SUPPORTING INFORMATION

Vat 3D Printing of Full-Alginate Hydrogels via Thiol-Ene Reactions towards Tissue Engineering Applications

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Summary:

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References

1. Thiol-ene chemical crosslinking scheme



Figure S 1: Thiol-ene chemical crosslinking.

2. Thiolation synthetic conditions

Ring oxidation

Table S 1: Synthesis conditions of TOSA (*At = Activation time, T = Temperature, FA = Functional agent, Rt = Reaction time)

Product	Atm*	Oxidation				Reaction				
		Molar ratio	Rt	Т	рН	FA*	Molar ratio	Rt (h)*	Т	рН
TOSA	Air	SA: NaIO ₄ 5:1	2	3 RT	7.4	CYS	OSA:CYS 1:4	24	RT	7.4
		10:1 20:1	3			NaBH ₄	TOSA:NaBH4 1:1	15	RT	7.4

Carbodiimide chemistry

Table S 2: Synthesis conditions of SA-CSA and SA-CYS(*At = Activation time, T = Temperature, FA = Functional agent, Rt = Reaction time)

	Atm*	Activation a	Reaction							
Product		Molar ratio	At* (h)	Т	рН	FA*	Molar ratio	Rt* (h)	Т	рН
SA-CSA	N ₂	SA:EDC 1:4 SA:NHS 1:4	2	RT	4	CSA	SA:CSA 1:4	24	RT	4
SA-CYS	N ₂	SA:EDC 1:4 SA:NHS 1:4	2	RT	4	CYS	SA:CYS 1:4	24	RT	4

3. Alkene functionalization synthetic conditions

Table S 3: Synthesis conditions of SA-NOR (*At = Activation time, T = Temperature, FA = Functional agent, Rt = Reaction time)

Product	Atm*	Activation ag	Reaction							
		Molar ratio	At (h)*	Т	рН	FA*	Molar ratio	Rt (h)*	Т	рН
SA-NOR	Ar	SA:EDC 1:2.4 SA:NHS 1:0.8	2	RT	4	NOR	SA:NOR 1:1	24	RT	8.5

4. Potentiometric titration of aldehydes

The aldehydes content was evaluated by potentiometric titration. The analytical reaction is as follows:

Reaction

 $SA-(CHO)_n + H_2N-OH * HCl = SA-(CH=N-OH)_n + H_2O + HCl$ (1)

Where $SA-(CHO)_n$ is OSA and $H_2N-OH * HCl$ is the hydroxylamine hydrochloride reagent.

Titration

 $HCl + NaOH = NaCl + H_2O$

The aldehyde content was calculated by recording the amount of sodium hydrochloride consumed from the following equation:

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(2)

$$D0\% = \frac{mol CHO}{mol SA} * 100 = \frac{mol NaOH}{Wt/_{SA Mw}} * 100$$
(3)

Where:

Mol NaOH= moles of NaOH into volume of solution at inflection point

Wt= sample weight

SA Mw= molecular weight@ of SA repeating unit

Figure S2 A represents a typical plot of the pH variation over the volume of NaOH solution added in the OSA solution. The inflection points on this curve as well as the respective maximum on the first derivative reflect the necessary NaOH volume needed to neutralize the HCl in the solution (Figure S2 B), which allows the calculation of the aldehyde content into the alginate backbone using the formula (3) ^{1, 2}. Moreover, the red-to-yellow methyl orange change of color at 4.3 pH, clearly evident in Figure S2 C, can give a first indication of the inflection point ³.



Volume of 0,1 NaOH solution

Figure S 2: Potentiometric titration of OSA 1 (A) and its first derivative (B). The image (C) shows the color change of methyl orange during NaOH titration, compared with a control sample.

5. Thiol content evaluation (Ellman reagent titration)

The concentration of the thiol groups is obtained using the Lambert-Beer law:

A=ɛ·l·c

(4)

Where A is the difference in absorption between the blank and TOSA/SA-CSA/SA-CYS samples at 412 nm, ε is the molar attenuation coefficient (1562 M⁻¹ cm⁻¹ obtained by cysteine UV-VIS spectra calibration, Figure S3) and c is the pathlength (1 cm). Once obtained the concentration and the respective mol of thiol groups, the degree of substitution is obtained by:



Figure S 3: Ellman reagent reaction in presence of thiol groups (A), Calibration curve using cysteine (B).

6. SA-CSA, SA-CYS and SA-NOR characterization

The characterization of SA-CSA and SA-CYS has been performed by ATR-FTIR, ¹H-NMR and TGA measurements. In the ATR-FTIR spectra for SA and the thiolated alginates (SA-CSA and SA-CYS) (Figure S4), the broad absorption band centred at 3300 cm⁻¹ corresponds to O–

H stretching vibration. The intense bands observed at 1597 and 1400 cm⁻¹ for sodium alginate (SA) are due to stretching vibrations of COO- (asymmetric) and COO- (symmetric), respectively ⁴. After reaction with CYS or CSA, additional new absorption bands appear at 1734 cm⁻¹, 1662 cm⁻¹ (amide I), 1544cm⁻¹ (amide II) and 1240 cm⁻¹ (amide III)⁵ for both thiolated polymers. The peak at 1743 cm⁻¹ is assigned to COOH stretching vibrational band ⁶.



Figure S 4: ATR-FTIR of alginate (SA), SA-CSA and SA-CYS.

The SH stretch band typical of mercaptans could not be clearly distinguished in the infrared spectra, as it usually happens for thiolated alginates, but the presence of the cysteamine residue is clearly seen in its ¹H-NMR spectra: signals at 3.44 y 2.44 ppm corresponding to the methylene groups, as well as the triplet at 1.22 ppm corresponding to the SH proton (Figure S5) ⁷. The assignment of this signal to the –SH proton has been done with the help of a COSY experiment (Figure S6).



Similarly, ¹H-NMR spectrum confirms the modification with cysteine of SA-CYS ⁷ (Figure S7).



TGA thermograms for SA, SA-CSA and SA-CYS are shown in Figure S8.



Figure S 8: Thermogravimetric and first derivative plots of SA, SA-CYS and SA-CSA.

Sodium alginate degradation occurs in two steps in agreement with previous publication⁸, corresponding to multiple decomposition processes, with the maximum weight loss at 228° C. A second degradation step of the main chain at 619° C, and the total remaining ash of about 30% of the initial weight, are also concordant with alginate degradation. In the case of SA-CSA and SA-CYS, the first degradation step was shifted to lower temperature (around 200° C), and the complete degradation of the skeleton turned more complex also in agreement with similar modified alginates already described ⁹. Similar results are found for SA-CYS polymer.

Integrated ¹H-NMR spectrum for SA-NOR is shown in Figure S9. The assignment of the proton signals corresponding to norbornene double bond has been done with the help of a COSY experiment (Figure S10).



Figure S 9: ¹H-NMR spectra acquired for SA-NOR in D_2O .



7. Viscosity measurements



Figure S 11: Viscosity measurements on the thiol-ene formulations at different concentrations.

8. Printing parameters

Table S 4: Printing parameters of the TE hydrogel formulation.

	Light intensity (mW/cm²)	Layer thickness (µm)	Burn-in exposure time (s)	Layer exposure time (s)	
TE Hydrogel	35	50	8	4	

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