Valorization of Atlantic cod (*Gadus morhua*) by-products by isolation of collagen and native ECM envisaging wound healing and skin regeneration

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INTRODUCTION

In today's society there is an increased concern in the sustainable use of natural resources as well as on waste management, together with the implementation of the Circular Economy concept. The fish industry by-products account for approximately 75% of the total fish caught, which despite their high value, namely for the production of relevant compounds and materials, are still poorly explored, being mainly directed for animal feed[1,2]. With increasing of environmental education, in specific the conservation of the marine ecosystems, the scientific community has been working on a sustainable exploration of marine biological resources. In particular, recent biotechnology advances have been made to discover, produce or transform compounds from marine sources to be incorporated as functional biomaterials or bioactive compounds for biotechnological, pharmaceutical or cosmetic application[1,3].

The use of marine origin protein in particular can have an added advantage by contribution to a more sustainable use of marine resources with a proposal of high added value application for medical and cosmetic sector. Collagen is the most abundant protein in mammalian extracellular matrix (ECM), representing an important subset of fibrous structural proteins[1,2].

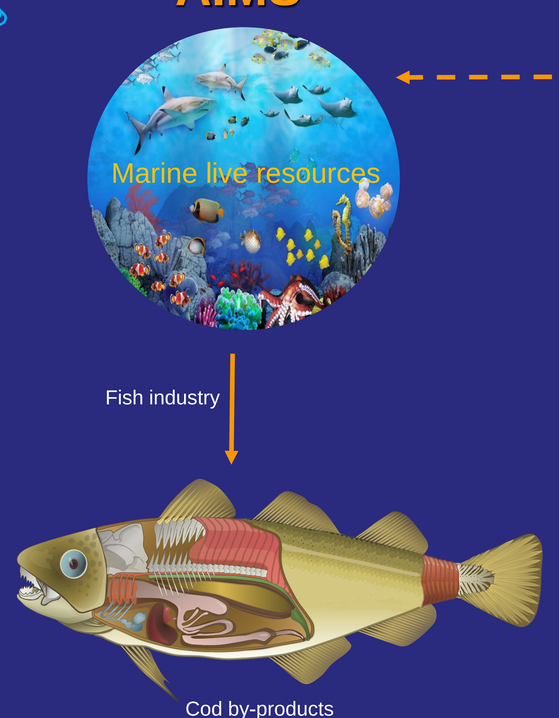
Cod skin and swim bladders are rich in collagen, namely type I collagen, but most are still regarded as waste, from which the isolation of a highly relevant biopolymer – collagen – may represent an important increase on the economic value of the fish by-product[1,2,4]. Marine origin protein has gained more attention because of their high yield and availability, no risk of pathogen infection compared to land-based animals (cows, pigs, poultry, etc.) and no religious barriers. These proteins can be recovered and used in the whole form, or hydrolysed into peptides, either in cosmetics, for the reinforcement of skin elasticity and hydration, or in biomedicine, for the production of grafts and for bone and skin regeneration[1,5–7].

Research on biomaterials for skin tissue engineering based on collagen has been exploring processing strategies to yield tri-dimensional templates for recovering lost tissues by guiding cell growth and re-establishing original tissue design[2,4,7,8]. Besides the production on new biomaterials, decellularized native ECM as scaffolds is a growing subject by representing the secreted product of the cells comprising each tissue and organ, they provide a unique biological material and microenvironment[8-10]. Typical decellularization protocols include combinations of detergents, organic solvents, and enzymatic solutions[9,10]. In this research work, supercritical carbon dioxide will be explored, as a promising alternative that includes the use of an inert substance for cell removal and minimal alteration of ECM mechanical properties.

AIMS

Marine Resources · Circular Economy · Blue Biotechnology · Biopolymers · Marine Biomaterials · Tissue Engineering

This research project will be based on the isolation of collagen from selected cod by-products, particularly from fish skin and swim-bladders, which may represent an environmentally friendly and sustainable approach for their valorization. This compound will be used for the production of new marine biomaterials, namely addressing its crosslinking and combination with marine origin glycosaminoglycans to develop dressings for the management of skin wounds. Moreover, the decellularization of cod skin using supercritical fluids technology will be addressed to isolate the native ECM, being hypothesized that such natural biomaterial could enhance skin healing in wound care approaches.



MATERIALS AND METHODS

Collagen extraction

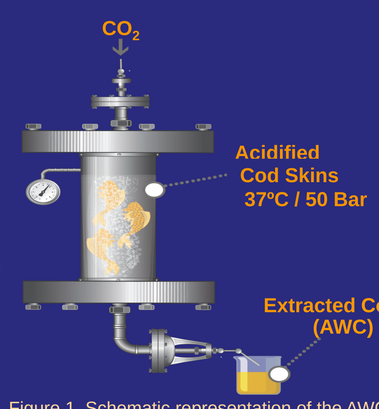


Figure 1. Schematic representation of the AWC extraction process.

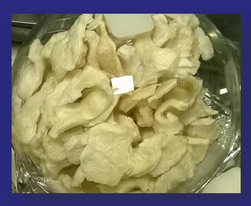
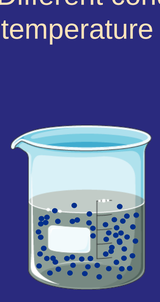


Figure 2. Lyophilized Atlantic cod (*Gadus morhua*) swim bladders.

Cod swim bladders → Remove non-collagen proteins (✓0.15M NaOH - 3h; ✓Water – neutral ph) → Remove fat (✓10% 2-propanol – 24h; ✓Water) → Lyophilized → Acid extraction → Precipitacion with NaCl → Dialyses → Freeze drying

Marine Based Biomaterials

Different conditions (pH, ionic strength, temperature and concentrations of the collagen)



Collagen dissolution in Acetic Acid or HCl

Gelation

Membranes

Gels

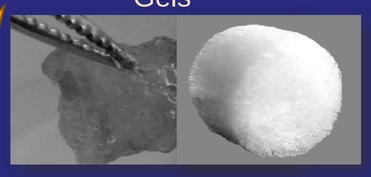


Figure 3. Scheme of the production of Collagen-based biomaterials.

Isolation of native ECM by decellularization of cod skins using high-pressure reaction apparatus.

Figure 4. Atlantic cod (*Gadus morhua*) skins.

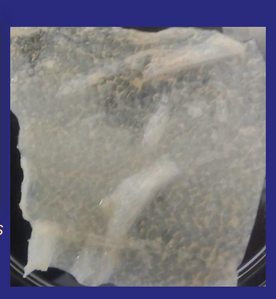


Figure 5. Descellularized cod skin.

Characterization

Physicochemical:
- ✓ SDS-PAGE;
- ✓ FTIR;
- ✓ Amino acid analysis;
- ✓ CD;
- ✓ XRD;
- ✓ GPC-SEC;
- ✓ Micro-DSC.

Biological performance:
- ✓ MTS assay.

Mechanical:
- ✓ Rheology;
- ✓ Tensile tests;

Morphological:
- ✓ SEM.

Efficiency:
- ✓ H&E histology;
- ✓ DAPI (staining cell nuclei – blue);
- ✓ DNA quantification

Biomedical approach

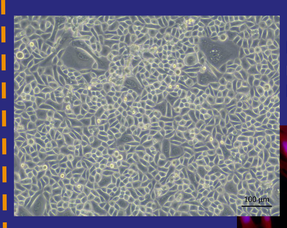


Figure 6. Bright field microscopy image of Keratinocytes.

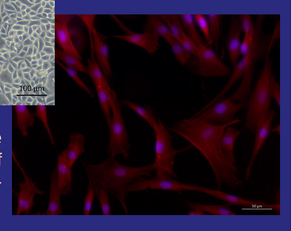


Figure 7. Fluroscence microscopy image of fibroblastos (Phalloidin-DAPI).

The *in-vitro* biological performance regarding skin regeneration will be accomplished using epidermal and dermal cells to assess cell viability, adhesion and proliferation.

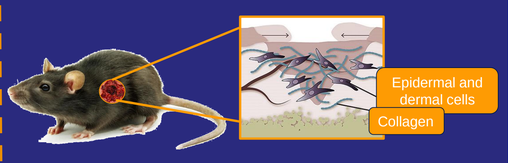


Figure 8. Mice wound healing model.

The materials that perform better will be further tested in a mice full-thickness excisional wound model.

EXPECTED RESULTS

By investing in marine research and innovation using biotechnological tools, the production of biologically active molecules of marine-based origin will be established, particularly regarding collagens, contributing to a more sustainable use of marine resources and derived by-products, under the scope of the circular economy concept.

The produced collagens will be used as building blocks for the development of new biomaterials, with promising application in skincare and skin regeneration.

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Acknowledgments: Financial support from European Regional Development Fund (ERDF), through European Union Transborder Cooperation Programme Interreg España-Portugal 2014-2020 (POCTEP), under the scope of project 0302_CVMAR_I_1_P and under the scope of Doctoral Program in Advanced Therapies for Health, funded by "Fundação para a Ciência e Tecnologia" (FCT, Portugal) (PD/169/2013).