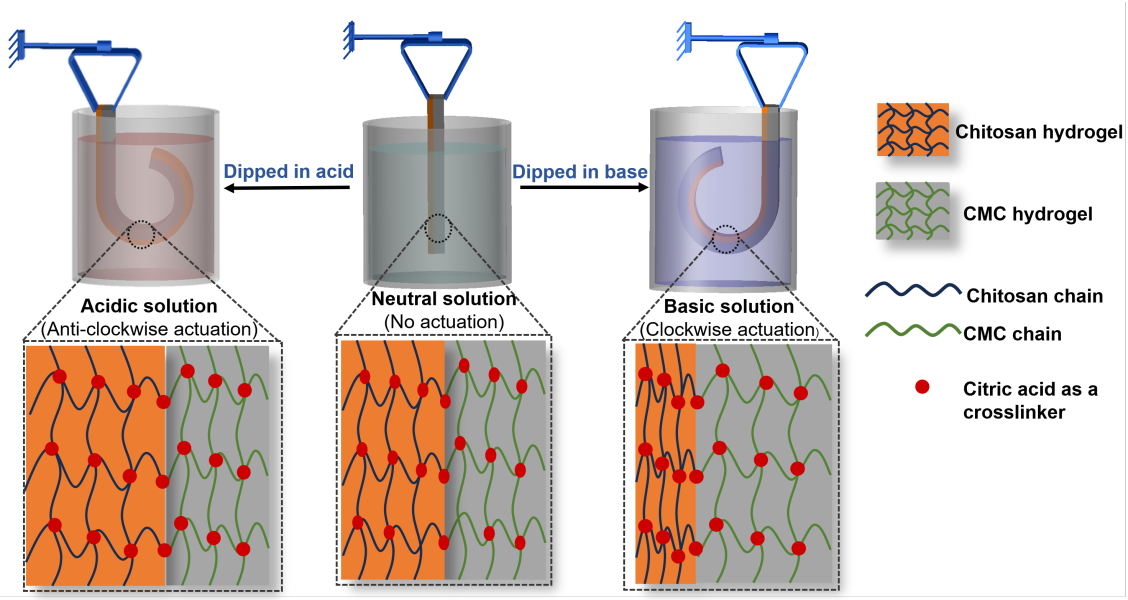


Figure S4: Schematic representation of actuation experiment in pH solvent showing the direction of actuation of 4D printed CS/CMC bilayer structure: the bilayer structure actuates in anticlockwise direction in low pH, does not actuate in neutral pH and actuates in clockwise direction in high pH

S5. Mechanical Tests

A Universal Material Testing Machine (1ST Tinius Olsen) equipped with a 1kN load cell was employed to examine the mechanical properties of dog-bone-shaped hydrogel specimens with a crosshead speed of 1 mm/min. All the experiments were repeated three times, and the average value was taken. The tensile strength of uncrosslinked 3D printed CS, CMC, and CS/CMC samples were 8.3MPa, 1.3MPa, and 18.6MPa, respectively. Furthermore, the tensile strength of the crosslinked 3D printed was found to be respectively 8.3MPa, 2.2MPa, and 10.4MPa for CS, CMC, and CS/CMC.

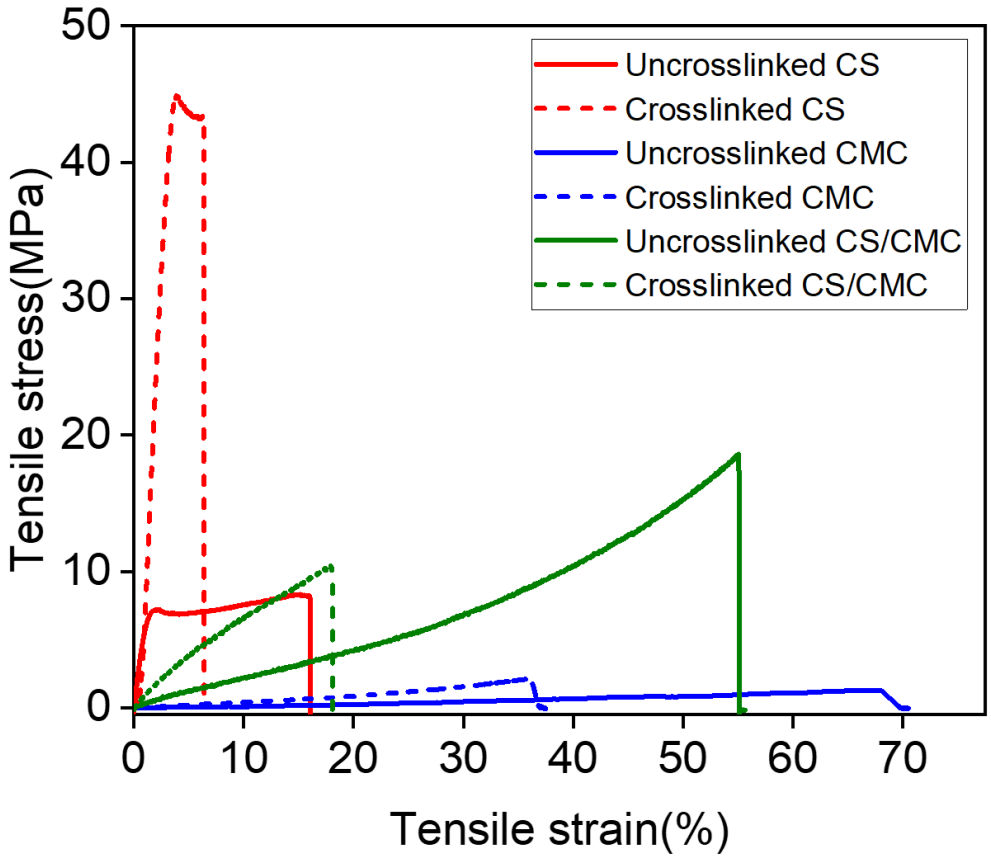


Figure S5: Stress-strain curves of 3D printed CS and CMC samples

S6. Adhesion Tests

Interfacial toughness between two hydrogel material systems was determined by T-peel tests (ASTM D 3359) using a Universal Material Testing Machine (1ST Tinius Olsen). First, a single layer CMC of 50mm x 50mm was printed on an acrylic substrate and was then allowed to partially dry for 6–8 hours. Then, a thin film of PMMA was placed on the CMC layer covering 10mm of the printed sample. A chitosan layer is printed on the uncovered and covered part of the printed CMC. The samples were dried and crosslinked, as explained in section 3.2. The PMMA film is removed, which creates an interface between the two adherends at one end. The samples were cut into 50mm x 10mm size. Double-sided tapes were applied to act as a stiff backing for the adhered hydrogels. The two open ends of length 10mm of the adherends were then gripped to two ends of the UTM and subjected to T-peel testing with a load cell of 1kN. All data were acquired at a stable peel rate of 1 mm/min, with test force plateauing when the peeling process approached a steady state. Interfacial toughness (Γ) was determined by dividing the plateau force, $F_{plateau}$ by the width of the sample, W.

$$\Gamma = \frac{F_{plateau}}{W} \tag{2}$$

It can be observed that the interfacial toughness was $71.814 J/m^2$ revealing a tough adhesion between the CS and CMC hydrogel, which is in good agreement with the literature[1].

S7. Optimised printing parameters

Hydrogel	Nozzle dia	Printing pressure (bar)	Printing speed (mm/sec)	Infill density
Chitosan	21G	5	5	100
Carboxymethyl cellulose	21G	6	5	100

Table 1: Optimised printing parameters for printing CS/CMC bilayer structure

References

[1] X. Ni, Z. Yang, J. Li, Scaling behavior of fracture properties of tough adhesive hydrogels, ACS Macro Letters 10 (2021) 180–185.