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Exploiting the tools of NMR spectroscopy for the analysis of derivatized biopolymers

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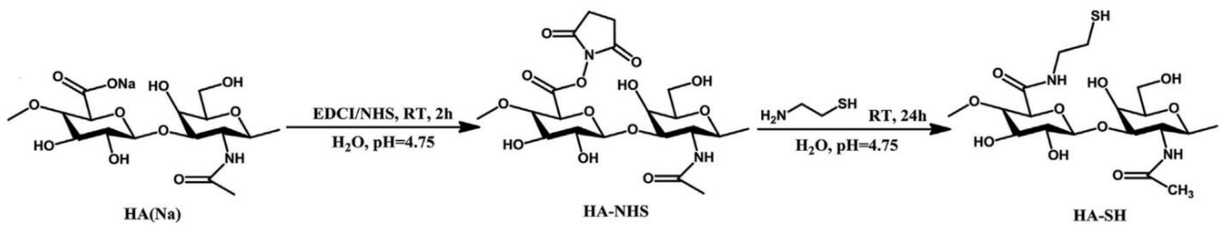
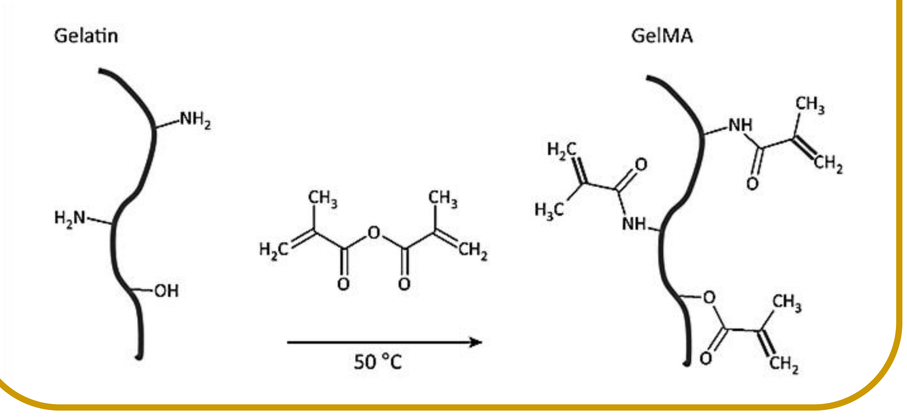
Introduction

Modeling the distinctive features of the central nervous system *in vitro*, in terms of cellular and extracellular composition and organization, is essential to effectively reproduce neural tissue architecture and functions towards the understanding of biological events occurring after injuries hampering the recovery of its physiological functions. Natural-based soft hydrogels have been widely employed to develop 3D neural constructs, recreating artificial environments for neural cell growth and maturation [1-4].
In 3DBIOSAME project, Hyaluronic Acid (HA) and Gelatin were derivatized to obtain thiolated hyaluronic acid (HA-SH) and gelatin methacrylate (GelMA). Both HA-SH and GelMA were characterized by using NMR spectroscopy (700 MHz and 80 MHz).

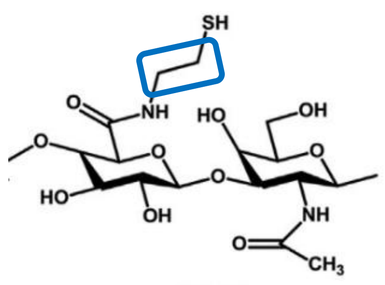
Synthesis of HA-SA



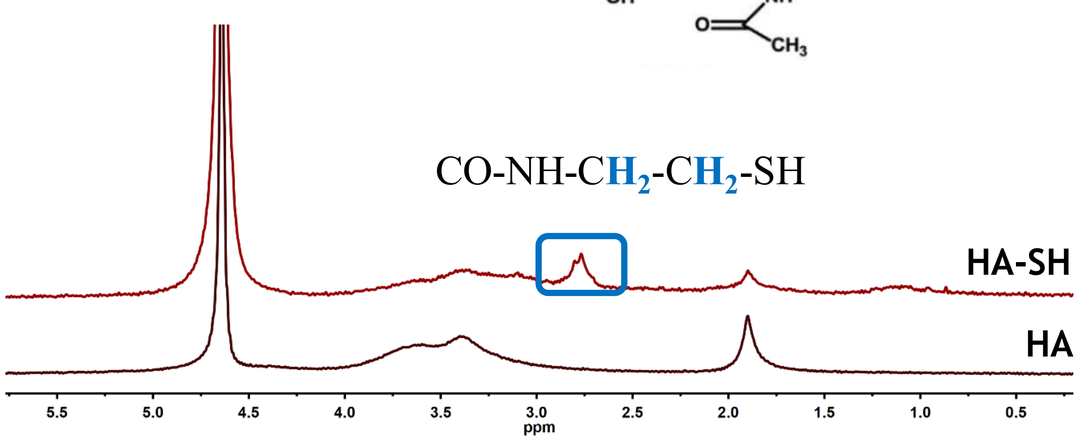
Synthesis of GelMA



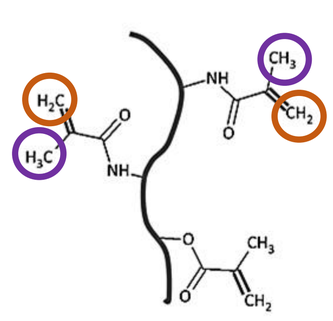
NMR analysis: 80 MHz

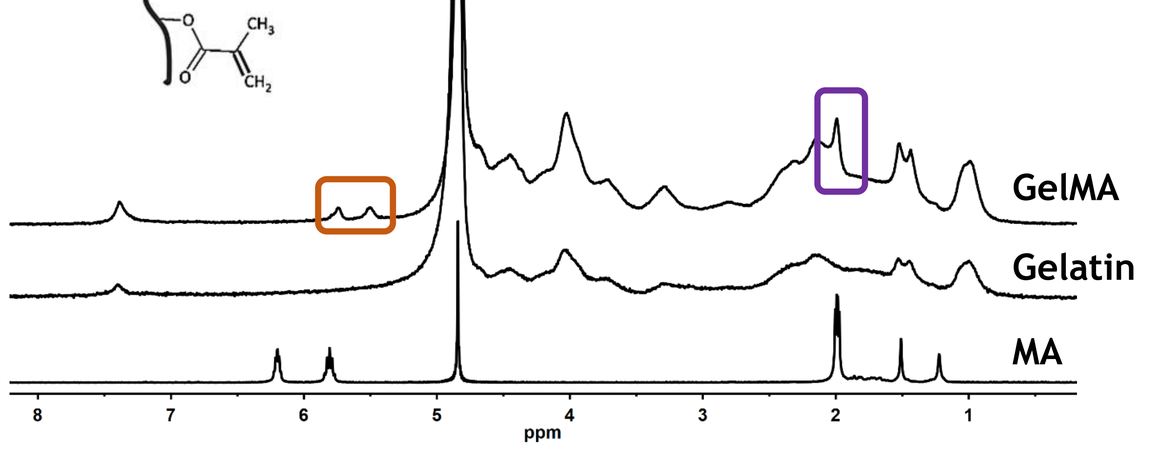


CO-NH-C**H**$_2$-C**H**$_2$-SH



^{1}H NMR (80 MHz, D_2O, 298K) spectra of HA and HA-SH.

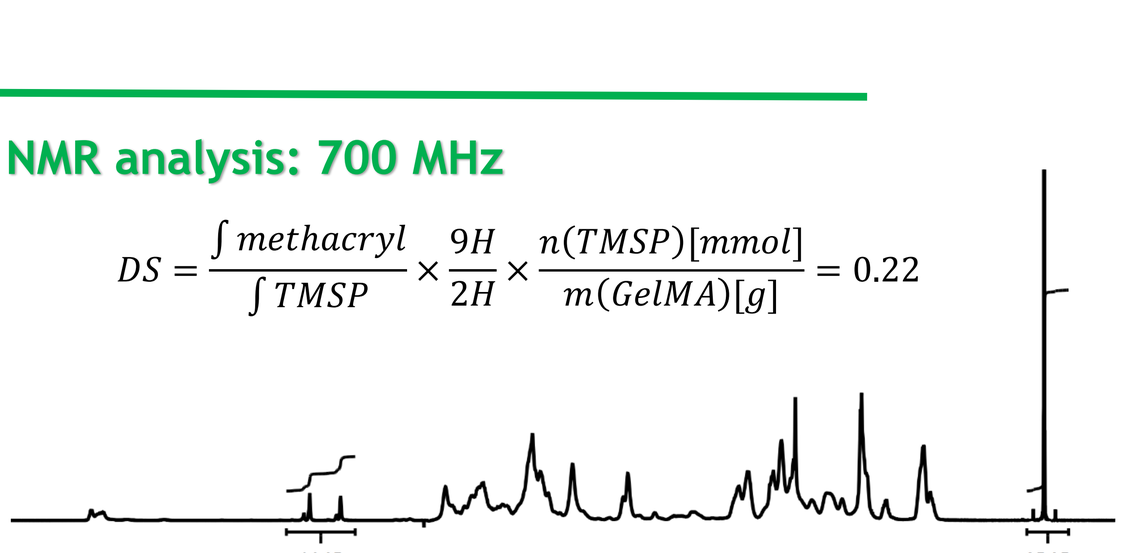




^{1}H NMR (80 MHz, D_2O, 298K) spectra of methacrylic anhydride (MA), gelatin and GelMA.

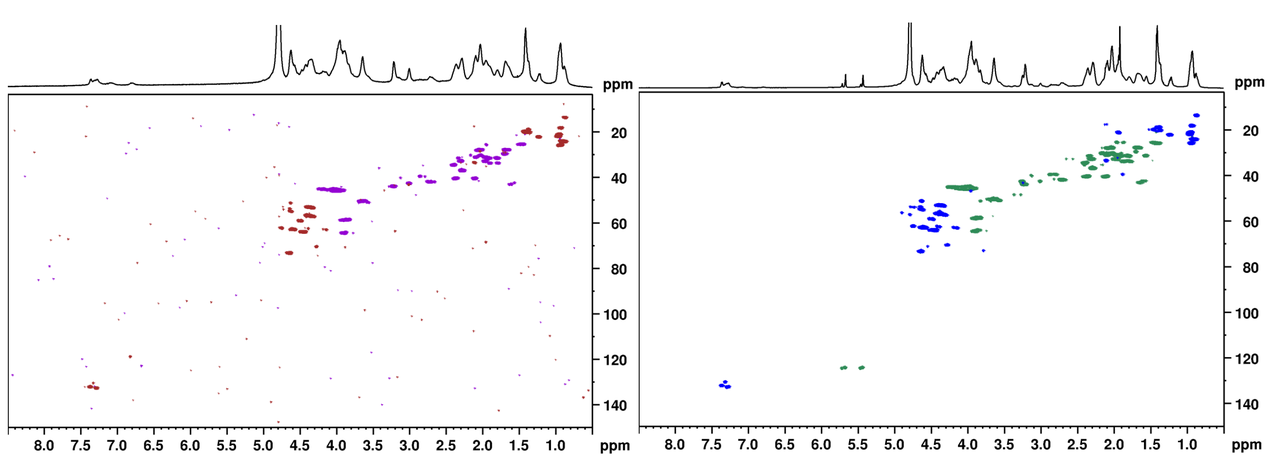
NMR analysis: 700 MHz

$$DS = \frac{\int methacryl}{\int TMSP} \times \frac{9H}{2H} \times \frac{n(TMSP)[mmol]}{m(GelMA)[g]} = 0.22$$



^{1}H NMR (700 MHz, D_2O, 298K) spectrum of GelMA with TMSP (1 mg/mL)

The addition of TMSP as internal standard [5] allowed determininig the degree of substitution (DS) of GelMA, equal to 0.22.



HSQC (700 MHz, D_2O, 298 K) maps of (left) underivatized gelatin and (right) GelMA with TMSP (1 mg/mL).

Amino acids found in raw gelatin [5]: Ala, Arg, Asn, Asp, Gly, Glu, Gln, Lys-OH, Pro-OH, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Tyr, Val

Amino acids showing variations in chemical shift in GelMA: Lys, Lys-OH

Conclusions

Benchtop NMR can be successfully used for confirming the derivatization of biopolymers. New signals were found in HA-SH and GelMa, and their linewidth suggested the attachment to the biopolymers chain.

High-field NMR provided information on the derivatization degree of GelMA and allowed identifying the main amino acids present in raw gelatin. Comparison of HSQC maps of gelatin and GelMA allowed determining the amino acids involved in the derivatization process.

References

[1] J. Biomed. Sci. 2018, 25, 1-21.
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[3] Front. Chem. Eng. 2022, 4, 1-21.
[4] Biofabrication 2024, 16, 015011.
[5] Biomacromolecules 2018, 19, 42−52.

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