Supramolecular hydrogels as a structural analogues of ECM proteoglycans

F. Duarte[1,2], V.Castro[1,2], R. L. Reis[1,2,3], R. A. Pires[1,2,3], I. Pashkuleva[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal;

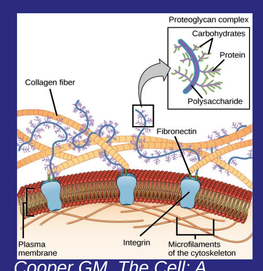
[2]ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal;

[3] The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal;

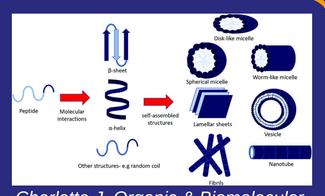
INTRODUCTION

Extracellular matrix (ECM) is a dynamic network which serves as a structural support for cell attachment but also as a bioinformation traducer that regulates many cell functions.[1] Glycosaminoglycans are main component of ECM: they are involved in protein glycosylation - a common modification of the proteins which can change their structure and function and, thus, modulate cell behavior. Supramolecular hydrogels that contains short glycopeptides have been proposed as ECM mimics because these molecules can code and present biochemical information in a dynamic and responsive manner just as the native matrix. [2],[3]

Herein, we generated very short glycopeptide amphiphile by the conjugation of a well-studied dipeptide amphiphile (N-fluorenylmethoxycarbonyl diphenylalanine, Fmoc-FF) and the monossacharide D-glucosamine-6-phosphate, to produce nanofibrous hydrogel.



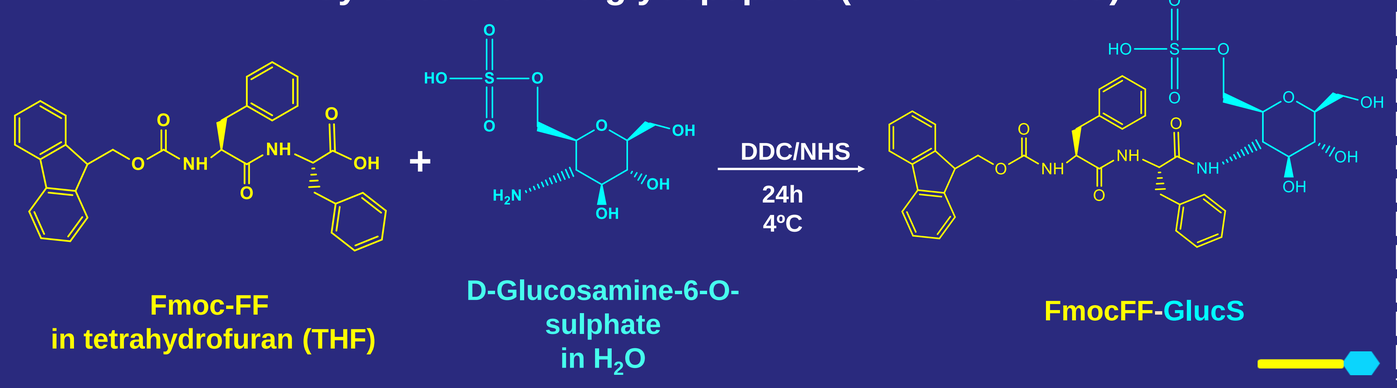
Cooper GM. The Cell: A Molecular Approach. 2nd edition. 2000



Charlotte J. Organic & Biomolecular Chemistry. 2017

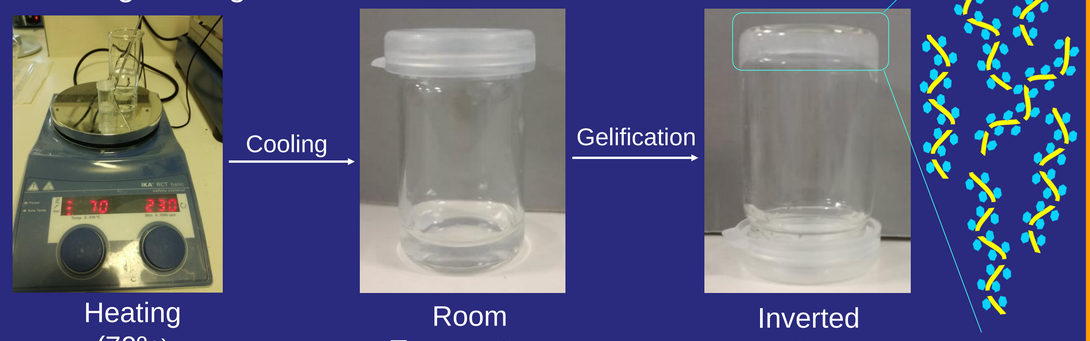
MATERIALS AND METHODS

Synthesis of the glycopeptide (FmocFF-GlucS)



Hydrogel preparation

Heating/cooling method

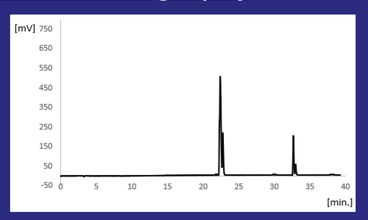


RESULTS AND DISCUSSION

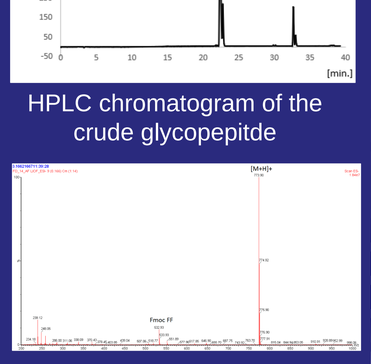
Purification of the glycopeptide

Column chromatography

AcOEt (ethyl acetate)
↓
AcOEt 9:1 MeOH
↓
AcOEt 8:2 MeOH
↓
AcOEt 7:3 MeOH
↓
AcOEt 1:1 MeOH



HPLC chromatogram of the crude glycopepitde

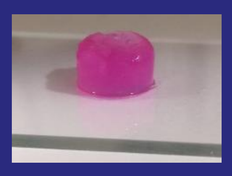


ESI-MS spectra of the FmocFF-GlucS before purification

Gelation

Different concentrations (4mM,8mM,12mM,15mM,20mM,25mM) in aqueous solution. (on the left). Gel was formed at 25mM only (on the right).

Stability test in culture medium



At 25mM the gel formed was immersed in D-MEM culture medium, stable for 14 days.(on the left)

Solutions at 8mM and 10mM was assembled into a gel after 1h in contact with D-MEM culture medium.

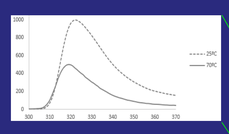
Characterization of the hydrogels

Circular Dichroism (CD)

CD signal ranging from 240nm to 300nm corresponding to the π-π stacking of the FMOC tails

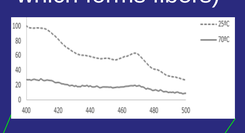
CD signal at ~210nm corresponding to the β-sheet arrangement

Fluorescence spectrometry



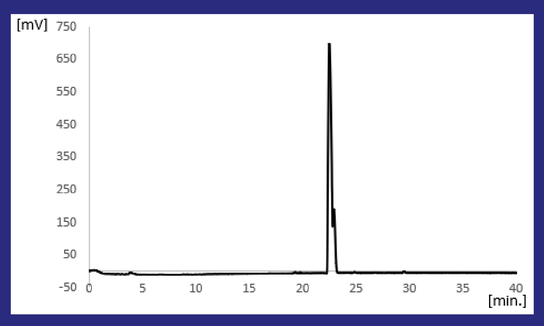
Upon gelation a red shift was observed (~319nm to ~322nm) which indicates the π-interactions of the FMOC groups.

Around ~470nm small peak can be observed indicating long range interactions (π-π or CH-π interactions which forms fibers)

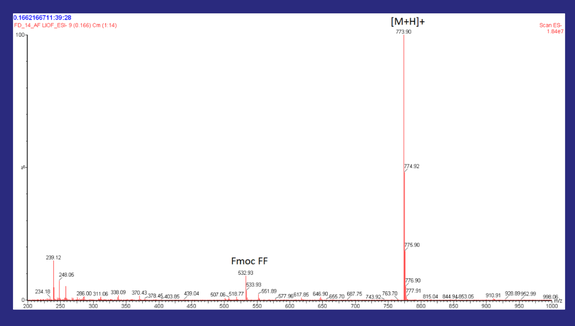


Around ~420nm, a small peak suggests that other structures can be formed like micelles

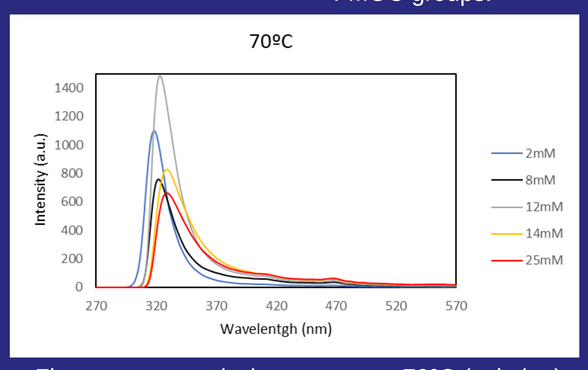
Characterization of the glycopeptide



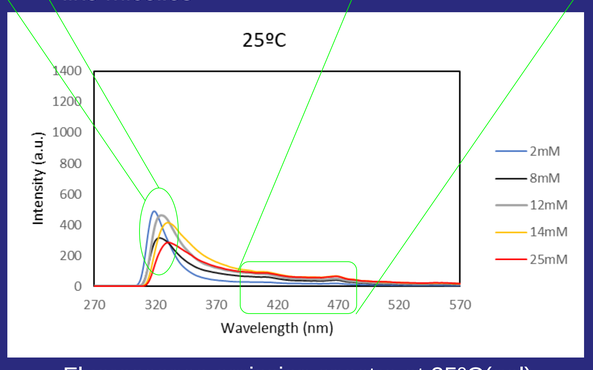
HPLC chromatogram of the pure glycopepitde (98,8%)



ESI-MS spectra of the FmocFF-GlucS after purification



Fluorescence emission spectra at 70ºC (solution)



Fluorescence emission spectra at 25ºC(gel)

CONCLUSIONS AND FUTURE WORKS

Main conclusions

- The glycopeptide was successfully synthesized and purified with 98,8% of purity degree.
- The hydrogels at 25mM were assembled, in aqueous environment, via hydrogen bonding interactions between the peptide backbones (β-sheet conformation) and π-stacking of FMOC tails, confirmed by CD and fluorescence spectrometry.
- The hydrogels at lower concentrations was assembled after the contact with D-MEM culture medium due to the interactions with calcium ions present in the medium and also the pH change.

Future works

- SEM analysis will be performed to study the morphology of the hydrogels and rheometer to measure the mechanical properties.
- The system can be further extended to similar systems by varying the gelation process and the saccharide units.
- Cell encapsulation and targeted delivery will be further explored.

References:

1. Geckil H, Xu F, Zhang X, et al (2010) Engineering hydrogels as extracellular matrix mimics. Nanomedicine (Lond) 5:469–84 . doi: 10.2217/nnm.10.12

2. Goor OJGM, Hendrikse SIS, Dankers PYW, Meijer EW (2017) From supramolecular polymers to multi-component biomaterials. Chem Soc Rev 46:6621–6637 . doi: 10.1039/c7cs00564d

3. Du X, Zhou J, Shi J, Xu B (2015) Supramolecular Hydrogelators and Hydrogels: From Soft Matter to Molecular Biomaterials. Chem Rev 115:13165–13307 . doi: 10.1021/acs.chemrev.5b00299

Acknowledgments:

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