Marine-based Collagen Membranes by electrospinning technique as Biomaterial for Tissue Engineering Applications

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INTRODUCTION

Tissue Engineering strategies seek the development of an ideal scaffold that can perfectly replicate the extracellular matrix (ECM) of the targeted tissue, giving to cells the 3D support they need for the biosynthesis of new tissues by biological stimulation on molecular level, i.e. support cell attachment, proliferation, differentiation and organization. To achieve this goal, electrospinning rises as an effective approach to fabricate scaffolds that can mimic ECM [1]. Through the control of electrospinning parameters and polymer choice, both synthetic (e.g. poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA)) and natural (e.g. collagen, chitosan, keratin), submicron- or nanometre-scale fibres can be produced.

In the present work, we propose the use of natural polymers or blends of synthetic and natural polymers on the development of electrospun membranes to produce new biomaterials envisaging Tissue Engineering (TE) applications such as corneal, bone and wound regeneration. For bone regeneration applications, a combination of ceramic and polymer is needed taking into account the natural bone tissue composition. Formulations composed by hydroxyapatite (**HAp**, the major inorganic component of human bone), polycaprolactone (**PCL**, a biodegradable synthetic polyester) and marine-derived Collagen (**Col**) and Gelatin (**Gel**) are being studied. Marine derived collagen and gelatin stand up as an alternative to the mammals sources, which present religious and social/life style constraints, disease transmission-connected reasons (e.g. bovine spongiform encephalopathy or BSE) and potential allergenic behavior, as well as to human collagen produced by recombinant technologies, associated to high costs [2, 3, 4].

In this work, Blue shark and Atlantic cod skins, by-products from Galicians and Portuguese fishing industries, were used as raw-materials for the production of marine collagen and gelatin, further characterized to assess their purity and biochemical features. The ceramic component chosen (β-TCP/HAp) was synthesized by our group for the establishment of processing methodologies, but marine origin HAp will be further used. The different materials were electrospun in different combinations (PCL; Col/Gel; PCL + Col/Gel; PCL+HAp; PCL + Col/Gel + HAp) and characterized to address morphological (SEM), chemical (FTIR) and physical (water content) characteristics. Mechanical performance upon tensile stress will be studied, as well as biological evaluation with different cell types according to the TE application.

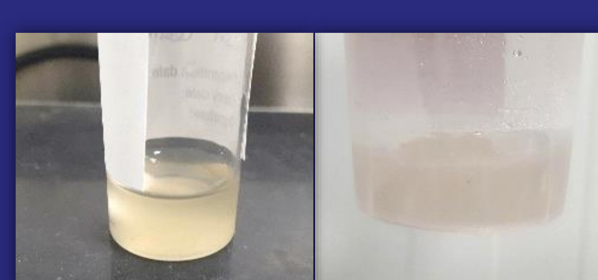
MATERIALS AND METHODS

❑ Samples and solutions

Collagen and Gelatin extracted from Blue Shark by collaborators from IIM-CSIC, Vigo.

Blue Shark Gelatin

Blue Shark Collagen



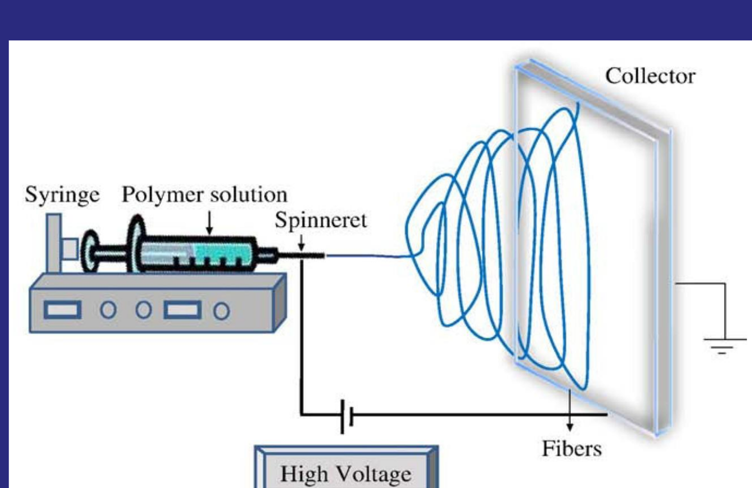
- 20% (w/v) Collagen and Gelatin were dissolved in 40% acetic acid under stirring.

- 20% (w/v) PCL were dissolved in Glacial acetic acid under stirring and further mix with 5% (w/v) of HAp.

❑ Electrospinning set-up

Membranes for cornea regeneration

Horizontal apparatus to obtain random electrospun fibers. Adapted [5].



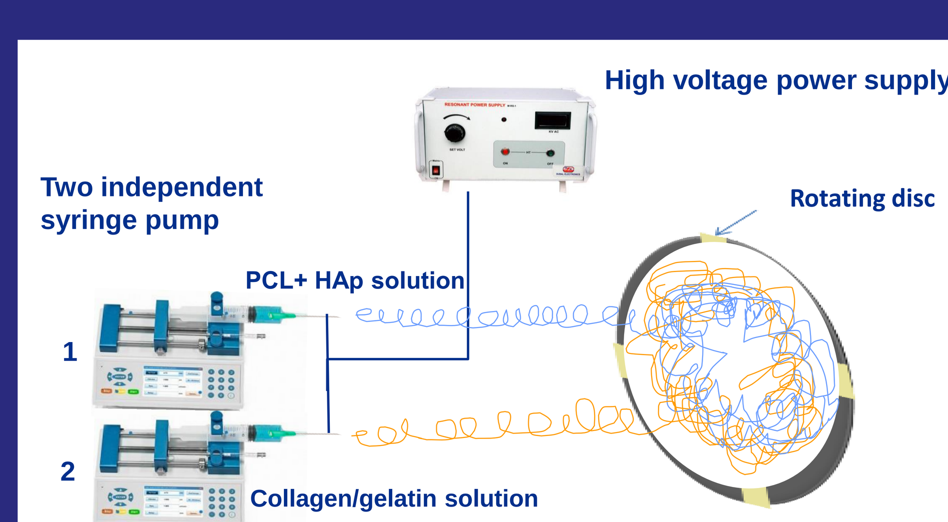
Electrospinning of collagen/gelatine solution.

Set-up conditions:

16 kV
14 cm
1 ml/h

Membranes for bone/periosteum

Horizontal apparatus with two independent syringe pumps and a rotating disc as a collector.



Syringe 1: 20% (w/v) PCL + 5% (w/v) HAp solution.

Syringe 2: 20% (w/v) Collagen/gelatine solution.

❑ Chemical & Physical Characterization

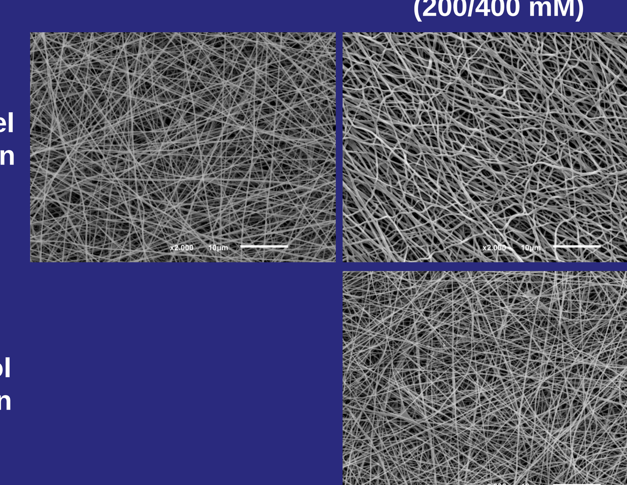
SEM
FTIR
Water content

RESULTS AND DISCUSSION

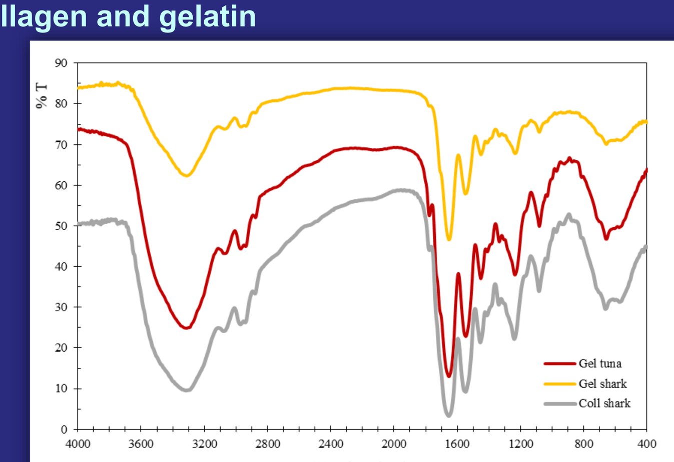
❑ Chemical & Physical characterization

Membranes for cornea regeneration

SEM images of gelatin and collagen nanofibers produced by electrospinning.



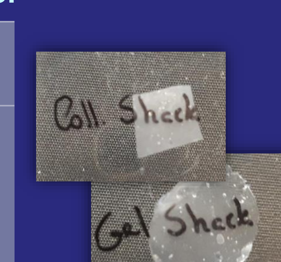
FTIR spectra showed the characteristic bands for collagen and gelatin



- Both collagen and gelatin exhibited comparable IR absorptions.
- Characteristic bands of collagen are present (Amide A, Amide B, Amide I, Amide II and Amide III).

Values for water content (%)in 0,1M PBS.

Condition	Water content (%)
20% Gel Blue Shark in AcOH	88
20% Coll Blue Shark in AcOH	79
Human Cornea	80

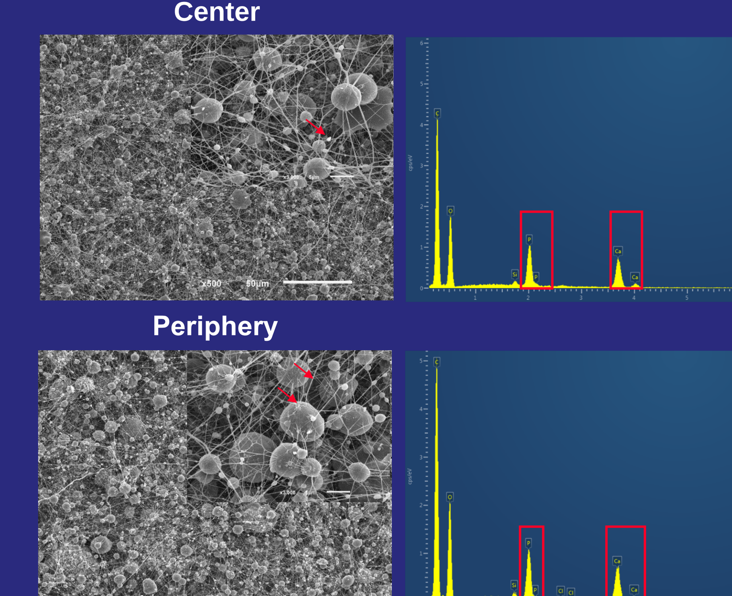


- All conditions showed similar values of water content to Human Cornea.
- When hydrated membranes are easy to handle and transparent.
- Stable in water (4 months) and PBS (2 months).

Membranes for bone/periosteum

SEM images of blending of Gel+PCL+HAp nanofibers produced by electrospinning.

Center



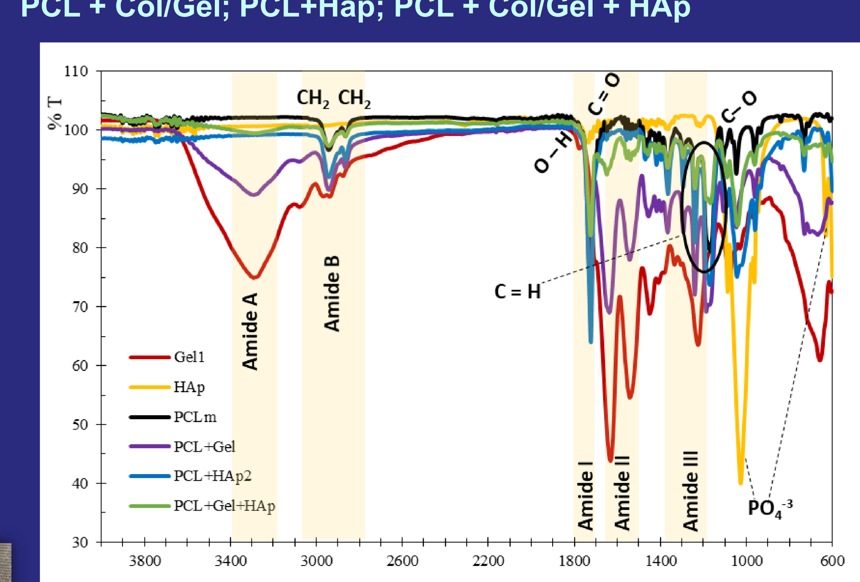
Ceramic content (wt%)
P = 8.3
Ca = 16.2

Periphery

Ceramic content (wt%)
P = 7.5
Ca = 13.9

- Membranes are fragile and very difficult to detach from parchment paper.
- The membranes present a lot of PCL beads and HAp agglomerates.
- It was observed the presence of HAp particle on surrounding the PCL fiber but also impregnated the fiber.

FTIR spectra showed the characteristic bands for PCL; Col/Gel; PCL + Col/Gel; PCL+Hap; PCL + Col/Gel + HAp



- Characteristic bands of gelatin are present (Amide A, Amide B, Amide I, Amide II and Amide III).
- The C–O, C=O, and C=H bands were related to PCL and the P – O and O – H bands were attributed to HAp.

FINAL REMARKS AND CONCLUSION

Membranes for cornea regeneration

- ❖ Optimize the crosslinking strategy;
- ❖ Evaluate the mechanical properties and optical features of the membranes;
- ❖ Analyse the cytotoxicity and bioactivity of these membranes using corneal-derived cells.

Membranes for bone/periosteum

- ❖ We think that acetic acid is degrading the PCL and HAp particles.
- ❖ Change the solvent of PCL for Chloroform : DMF to get better fibbers.
- ❖ Evaluate the mechanical properties and optical features of the membranes;
- ❖ Analyse the cytotoxicity and bioactivity of these membranes using osteoprogenitor cells.

The obtained results confirmed the successful electrospinning of marine collagen/gelatin membranes with smooth surface and porous structures. The membranes for corneal applications showed a water content value close to the Human cornea, demonstrating that could be good to retain tissue fluid and keep the nutrients in corneal tissue.
This work showed the potential of using codfish skin collagen as a sustainable and low-cost platform for biotechnological valorization of codfish by-products towards biomedical applications.

References:
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