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Extended Abstract

Evaluation of Fish Hydrolyzate Interaction with Skin Cells †

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Fish proteins and peptides are well-known for a plethora of biological activities and medicinal value. Fish bones and scales contain mainly type I collagen fibers and hydroxyapatite $Ca_{10}(OH)_2(PO_4)_6$ [1] and represent one of the major source of solid waste generated by the fish processing industry. Recovery of bioactive peptides from these fish by-products brings new perspectives on the wound healing process and is part of developing bioeconomy. In this study, we have evaluated the interaction of fish-derived peptides with fibroblasts and keratinocyte cells, in experimental models in vitro, to show their biomedical potential use for skin tissue regeneration.

Fish hydrolyzate was enzymatically obtained by papain digestion of minced and decalcified bones of *Hypophthalmichthys molitrix* (silver carp). The solution was subjected to centrifugal filtration using membranes with a molecular weight cut-off of 3 kDa, and its protein content was determined by BCA assay. The cytotoxicity of peptides was tested, at different concentrations, in NCTC mouse fibroblasts and HaCaT human keratinocytes cultivated with or without fetal serum, using MTT assay [2]. Their effect on skin cell migration was measured using in vitro scratch assay, after 24 h of cultivation in standard conditions [3]. Cellular and cytoskeleton morphology changes were analyzed by immunofluorescence microscopy after cells were stained with TRITC-phalloidin and anti-tubulin antibodies. Statistical analysis was performed using one-tailed paired Student t-test.

Fish hydrolyzate was not cytotoxic in a wide range of concentrations. At low concentrations, both skin cells cultivated in the presence of fish peptides presented a significantly higher ($p < 0.05$) cell proliferation, compared to untreated cells (control). The images captured overnight, after scratched cell cultures incubation with fish peptides, showed that the cell monolayer was more rapidly formed in treated cell cultures. These observations indicated that fish hydrolyzate stimulated the migration of skin cells, probably due to the presence of Gly-Pro-X and Gly-X-Hyp sequences, frequently encountered in the collagen molecule, and known to be involved in fibroblast activity [4]. No alterations in cell morphology and cytoskeletal structures were observed.

Taken together, our results demonstrated that the enzymatic fish hydrolyzate was efficient in wound healing models in vitro, and had valuable properties which recommend it as a promising solution for tissue regeneration applications.

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