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

F. Duarte1,2, D.N.Carvalho1,2, R.L. Reis1,2,3, T.H. Silva1,2

13B’s Research Group, 3B’s – Research Institute on Biomaterials, Biodegradables and Biocomposites, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciências e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal;

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

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

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. 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. On the other hand, bioactive peptide sequences present in different ECM-proteins have 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.

MATERIALS AND METHODS

Peptide Synthesis

High Performance Liquid Chromatography (HPLC)

Electrospray Ionization Mass Spectrometry (ESI-MS)

RESULTS AND DISCUSSION

Peptide Synthesis

The peptide 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)

The conjugate was analysed by FTIR, which confirmed the peptide-polymer bond through an increase in 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).

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 successfully, 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 subsidies;
- Functionalized chitosan will be also analysed by 1H 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:

Acknowledgments:
This work is being partially funded by European Regional Development Fund (ERDF), through European Union Transborder Cooperation Programme INTERREG V A España-Portugal 2014-2020 (POCTEP), under the scope of project 0206, CVMAR_3.1.1, and through NORT2020, within the Portugal 2020 Partnership Agreement, under the scope of projects NORTE-01-0145-FEDER-000021 and NORTE-01-0145-FEDER-000022.