Functionalization of squid chitosan with angiogenic peptides for the development of new biomaterials

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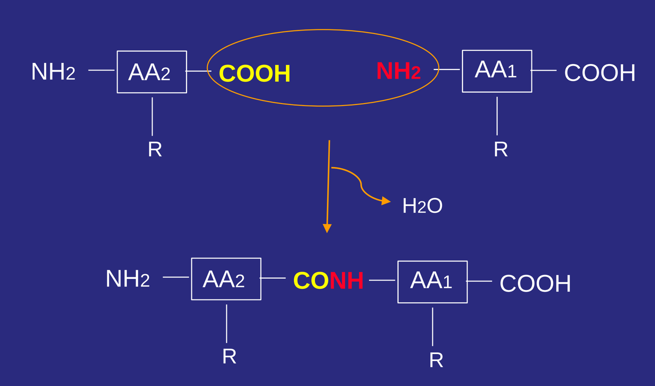
INTRODUCTION

The study and development of materials of marine origin has been growing over the years due to their interesting and sometimes unique characteristics and availability in nature.[1] Chitosan, produced by deacetylation of chitin, the second most abundant natural polymer, has a great potential for biomedical applications due to its biocompatible, biodegradable and antimicrobial properties. Moreover, due to its chemical variability, chitosan can be also processed into different forms, such as membranes or hydrogels, which can be used for instance in burn treatments and tissue regeneration.[2] On the other hand, bioactive peptides sequences present in different ECM-proteins also been exploited in the development of biomaterials, for instance through functionalization of biopolymers, aiming to modulate cell behaviour, namely promoting cell adhesion, growth or differentiation, supporting mineralization of angiogenesis, among others. Here, we propose to develop new biomaterials based on marine origin chitosan functionalized with a bioactive peptide capable to promote the formation of new blood vessels, which can modulate the inflammatory response and improve the wound healing process.[3]

MATERIALS AND METHODS

Peptide Synthesis

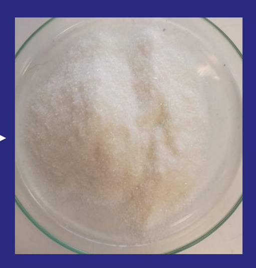


Pep12 (VEGF mimic)
sequence : NYLTHRQ
MW= 931,01g/mol
scale: 1mmol

Chitosan extraction

Squid pens of *Dosidicus gigas*

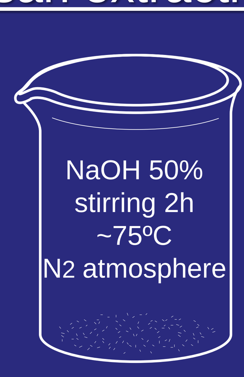
Trituration



125 < µm < 250

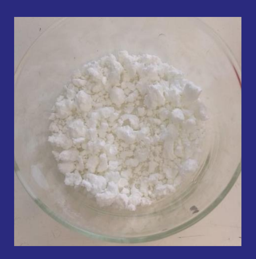
Deacetylation of chitin

NHCOCH3→ NH2

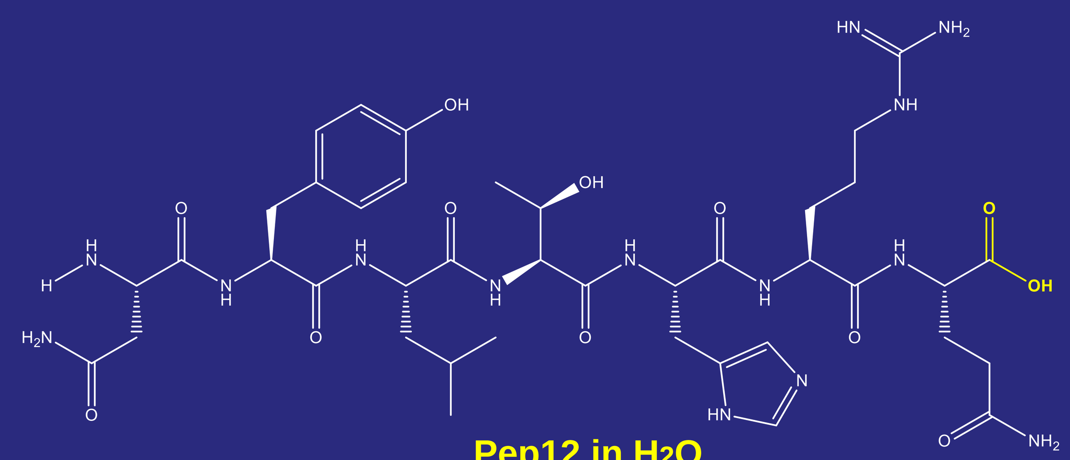


Wash and filtration

Freeze-dry

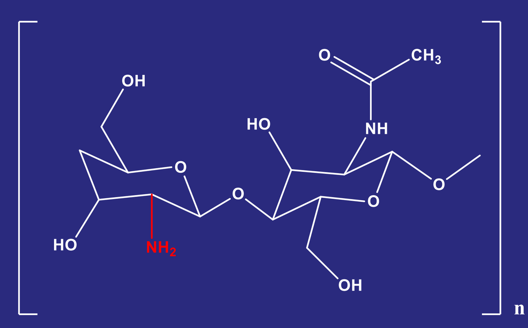


Functionalization



Pep12 in H_2O

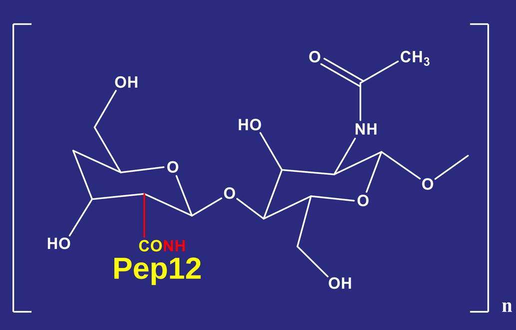
+



Chitosan in acetic acid (2%)

EDC/NHS

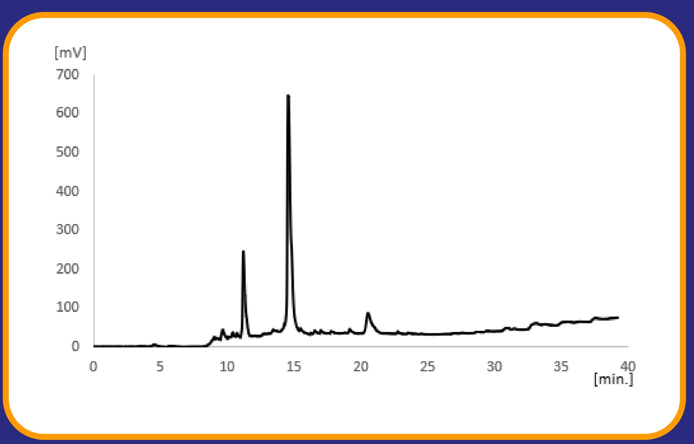
Room Temperature o/n



RESULTS AND DISCUSSION

Peptide Synthesis

High Performance Liquid Chromatography (HPLC)

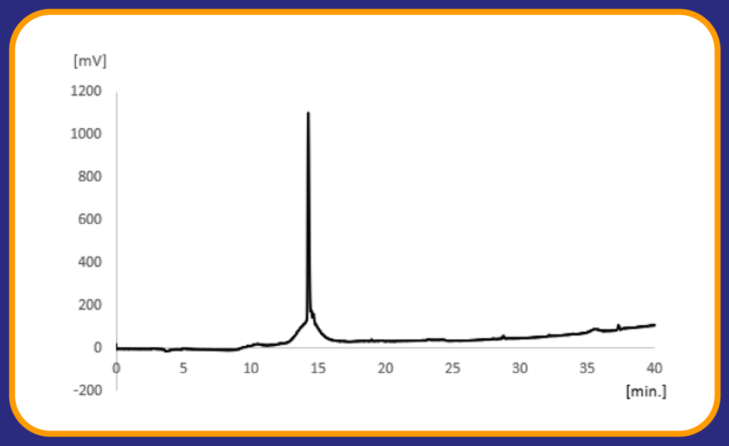


Chromatogram of the crude peptide

Purification

Column: Atlantis T3 5µm, 4.6x250mm

λ=280nm

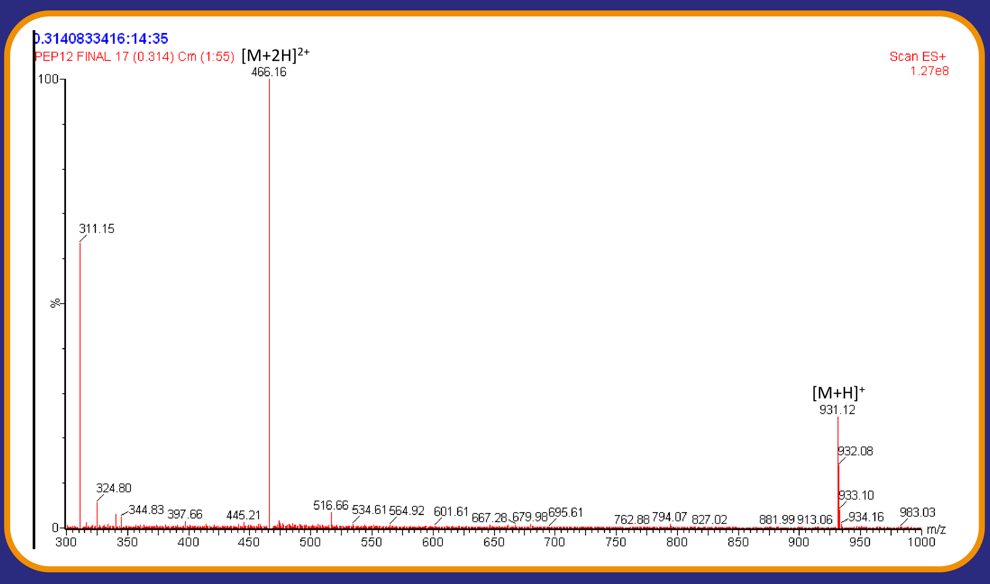


Chromatogram of the pure peptide (97,8%)

The peptide was analysed through analytical HPLC to measure the purity and identified through their molecular weight by Electrospray Ionization Mass spectrometry (ESI-MS)

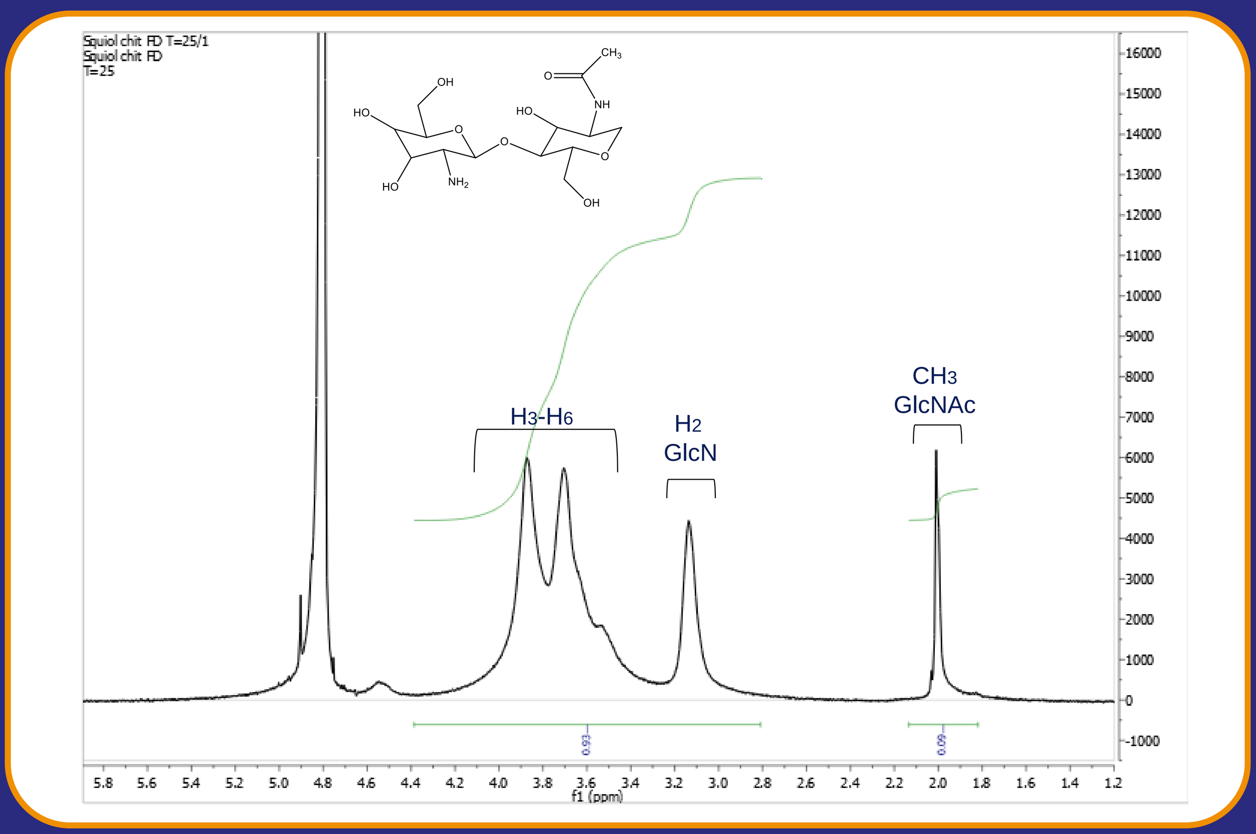
The peptide corresponding to the main peak in the chromatogram was isolated through preparative HPLC with a gradient 0-80%ACN in H2O (0.1% TFA) and a flow of 25 ml/min. The degree of purity was 97.8%.

Electrospray Ionization Mass Spectrometry (ESI-MS)



Chitosan extraction

Nuclear Magnetic Ressonance (^{1}H NMR)



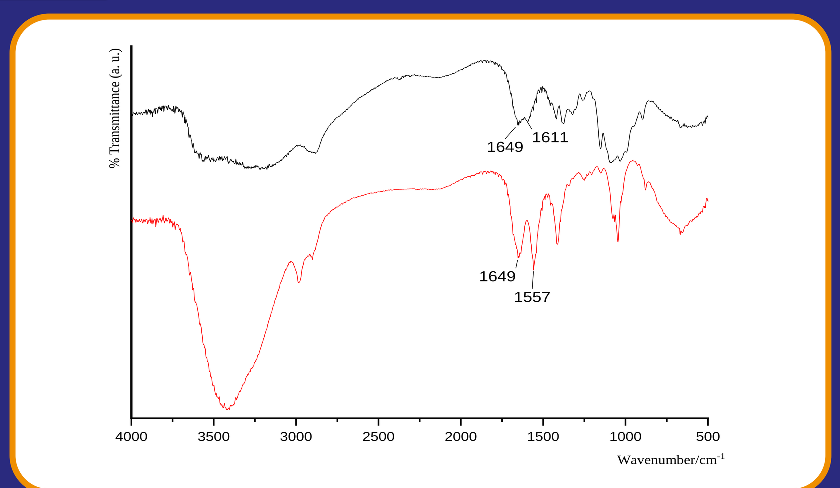
^{1}H NMR spectrum of the extracted chitosan

Degree of Acetylation:

$$DA=\frac{I(Ch3)/3}{I(H2-H6)/6} \times 100 = \frac{0{,}09/3}{0{,}93/6} \times 100 = 19{,}4\%$$

Functionalization

The conjugate was analysed by FTIR, which confirmed the peptide-polymer bond through an increase on the intensity of the amide bands, namely amide I at 1649 cm-1 (C=O stretching) and amide II at 1557 cm-1 (N-H bending), for modified chitosan (red line)



FTIR spectrum of the unmodified chitosan (black line) and chitosan functionalized with peptide (red line)

CONCLUSIONS AND FUTURE WORKS

Main conclusions

- The peptide was successfully synthesized and purified with 97,8% of purity;
- The extracted chitosan had a yield of 10%, without the need of purification;
- The functionalization of the chitosan occurred successsfully, confirmed by FTIR, which demonstrate the insertion of the peptide chain (amide bands).

Future works

- Different peptides will be synthesized to investigate their influence on the angiogenic sutdies;
- Functionalized chitosan will be also analysed by ^{1}H NMR to measure the yield of the reaction;
- The angiogenic potential of peptides, the conjugates and derived biomaterials will be further studied by assessing the effect over endothelial cells or on vascularization using a CAM assay.

References:

1. Silva TH, Alves A, Ferreira BM, et al (2012). Int Mater Rev 57:276–306 . doi: 10.1179/1743280412y.0000000002
2. Reys LL, Silva SS, Oliveira JM, et al (2013). Biomed Mater 8: . doi: 10.1088/1748-6041/8/4/045002
3. Van Hove AH, Benoit DSW (2015). Front Bioeng Biotechnol 3:1–18 . doi: 10.3389/fbioe.2015.00102

Acknowledgments:

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