Deposition of Chitin Nanofibrils on different substrates by Electrospray Technique

Bahareh Azimi

[1]Dept. of Civil and Industrial Engineering, University of Pisa, Pisa, Italy.
[2]Interuniversity National Consortium of Materials Science and Technology (INSTM), Florence, Italy.

Bahareh.azimi@ing.unipi.it

University of Pisa
Department of Civil and Industrial Engineering,
(Aerospace, Mechanical, Civil Engineering and **Chemical Engineering and Materials Science)**

Bio-based Industries Consortium
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European Union Funding
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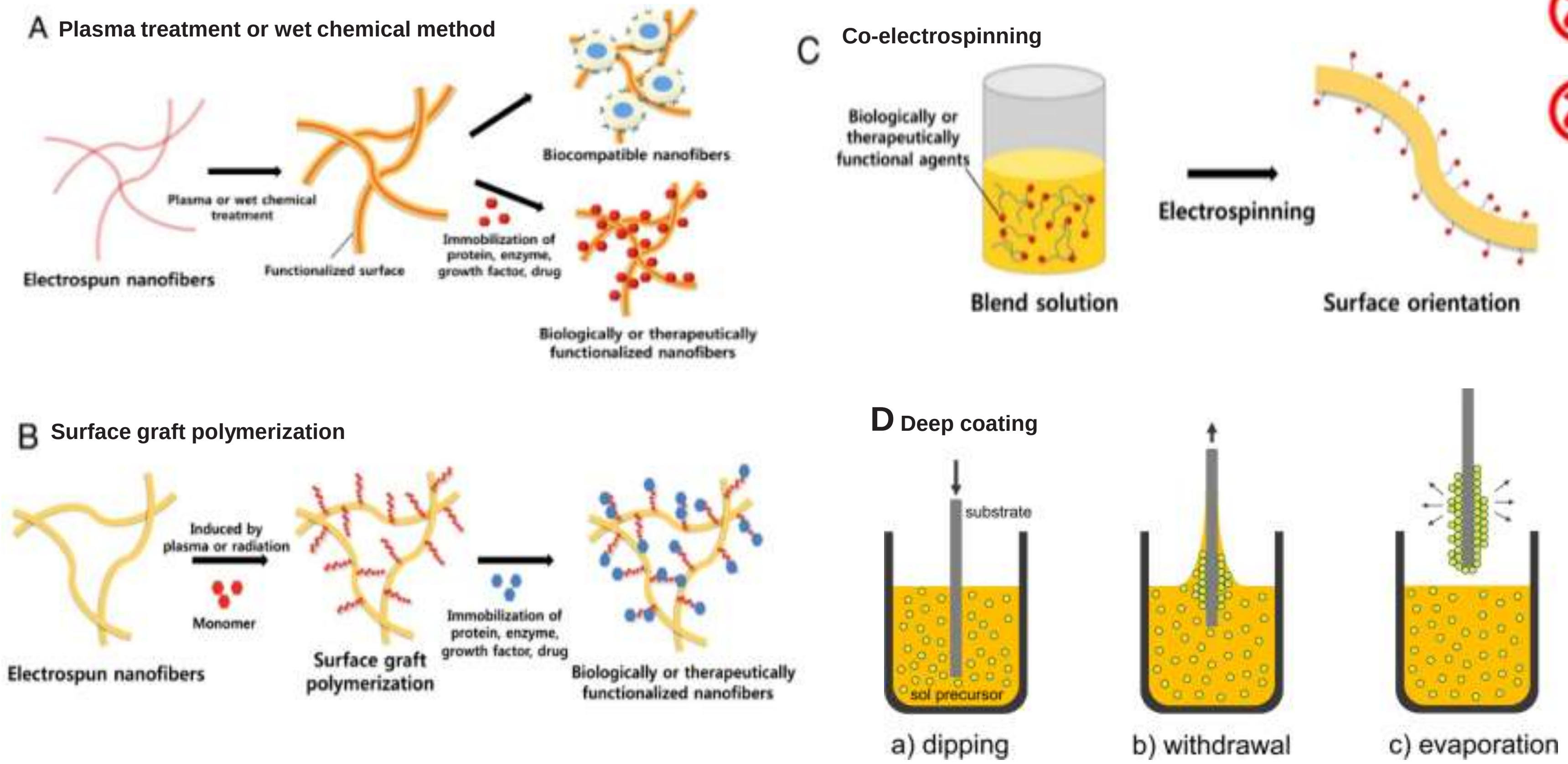
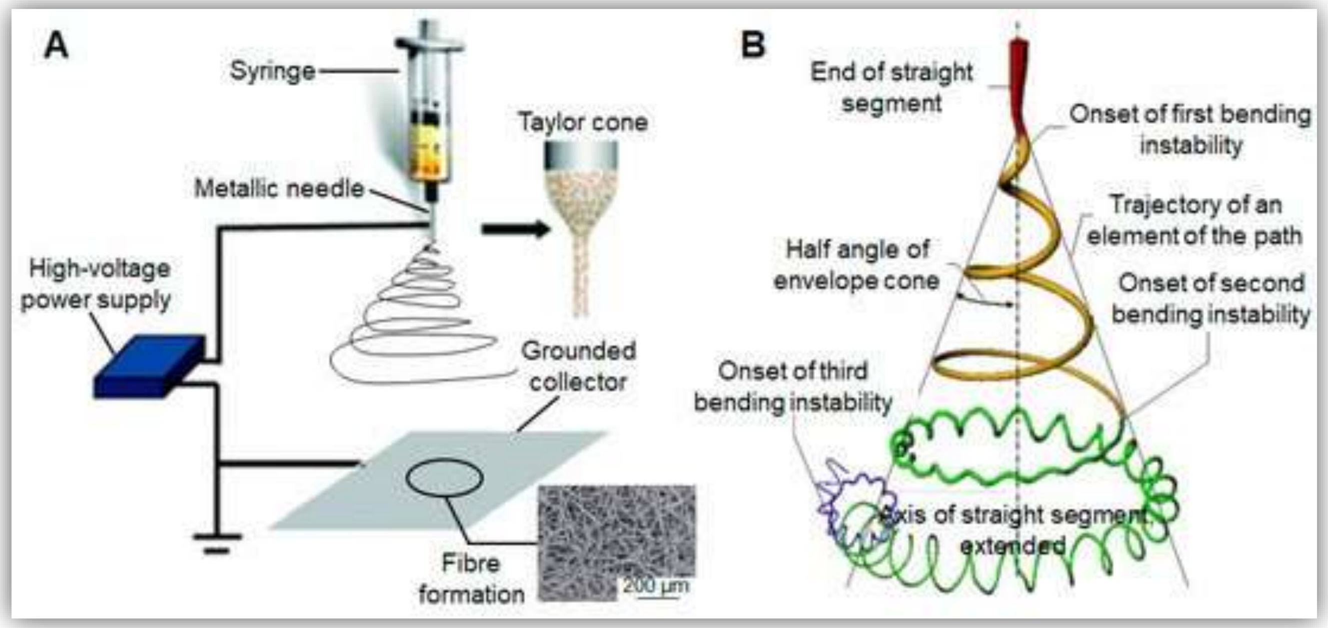
Various surface modification techniques

Several techniques such as gamma irradiation, plasma treatment, dip-coating, and chemical modification have been used over the past several years to produce biocompatible coatings on different substrates.

- Poor control of adhesion and composition
- Rigorous processing conditions
- Long reaction times



Hyuk SangYoo et al. Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery, doi:10.1016/j.addr.2009.07.007



the charged liquid jet is elongated and experiences a whipping instability stage during its flight to the collector

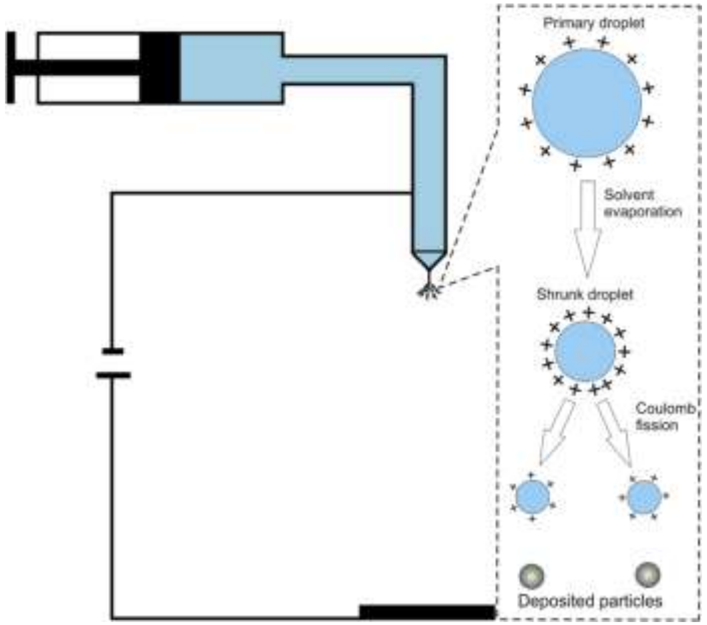
https://www.youtube.com/watch?v=Dn6r1Ag1npE

https://www.youtube.com/watch?v=5B5iyyO2RRE&t=28s

Martins, P. L., A. C.; Lanceros-Mendez, S. (2014). "Electroactive Phases of Poly(Vinylidene Fluoride): Determination, Processing and Applications." Prog. Polym. Sci 39: 683–706.

Working parameters are very important to understand not only the nature of electrospinning but also the conversion of; polymer solutions into nanofibers through electrospinning.

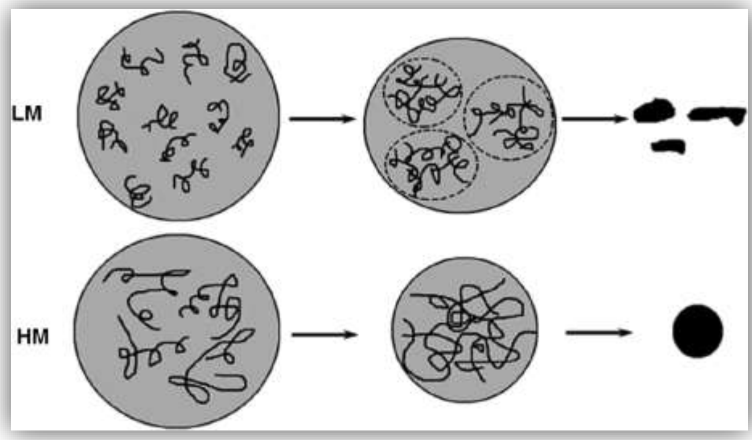
- **Solution parameters** (Type of polymer and solvent system, Solution concentration, molecular weight, viscosity, surface tension, conductivity/surface charge density)
- **Process parameters** (The strength and uniformity of the applied electric field, flow rate, collectors, distance between the collector and the tip of the syringe)
- **Ambient parameters** (Humidity, temperature)

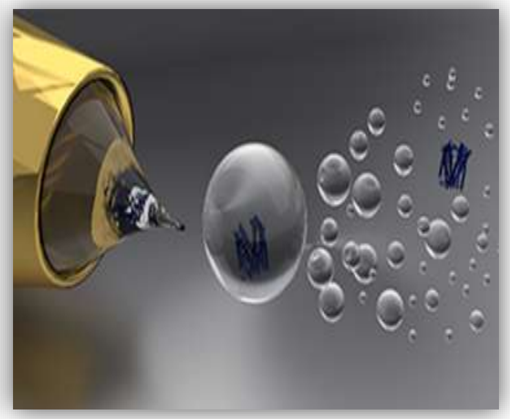


The charged liquid jet, at some point, will break up into droplets. During their flight to the collector, the solvent evaporation makes the primary droplets to shrink which leads to the increase in charge concentration so the primary droplets finally will break up into smaller offspring.

Nguyen, D.N et.al. (2016). "Review Pharmaceutical Applications of Electrospraying." Journal of Pharmaceutical Sciences 105: 2601-2620.

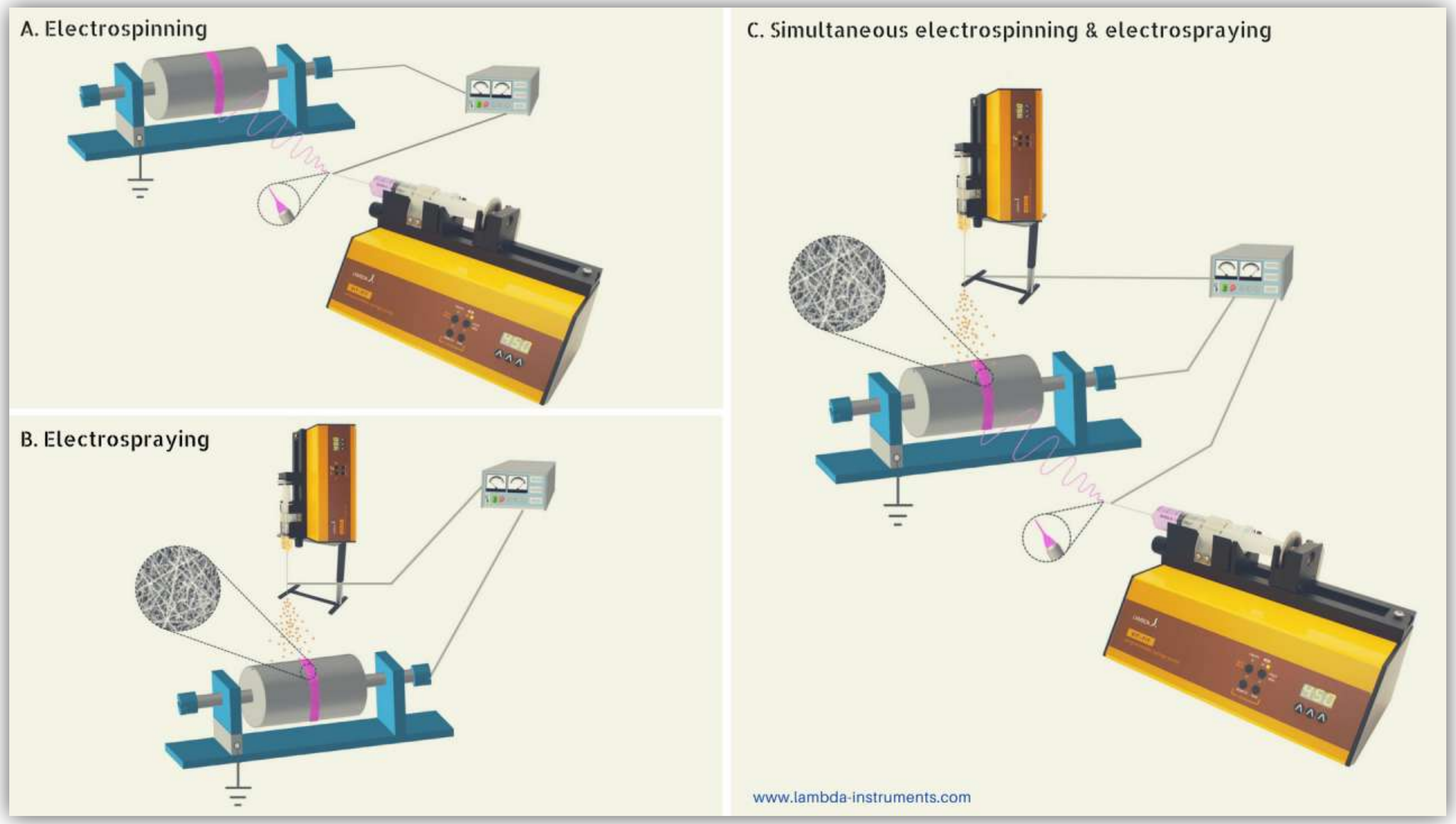
1. Solvent evaporation
2. Polymer diffusion
3. Chain entanglement (the number of entanglements per chain in solution)
   - Sufficiently molecular weight of polymer chains (lower polymer molecular weights: 1-2.5 entanglements per chain)
   - Reasonable polymer concentration (Critical overlap concentration (C*))
     - Molecular weight
     - Solvent quality f or the specific polymer





No chain entanglement during electrospraying ≤ **Critical overlap concentration (C*)** ≤ Highly viscous solutions, the axial tension becomes too high for electrospraying and beaded fibers or fibers are generated instead (electrospinning).

The recommended highest possible concentration of polymer should not be larger than 3 times C* for lower molecular weights polymers and even lower for polymers with higher molecular weights.
In short, a polymer solution used for electrospray process should be sufficiently diluted so that a low enough viscosity allows the solution to breakup into droplets at the same time should not be too viscose to form the fibers.


A. Electrospinning
B. Electrospraying
C. Simultaneous electrospinning & electrospraying
www.lambda-instruments.com

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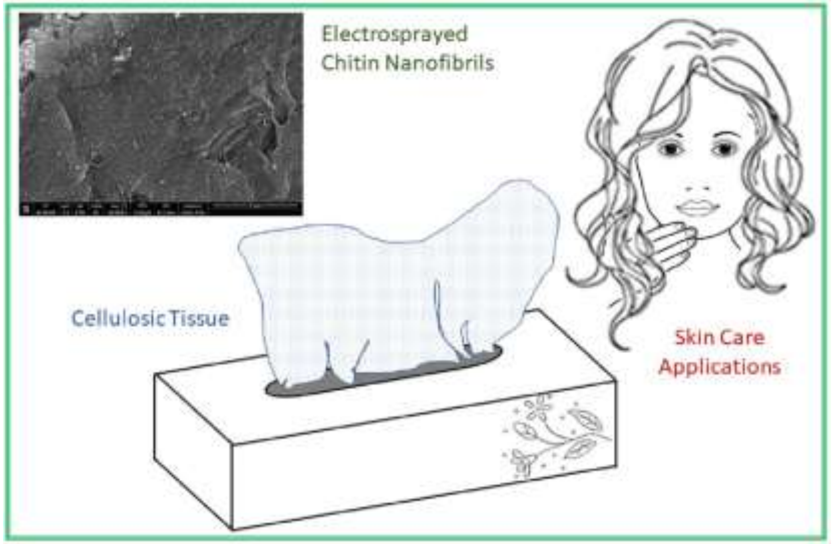
Bio-based Industries Consortium

The overall objective of ECOFUNCO is to select, extract and functionalise molecules such as proteins, cutin, polysaccharides, polyphenols, carotenoids, and fatty acids from readily available, low-valorised biomass sources. This will be used to develop new bio-based coatings for application on two different substrates, specifically cellulosic and plastic based. This will deliver materials for food and personal hygiene use that will offer better performance than currently-available products, as well as delivering more sustainable end of life options.

BBI JU · INSTM · IRIS Technology Group · Fraunhofer · SSICA · condensia · The University Of Sheffield · LUCENSE · bio-mi Sustainable Solutions · Kneia · TIPA Sustainable Packaging · PARMA · Planet Bioplastics srl · celabor · Organik Kimya · ARCHA · Huhtamaki · Universitat d'Alacant Universidad de Alicante



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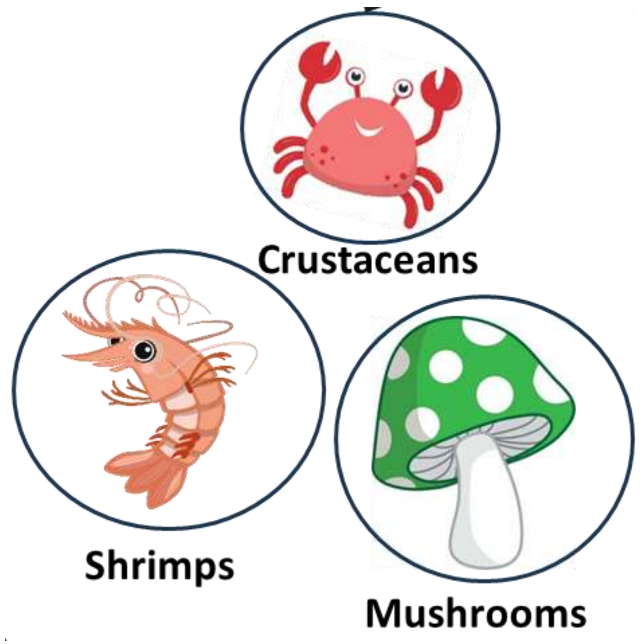
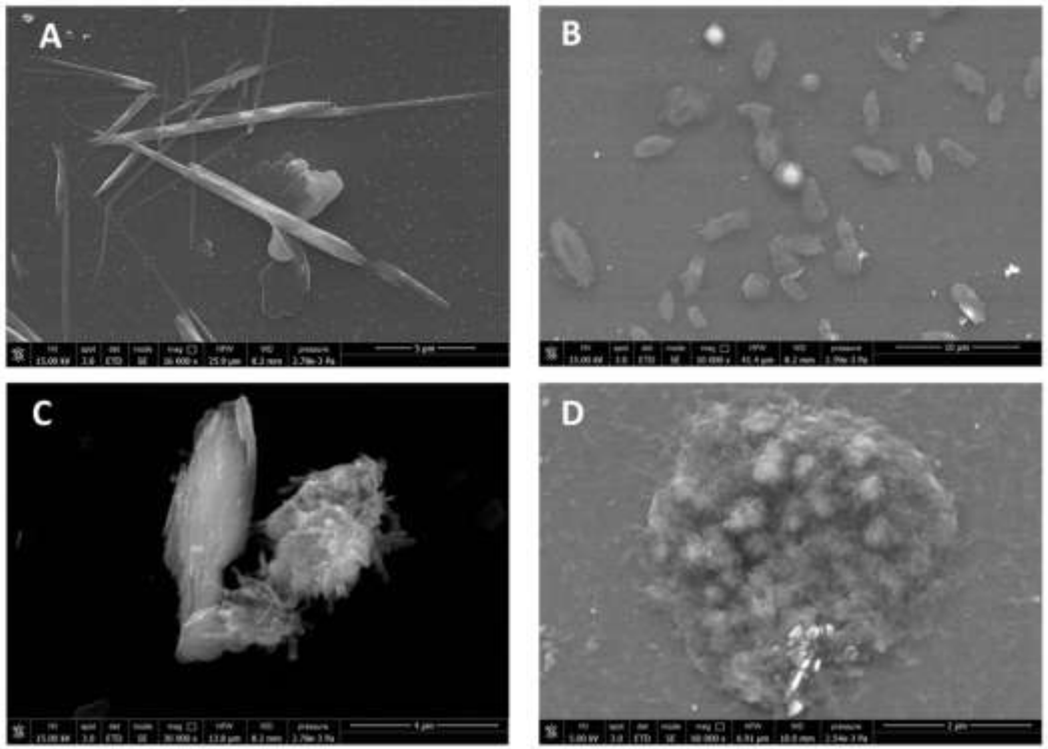
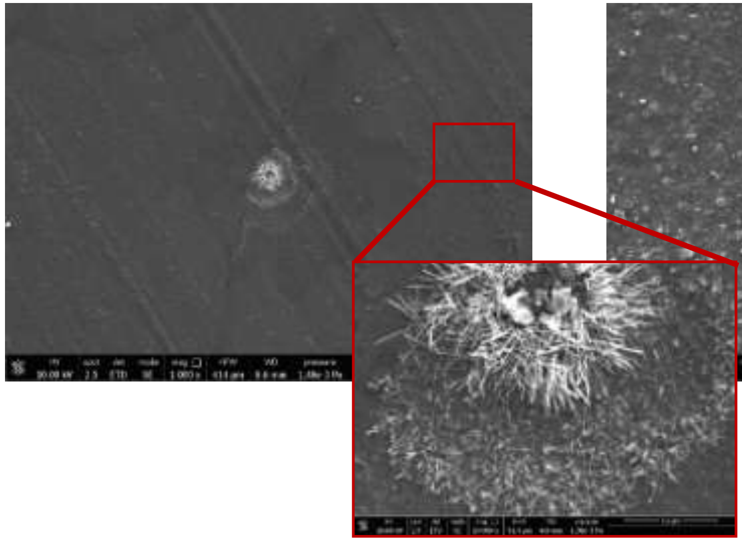
Chitin and lignin, byproducts of fishery and plant biomass, show antimicrobial and anti-inflammatory activity on the nanoscale. Due to their polarities, chitin nanofibril (CN) and nanolignin (NL) can be assembled into micro-complexes, which can be loaded with bioactive factors, such as the glycyrrhetinic acid (GA) and CN-NL/GA (CLA) complexes, and can be used to decorate polymer surfaces.



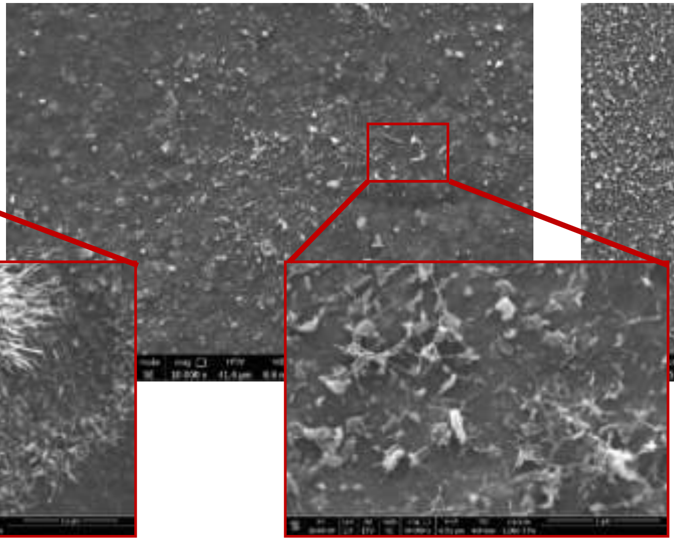


Electrospray of chitin nanofibrils (shrimp-based) on the surface of AL foil

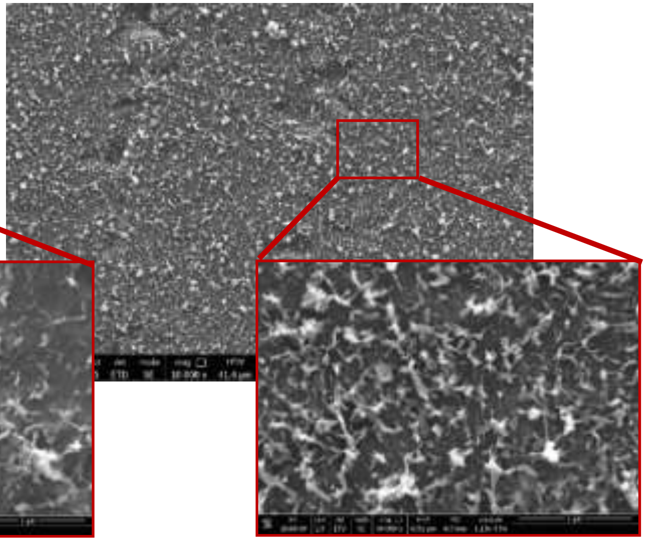
Distilled water



Distilled water: acetic acid
((50:50) w/w)



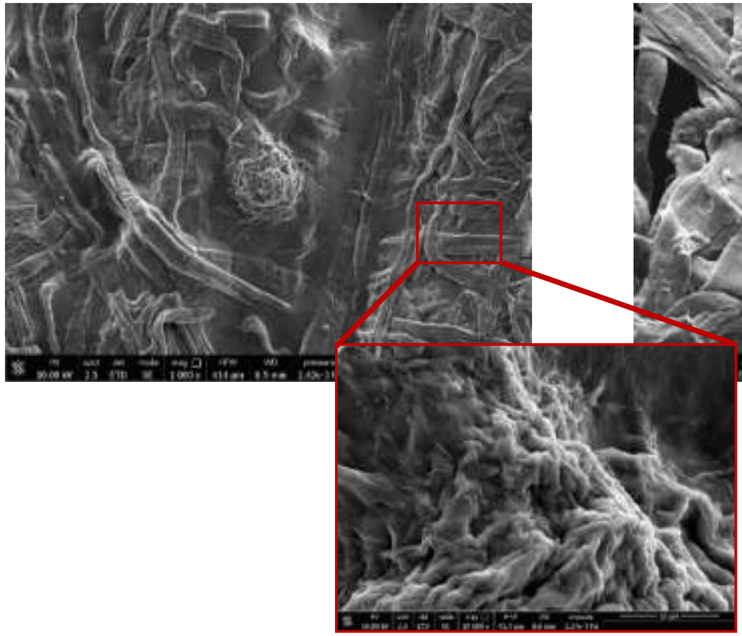
Distilled water: HFIP
((3:2) w/w)



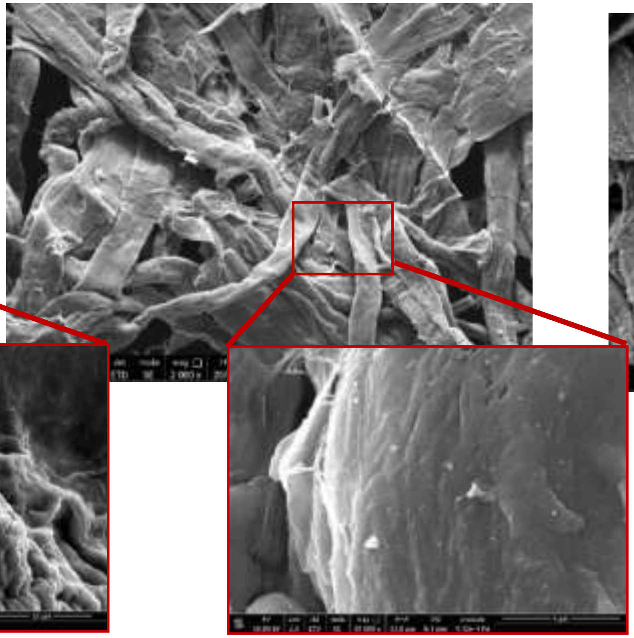
Azimi et al, Electrosprayed Shrimp and Mushroom Nanochitins on Cellulose Tissue for Skin Contact ApplicatioMolecules, 26, 4374

Electrospray of CN (shrimp-based) on the surface of cellulose substrate

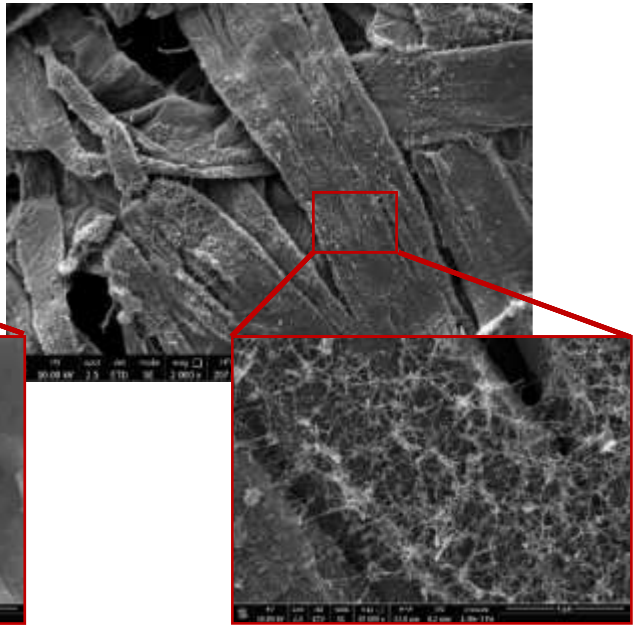
Solvent: Distilled water



Distilled water: Acetic acid
((50:50) w/w)



Distilled water: HFIP
((3:2) w/w)



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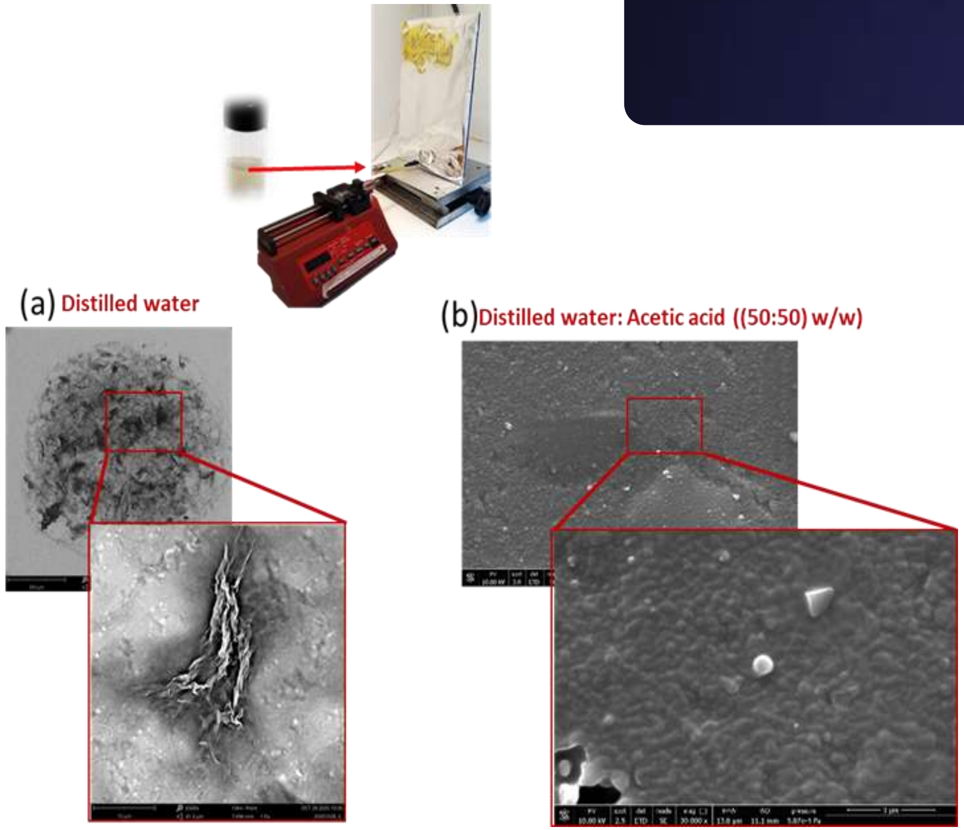
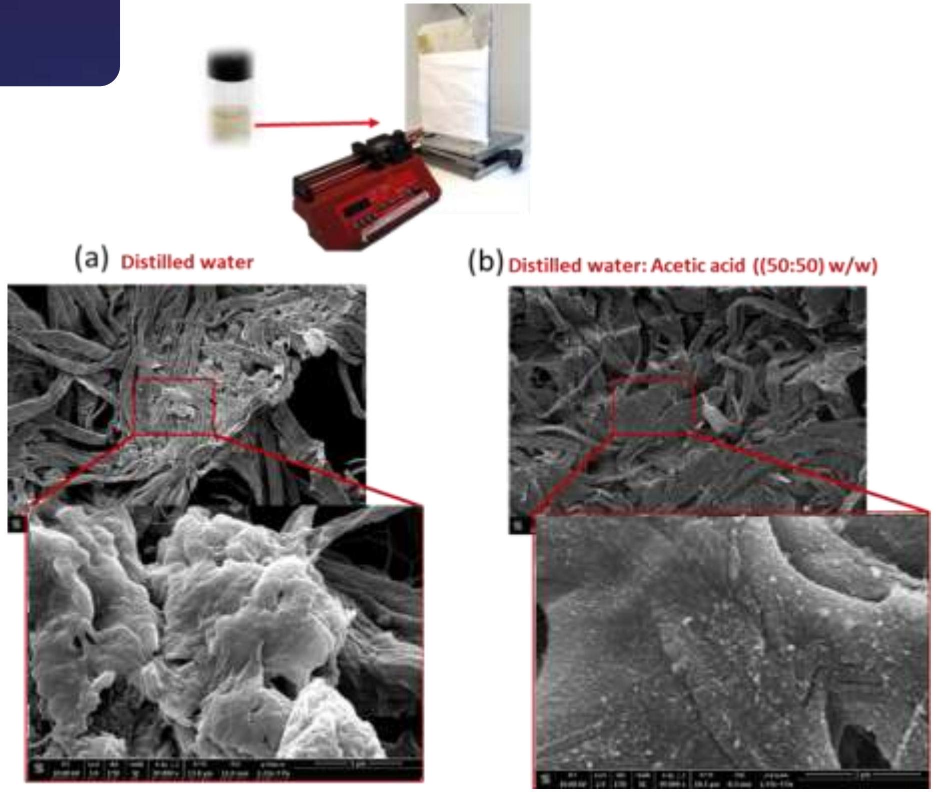
Electrospray of CN (Mushroom-based) on AL foil and cellulose substrate





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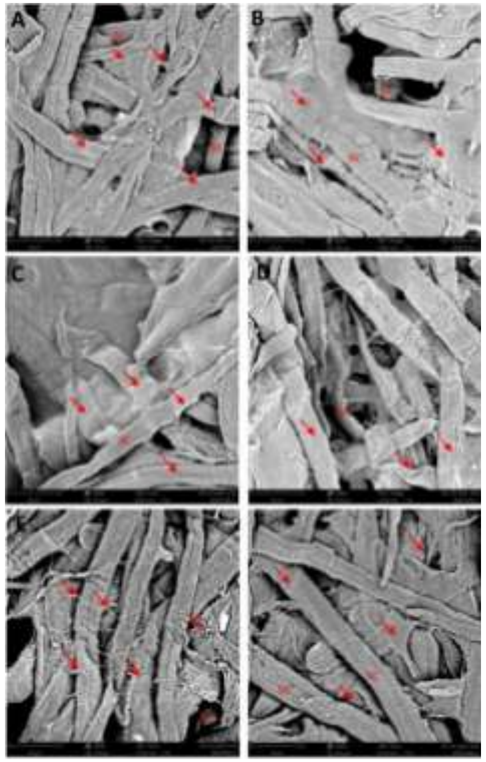
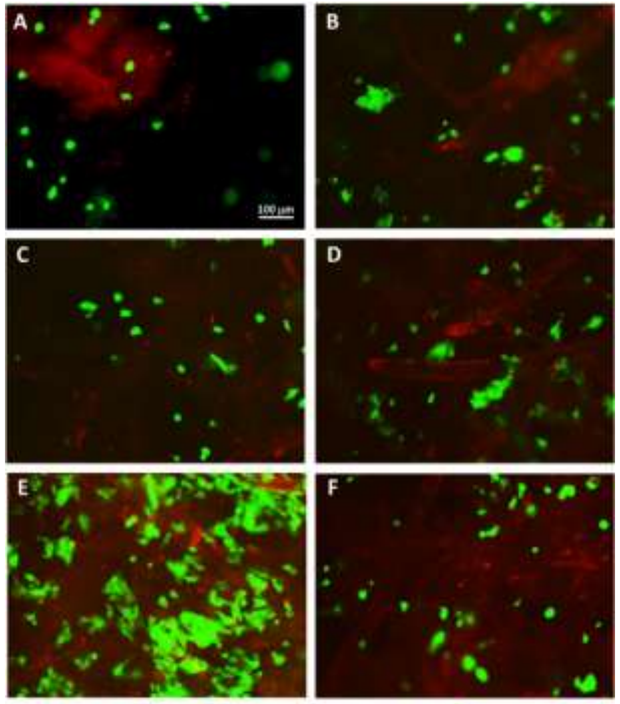
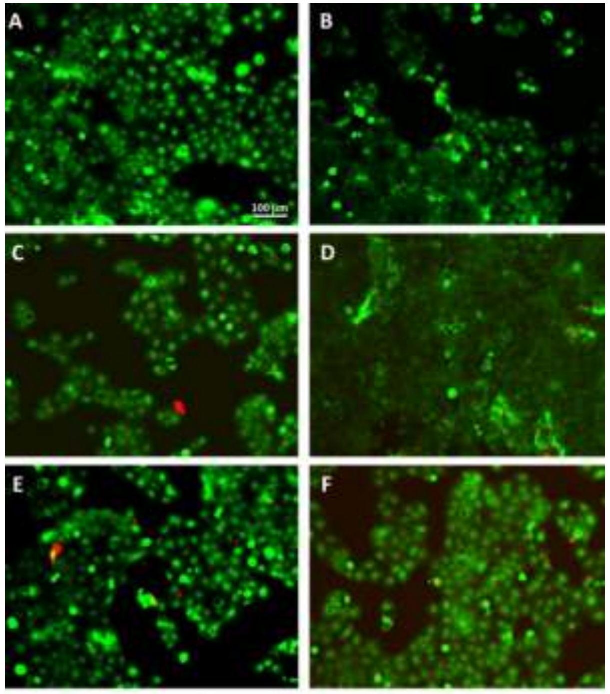
Direct and indirect cytotoxicity test: Live/Dead viability test performed on HaCaT cell line seeded on different scaffolds



Direct cytotoxicity test



Indirect cytotoxicity test



(a) sCNs (water); (b) sCNs (water/acetic acid); (c) sCNs (water/HFIP); (d) mCNs (water); (e) mCNs (water/acetic acid). (f) Pristine cellulose tissue.

Biological analysis revealed that all treated samples are suitable for skin applications since human dermal keratinocytes (HaCaT cells) successfully adhered to the scaffolds and were completely viable after being in contact with released substances in culture media.

These results indicated that the use of solvents did not affect the final cytocompatibility due to their effective evaporation during the electrospray process. These treatments did not affect antimicrobial characteristics of pure cellulose.

Azimi et al, Electrosprayed Shrimp and Mushroom Nanochitins on Cellulose Tissue for Skin Contact ApplicatioMolecules, 26, 4374

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SANITARY

DIAPER

- Flat die extrusion of topsheet
- SAP production by polysaccharide modification
- Topsheet surface texturing and antimicrobial modification
- Advanced in vitro testing

COSMETICS

BEAUTY MASK

- Bacterial fermentation (PHA)
- Film extrusion / casting
- Non woven production via electrospinning
- Impregnation with natural anti oxidant nanoparticles
- Advanced in vitro testing

WOUND CARE

WOUND DRESSING

- Bacterial fermentation (PHA)
- Non woven production via electrospinning
- Fibre modification
- Impregnation with natural anti oxidant nanoparticles
- Advanced in vitro testing

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BBI JU, European bioplastics, Fibroline, exergy, ARMINES, Innoconsult International, TEXOL, Universiteit Gent, University of Westminster, IRIS, mavi, Bioinicia

Increasing the knowledge and understanding of smart nanomaterials and nanotechnologiesas applied to biopolymers and validating them in the market.

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WOUND CARE

WOUND DRESSING

Bacterial fermentation (PHA)

Non woven production via electrospinning

Fibre modification

Impregnation with natural anti oxidant nanoparticles

Advanced in vitro testing

Developing a biodegradable and at least 90% bio-based nanostructured biocompatible non-woven tissue for use in wound dressings.

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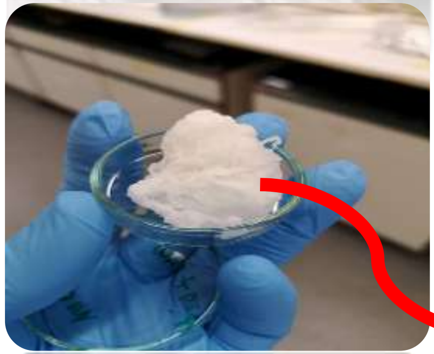
P(3HO-co-3HD/ PHB) fiber

POLYBIOSKIN
WOUND CARE

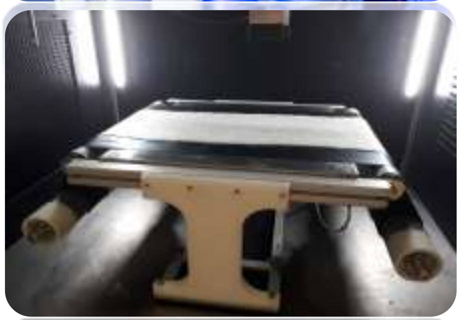
The University Of Sheffield.

Bacterial fermentation method

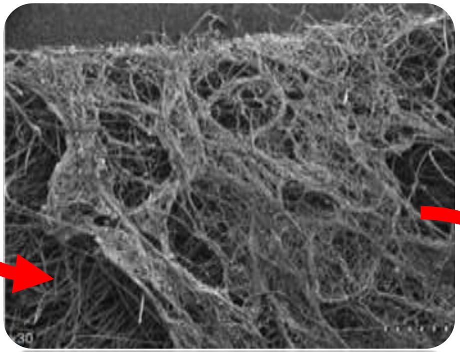


Polyhydroxyalkanoates (PHAs)

Bioinicia
Innovative Polymer Applications

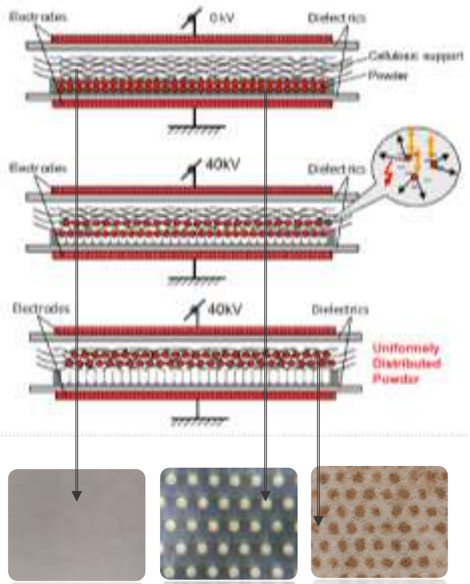


Electrospinning (FluidnatekTM LE-500 production equipment)



PHAs electrospun nanofibers

FIBROLINE



Chitosan coated by P(3HD-3HO)

Engraved plate filled of CLA

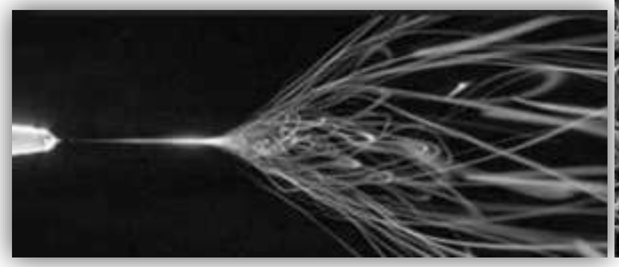
Chitosan coated by P(3HD-3HO) impregnated with CLA

Impregnation process to incorporate the bioactives into the wound dressing.

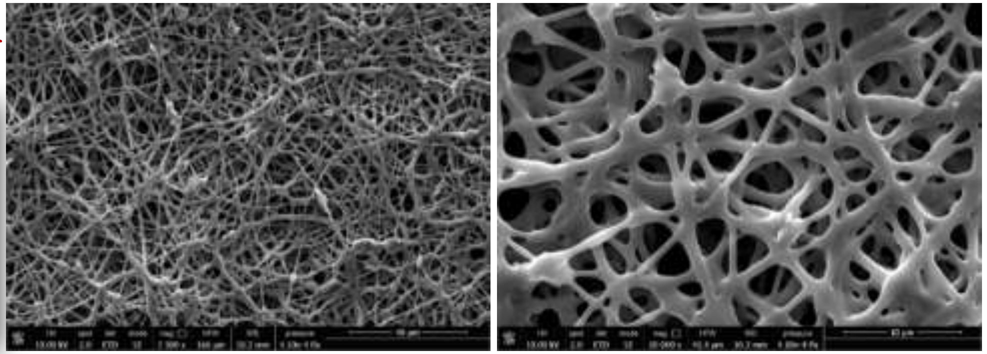
Azimi, B. et.al., *Electrosprayed Chitin Nanofibril/Electrospun Polyhydroxyalkanoate Fiber Mesh as Functional Nonwoven for Skin Application*, J. Funct. Biomater. 2020, 11, 62

P(3HO-co-3HD/ PHB) fiber

Electrospinning of Poly(3-hydroxyoctanoate-co-3-hydroxydecanoate)/Polyhydroxybutyrate

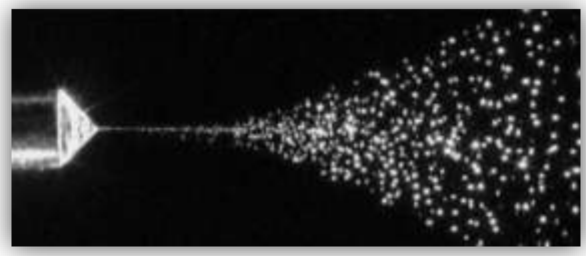


(P(3HO-co-3HD/ PHB) electrospun fiber mesh

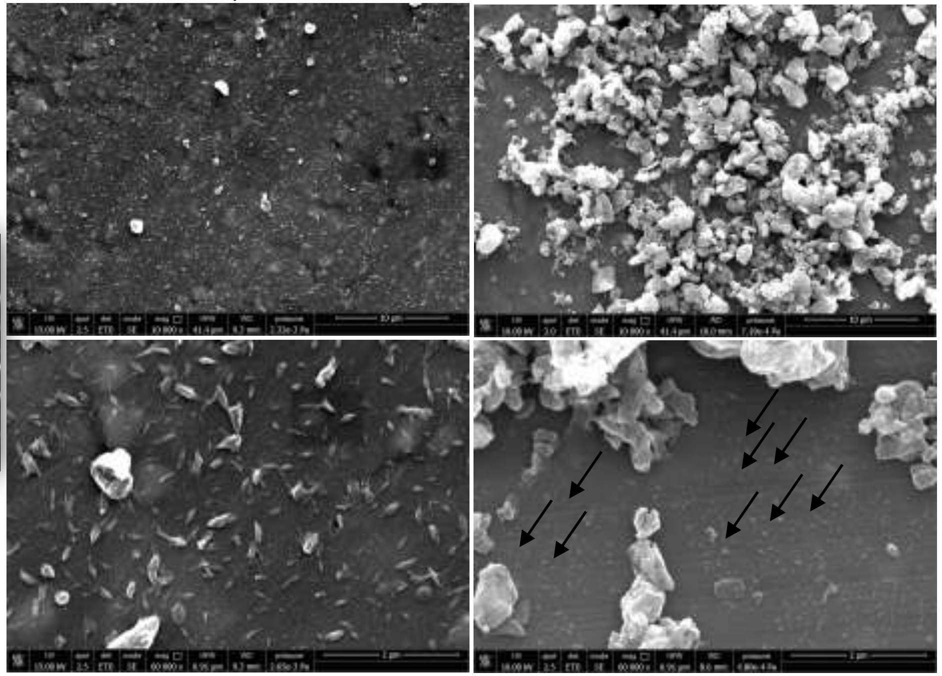


Solution properties: concentration (11 w/w%), PHB/PHOHD (1:10), solvents: (chloroform/2-butanol)(70:30 (v/v)), additive: 0.002 g/mL $LiBr_2$
Electrospinning conditions: voltage: 40 kV, flow rate: 0.5 mL/h, distance from needle tip to the static collector of 40 cm.
Ambient condition: Humidity: 40%, temperature: 20 °C, Average fiber diameter: 1.28 ± 0.58 µm

Electrospraying of chitin nanofibril (CN) and chitin nanofibril/nanolignin/glycyrrhetinic acid (CLA) complex



Electrosprayed CN　　Electrosprayed CLA nanofibrils



Solution properties: concentration (0.52 w/w%), solvents: aqueous acetic acid (50:50 w/w) for CN and distilled water for CLA

Electrospinning conditions: voltage: 15 kV, flow rate: 0.298 mL/h , distance from needle tip to the static collector of 10 cm

Average CN diameter: 180 nm ± 47 nm, Average CLA diameter: 65 ± 20 nm (shown with arrows) and 1239 ± 626 nm

P(3HO-co-3HD/ PHB) fiber

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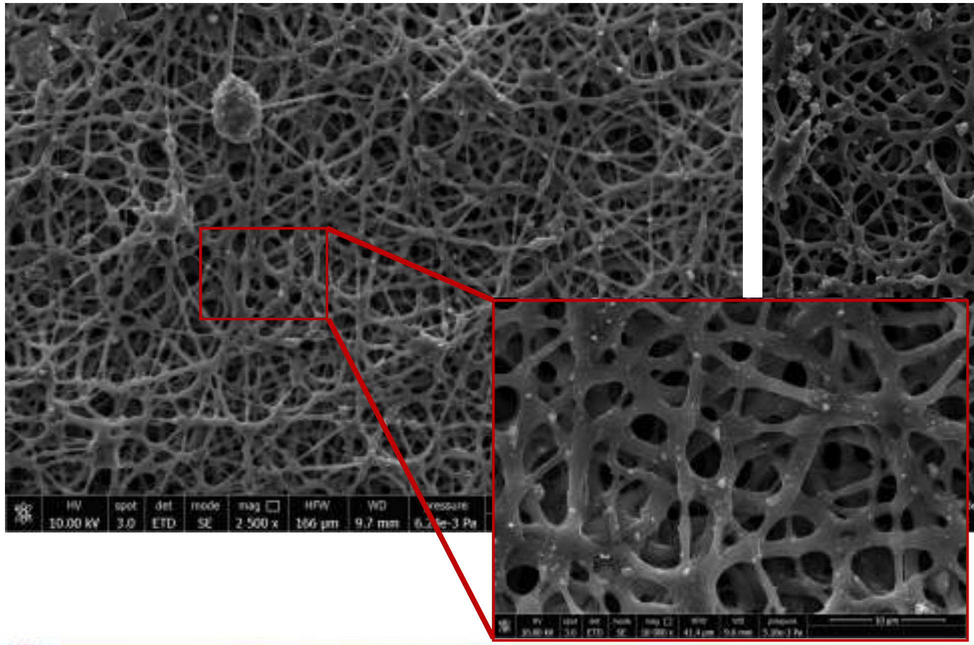
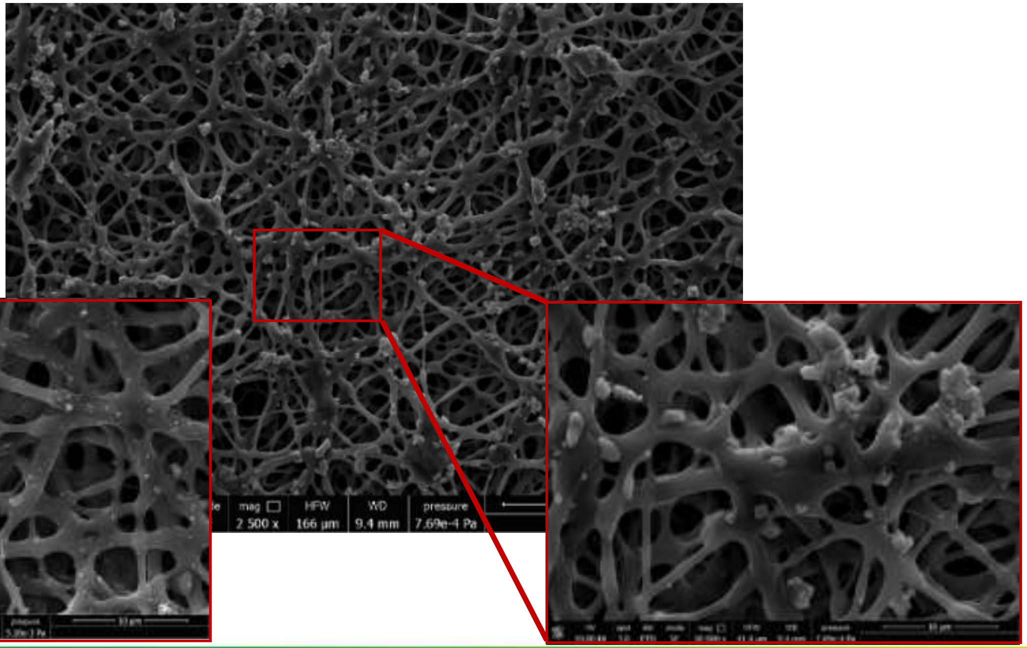
Electrosprayed CNs on the surface of PHOHD/PHB fibers



Electrosprayed CLAs on the surface of PHOHD/PHB fibers



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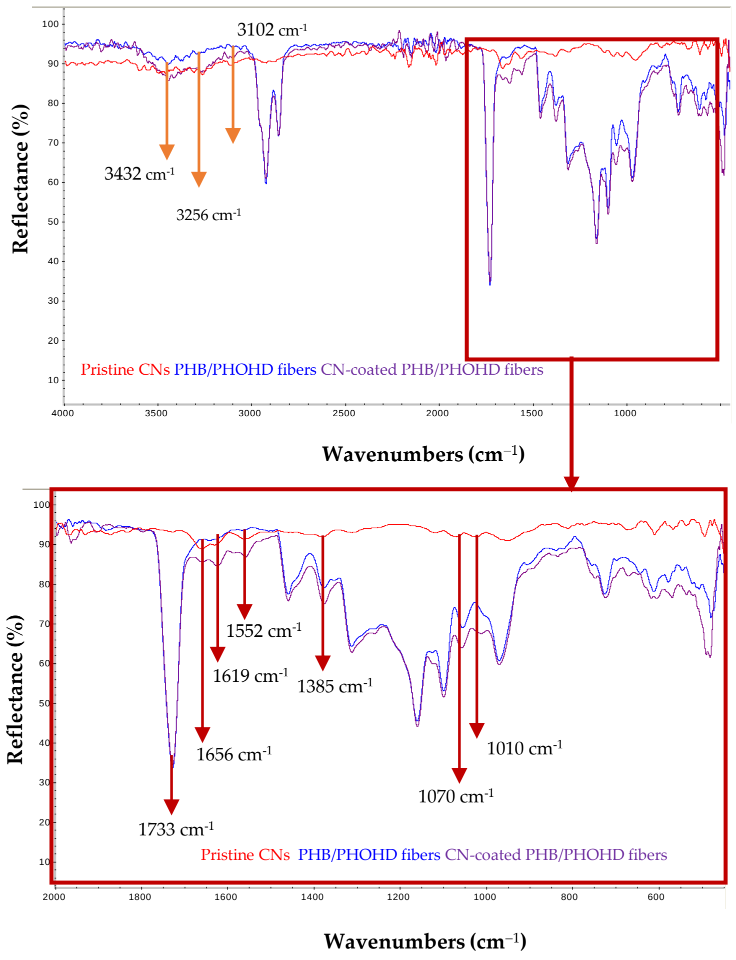
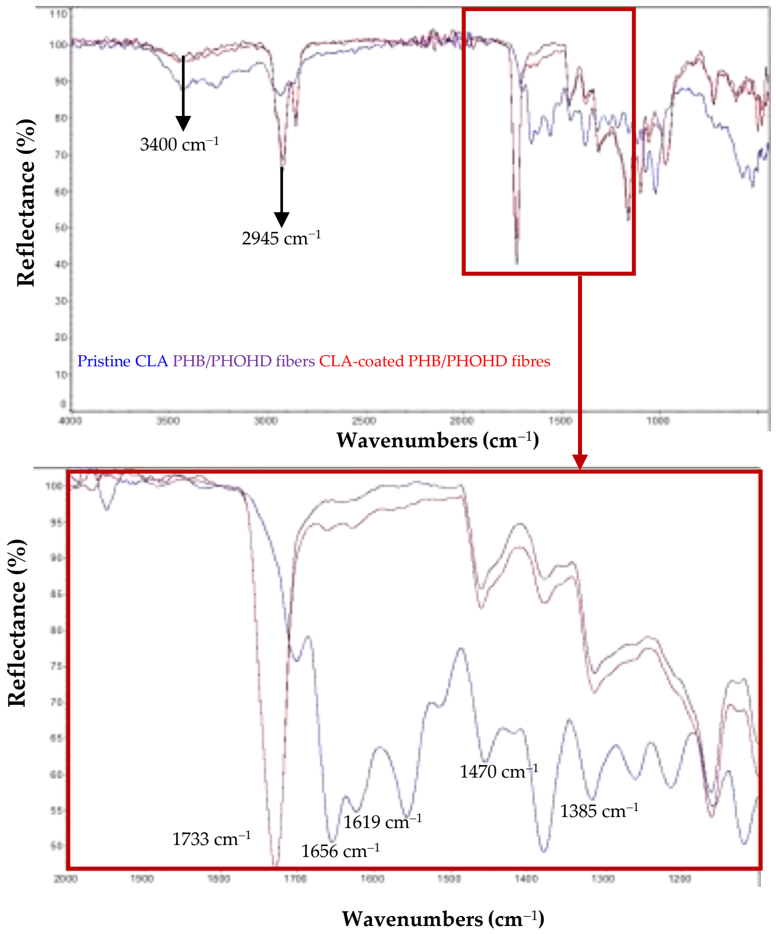
POLYBIOSKIN WOUND CARE

FTIR spectrum of CN-Coated PHB/PHOHD fibres



FTIR of CLA-Coated PHB/PHOHD fibres



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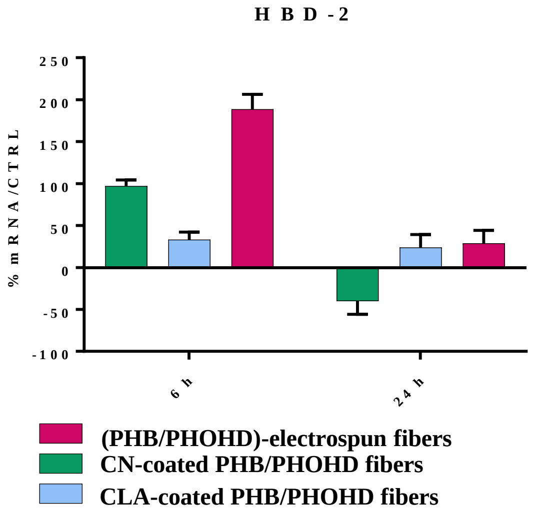
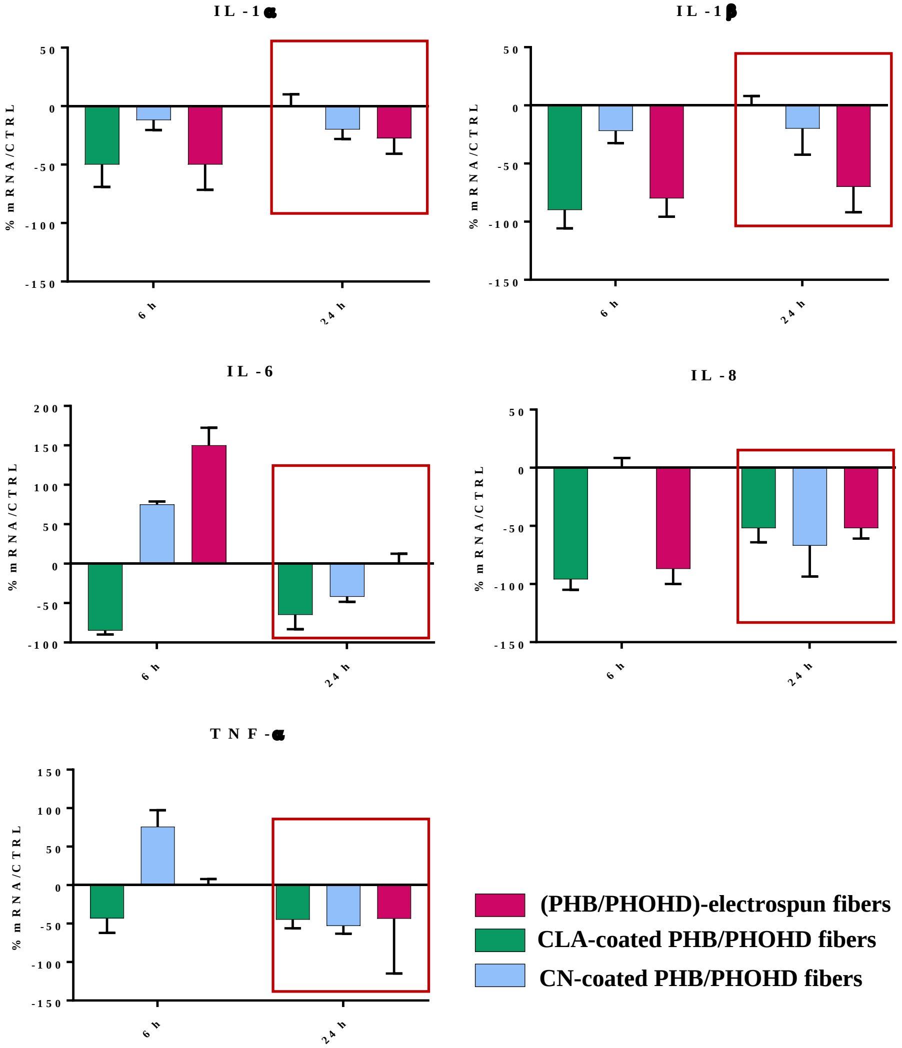
POLYBIOSKIN
WOUND CARE

AlamarBlue® test

SAMPLE	%AB$_{RED}$
PHB/PHOHD fiber mesh	76
CLA-coated PHB/PHOHD fiber mesh	64
CN-coated PHB/PHOHD fiber mesh	69





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4NanoEARDRM

4NanoEARDRM
is aimed at synergising different nanotechnologies for an optimal eardrum restoration, including acoustic, regenerative, and pathologic cues, to achieve a durable and effective performance in implanted patients.

INSTM

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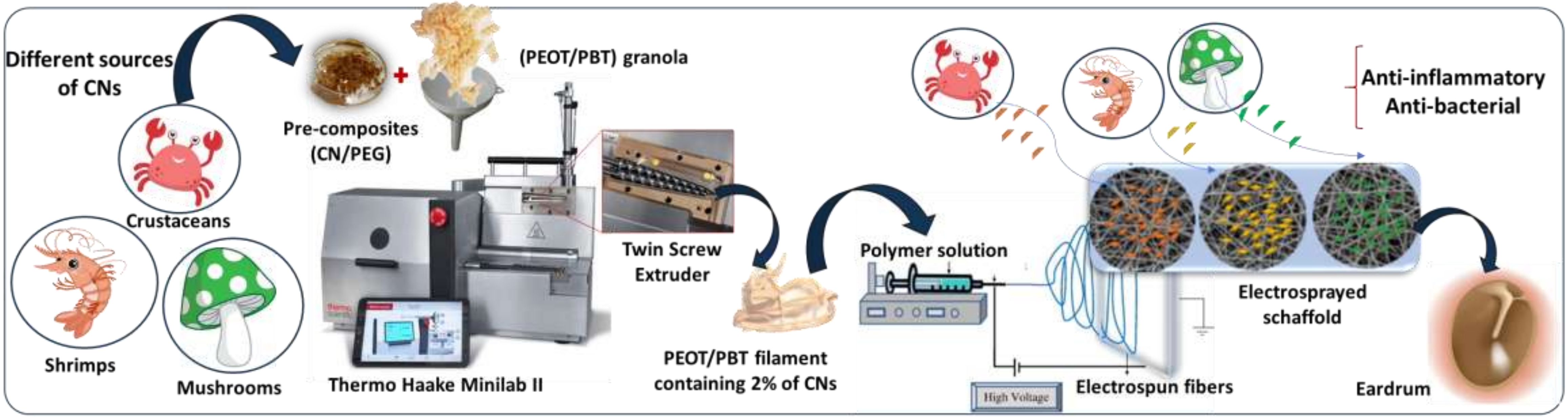

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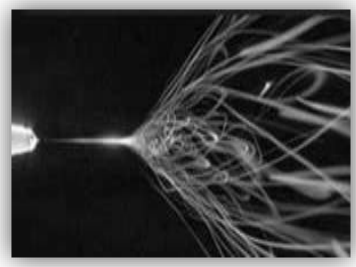
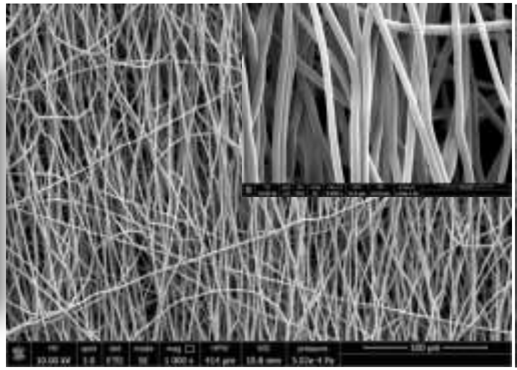
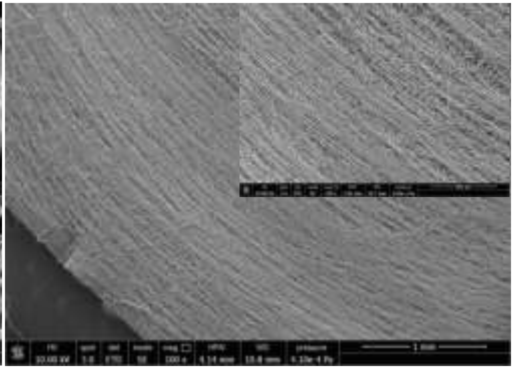
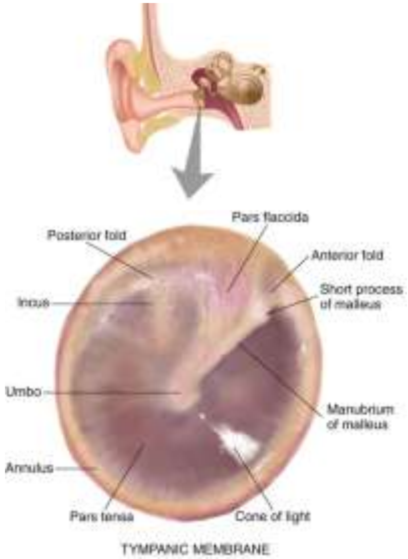
Radial fibers

Circular fibers

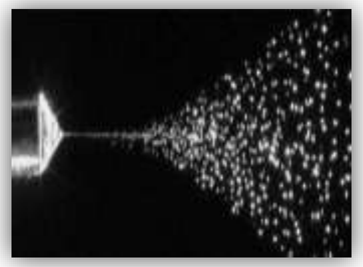


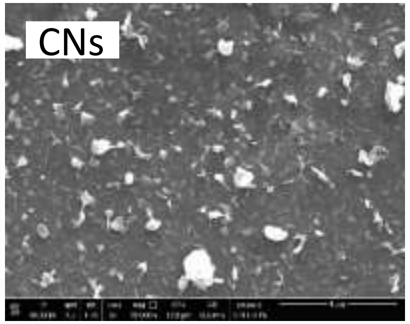


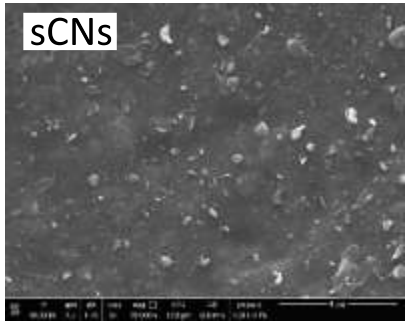


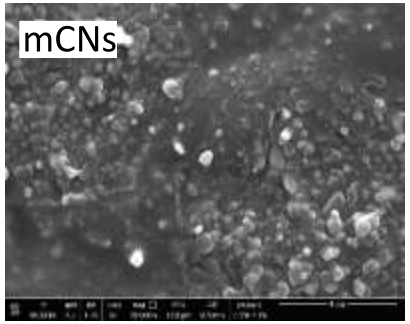


Electrospaying of CN nanoparticles



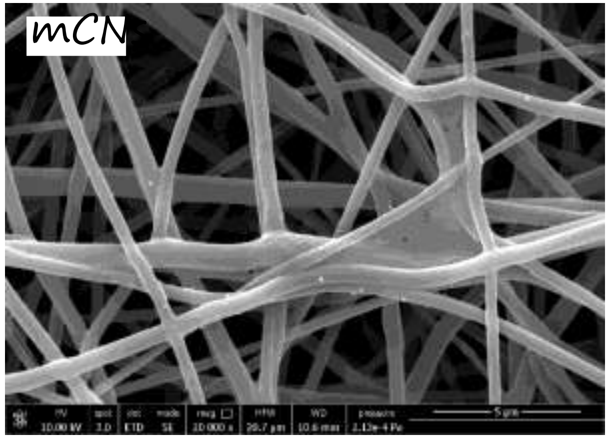


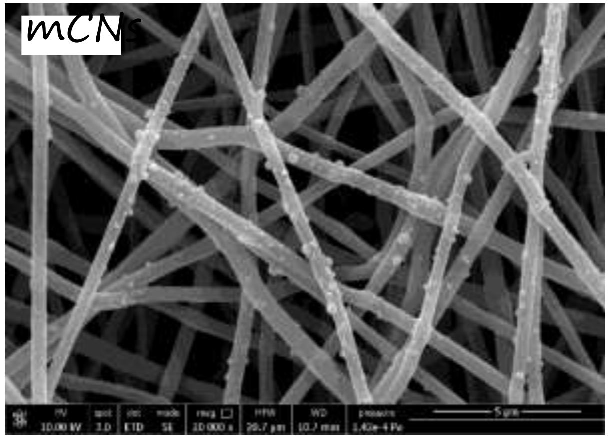




Concentration: 0.5% w/v PLGA-Ciprofloxacin HCl solution
Flow rate: 1 µl/min, **Working distance**: 30 cm, **Voltage**: 40 kV
Temperature: 25°C
Humidity: 40%

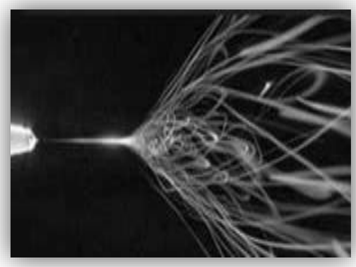
Electrospray of CN on the surface of (PEOT/PBT)/CN composite fibers



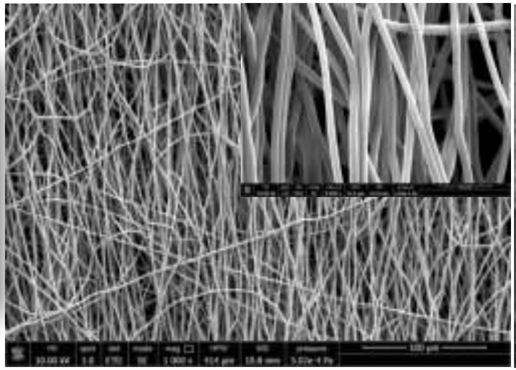


SEM images of electrosprayed mCNs on (left) PEOT/PBT and (right) PEOT/PBT/ (CN/PEG 50:50) scaffolds.

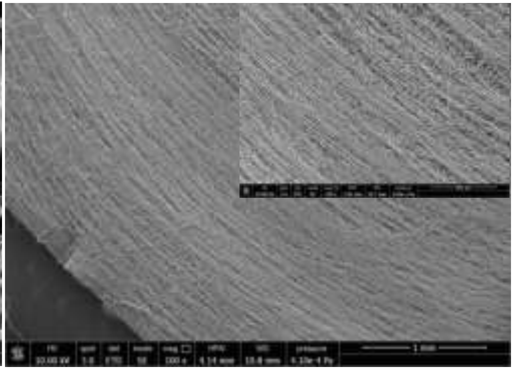
Electrospinning of (PEOT/PBT) /CN composites fibers

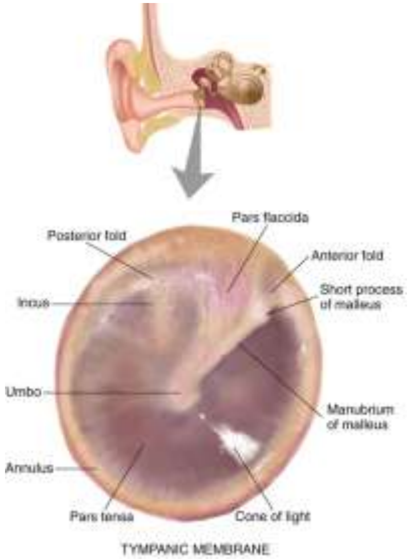


Radial fibers

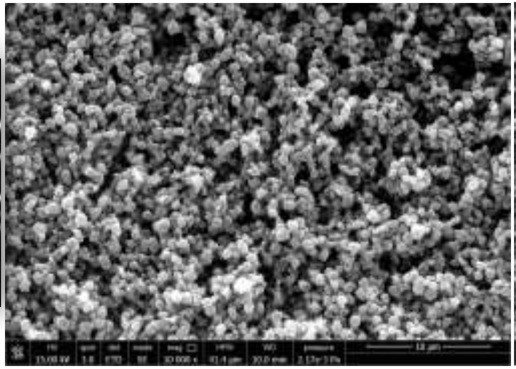


Circular fibers

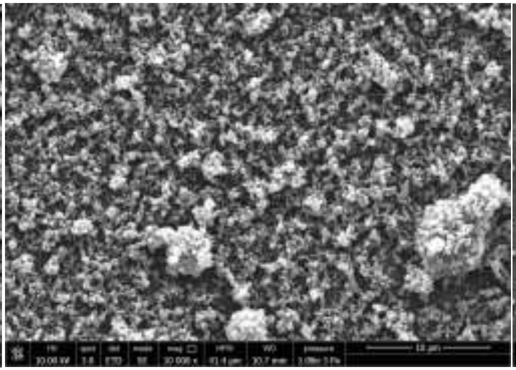




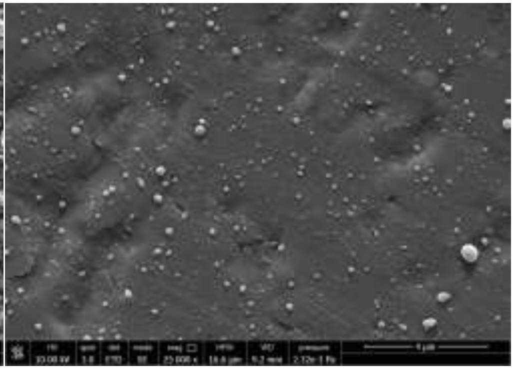
Electrospaying of drug-loaded PLGA Nanoparticles



PLGA nanoparticles



Rhodamine -Loaded PLGA particles



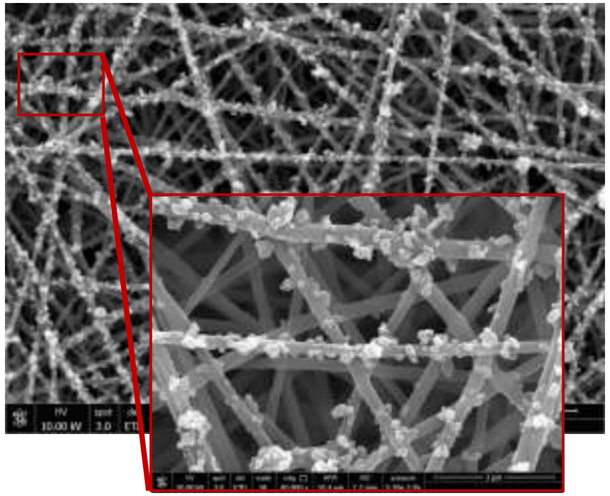
Ciprofloxacin -Loaded PLGA particles

(particle diameter:500-900 nm)

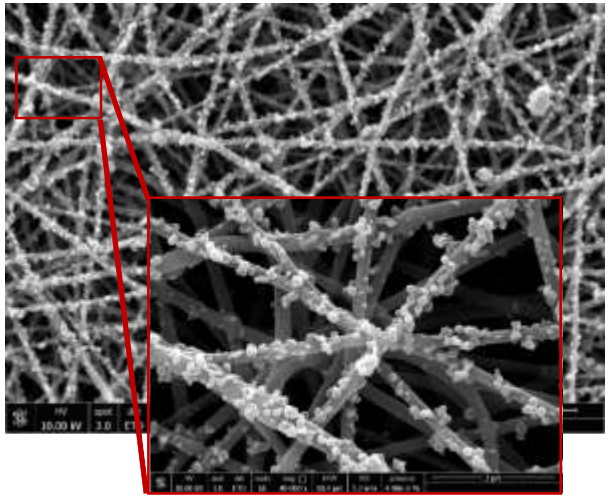
Concentration: 0.5% w/v PLGA-Ciprofloxacin HCl solution
Flow rate: 1 µl/min, **Working distance:** 30 cm, **Voltage:** 40 kV
Temperature: 25°C
Humidity: 40%

Electrospray of PLGA NPs on the surface of (PEOT/PBT)/CN composite fibers

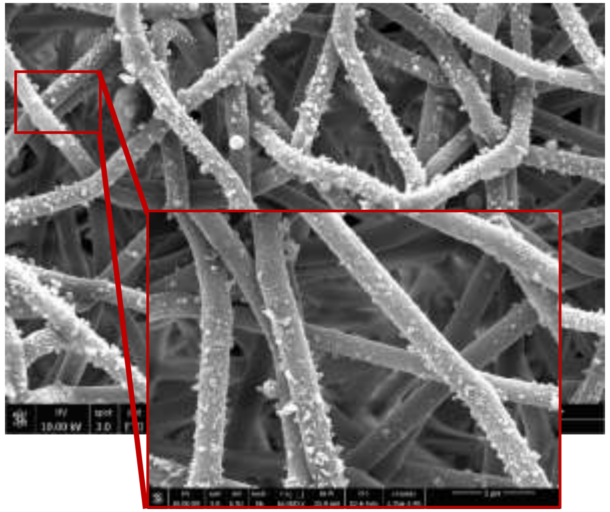
4NanoEARDRM



Rhodamine -Loaded PLGA particles on the surface of non-plasma treated fibers



Rhodamine -Loaded PLGA particles on the surface of Argon plasma treated fibers



Ciprofloxacin-Loaded PLGA particles on the surface of non-plasma treated fibers

Università di Pisa

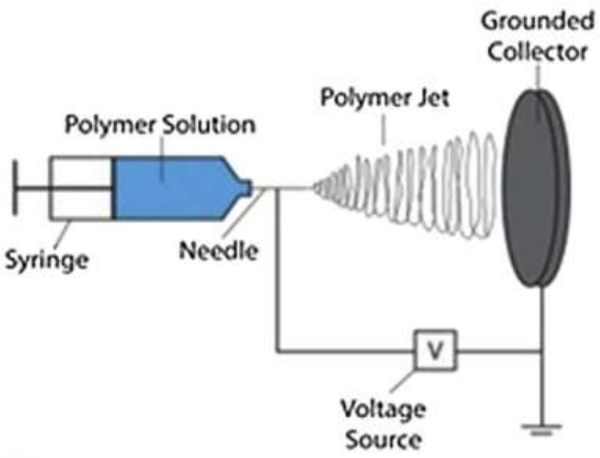
NanoCell

The overall objective of NanoCell is selecting novel ionic liquid with specific properties for cellulose dissolution and producing continues cellulose nanofibers via electrospinning as a simple and versatile method for nanofiber production. CNs will be used for surface modification of electrospun cellulose nanofibers via electrospray all the way to the structure of the product to fully enable it to provide required properties for two predetermined targeted applications (wound healing and tympanic membrane healing).

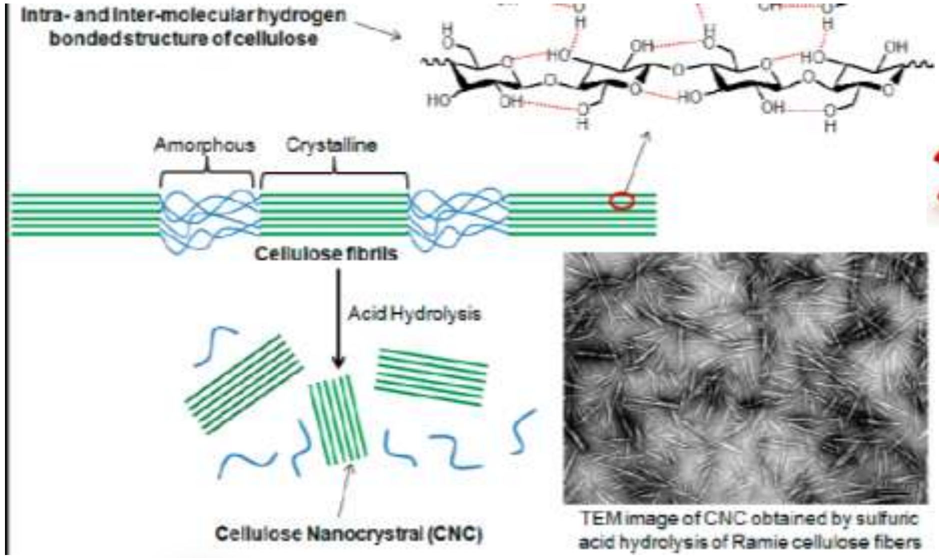
ICAN

IRAN Nanotechnology Innovation Council (INIC)

UNIVERSITÀ DI PISA



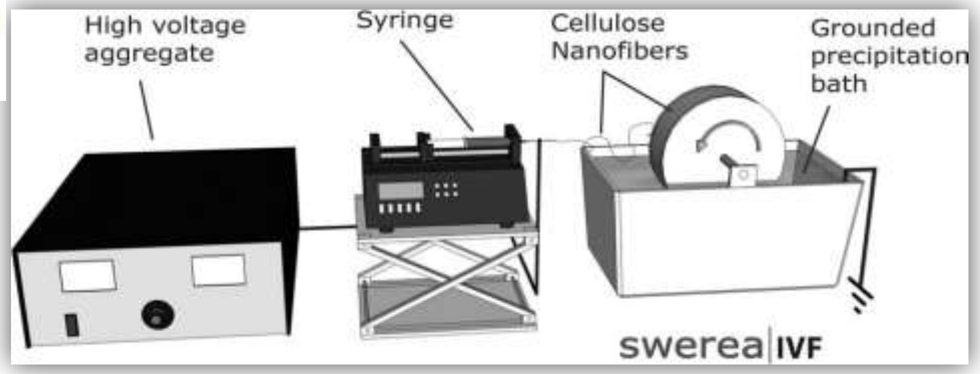
Sharma, A., M. Thakur, et al. (2019). Biotechnology Reports: e00316.



TEM image of CNC obtained by sulfuric acid hydrolysis of Ramie cellulose fibers

Chakrabarty, A. and Y. Teramoto (2018). Polymers 10(5): 517.

NanoCell



Room-temperature Ionic liquids (RTILs)

- Excellent dissolving capability
- low vapor pressure
- chemical and thermal stability
- non-flammability
- recyclability
- variety of structure

For industrial use, any IL selected for cellulose dissolution should be:

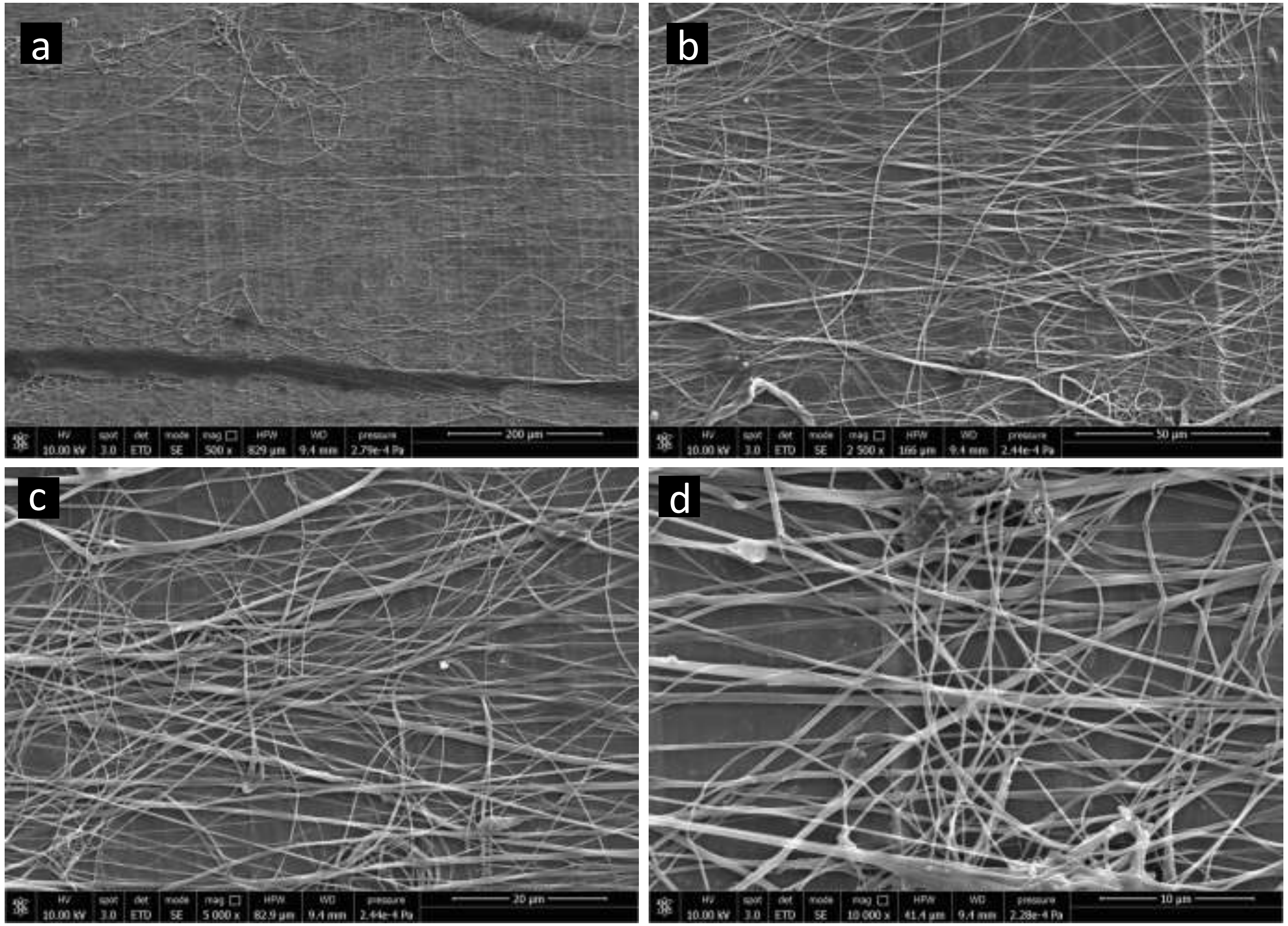
Easy to produce
Recyclable in high amount (>99.5)
possess the lowest possible toxicity
Low vapor pressure
Low melting point
Low side reactions and degeneration
High dissolution capability for different pulp sources

Zhang, J., J. Wu, et al. (2017). Materials Chemistry Frontiers 1(7): 1273-1290.

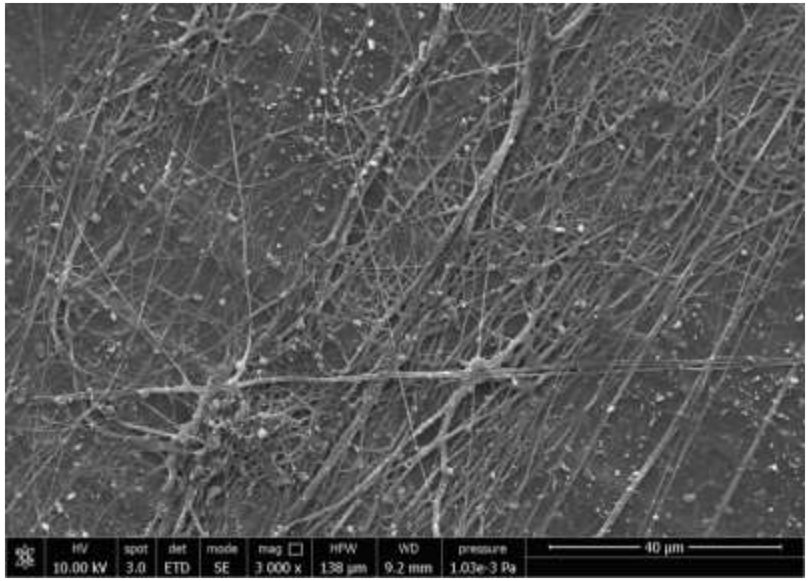
NanoCell

NanoCell

50 rpm fiber diameter:375± 118



NanoCell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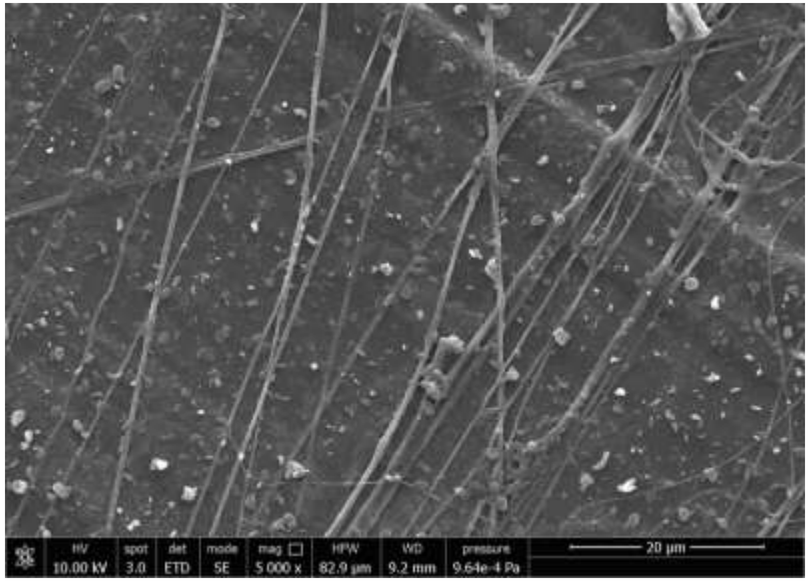
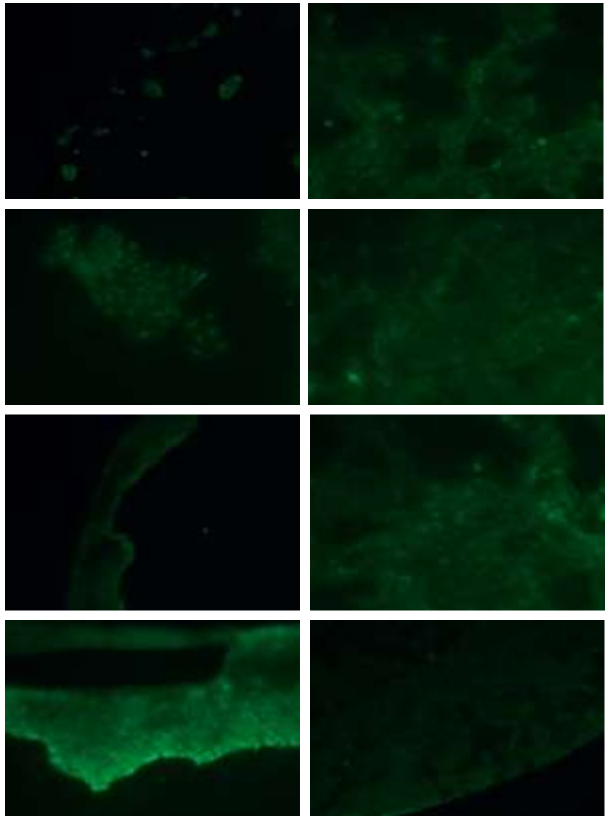
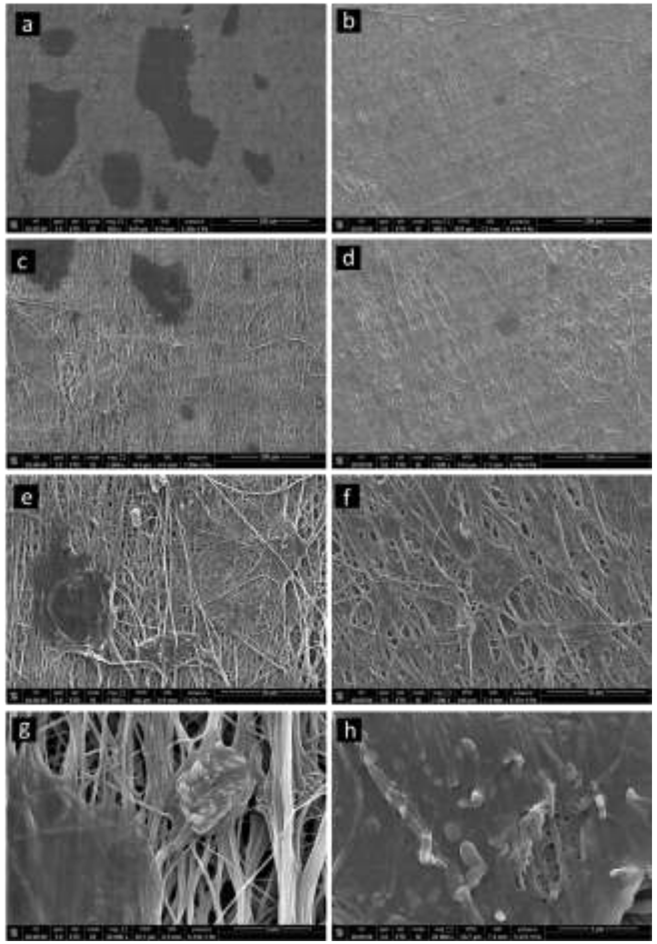
spot 3.0
det ETD
mode SE
mag 3 000 x
HFW 138 µm
WD 9.2 mm
pressure 1.03e-3 Pa
40 µm

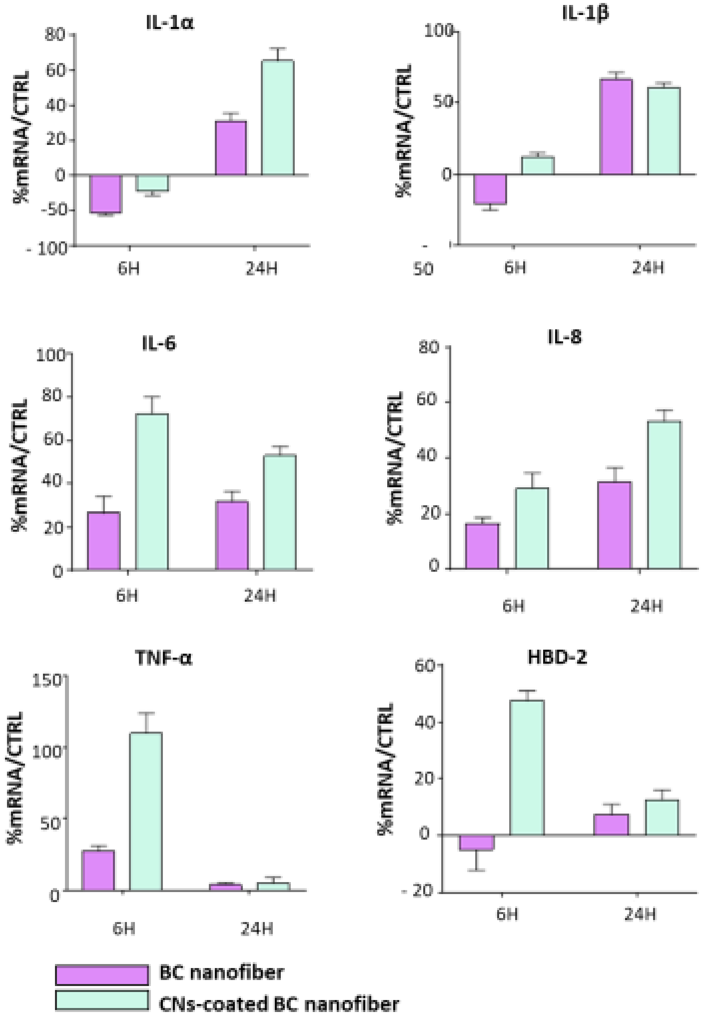

HV 10.00 kV
spot 3.0
det ETD
mode SE
mag 5 000 x
HFW 82.9 µm
WD 9.2 mm
pressure 9.64e-4 Pa
20 µm

BC nanofiber | CN-coated BC nanofiber



BC nanofiber | CNs- coated BC nanofiber





In addition, the fiber coated with chitin nanofobril is also able to upregulate the expression of HBD-2, so we can hypothesize that it is endowed with indirect antibacterial activity.

- Electrospray is an interesting and effective method for surface-decorating of different substrate including electrospun nonwovens.

- Chitin nanofibrils are intersecting materials for surface modification of different substrate due to their anti-microbial and anti-inflammatory properties.

- Thanks to electrospray technology, it was possible to decorate the surface of different substrates including cellulose tissue, electrospun PHAs nanofibers, electrospun PEOT/PBT electrospun nanofibers and cellulose nanofibers to improve some specific properties such as antibacterial and anti inflamatory properties.

Research Team

THANK YOU