Development of hydrogels-based on marine biopolymers envisaging cartilage tissue engineering and regenerative medicine

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Introduction & Aims

In the recent decade, marine origin products have been growingly studied as building blocks complying to the constant demand from the biomedical sector for new materials regarding the development of improved devices for clinical applications and new therapeutical approaches [1, 2]. The advantages of marine products are the reduction or elimination of risks associated with zoonosis, as well as overcoming social/religious-related constraints when compared to the mammal sources for some compounds [3]. Equally important, their production methodologies are commonly associated to low-cost processes, corresponding in many cases to valorization of by-products, with inherent environmental and economic benefits [4].

The present work addresses the synthesis of hydrogels using a combination of marine origin biopolymers (collagen, chitosan and fucoidan) at different ratios, freezing at -80 ºC and further slowly thaw, for tissue engineering and regenerative medicine applications. The produced hydrogels were characterized by scanning electron microscopy to address morphological features, by rheology to access mechanical properties and by *in vitro* tests with cell lines to evaluate cytocompatibility and their capacity to support cell proliferation envisaging new human tissue formation.

Methodology

Figure 1. Schematic representation of the biopolymers extraction from different marine sources and the development of new cryo-biomaterials using polyelectrolyte procedures. The low temperatures promote the natural cross-linking between polymers.

Results

1. Biopolymer characterization

Amino acid	COL (mol %)
Asp	88,22
Thr	35,73
Ser	52,08
Glu	98,51
Gly	**293,73**
Ala	87,61
Cys	0,00
Val	25,84
Met	12,71
Ile	16,15
Leu	32,50
Nleu	17,97
Tyr	8,88
Phe	18,77
OHlys	38,96
His	3,43
Lys	29,47
Arg	46,43
Hyp	**61,51**
Pro	**119,11**
Total	1000

Table I. Amino acid composition of collagen (Col) from jellyfish *Rhizostoma pulmo* (residues/1000 residues).

The high presence of glycine (Gly), proline (Pro) and hydroxyproline (Hyp) agrees with the typical composition of collagen.

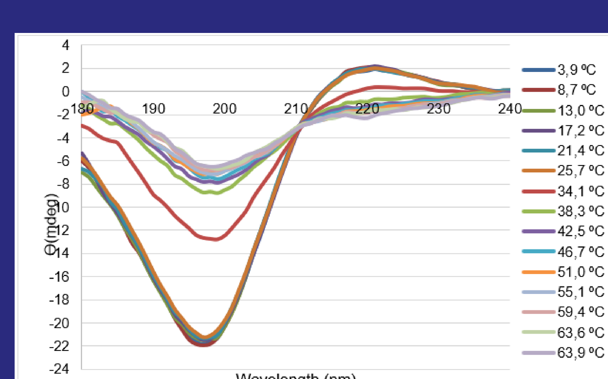

Figure 2. Circular Dichroism spectra of the collagen obtained at different temperatures. Denaturation temperature determined to be 35.3 ºC.

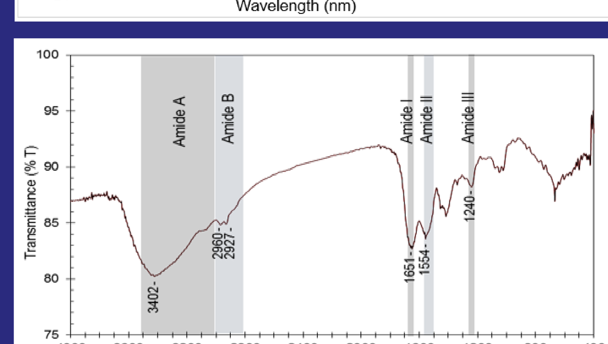

Figure 3. Fourier Transform InfraRed (FTIR) spectra of jellyfish collagen. Typical spectra of collagen.

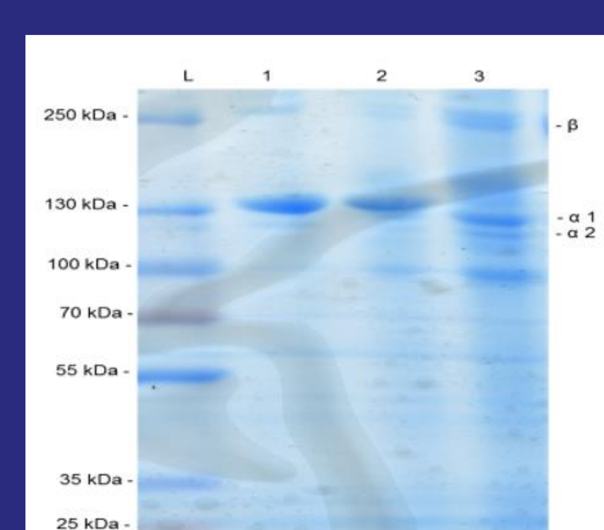

Figure 4. SDS-PAGE (9%) pattern of analyzed collagen samples. L: Page Ruler Prestained protein ladder – 0 to 250 kDa; 1: type I collagen from bovine skin; 2: type II collagen from chicken; 3: Col. The results indicate the Col are collagen type II.

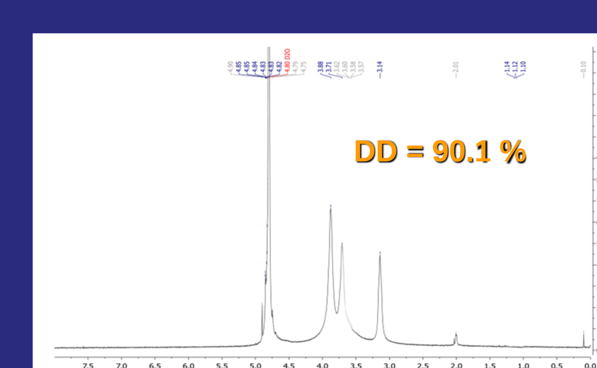

Figure 5. ^{1}H-NMR spectra obtained for the chitosan at temperature of 25 ºC to obtain the Deacetylation Degree (DD).

Samples	Mn (KDa)	Mw (KDa)
Col	113,1 (± 12,2)	144,4 (± 9,7)
Chi	186,7 (± 0,5)	348,2 (± 60,6)
Fu	49,7 (± 0,1)	120,0 (± 5,6)

Table II. The weight average molecular weight (Mw) and the number average molecular weight (Mn) of collagen (Col), chitosan (Chi) and fucoidan (Fu).

2. Cryo-biomaterial characterization

Samples	Abbreviation of each composition	Composition		
Hydrogel 1	C1	3 % Col	3 % Chi	5 % Fu
Hydrogel 2	C2	3 % Col	3 % Chi	10 % Fu
Hydrogel 3	C3	5 % Col	3 % Chi	5 % Fu
Hydrogel 4	C4	5 % Col	3 % Chi	10 % Fu

Table III. Composition of the four biomaterial (hydrogels) by the ratio of the biopolymers.

Samples	Ratio $\frac{Sulfur\ (S)}{Carbon\ (C)}$	Ratio $\frac{Sulfur\ (S)}{Nitrogen\ (N)}$
C1	0,017	1,783
C2	0,010	0,755
C3	0,021	0,651
C4	0,018	0,294

Table IV. Ratios between sulfur/carbon and sulfur/nitrogen atomic concentrations in the studied cryo-biomaterial. Presence of sulfur (fucoidan) and the nitrogen (chitosan and collagen).

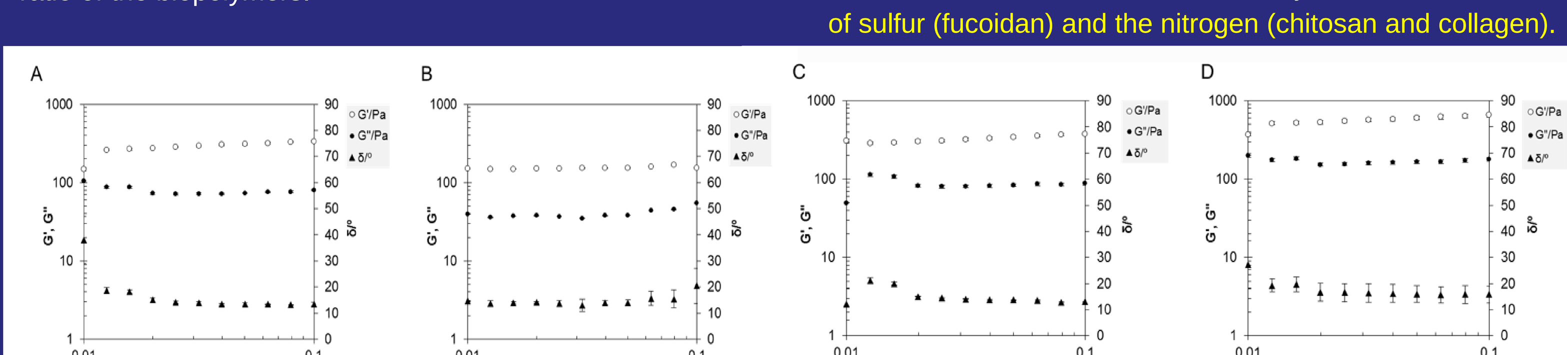

Figure 6. Average of elastic (G´), viscous (G") and viscoelastic grade (δ/º) modulus as a function of the frequency for different biomaterials (A-C1; B-C2, C-C3 and D-C4). The results indicates a strong elastic-solid character.

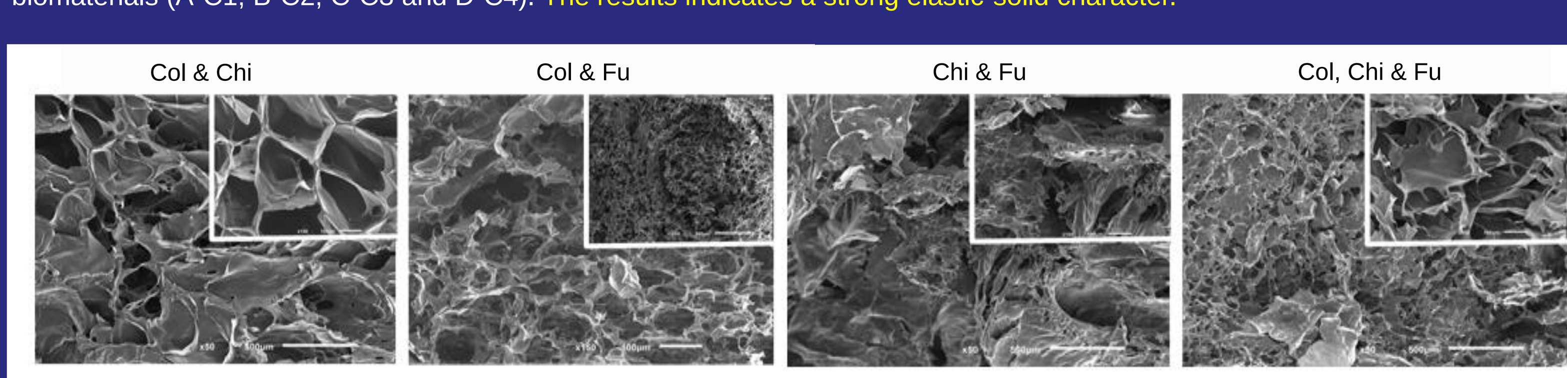

Figure 7. Scanning electron microscope (SEM) images of combination of two biopolymers (3% Col & 3% Chi); (3% Col & 5% Fu) and (3% Chi & 5% Fu) and one condition biomaterial (5% Col, 3% Chi & 5% Fu) . All images at the magnification of 50x, scale bar: 500 µm (and inserts with magnification of 150x, scale bar: 100 µm).

3. Cryo-biomaterial *in vitro* assessment

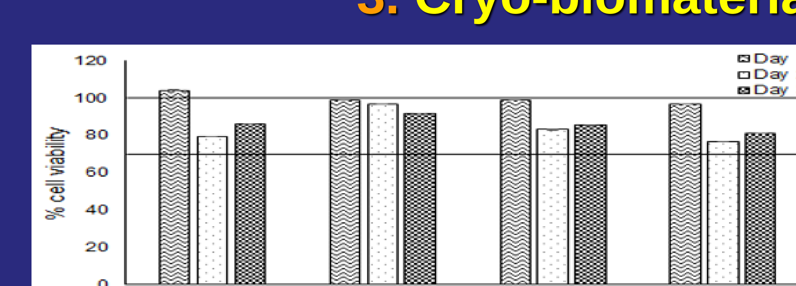

Figure 8. Cytotoxicity assessment using MTS assay in biomaterials (C1, C2, C3 and C4) with L929 cell lines. The percentage of the cell metabolic activity in respect to control (>70%) suggest the viability of the developed cryogels.

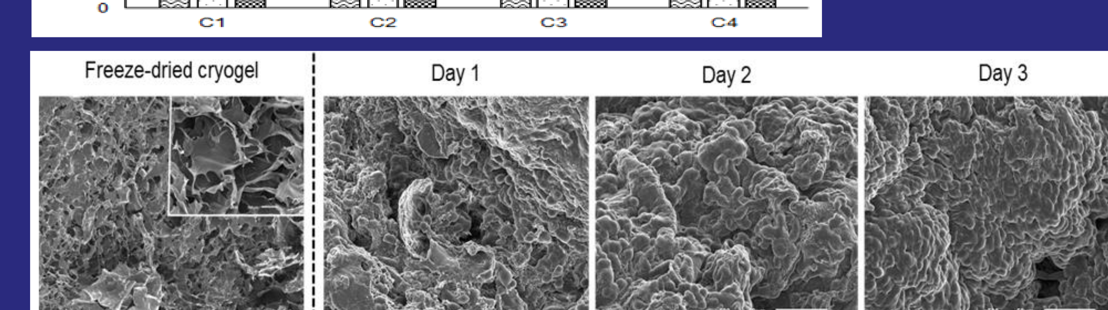

Figure 9. Scanning electron microscope (SEM) images of C1 – freeze-dried biomaterial at the magnification of 50x, scale bar: 500 µm (insert with magnification of 150x, scale bar: 100 µm) and of the fixated biomaterial with L929 cells after 3 days of culture.

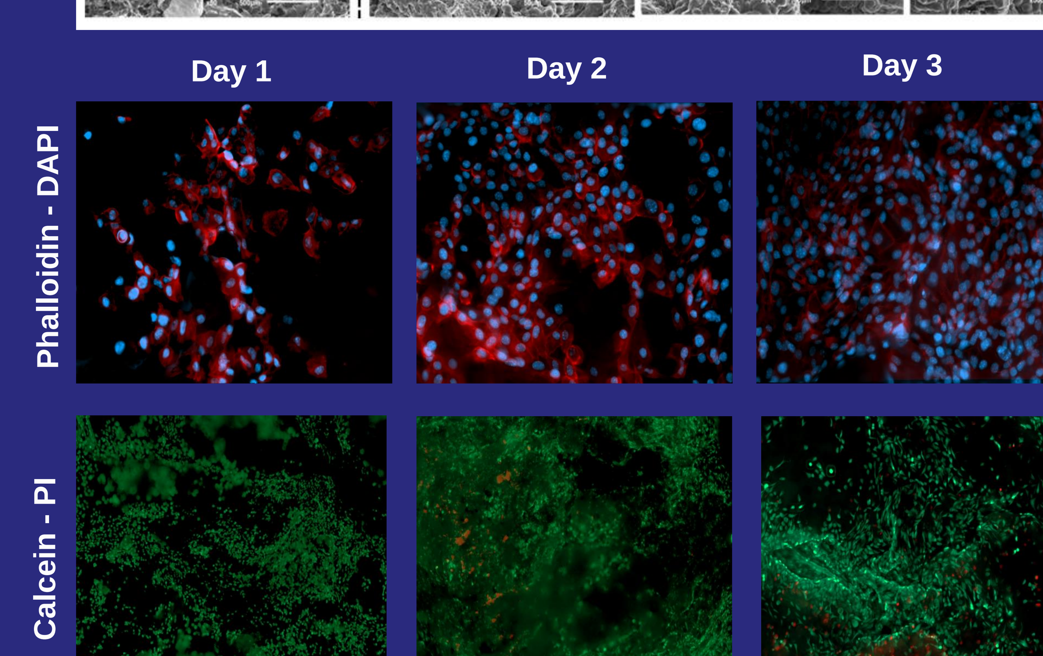

Figure 10. Schematic representation of the fluorescence microscopy images obtained in Live/dead assay (Calcein-PI) and in the assessment of cell morphology (Phalloidin-DAPI) during up to three days of cell culture.

The structures provide a good microenvironment for cellular viability during the culture time.

Future Perspectives

The marine origin materials under study are an economically viable alternative to mammal-origin materials, supporting the production of hydrogels as biomaterials for cell culture envisaging tissue engineering, having similar cytocompatibility, mechanical stability, non-cytotoxic behaviour, arising as potential providers of a proper microenvironment for cell proliferation.

These biomaterials can respond to the requirements of personalized treatments, including cartilage regenerative procedures in biomedical approaches.

References:

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[3] Hoyer, B. *et al.* (2013). Doi: 10,1016/j.actbio.2013.10.022.
[4] Ferraro et al. (2016). Doi: 10.1016/j.tifs.2016.03.006.

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