Green solvents extraction of sponge-origin collagen/gelatin for biomedical applications

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Statement of Purpose: Marine sponges are extremely rich in natural products and are considered a promising biological resource. The major objective of this work is the coupling of a green extraction process with a natural origin renewable raw-material to obtain sponge-origin collagen/gelatin, particularly for biomedical applications. Marine sponge collagen has unique physico-chemical properties but its application is hindered by lack of availability due to inefficient extraction methodologies. Traditional extraction methods are time consuming as they involve several operating steps and large amounts of solvents. Alternatively, the use of subcritical water for the extraction of collagen can appear as a highly relevant methodology. It has been proposed before for the extraction of collagen from fish scales. However, the process conditions are extreme (high temperature and pressure) conducting to the denaturation degradation of collagen, which occurs above 37ºC for human collagen. In this work, we propose a new extraction methodology, under mild operating conditions, in which water is acidified with CO_2 to promote the extraction of collagen/gelatin from different marine sponge species [1-5].
Methods: Marine sponge materials (*Timosea* sp., *Chondrosia reniformis, Chondrilla nucula* (wild and cultured) were grinded in small pieces. After that, the samples were placed in a high pressure vessel, with distilled water, at 40ºC and the vessel was pressurized with CO_2 to 50 bar, overnight. The extract obtained was filtered with a 0.22 µm filter and freeze dried to obtain the extracted collagen/gelatins. The powder obtained from the different extractions was observed by Scanning Electron Microscope (SEM). The isoelectric point of the different collagen/gelatin samples was determined by titration with NaOH and HCl. The quantification of collagen was performed using the Sircol Assay kit (Life Science Assays, UK). The composition of amino acid in the samples was determined by quantitative amino acid analysis and the molecular weight of the extracts was determined by gel permeation chromatography (GPC-SEC). The extracts were also characterized by FTIR, thermal denaturation ($T_{d)}$ and UV-Vis spectroscopy. Cytotoxicity studies were also executed in accordance with ISO/EN 10993.
Results: The extraction yields obtained with the proposed methodology (~17%) are significantly higher than the ones previously reported. *Addad et al*, report the extraction of collagen from different organs of jellyfish with lower recovery yields. Furthermore, the quantification of collagen (Sircol assay) revealed that the recovered extracts *Timosea* sp. and *Chondrilla nucula (wild)* are composed of up to 82% of collagen, The morphology of the powder obtained are presented in figure 1.

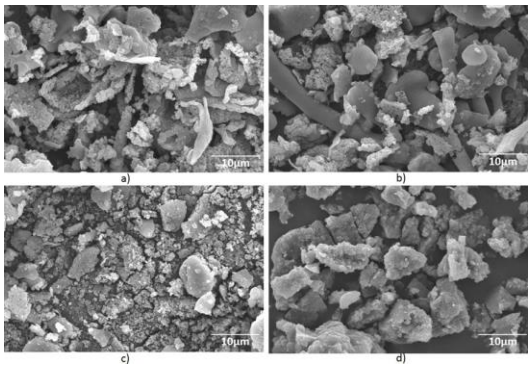

Figure 1. SEM micrographs of the powder obtained from a) *Chondrilla nucula* (cultured), b) *Chondrilla nucula* (wild), C) *Chondrosia reniformis* and d) *Timosea* sp..

Collagen has a triple helical structure with characteristic amino acid repetition, (Gly–Pro–Hyp) n, glycine (Gly) was the most abundant. The amino acid quantification for four samples corroborate the collagen composition, being the Gly the most abundant (2.17 – 4.00 nmol/mg). The peaks observed in FTIR and UV-Vis analysis confirms the chemical structure of collagen. Our measurements for the isoelectric point are in agreement with the reported by *Highberger*, in 1939, and may suggest the presence of a mixture between collagen and gelatin. The molecular weight (Mw) obtained for the collagen/gelatin extracted are in the range of 110.59 (8.75) KDa for *Chondrilla nucula* (wild) and 208.92 (14.96) KDa for *Chondrosia reniformis*. The T_d of all collagen samples was determined to be ~38ºC, which is close to that of calf skin collagen (40.8ºC). Cytotoxicity experiments have shown that the collagen extracted from *Chondrosia reniformis* and *Chondrilla nucula* is non-cytotoxic. The only exception was the extract from *Timosia* sp.. In this case, although 82% of the extract was determined to be collagen, there may be some cytotoxic compounds in the extract, responsible for the results obtained.
Conclusions: The extraction of sponge-origin collagen/gelatin with high pressure carbon dioxide acidified water was successfully achieved. The extracted material was confirmed to be a mixture of collagen and gelatin using different physical and chemical analytical techniques. Non-cytotoxic behavior demonstrated that the collagen obtained is thus a promising material for biomedical application.
References: (Laemmli UK. Nature 1970;227:680), (Addad S, Mar Drugs 2011;9:967-83), (Veeruraj A, J Mater Sci-Mater M 2012;23:1729-38), (Swatschek D, Eur J Pharm Biopharm 2002;53:107-13), (Highberger JH. J Am Chem S 1939;61:2302-3)