# Thermoresponsive properties of alginate-based graft copolymers

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## Introduction

Alginate is a natural polysaccharide which contains (1–4)-linked *β*-D-mannuronic acid (M) and *α*-L-guluronic acid (G) residues.[1] Due to its properties, such as biocompatibility and gelation, this biomaterial has various applications in biomedical science and engineering.[2] Alginate hydrogels retain a structural similarity to the extracellular matrices in tissues and as a result these gels have promising applications in drug delivery and tissue engineering.

The motivation of this work is the design and development of injectable hydrogels that behave as soft gels at room temperature and as strong ones at physiological temperature. For this reason, alginate-based graft copolymers were synthesized, using temperature responsive poly(N-isopropylacrylamide) (PNIPAM) side chains.[3] The enrichment of the PNIPAM chains with the hydrophobic comonomer N-tert-butylacrylamide (NtBAM) led to effective tuning of the sol-gel transition temperature and gel strengthening. Moreover, the shear- and thermo-responsiveness endowed the gel with injectability properties that render it a good candidate for cell transplantation applications, through injection strategies, which require a weak gel to protect the cells during injection and a stronger gel after injection to immobilize the created scaffold in the targeting position of the host tissue.[4]

Table 1: Characteristics of Alginate

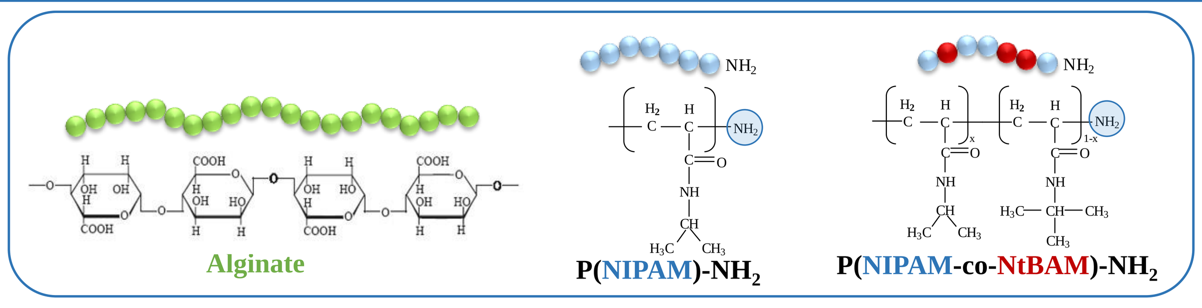
| Backbone | $\overline{M_v}$ (g/mol) | M/G ratio |
|---|---|---|
| Alginate | 140000 | 1.50 |

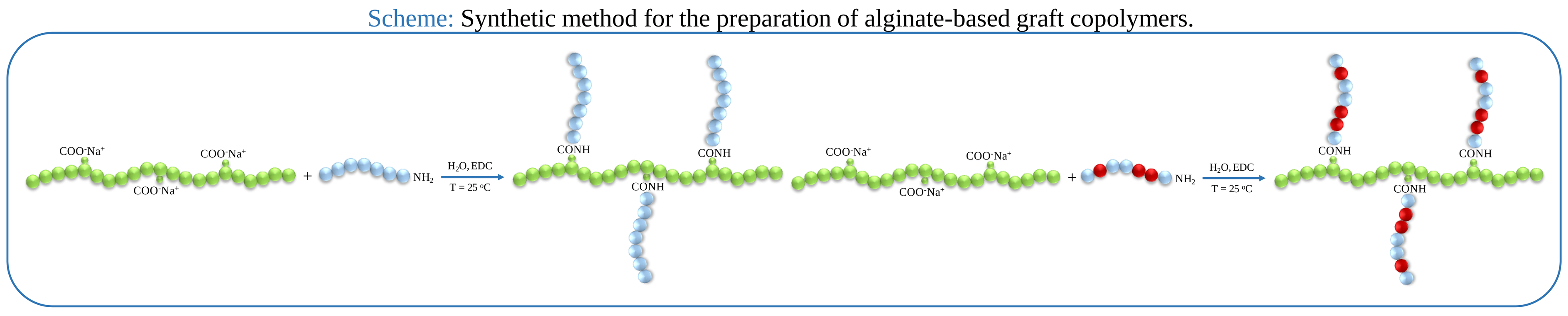
Table 2: Characterization of the side chains

| Side chains | $\overline{M_n}$ (g/mol) | mol composition NIPAM/NtBAM monomer (%mol) | Tcp (°C) |
|---|---|---|---|
| L-PNIPAM-$NH_2$ | 5500 | 100/0 | 32 |
| H-PNIPAM-$NH_2$ | 15300 | 100/0 | 32 |
| P($NIPAM_{90}$-co-$NtBAM_{10}$)-$NH_2$ | 12700 | 90/10 | 26 |
| P($NIPAM_{86}$-co-$NtBAM_{14}$)-$NH_2$ | 17000 | 86/14 | 23 |

*The numbers next to the monomers of the side chains denote the %mol composition found from $^1$H-NMR

**Materials**




Scheme: Synthetic method for the preparation of alginate-based graft copolymers.

Table 3: Graft copolymers characterization

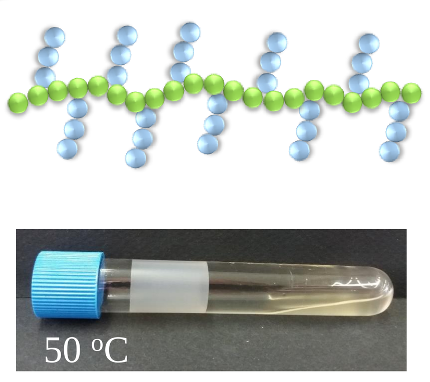
| Graft copolymers | mol composition NIPAM/NtBAM monomer (%mol) | weight composition Alg/side chain (%wt/wt) | mol composition Alg/side chain (%mol) | grafting density (number of side chains/alginate chain) |
|---|---|---|---|---|
| Alg-g-L-$P(NIPAM)_{10}$ | 100/0 | 71/29 | 59/41 | 10 |
| Alg-g-H-$P(NIPAM)_6$ | 100/0 | 62/38 | 48/52 | 6 |
| Alg-g-P($NIPAM_{90}$-co-$NtBAM_{10}$)$_{118}$ | 90/10 | 8/92 | 5/95 | 118 |
| Alg-g-P($NIPAM_{86}$-co-$NtBAM_{14}$)$_4$ | 86/14 | 69/31 | 56/44 | 4 |

*The numbers next to the monomers of the side chains denote the %mol composition found from $^1$H-NMR; the numbers outside the parenthesis of the side chains symbolize the number of side chains per alginate arm

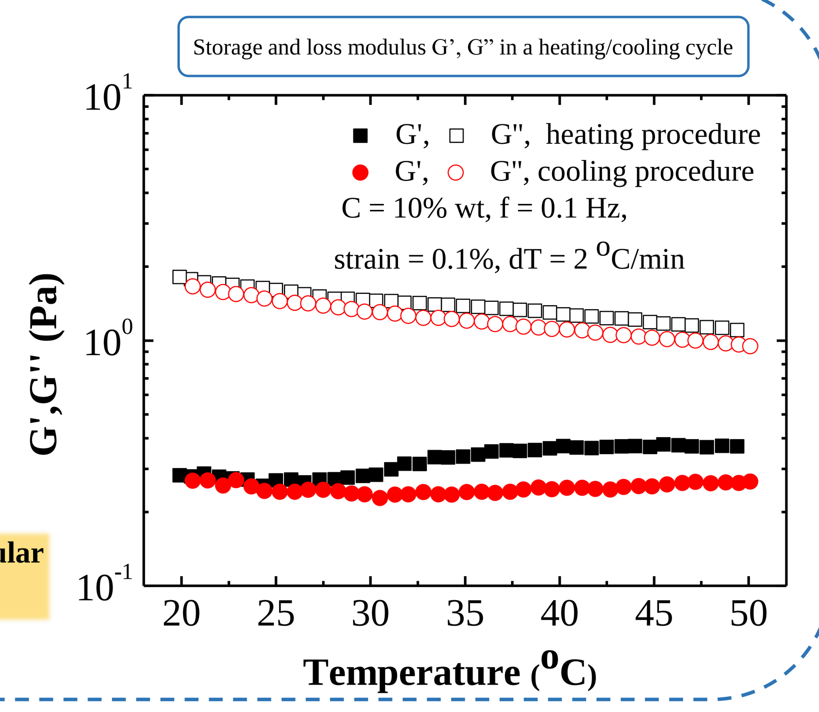
## Results

Effect of **low** and **high** molecular weight **PNIPAM-$NH_2$** side chains on gel formation

Alg-g-L-$P(NIPAM)_{10}$



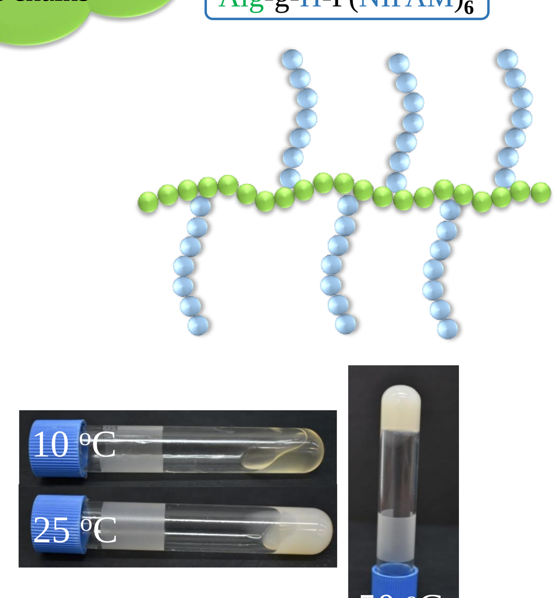

Storage and loss modulus G', G'' in a heating/cooling cycle



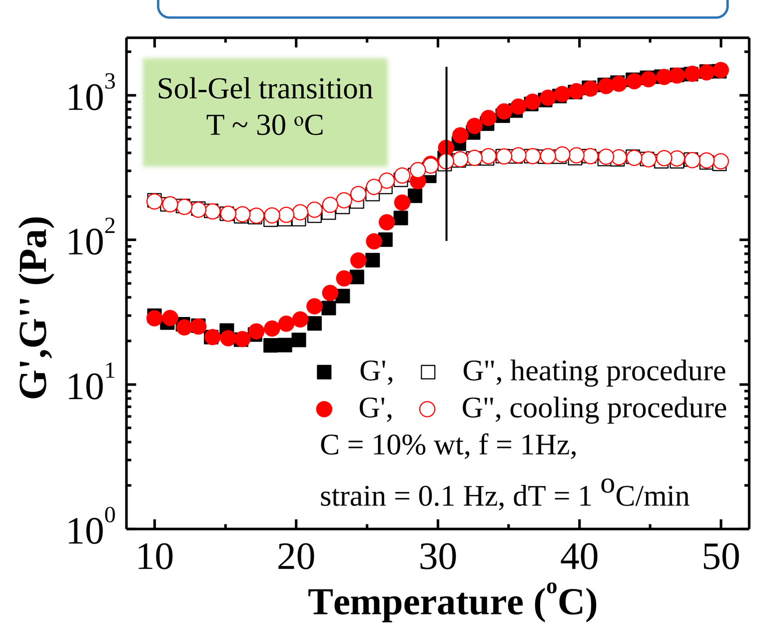

**No gel formation** with **low molecular weight** thermoresponsive side chains

Successful **gel formation** by **increasing the molecular weight** of the side chains

Alg-g-H-$P(NIPAM)_6$

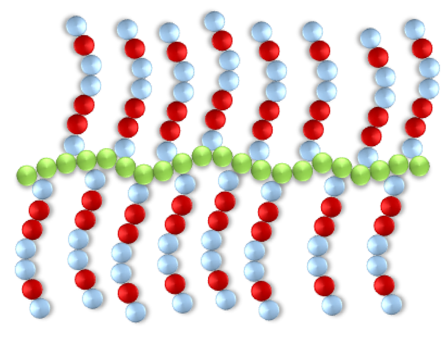



Storage and loss modulus G', G'' in a heating/cooling cycle

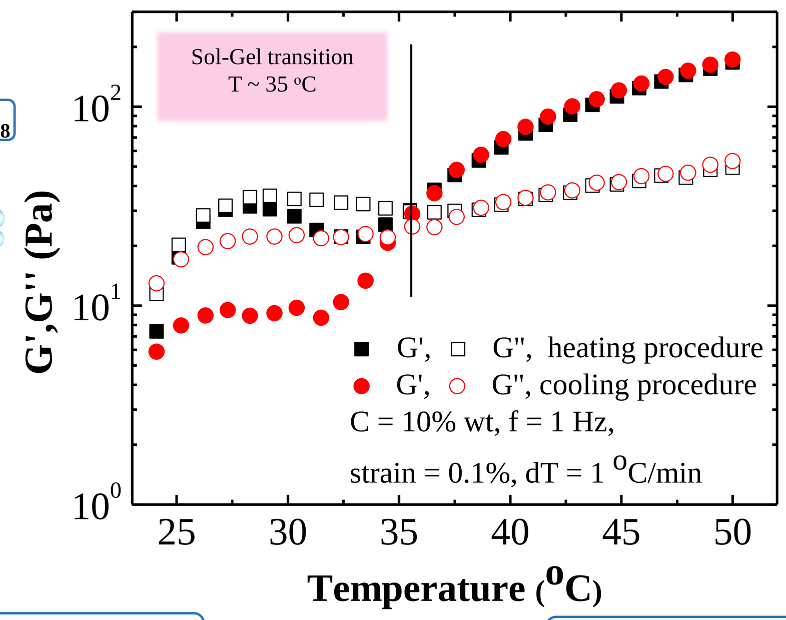



Enrichment of the thermoresponsive side chain with **hydrophobic** comonomer NtBAM

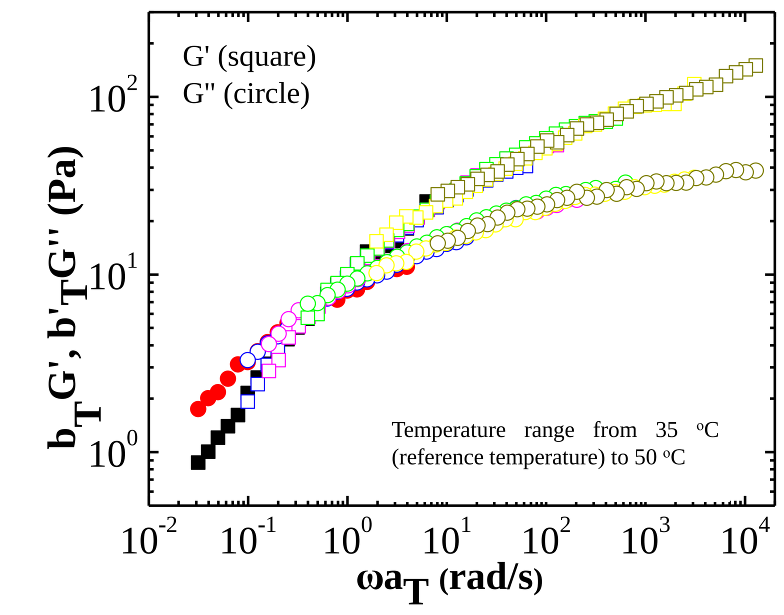
Alg-g-P($NIPAM_{90}$-co-$NtBAM_{10}$)$_{118}$



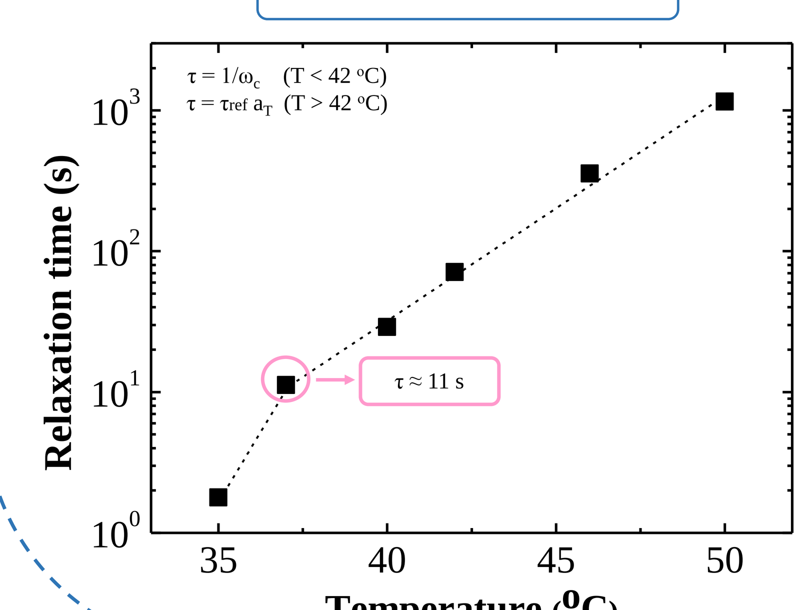
Storage and loss modulus G', G'' in a heating/cooling cycle



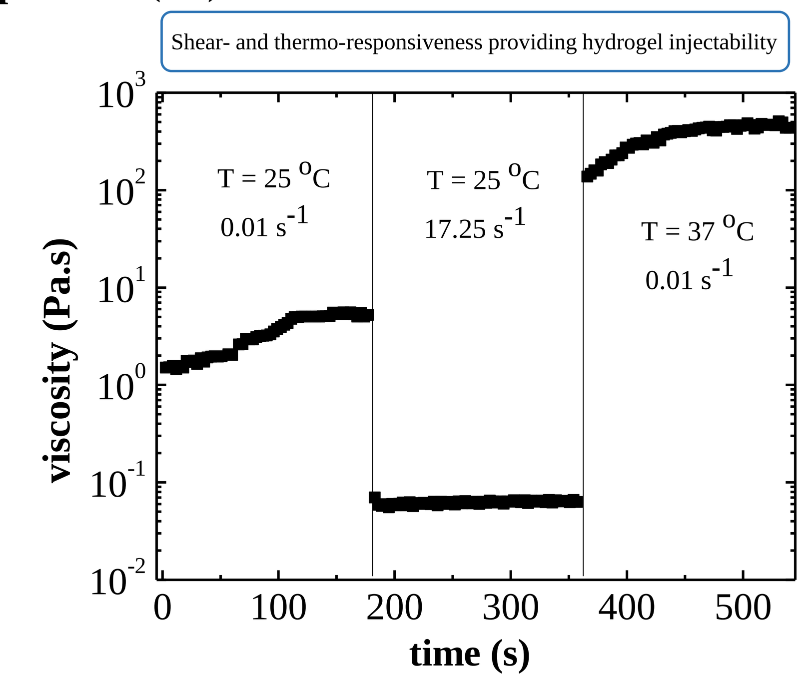

Time-Temperature superposition master curve

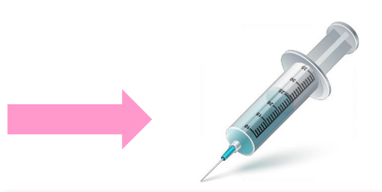



Temperature dependence of relaxation time

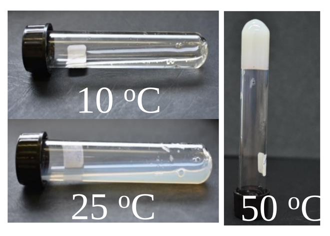



Shear- and thermo-responsiveness providing hydrogel injectability



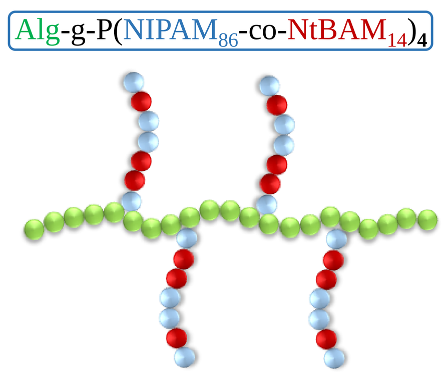



Suitable conditions for hydrogel injection through a 28-gauge syringe needle

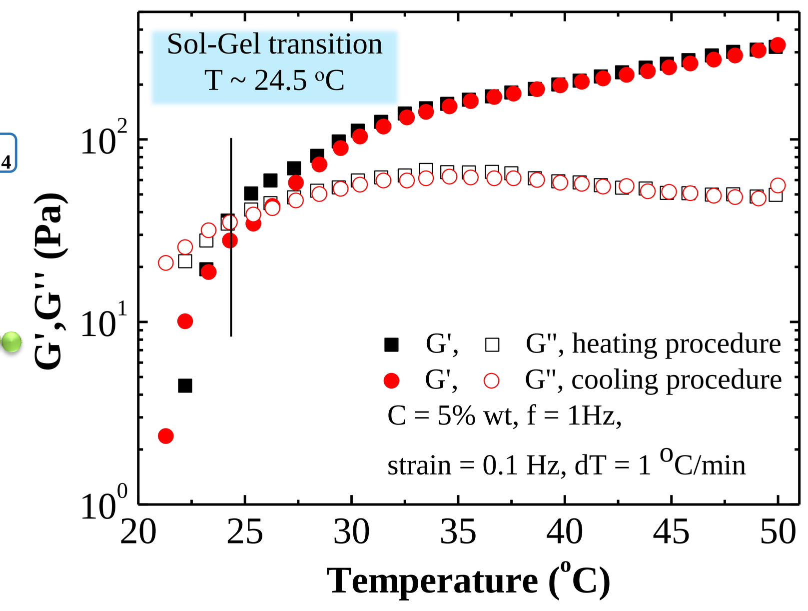



**Improvement** of the rheological properties by **increasing** the **hydrophobic ratio**

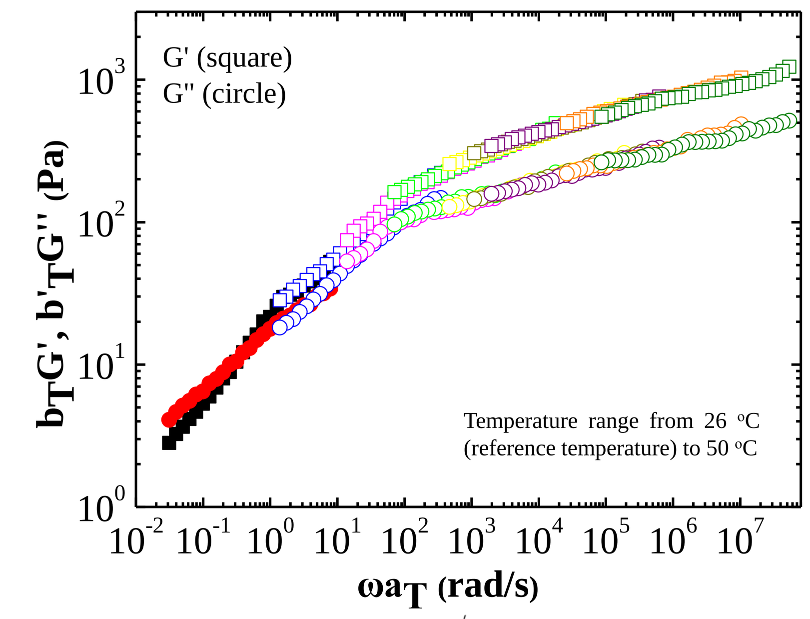
Alg-g-P($NIPAM_{86}$-co-$NtBAM_{14}$)$_4$



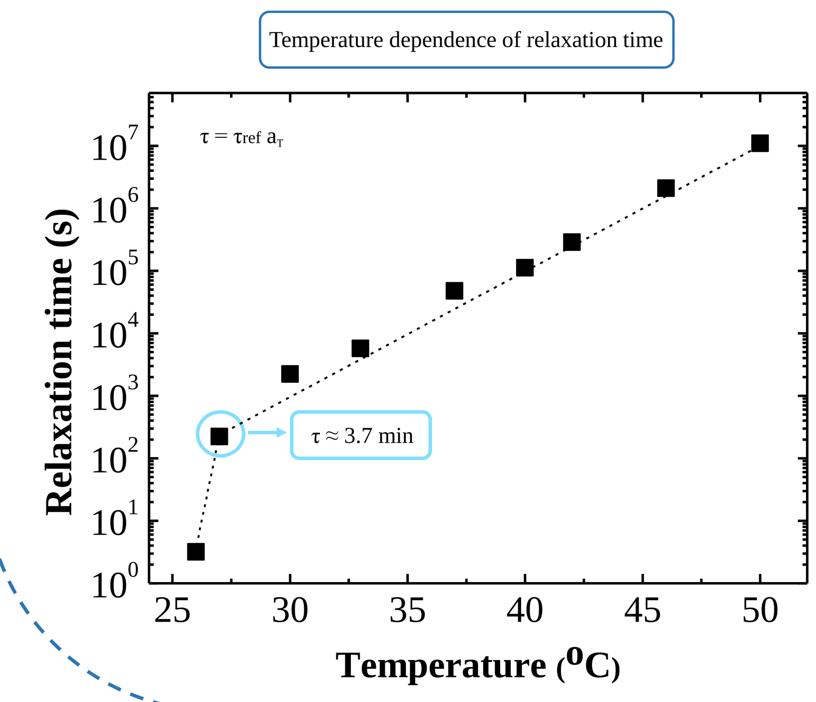
Storage and loss modulus G', G'' in a heating/cooling cycle



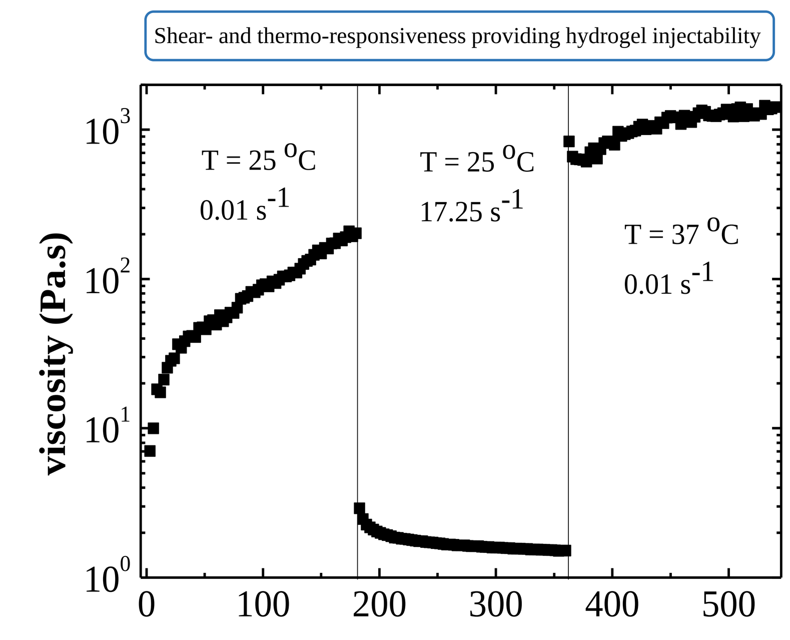

Time-Temperature superposition master curve

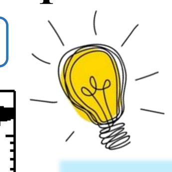



Temperature dependence of relaxation time

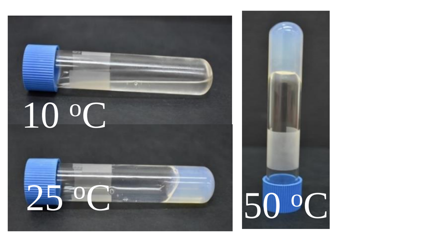



Shear- and thermo-responsiveness providing hydrogel injectability



Biodegradable, injectable hydrogel suitable for stem cells protected transfer to specific tissues




## Conclusions

- **The thermo-responsiveness and rheological properties of the alginate-based gelators depend on the grafting density, along with the length and the hydrophobic content of the grafted chains. Thus, the gelator properties can be designed at will by chemistry.**
- **The presence of hydrophobic moieties along the LCST-type thermoresponsive PNIPAM side chains affects the critical gelation temperature and the exchange dynamics of the stickers.**
- **The alginate-based gelators, grafted with P(NIPAM-co-NtBAM), exhibit thermo and shear-induced injectability. This combined property endows the formed hydrogel with a thermo-induced dynamic to "frozen" hydrogel transition. These kinds of hydrogels are suitable for stem cell transplantation through injection strategies.**
- **The ALG-g-P($NIPAM_{86}$-co-$NtBAM_{14}$)$_4$ hydrogel meets the requirements of a biodegradable injectable hydrogel for stem cell protected transfer, potentially suitable for regenerative medicine therapies.**

## References

[1] H. Guo, M. M. Goncalves, G. Ducouret, D. Hourdet, *Biomacromolecules* **2018**, *19*, 576-587.
[2] J. Sun, H. Tan, *Prog. Polym. Sci.* **2012,** *37*, 106-126.
[3] M.M.S. Lencina, Z. Iatridi, M.A. Villar, C. Tsitsilianis, *Europ. Polym. J.* **2014**, *61*, 33-44.
[4] C. Tsitsilianis, G. Serras, C.H. Ko, F. Jung, C.M. Papadakis, M. Rikkou-Kalourkoti, C.S. Patrickios, R. Schweins, C. Chassenieux, *Macromolecules* **2018**, *51*, 2169–2179.

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