Extraction and characterization of marine gelatine and its methacrylation (GelMA) towards the production of hydrogels for tissue engineering

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INTRODUCTION

Marine organisms are constituted by materials with a vast range of properties and characteristics that may justify their potential application within the biomedical field and have been receiving increasing attention as they are free from the transmissible diseases associated with the use of mammalian resources, as well as the fact that they may enable high production at a low cost.[1, 2]

Gelatine (Figure 1) is derived from the fibrous protein collagen, which is the principal constituent of animal skin, bone, and connective tissue, by denaturation and often partial hydrolysis.[3] It is applied in many industrial sectors, from food to pharma, and particularly in biomedical context can be utilized in tissue engineering/regenerative medicine for the development of scaffolds.[4]

Gelatine can be combined with methacrylic anhydride to give origin to methacrylated gelatine (GelMA), enabling the formation of hydrogels and 3D printing upon photocrosslinking.[5]

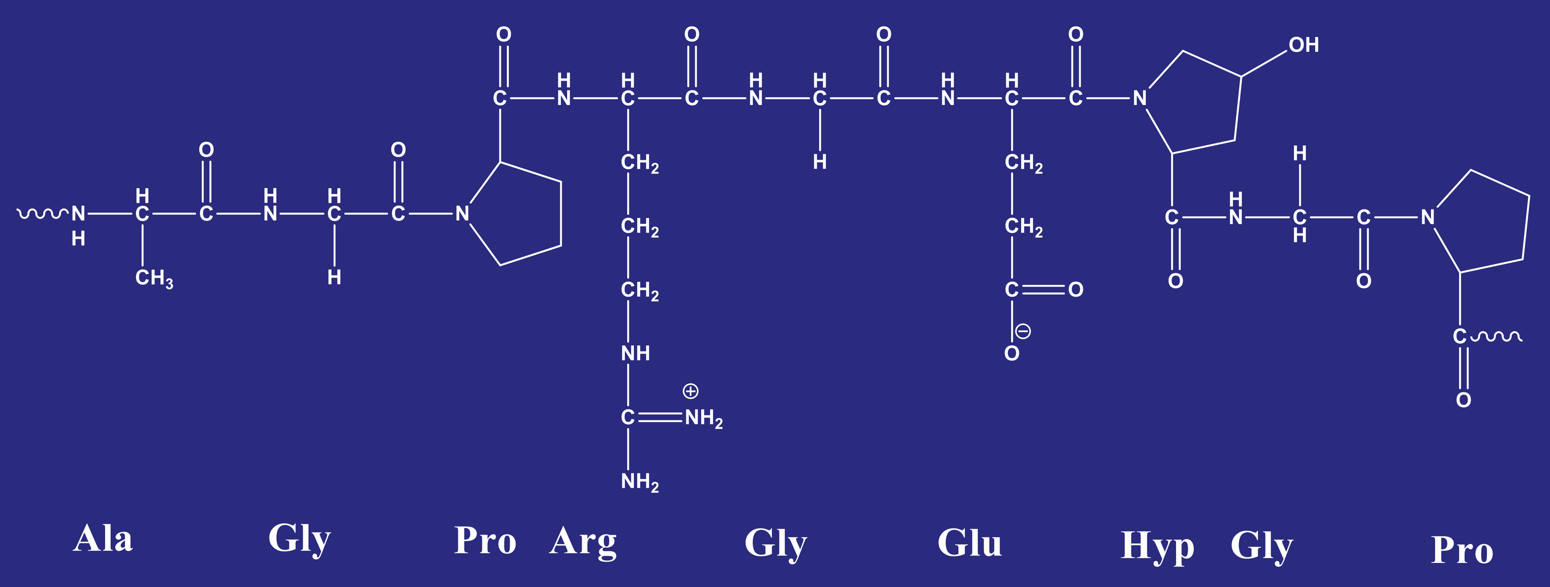

Figure 1 – Illustrative scheme of a part of the structure of gelatine.

MATERIALS AND METHODS

Production of gelatine from halibut fish skins

- Pretreatment with sodium hydroxide (0.04M)
- Wash with acidic solutions, namely, sulfuric acid (0.12M) and citric acid (0.005M)
- Extraction with hot water, promoting the partial hydrolysis of native collagen, involving the breaking of the triple-helix structure into random coils (figure 2).[6]

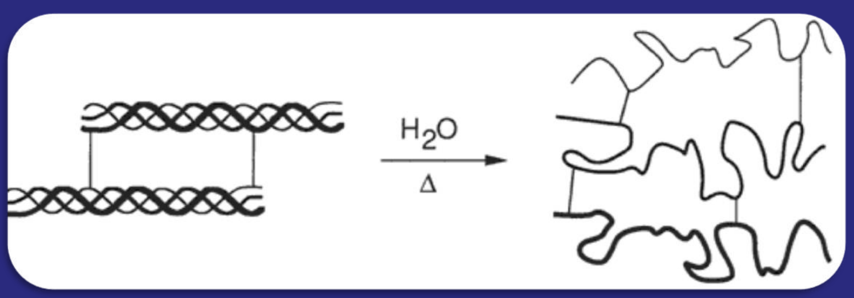

Figure 2 – Denaturation of collagen to obtain gelatine.

Physical and chemical characterization

- SDS-PAGE
- NMR spectroscopy
- Extraction yield
- CD spectroscopy
- DSC analysis
- FTIR spectroscopy

Functionalization of gelatine with methacrylate groups

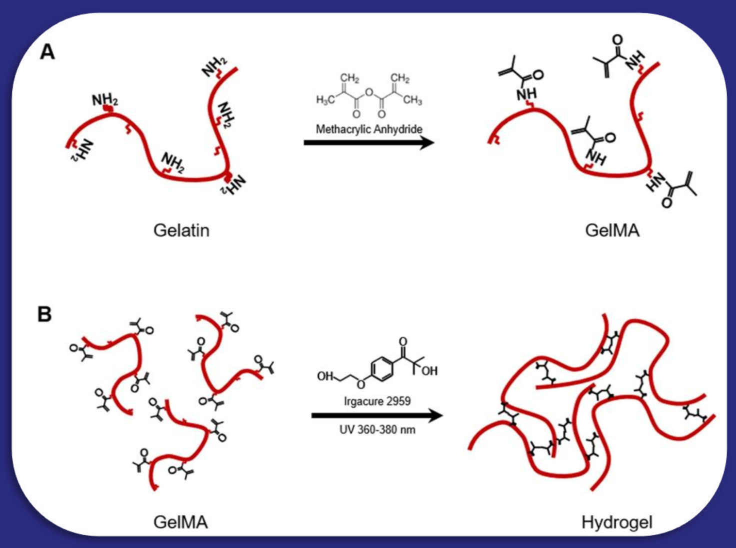

Figure 3 – (A) Gelatine is reacted with methacrylic anhydride to introduce a methacryloyl substitution group on the reactive amine and hydroxyl groups of the amino acid residues. **(B)** GelMA photocrosslinking to form a hydrogel matrix under UV irradiation. The free radicals generated by the photoinitiator initiated chain polymerization with methacryloyl substitution.[7]

RESULTS AND DISCUSSION

✓ **CD** spectrum are consistent with the presence of random coil conformation of gelatine and GelMA.

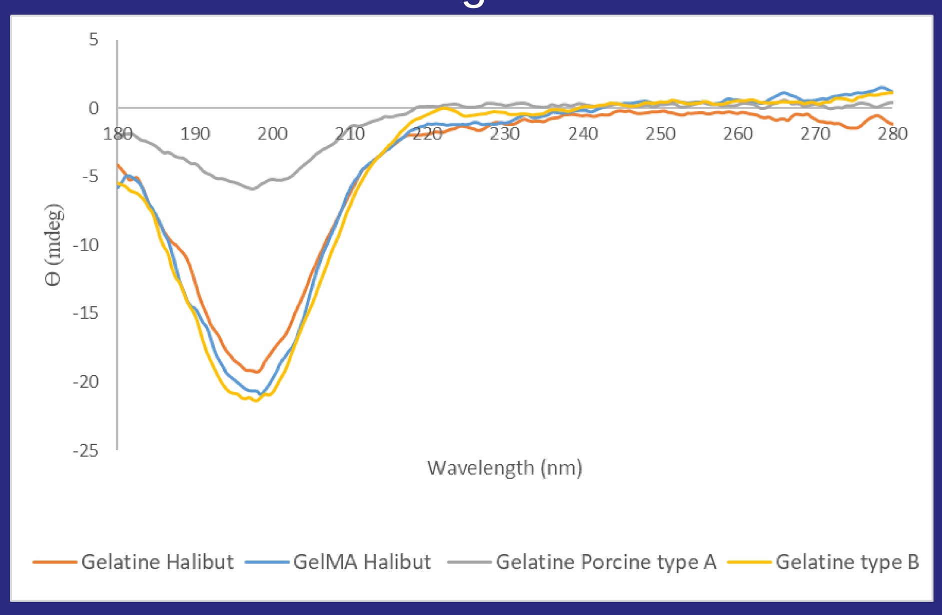

Figure 4 – CD spectra of gelatine and GelMA from halibut skin.

✓ **FTIR** spectrum exhibited the characteristic peaks of gelatine.

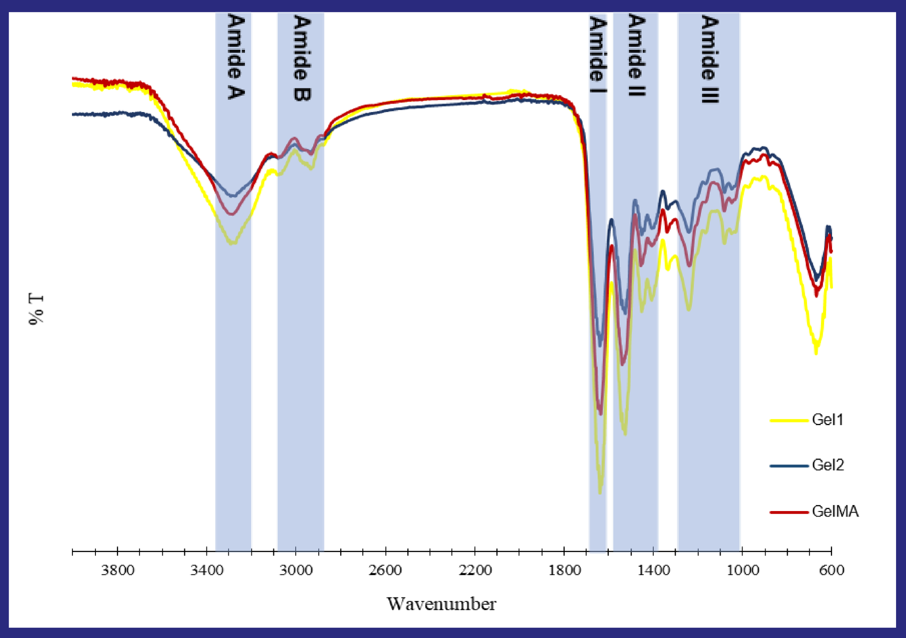

Figure 5 – FTIR spectra of gelatine and GelMA from halibut skin.

✓ **NMR** spectrum exhibited the characteristic peaks of gelatine and GelMA.

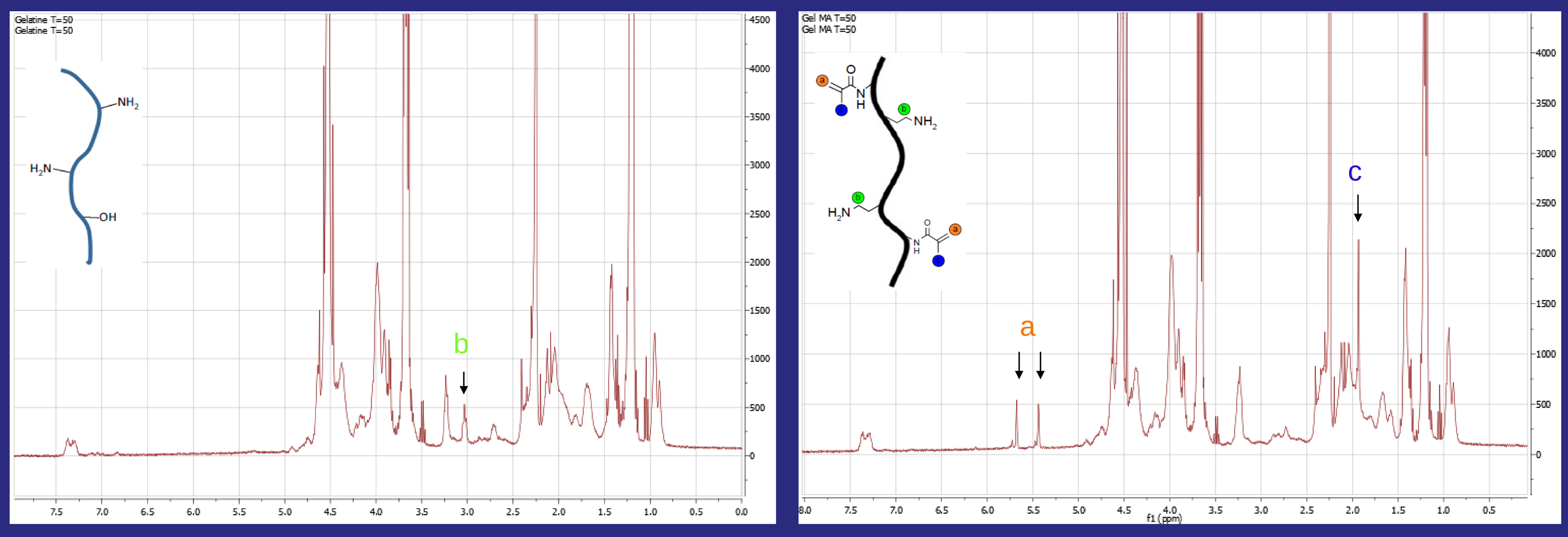

Figure 6 – NMR spectra of gelatine and GelMA from halibut skin.

✓ **Extraction yield:** ~5%, which is in accordance with the literature for extractions under the same conditions.

CONCLUSIONS

- ✓ Potential procedure for gelatine extraction because this is an environmentally sustainable process and represents a biotechnological strategy to valorize a fish by-product;
- ✓ According to the results from CD analysis, the gelatine extracted from halibut skins has a profile identical to commercial type B gelatine and besides these, FTIR also confirms that its structure is as expected;
- ✓ According to the results from NMR it is possible to verify that the methacrylation has occurred.
- ✓ As future work, the goal is to obtain a hydrogel through GelMA photocrosslinking.

References

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Acknowledgments

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