Sericin nanofibers:
from by-product to innovative resource for cosmetics

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BtBs Department of Biotechnology and Biosciences

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Fondazione CARIPLO

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Bicocca Biotech @BtBs

RITESSERE: Silk Sericin materials from textile industry by-products

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- **PI of the RITESSERE project: S. Vesentini**
- Degumming and sericin production.
- Elettrospinning.
- Economic and strategic analysis.

✓ Biomechanics Group (S. Vesentini, I. Dragojlov, R. Aad)
✓ Industry 4.0 lab (S. Terzi, P. Rosa)

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- Sericulture and cocoon production.

✓ Lab. Gelsibachicoltura (S. Cappellozza, A. Saviane)

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grant n° 2022-0529

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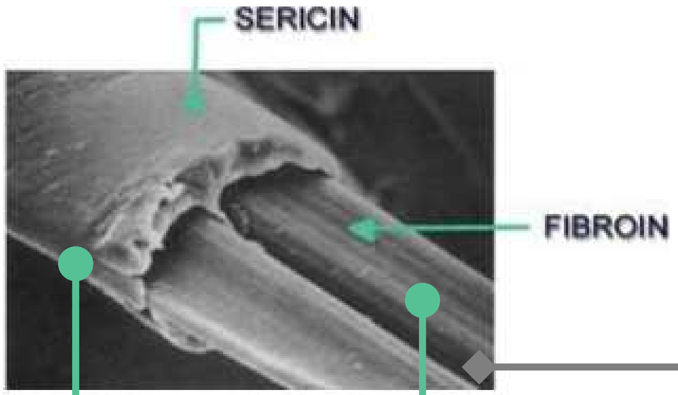
- Bio-analytical characterizations.
- Functionalizations and chemical modifications.

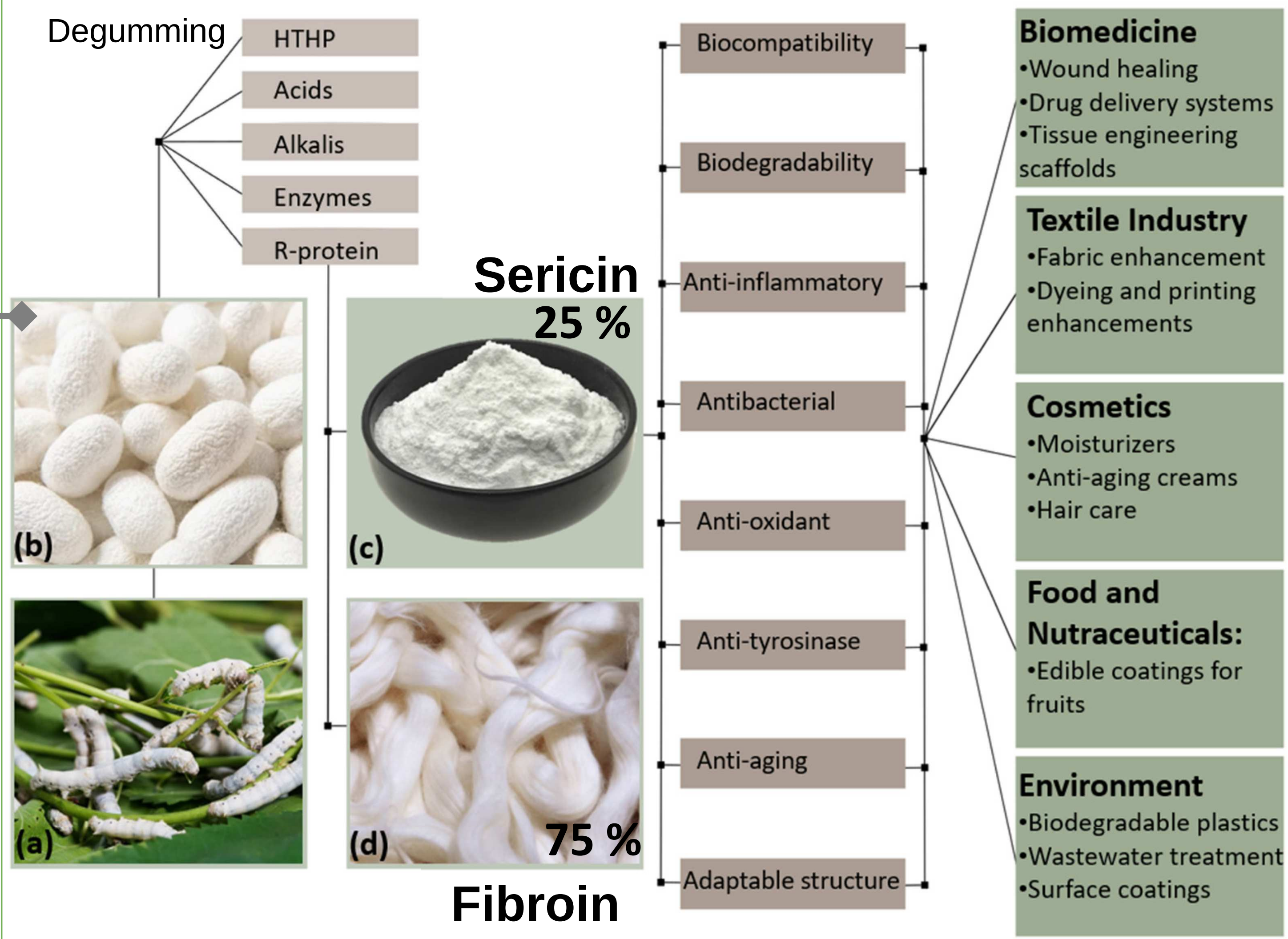
✓ Lab. Biophysics (A. Natalello, D. Ami)
✓ Lab. Organic Chemistry (L. Cipolla, S. Petroni)
✓ Lab. BioNMR (C. Airoldi)

Silk proteins



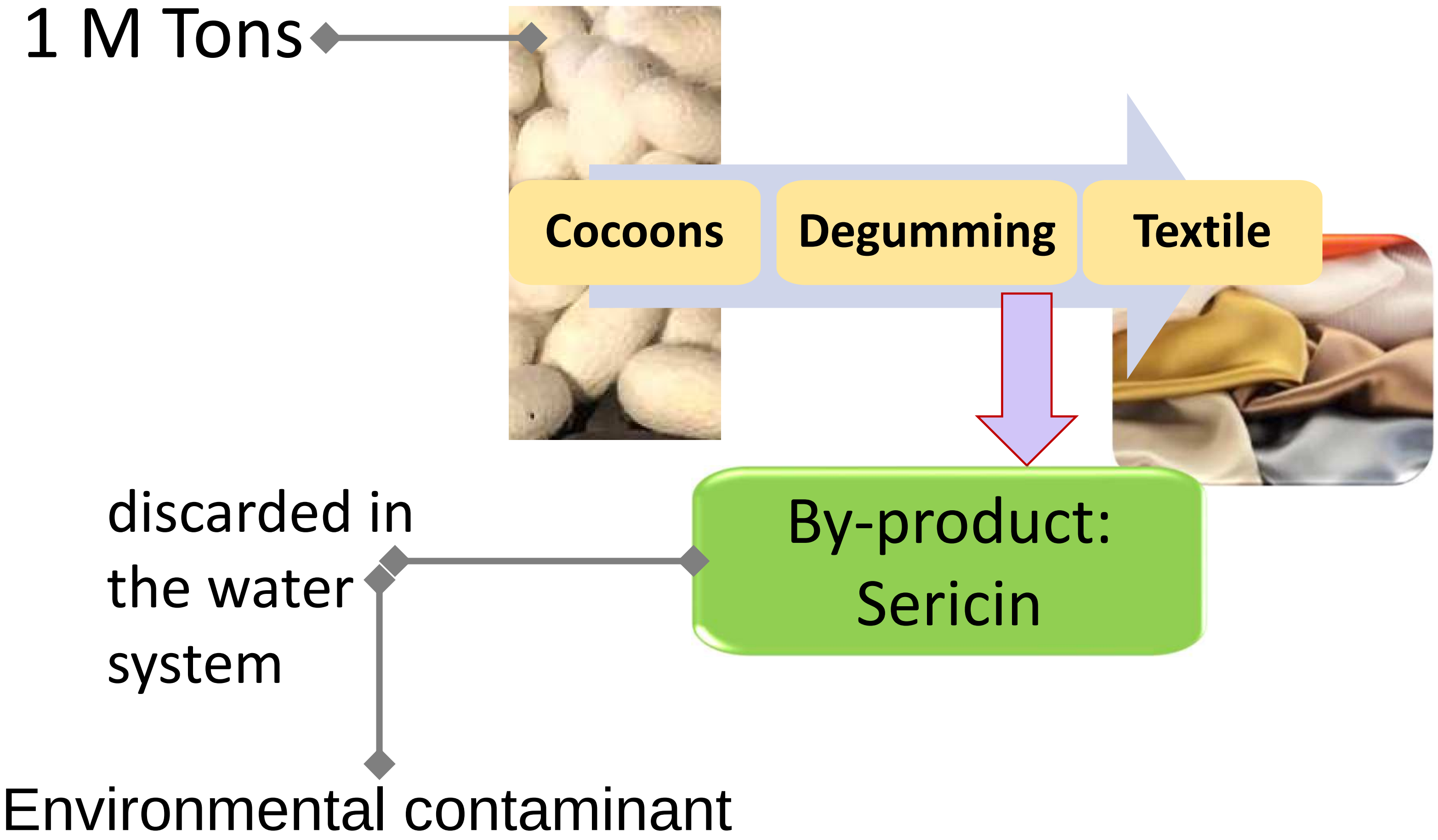
→By-product.
→Environmental contaminant (high oxygen demand for microbial degradation).

→The product of interest for the textile industry, medical applications,…

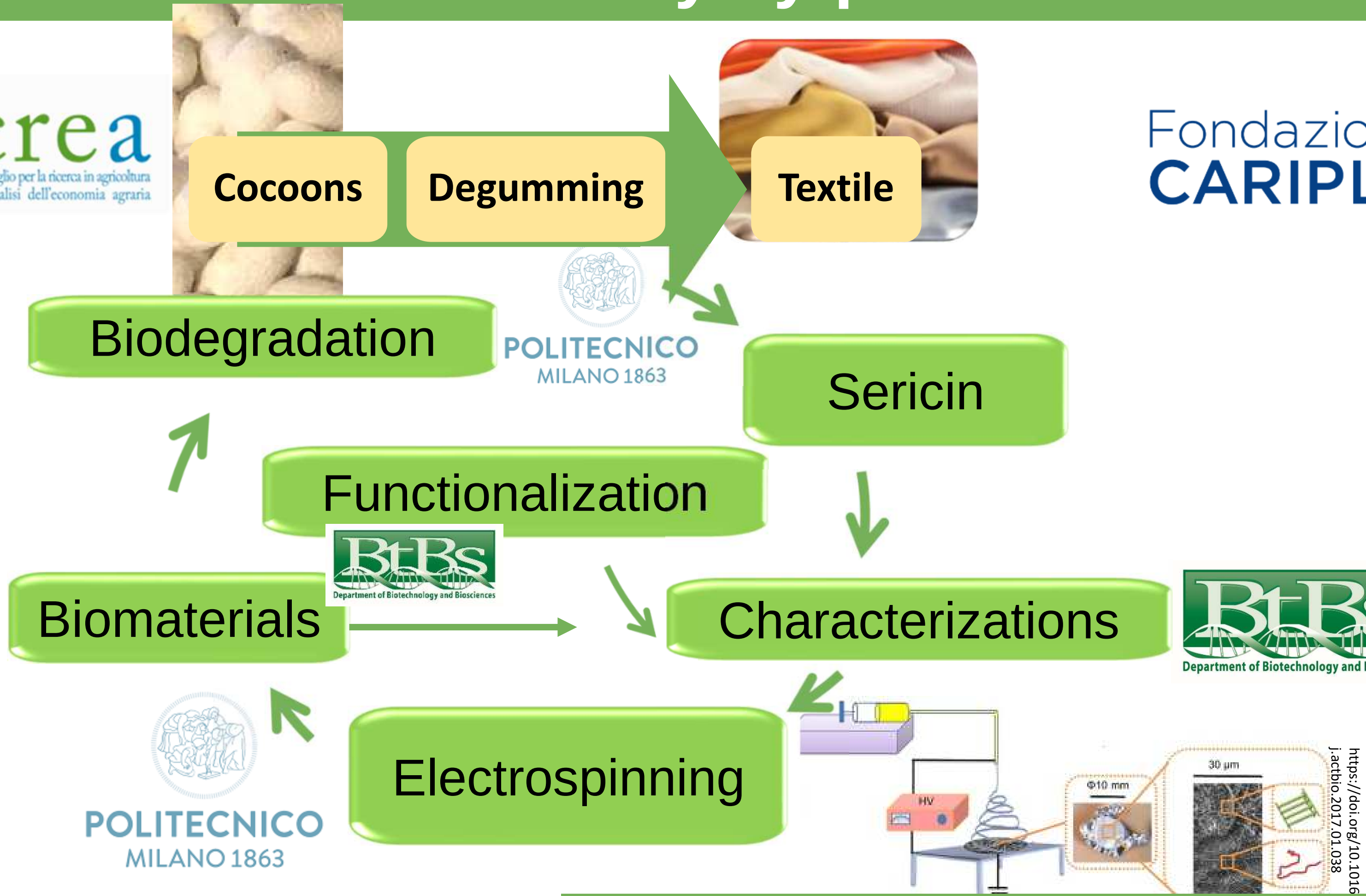


J. Funct. Biomater. **2024**, 15, 322

From the linear chain of silk



To RITESSERE: Silk Sericin materials from textile industry by-products



https://doi.org/10.1016/j.actbio.2017.01.038

Sericin molecular properties←→Biomaterials

- Different molecular weights (from 10 to > 400 kDa) and secondary structures depending on the extraction method, temperature, pH, pressure.
- AA composition:
Serine 41%
Glycine 13%
Aspartic acid 12%
Aromatic aa

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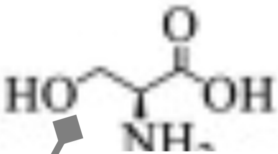
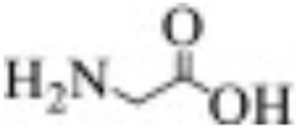
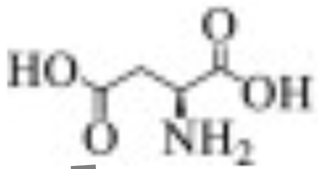


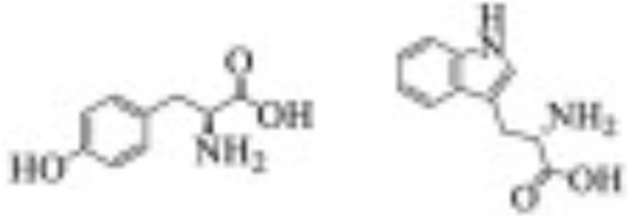
It provides hydrophilicity, aiding in water retention and moisturizing properties.



Contributes to flexibility and compactness



Adds negative charge, enhancing sericin's water solubility and binding with metal ions.



contribute to antioxidant properties

Sericin molecular properties←→Biomaterials

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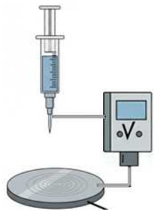
- Biological activities depend on molecular properties
- Spinnability depends on molecular properties



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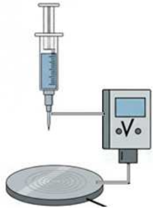

- It is NOT easily assembled into a stable membrane or film with good mechanical properties

Sericin molecular properties ←→ Biomaterials

Biophysical and Biochemical characterizations

Degumming method optimization

Electrospinning optimization

Functionalization and

Co-polymerization

- Different molecular weights (from 10 to > 400 kDa) and secondary structures depending on the extraction method, temperature, pH, pressure.
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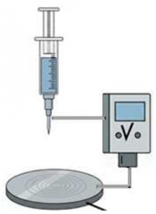

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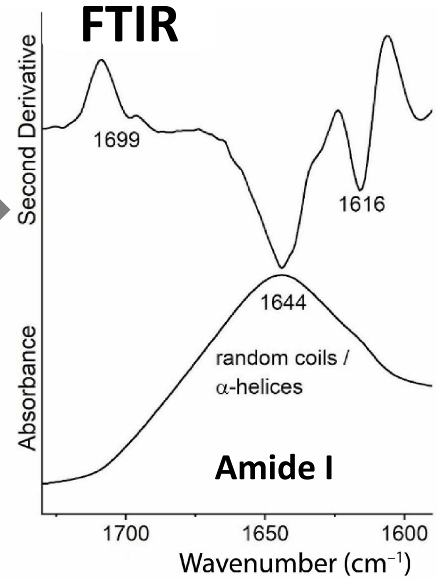
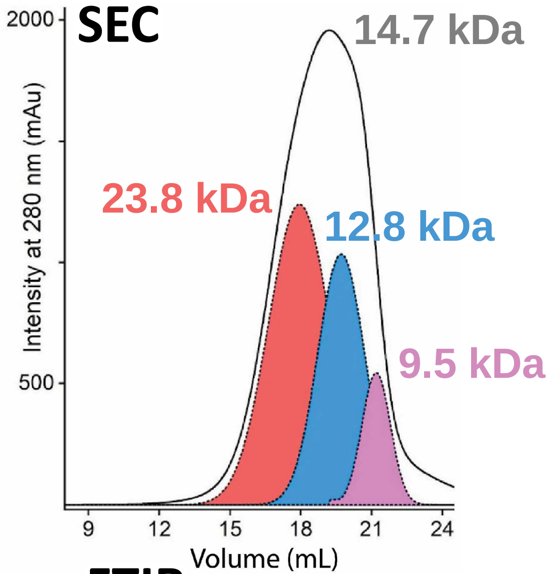
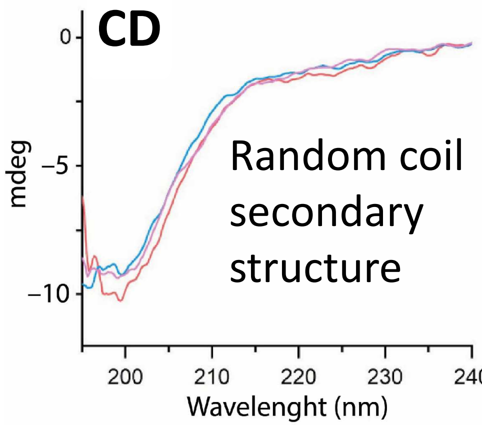
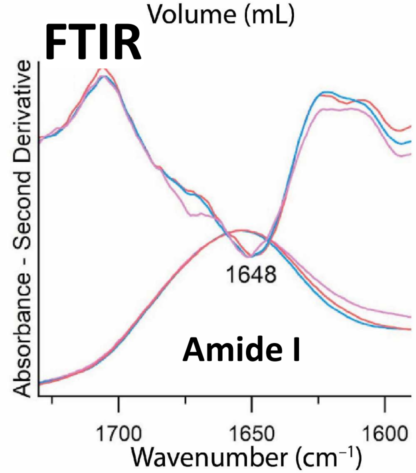
Characterizations of the HTHP sericin powder

SDS-PAGE and Mass Spectrometry:
Sericin 1



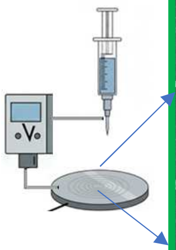


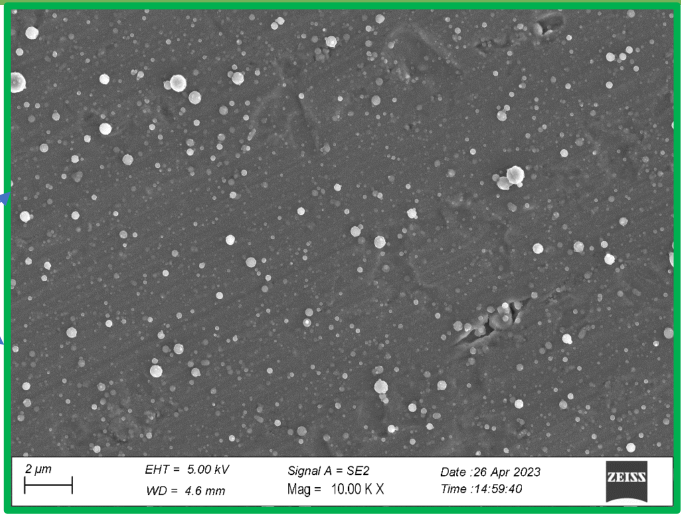


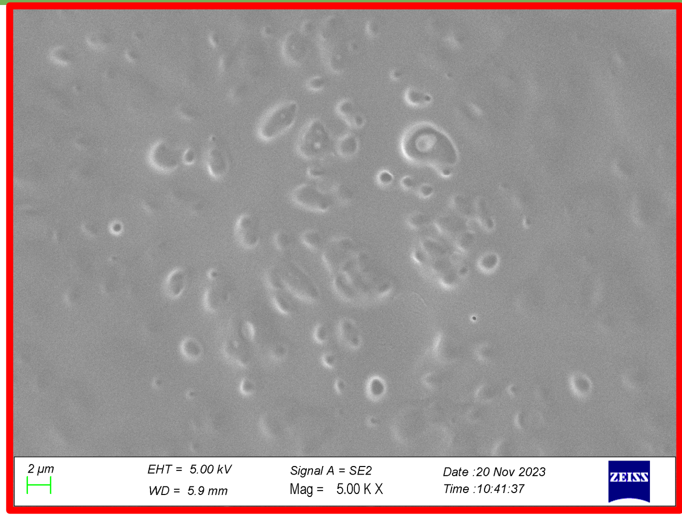


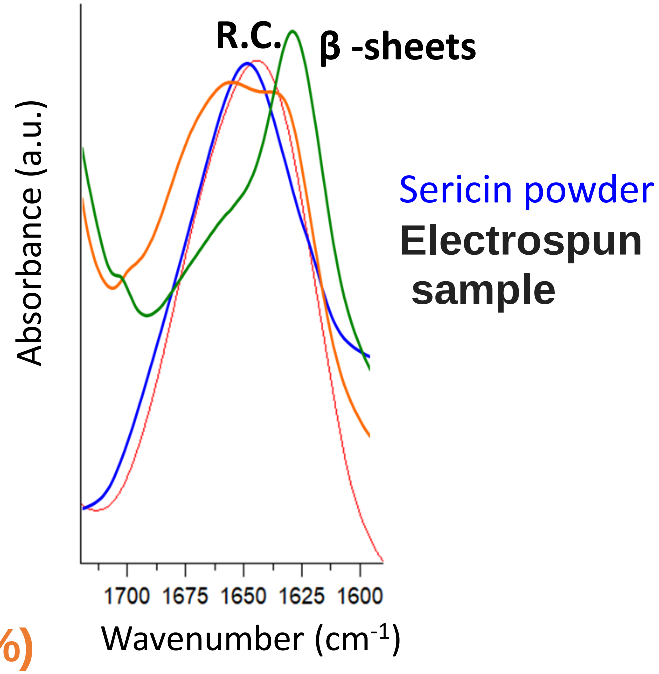
Molecules **2025**, *30*(3), 715; https://doi.org/10.3390/molecules30030715

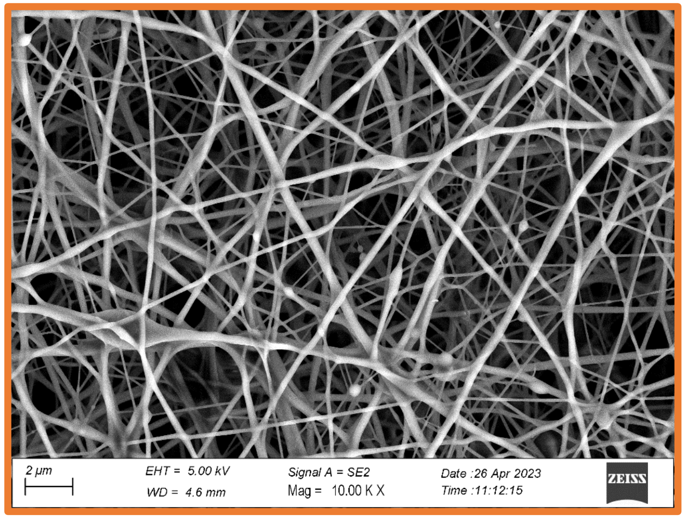
Optimization of the degumming and electrospinning (preliminary) conditions











from ↑R.C. to ↑β-sheets

from ↑R.C. to ↑ R.C.

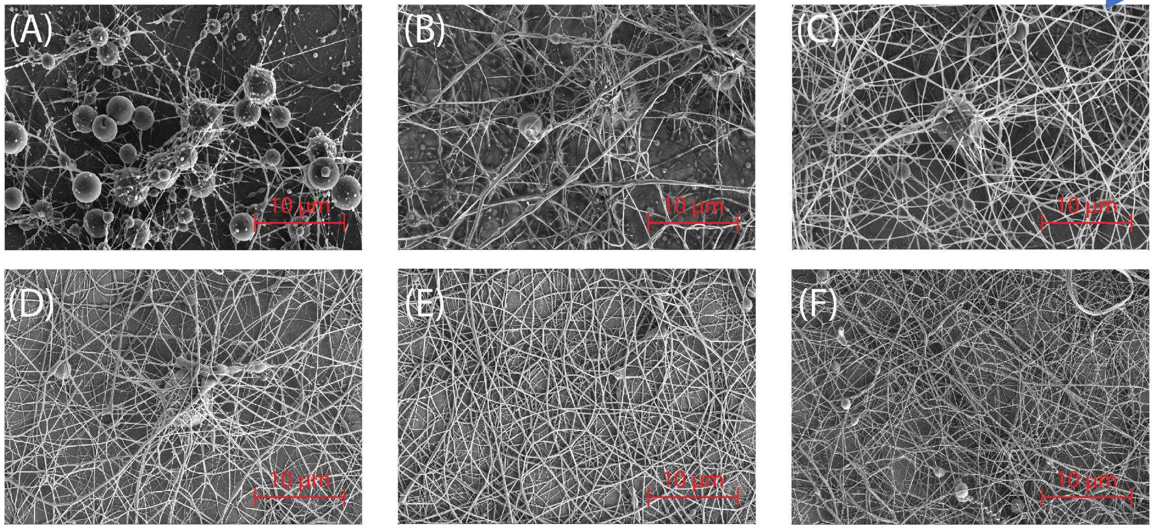
from ↑R.C. to R.C. + β-sheets (25-35%)

- ☑ The sericin source (i.e. molecular properties of the starting materials) and electrospinning conditions affect the possibility to obtain fibers in a three-dimensional matrix
- ☑ The internal structure of fibers in a three-dimensional matrix requires both R.C. and β-sheets

Morphological Properties of Sericin Electrospun Samples

Sericin concentrations

8 wt% 9 wt% 10 wt%



12 wt% 12 wt% 12 wt%

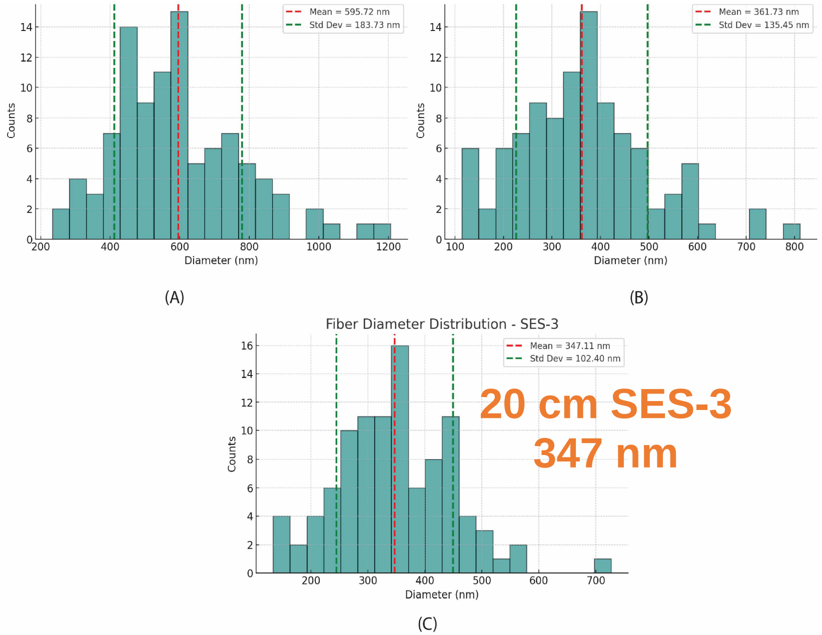
10 cm 15 cm 20 cm

Collector distances

Fiber diameters

10 cm SES-1
596 nm

15 cm SES-2
362 nm



20 cm SES-3
347 nm

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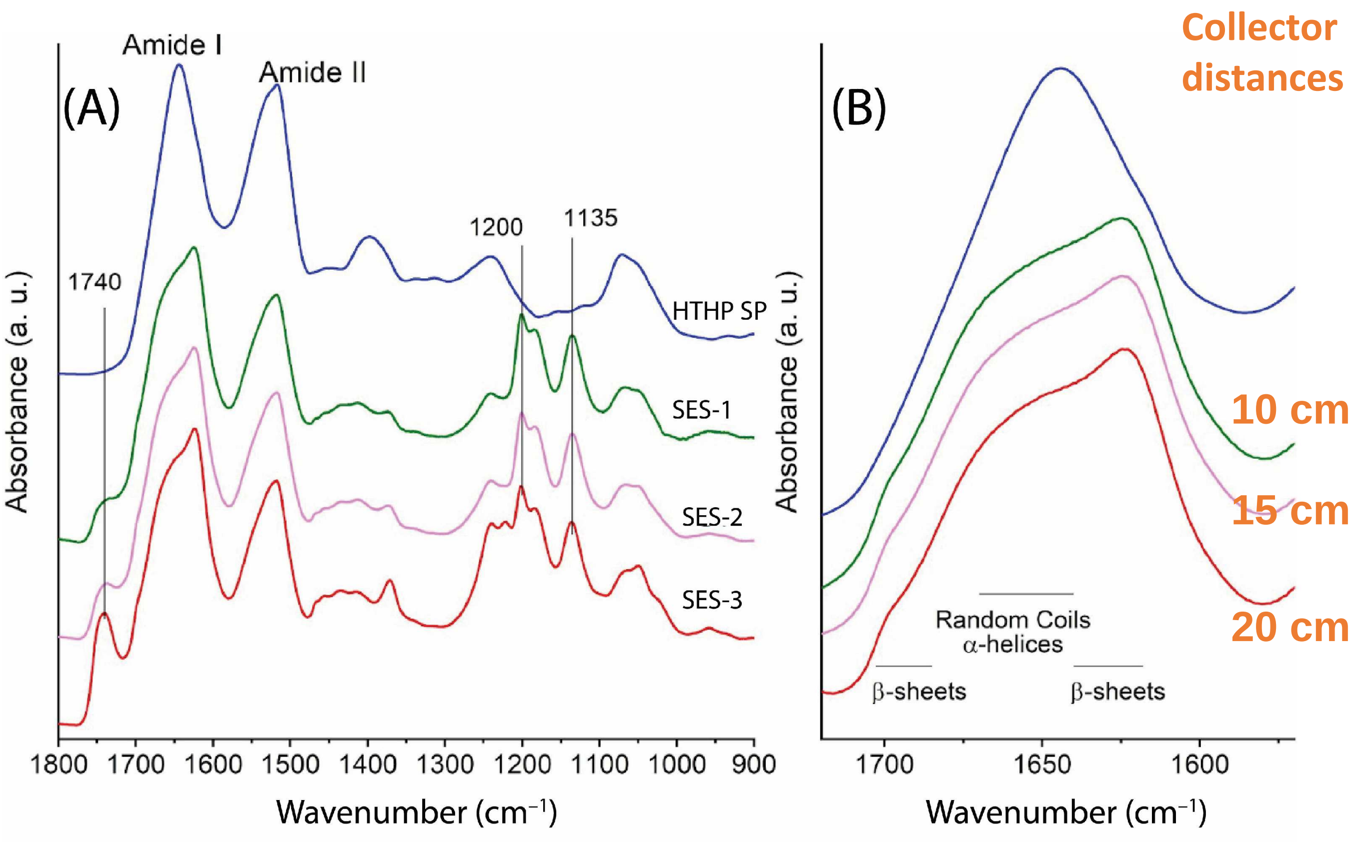
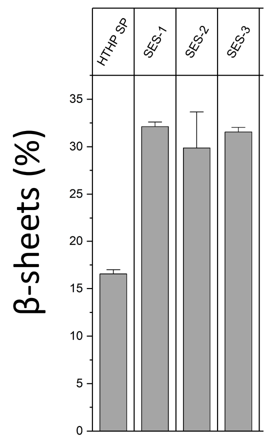
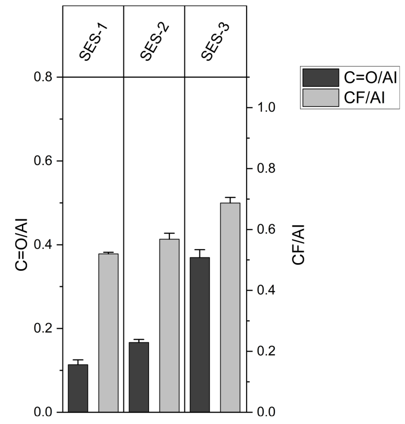
FTIR characterizations of <u>Sericin</u> Electrospun Samples

12 wt% Sericin







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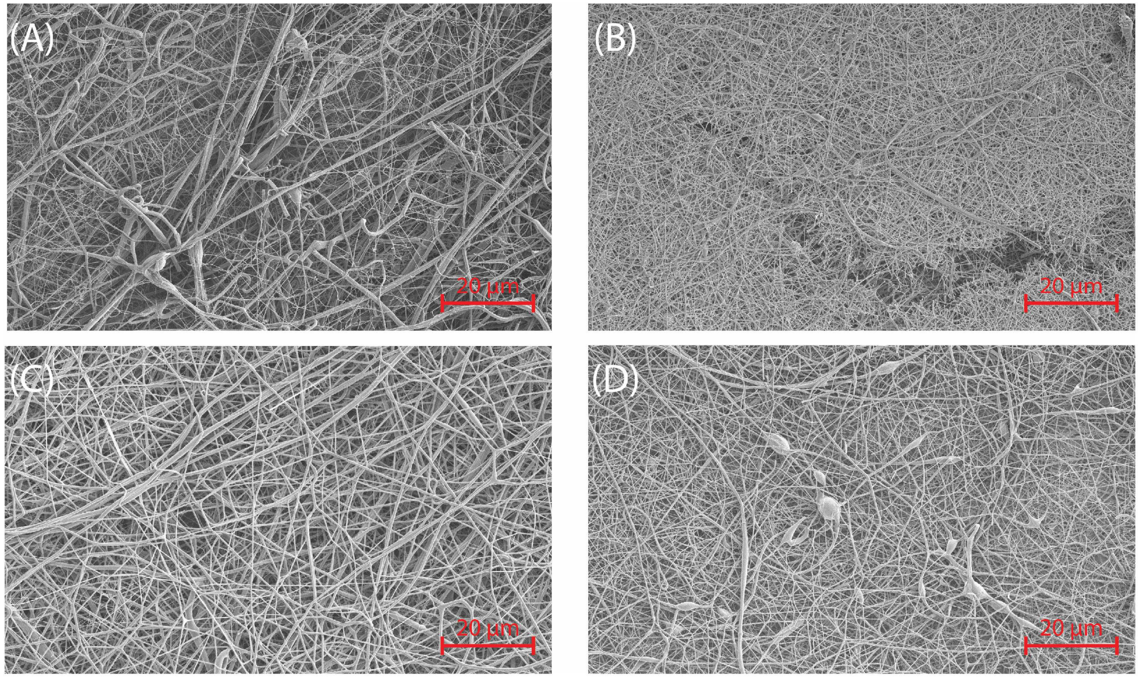
Morphological Properties of Sericin–Gelatin Electrospun Samples

SEM images of electrospun **sericin–gelatin (1:1)** fibers prepared at a **constant concentration of 12 wt%** with varying **collector distances and flow rates.**

10 cm at 1 mL/h (SGES-1) **15 cm at 0.8 mL/h (SGES-2)**



15 cm at 1 mL/h (SGES-3) **20 cm at 1 mL/h (SGES-4)**

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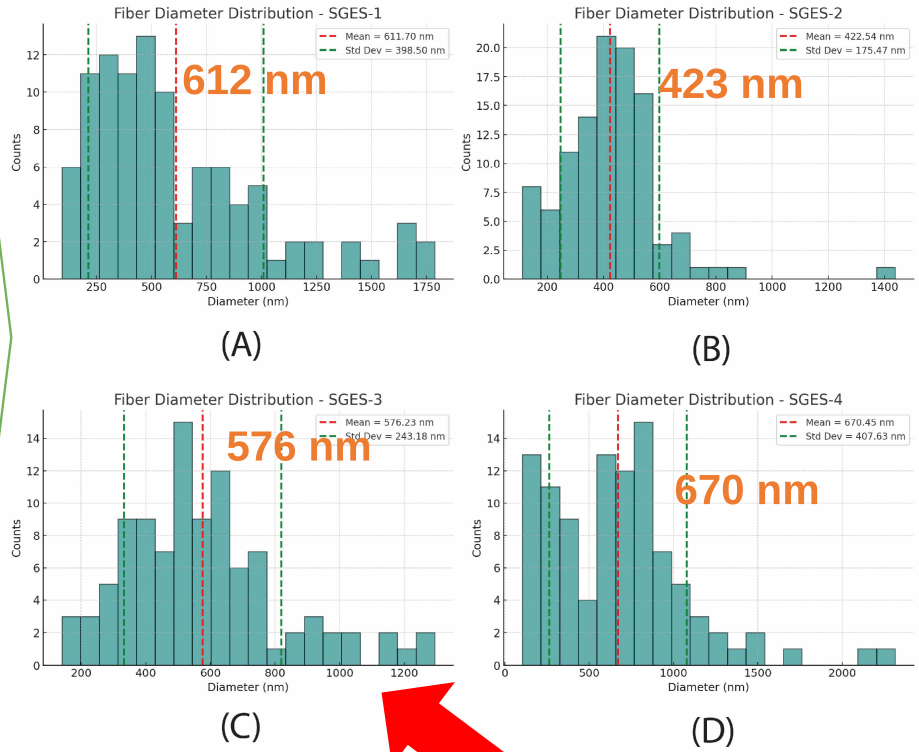
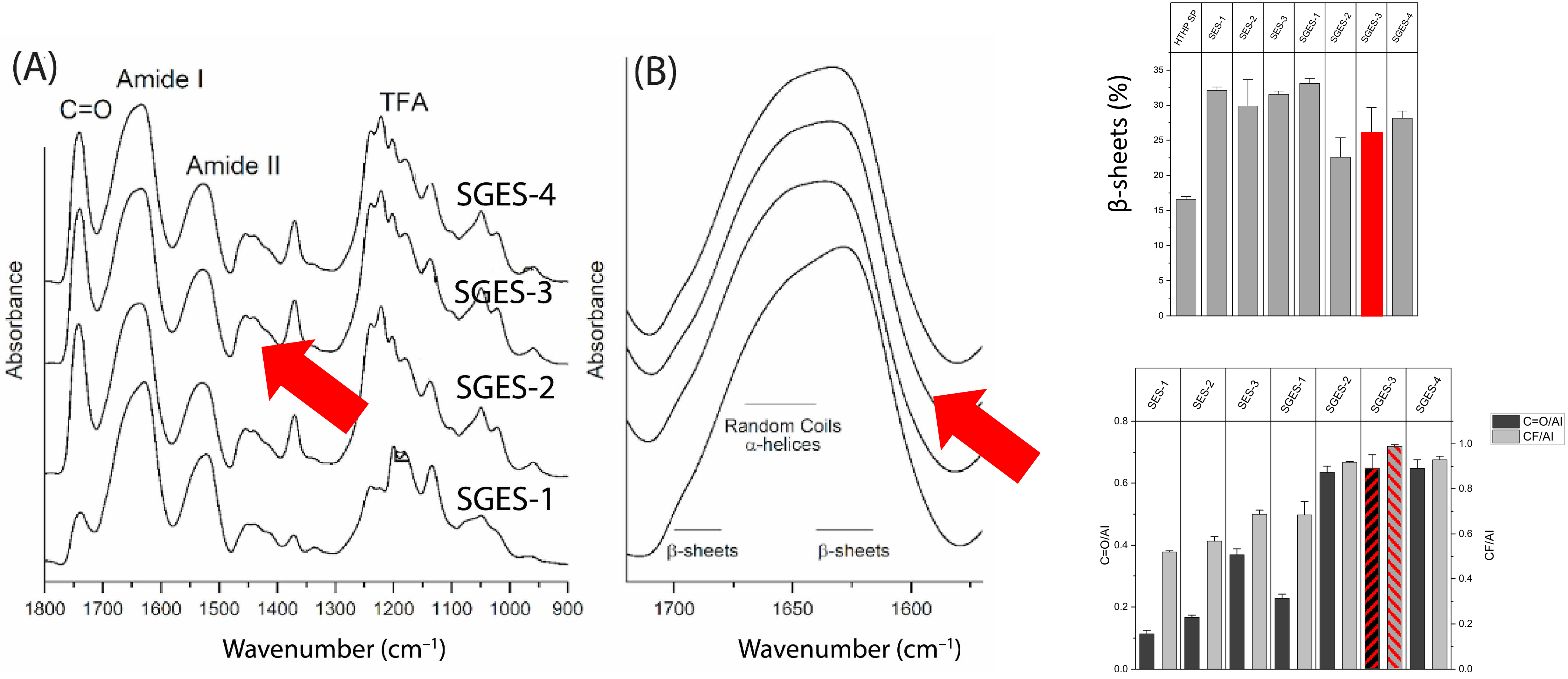
Fiber diameters

10 cm at 1 mL/h **15 cm at 0.8 mL/h**



15 cm at 1 mL/h **20 cm at 1 mL/h**

FTIR characterizations of Sericin–Gelatin Electrospun Samples



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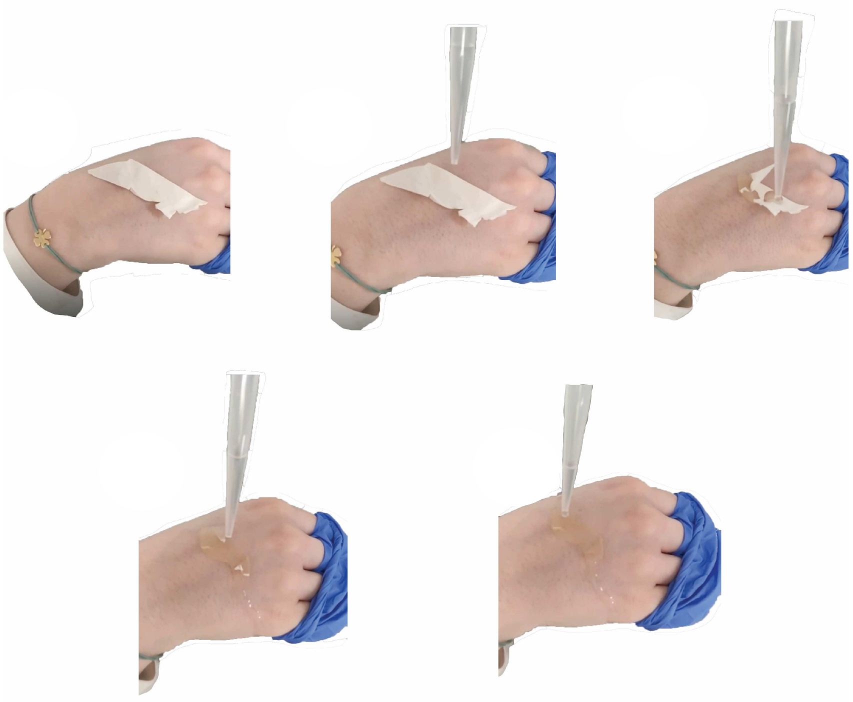
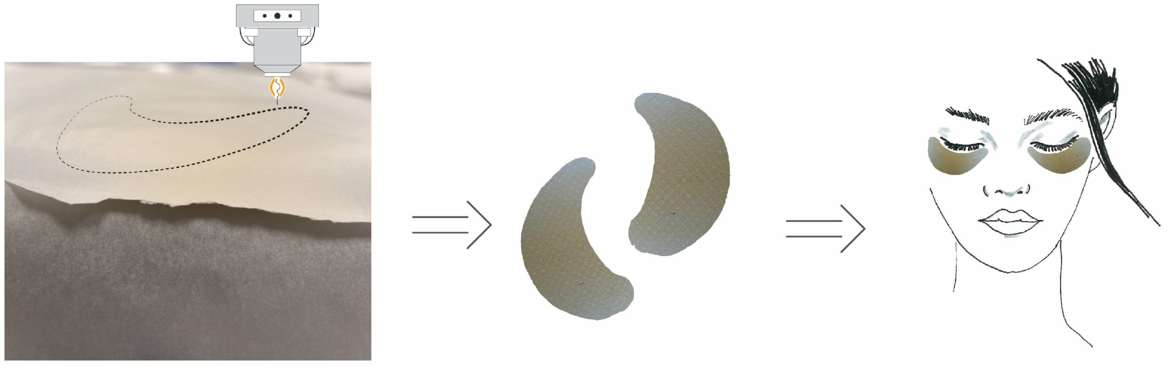
Eye contour patches prepared from sericin–gelatin electrospun films (SGES-3)





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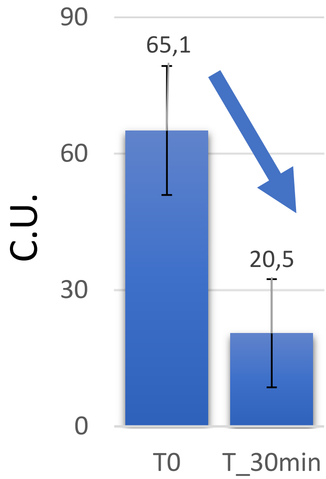
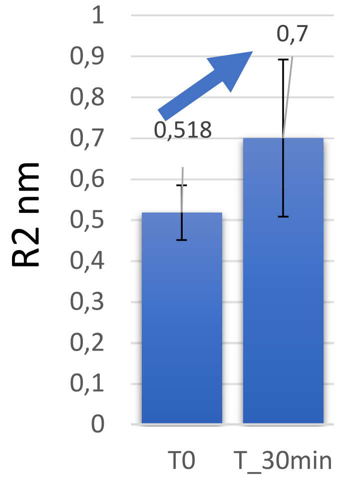
Cosmetic performance of sericin–gelatin electrospun films

Skin Hydration
(capacitance)

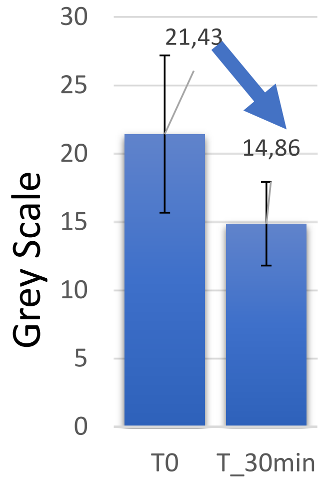


Skin gross Elasticity
(vertical deformation under controlled suction and release)

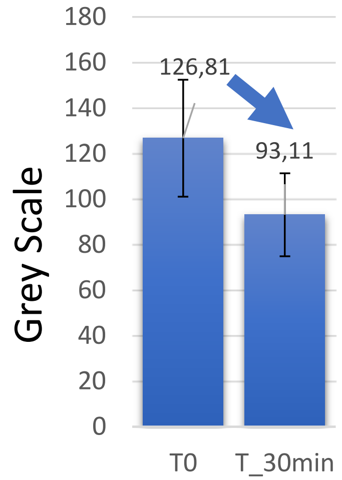


Skin Roughness
(image analysis)

Average



Average maximum



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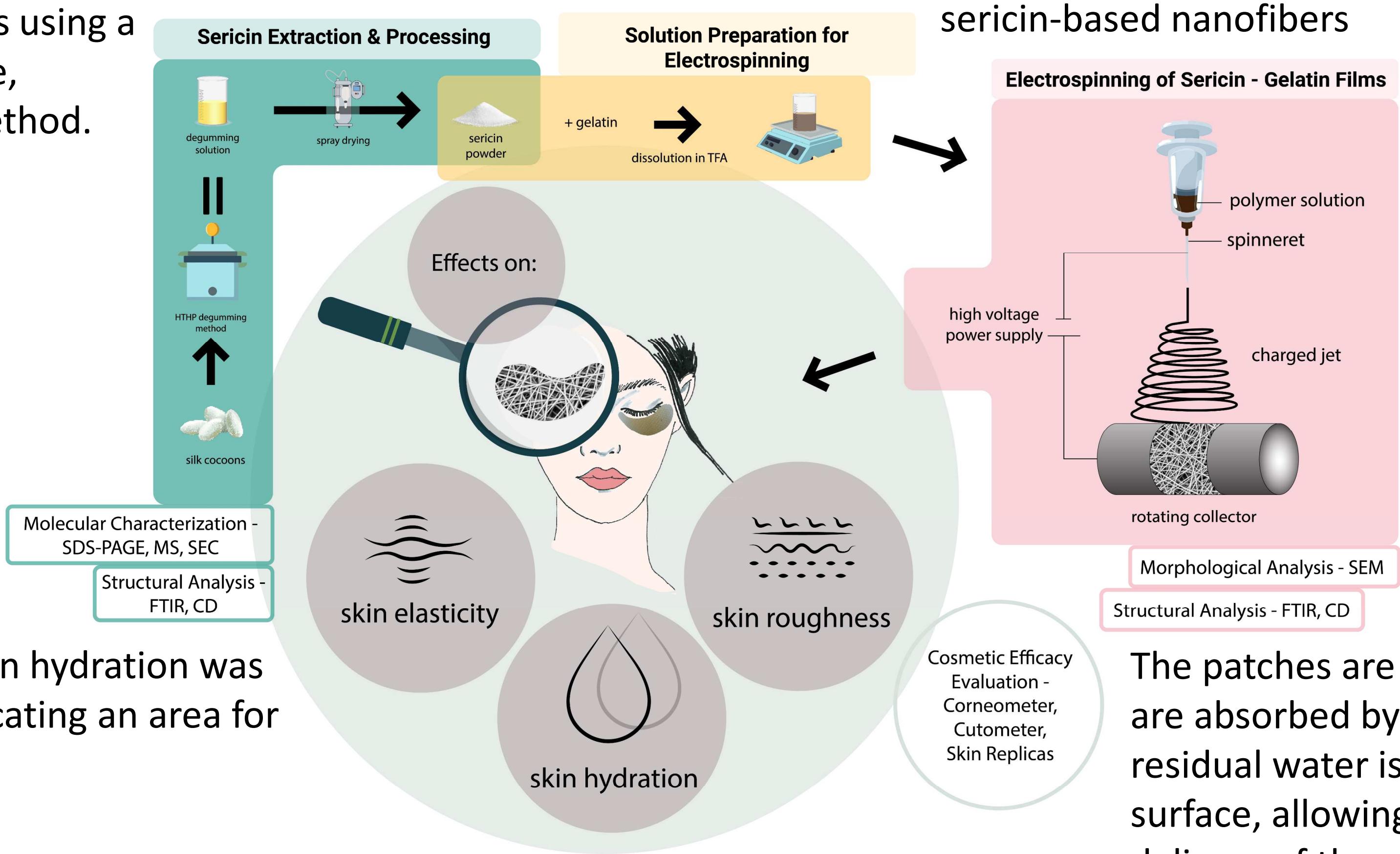
Conclusions

Silk sericin was successfully isolated from Bombyx mori silkworm cocoons using a high-temperature, high-pressure method.

Electrospinning was employed to fabricate sericin-based nanofibers



The electrospinning process significantly altered the secondary structure of sericin, leading to an increase in β-sheet content compared to the raw sericin powder.

A decline in skin hydration was observed, indicating an area for optimization

The patches are water-soluble and are absorbed by the skin when residual water is present on the surface, allowing for effective delivery of the protein to the target area.

The sericin/gelatin nanofiber patches demonstrated positive elasticizing and smoothing effects in the cosmetic evaluations.

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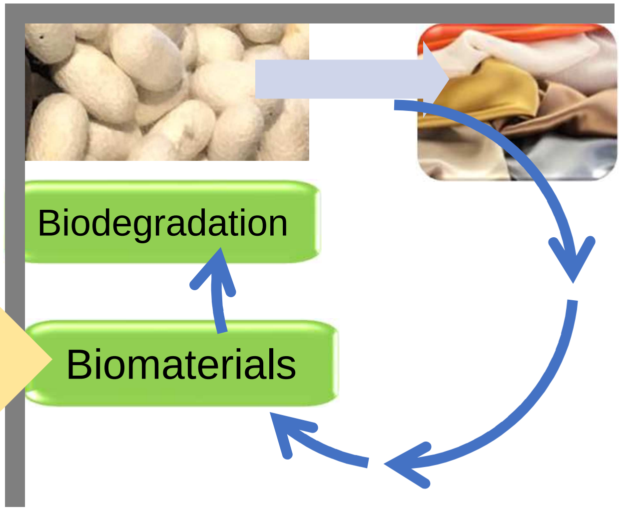
RITESSERE: Demonstration Activities

Sericin starting from certified silk cocoons

Optimizing the extraction process and electrospinning conditions

- ✓ **Facial sheet mask composed by sericin**
- ➢ **3D scaffold based on sericin for new cell culture substrates**
- ➢ **Sustainable packaging films based on sericin**



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Acknowledgements

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molecules

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MDPI

Article

Silk Sericin-Based Electrospun Nanofibers Forming Films for Cosmetic Applications: Preparation, Characterization, and Efficacy Evaluation

Ivana Dragojlov [1], Rony Aad [1], Diletta Ami [2], Marco Mangiagalli [2], Antonino Natalello [2] and Simone Vesentini [1,*]

1 Department of Electronics, Information, and Bioengineering, Politecnico di Milano, 20133 Milan, Italy; ivana.dragojlov@polimi.it (I.D.); rony.aad@polimi.it (R.A.)
2 Department of Biotechnology and Biosciences, University of Milano-Bicocca, 20126 Milano, Italy; diletta.ami@unimib.it (D.A.); marco.mangiagalli@unimib.it (M.M.); antonino.natalello@unimib.it (A.N.)
* Correspondence: simone.vesentini@polimi.it

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2 Department of Biotechnology and B...iences, ...iversit... Milano-Bicocca, 20126 Milano, Italy; diletta.ami@unimib.it (D.A.); marco....ngiaga...unimib... M.M.); antonino.natalello@unimib.it (A.N.)

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Thank you!!!

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- ✓ Lab. Organic Chemistry (L. Cipolla, S. Petroni)
- ✓ Lab. BioNMR (C. Airoldi)

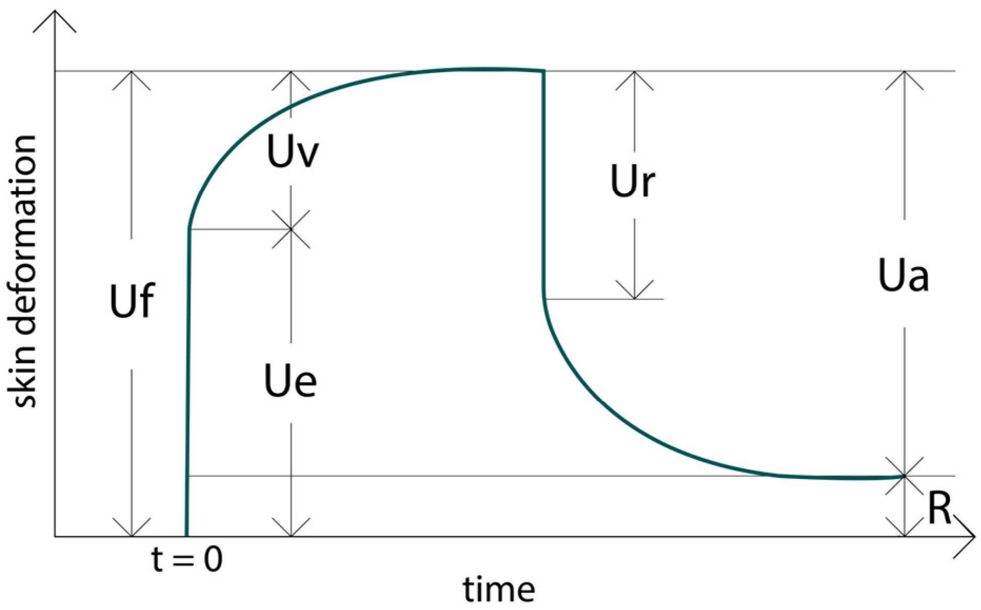
•Hydration: Assessed with the Corneometer® CM 825 to quantify water content in the stratum corneum.

The assessment of skin hydration levels was conducted using the Corneometer® CM 825, a widely utilized instrument in dermatological and cosmetic studies. The Corneometer operates on the principle of capacitance measurement, utilizing a square-shaped sensor with an area of 49 mm^2, covered by a specialized glass and mounted on a movable axis. This configuration facilitates accurate measurements by ensuring stable sensor–skin contact.

•Elasticity: Evaluated using the Cutometer® MPA 580 to measure biomechanical properties of the skin.

During the assessment, the skin was subjected to a constant air depression of 350 mbar for a duration of 3 s using the probe's opening, followed by a release phase of equal duration (3 s). This suction–release cycle was repeated three times on the same skin site to ensure accuracy and reproducibility of the measurements.

The deformation of the skin, expressed in millimeters (mm), was plotted as a function of time (in seconds), generating characteristic deformation curves for each cycle.



The graph displays the following components:

•UF (R_0, Skin Extensibility): The initial deformation of the skin caused by the applied force, reflecting its passive behavior under stress.

•UE (Elastic Deformation): The portion of deformation that occurs immediately due to the application of stress.

•UV (Visco-Elastic Creep): The time-dependent deformation that continues to occur after the initial elastic deformation.

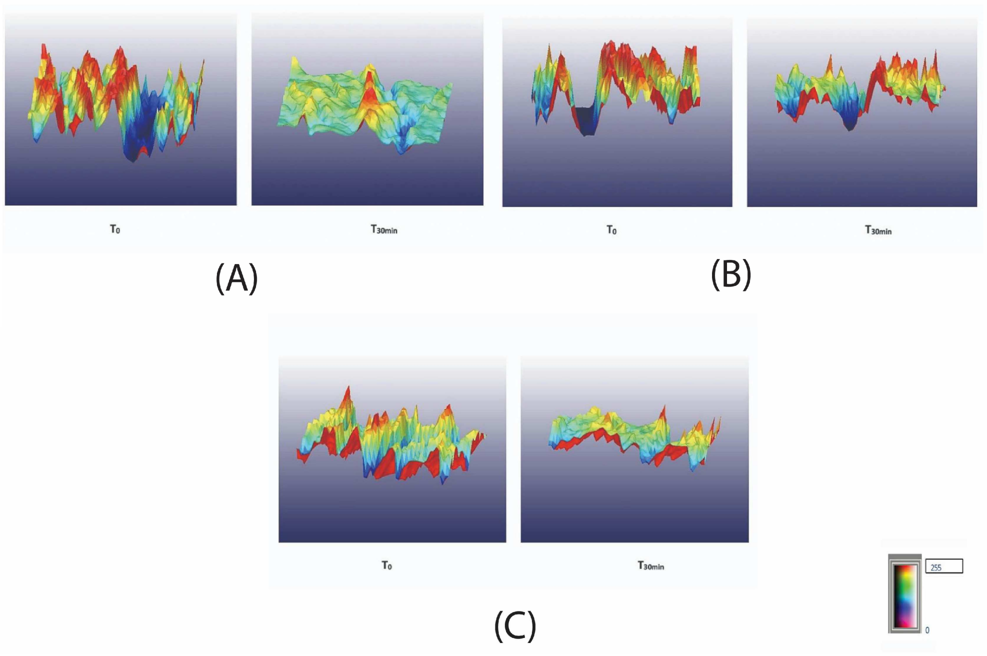
•UR (Immediate Recovery): The elastic recovery of the skin due to the removal of stress.

•UA (Total Recovery): The overall recovery at the end of the stress-off phase, quantifying the skin's ability to return to its original position.

•R (Residual Deformation): The amount of deformation that is not recovered at the end of the stress-off time.

Skin roughness was evaluated using skin replicas and advanced image analysis techniques with specialized equipment and materials, including fast-hardening synthetic polymer (SILFLO, Monaderm, Monaco) and adhesive discs (3M, 24 × 40). A precise negative imprint of the skin surface was created using these materials.
The procedure began by applying an adhesive disc to the subject's skin, ensuring a well-defined area for investigation while minimizing skin stretching during the polymer application. A small amount of the synthetic polymer was then carefully spread within the circular area defined by the adhesive disc. The polymer was left to dry until fully hardened, after which the disc was gently removed, leaving an accurate duplicate of the skin's surface.
The obtained skin replicas were analyzed using the image processing software Quantilines (Version 1.1.7.0, Monaderm), enabling a comprehensive evaluation of the skin's surface relief based on the method outlined by Corcuff [46].

- R_a (average roughness): Represents the mean deviation of the surface profile from a reference line, providing an overall measure of the skin's texture.
- R_z (average maximum roughness): Reflects the average difference between the highest and lowest points over five sections of the profile, indicating the depth of wrinkles and furrows.



3D surface representation of skin roughness for: (**A**) subject 2 at baseline (T_0) and 30 min (T_{30min}), (**B**) subject 4 at baseline (T_0) and 30 min (T_{30min}), and (**C**) subject 17 at baseline (T_0) and 30 min (T_{30min}).