Supporting information

Injectable polyoxazoline grafted hyaluronic acid thermoresponsive hydrogel for biomedical applications.

Morgane Morel[a,b], Mathieu Madau [a], Virginie Dulong[a], Anne-Claire Groo[b], Aurélie Malzert-Fréon[b], Didier Le Cerf[a], Luc Picton[a*]

[a] Univ Rouen Normandie, CNRS, PBS UMR6270, F-76000 Rouen, France

[b] Univ Caen Normandie, CERMN, UR4258, F-14000 Caen, France

1. ^{1}H NMR characterization of HA-g-P(iPrOx-co-BuOx)

1H NMR analysis is used to monitor the evolution and grafting efficiency of the P(iPrOx-co-BuOx) copolymer on hyaluronic acid.

Firstly, an analysis is carried out at the end of the polymerization process (8 scans in $CDCl_3$ at 5g.L^{-1}) of the 2 oxazolines (2-n-isopropyl-2-oxazoline and n-butyl-2-oxazoline) to confirm whether the targeted DP and IPrOx/BuOx ratio have been achieved.

To produce the thermo-sensitive hydrogel, a polymerization degree of 30 and an iPrOx/BuOx ratio of 68/32 are targeted. The results of the 1H NMR polymerization are shown in Figure S1.

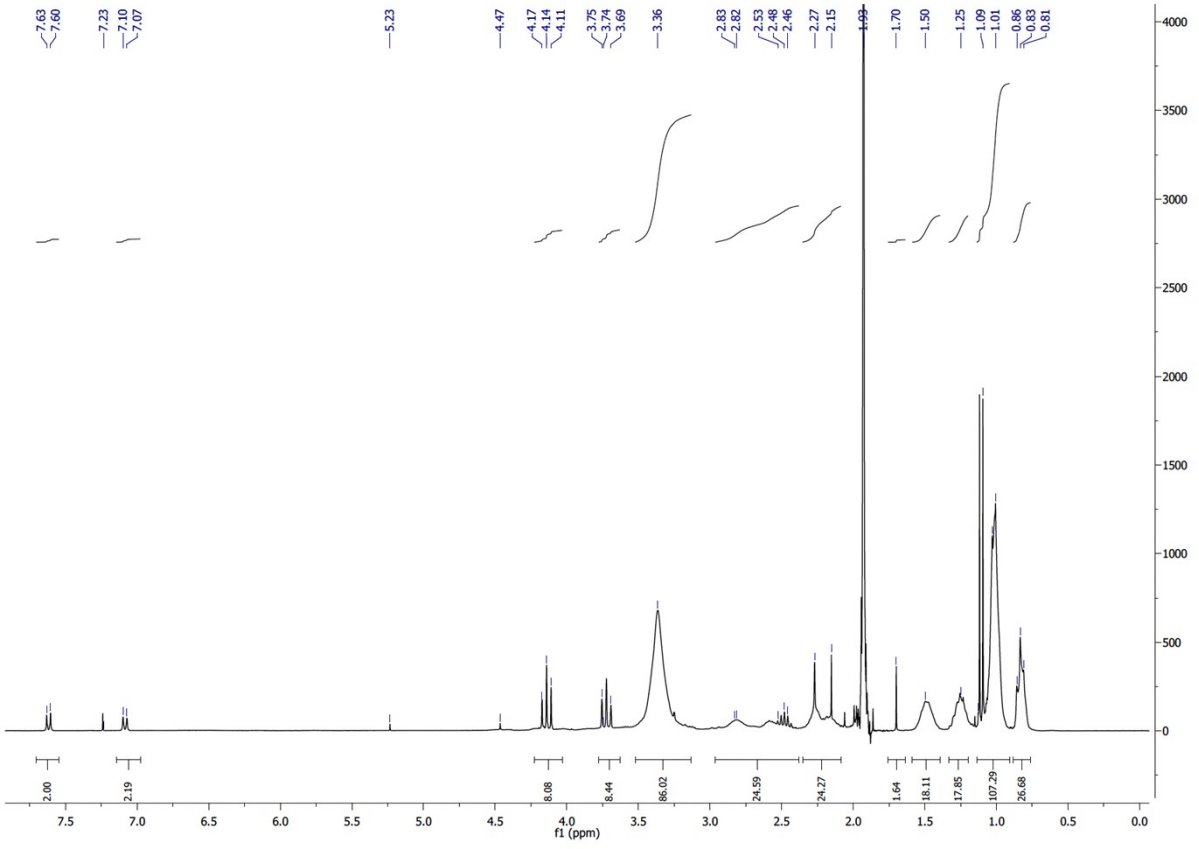


Figure S1 : 1H NMR spectrum of P(iPrOx-co-BuOx-67-33); DP= 21 in CDCl3 8 scans

Equations 1a, 1b, and 2 are used to calculate the IPrOx/BuOx ratio and DP.

$$\%\ IPrOx = \frac{\frac{I_{1.1ppm}}{6}}{\frac{I_{1.1ppm}}{6} + \frac{I_{0.83ppm}}{3}} \times 100 \quad \text{(Eq S1a)}$$

$$\%\ BuOx = \frac{\frac{I_{0.83ppm}}{3}}{\frac{I_{1.1ppm}}{6} + \frac{I_{0.83ppm}}{3}} \times 100 \quad \text{(Eq S1b)}$$

Where $I_{1.1}$ ppm is the pic integration of the 2 methyl groups from the isopropyl oxazolines after the polymerization and $I_{0.83}$ is the pic integration from the methyl group of the butyl oxazolines.

$$DP = \frac{I_{3.36\ ppm}}{4} \times \frac{2}{I_{7.60ppm}} \text{ (Eq S2)}$$

Where $I_{3.36}$ ppm is the pic integration of 2 methylene groups from the oxazoline backbone and where $I_{7.60}$ is the integration value of the 2 aromatic protons of the tosylate (initiator).
In a second step, a second ^{1}H NMR (64 scans in D_2O/NaOD-0.5% at 5g.L^{-1}) is performed on the final sample of HA-g-P(iPrOx-co-BuOx) after purification, in order to calculate the degree of substitution.

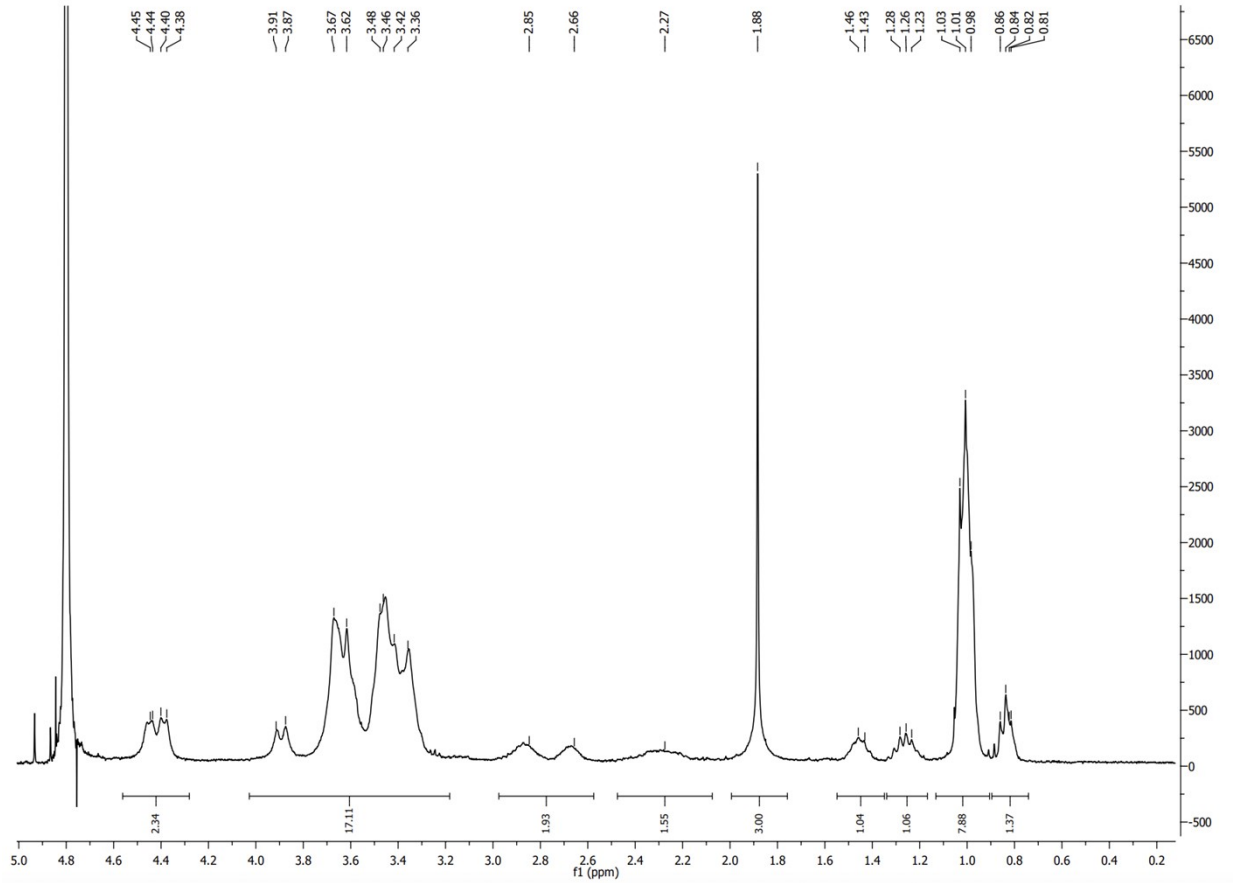
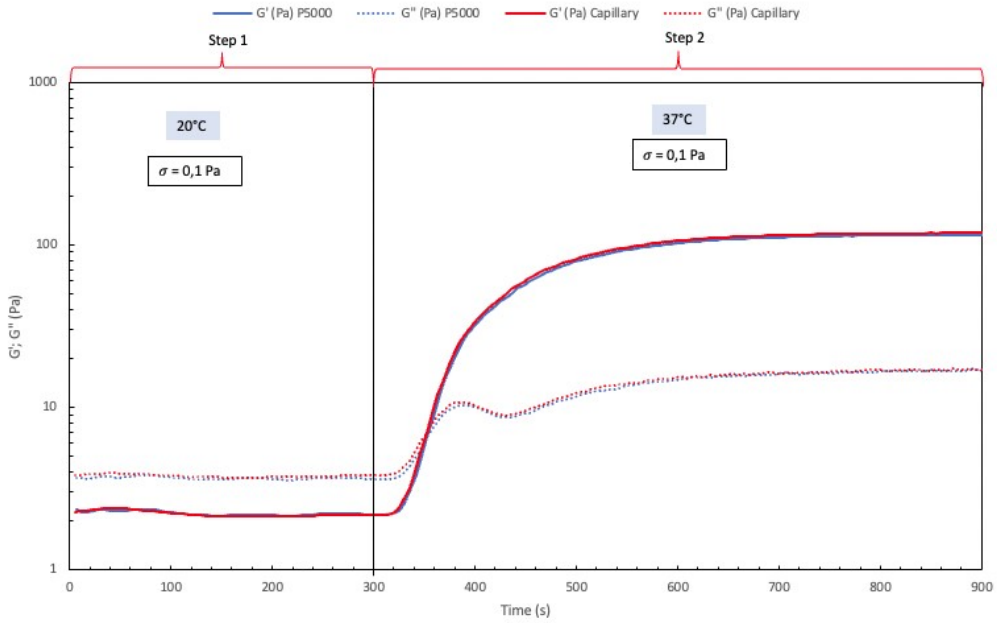
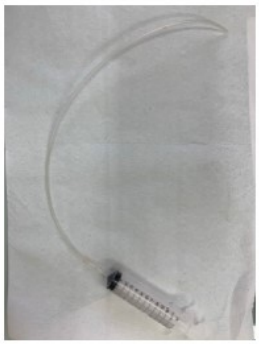


Figure S2 : ^{1}H NMR spectrum of HA-g-P(iPrOx-co-BuOx-67/33)-0,08 at 5g.L^{-1} in D2O (with 0.125M NaOD)

2. Hydrogel injectability (at 20°C)





Syringe used: 10mL
Capillary used: 30cm (length) et 1mm of diameter

Figure S3: Rheological in situ injection via syringe and capillary for HA-g-P(iPrOx-co-BuOx-66/34)-0.10 at 20°C to mimic the injection phenomenon (15g/L in NaCl 0.9%)

3. Incorporation of a secondary network (semi-interpenetrating network (IPN) (stability and injectability studies)

As with the hydrogel alone, hydrogel stability measurements were carried out. The results are shown in Figure S4.

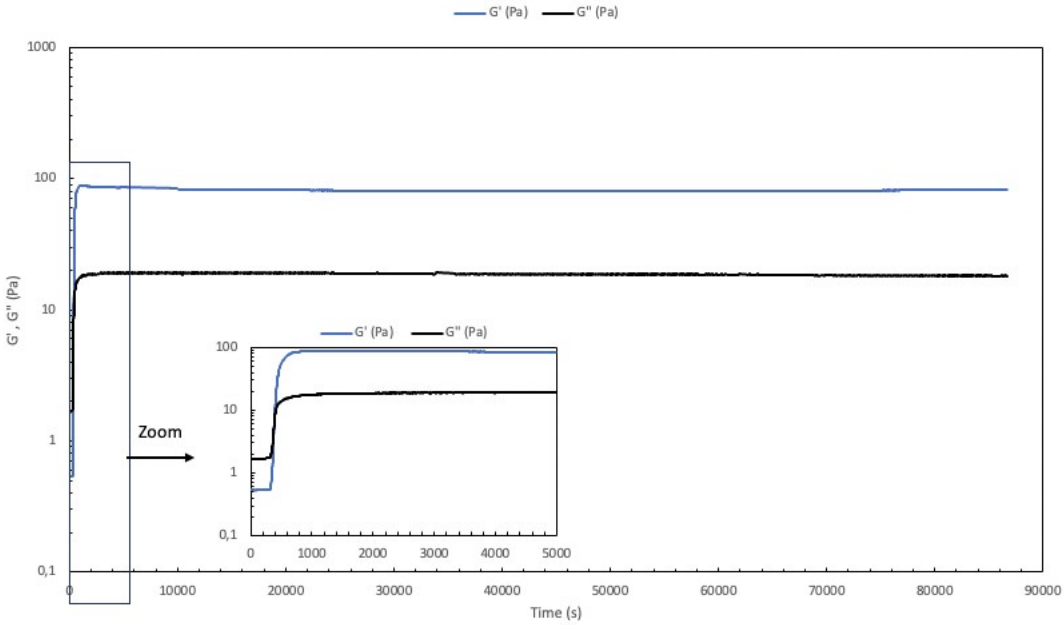


Figure S4: Stability studies of HA-g-P(iPrOx-co-BuOx-66/34)-0.10/native HA mixture (75/25 v/v) at 37°C (15g/L in NaCl 0.9%) for 24h (0.1Pa and 1Hz)

The results obtained are similar to those obtained for hydrogel alone. In fact, the mixture showed module stability at 37°C, over the 24-hour analysis period. So, the incorporation of a secondary network does not affect the stability of the hydrogel alone.

The hydrogel/HA mixture was also subjected to rheological injection simulation (as shown for the hydrogel alone). The same experiment (shown in Figures 5 and 6 of the article) was carried out; the results are below.

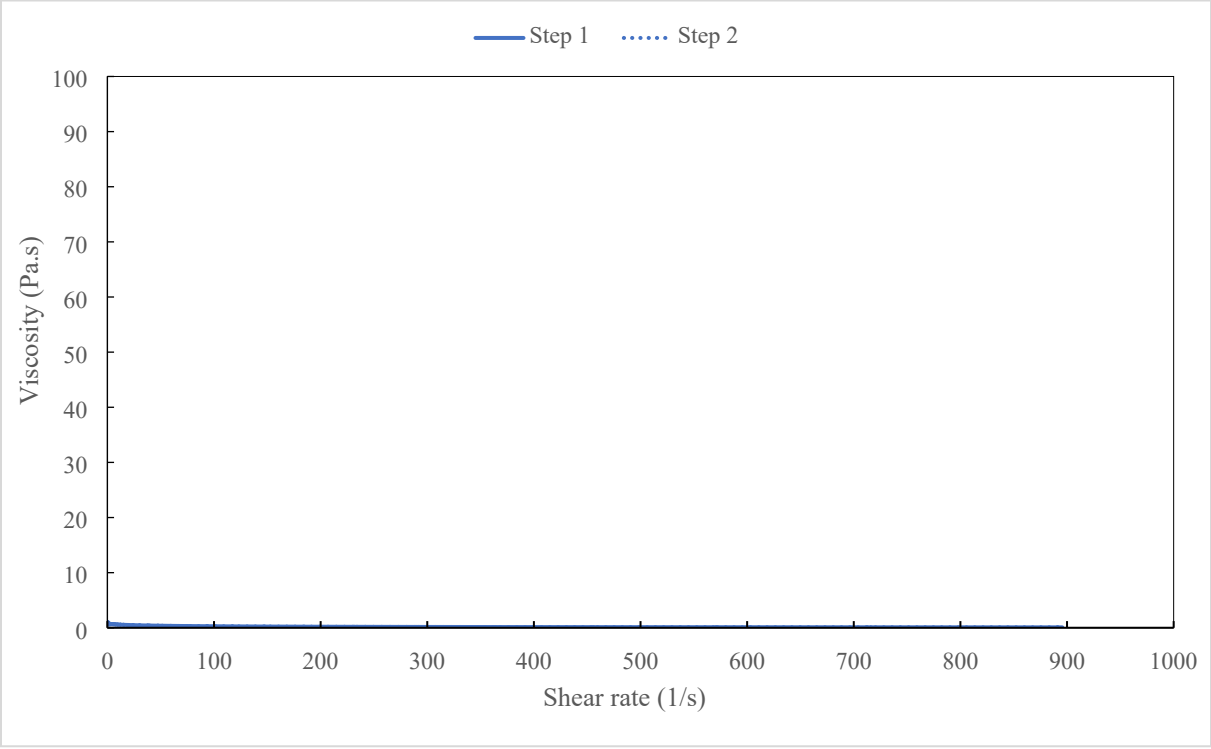


Figure S5: Flow curves for hydrogel/HA 75/25 mixture 15g.L-1 in NaCl 0.9% at 20°C

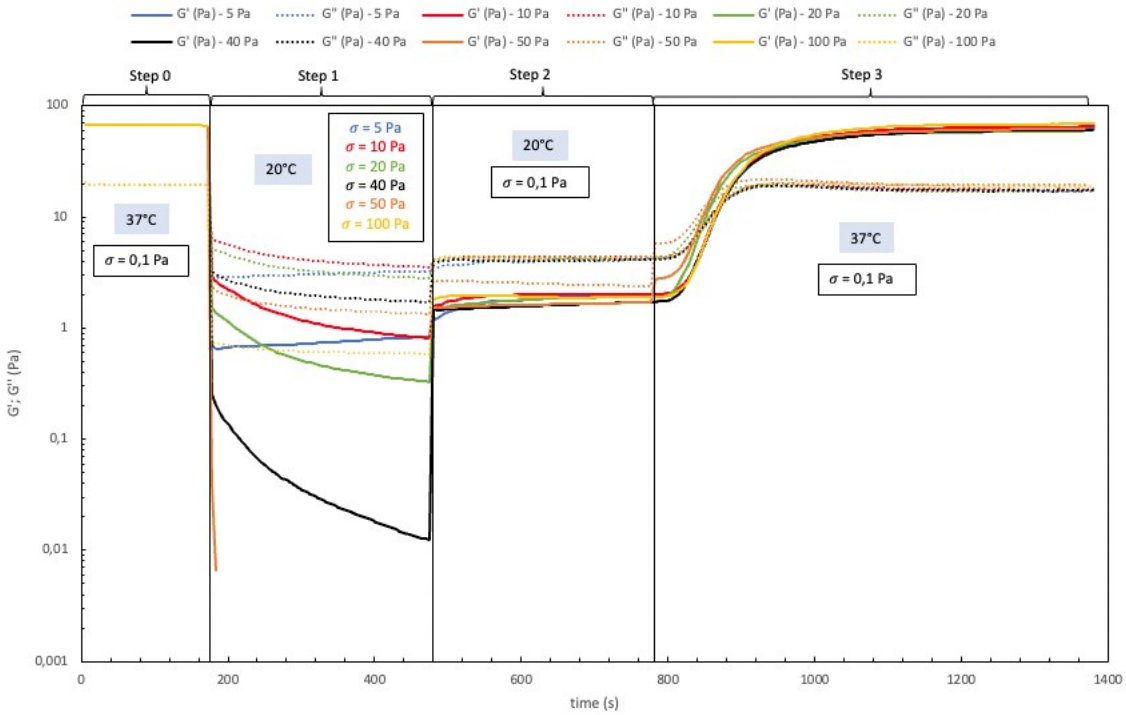


Figure S6: Injection simulation at different stresses of a hydrogel/HAsigma mixture (75/25) 15g/L in NaCl 0.9%

The results obtained for the injectability of the mixture are very similar to those obtained for the hydrogel alone. Indeed, at 20°C, the mixture flows at high shear rates, as shown for the hydrogel alone. In addition, the injection simulation clearly shows that when stress is applied at 20°C, the hydrogel/HA mixture will flow, and when the stress is stopped, the mixture regains its initial properties.