WORKSHOP

2021

BIOBASED MATERIALS RESEARCH: ADVANCES FROM ECOFUNCO AND BIONTOP EUROPEAN PROJECTS

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Bio-based Industries Consortium

Horizon 2020 European Union Funding for Research & Innovation

This project has received funding from the Bio Based Industries Joint Undertaking (JU) under grant agreement No 837863. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium.

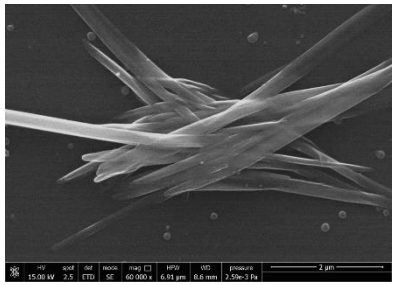
Biological data on chitin

Serena Danti, UNIPI

When fibrillated on the nanoscale,

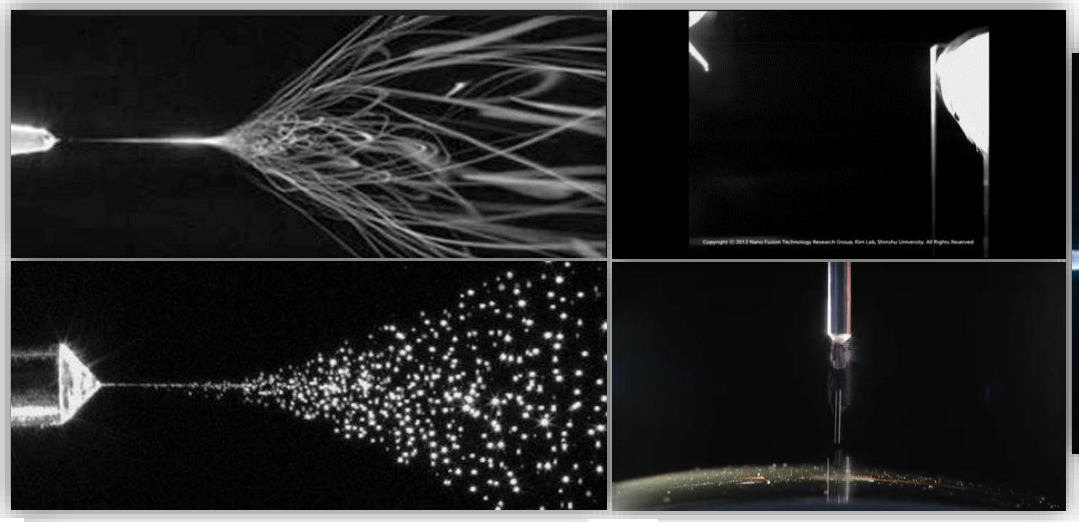
- chitin loses its pro-inflammatory and allergenic character
- is easily metabolized by the body's endogenous enzymes
- is able to proficiently interact with many cellular compounds in biological tissues thus is used in cosmetic dermatology
- controlling the crystalline structure and purity of CNF leads to improvement of its anti-bacterial, anti-inflammatory, cicatrizing, and anti-aging activity.
- excellent biocompatibility and biodegradability
- non-toxicity to human and environment (air, water and soil)
- antibacterial potency and low immunogenicity

Therefore, being considered as a superior material for a sustainable future of industrial development, chitin perfectly meets up the demands with diversified functionalities in applications



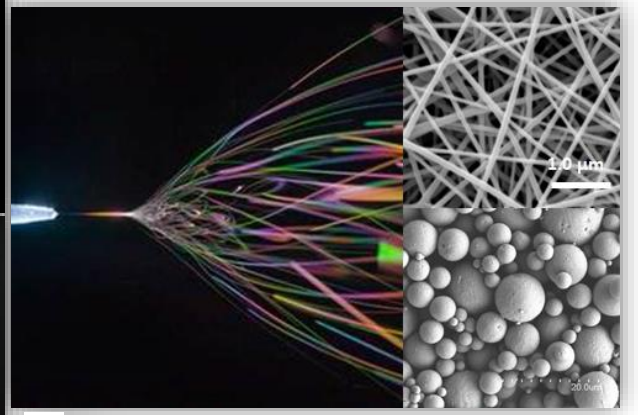
Danti S, Trombi L, Fusco A, et al. Chitin nanofibrils and nanolignin as functional agents in skin regeneration. Int J Mol Sci 2019;20. https://doi.org/10.3390/ijms20112669.

Thangadurai TD, Manjubaashini N, Thomas S, Maria HJ. Nanostructured Materials. Cham: Springer International Publishing; 2020. https://doi.org/10.1007/978-3-030-26145-0

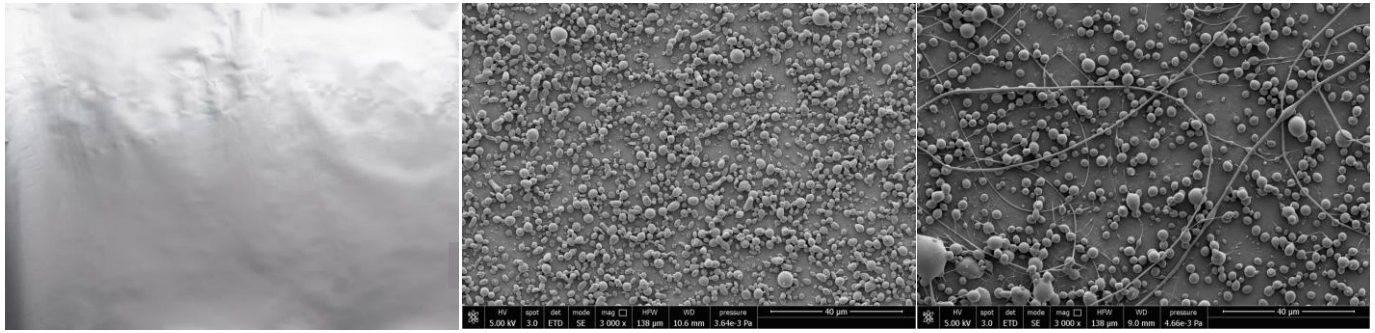


https://www.nanoscience.com/products/electrospinning-equipment/

https://www.youtube.com/watch?v=r6TGvG7RUyo&t=54s



https://www.prweb.com/releases/2017/08/prweb14593130.htm



No chain entanglement during electrospraying ≤ **Highest possible concentration of polymer should not be larger than 3 times critical overlap concentration (C*) for lower molecular weights polymers** ≤ Highly viscous solutions, beaded fibers or fibers are generated

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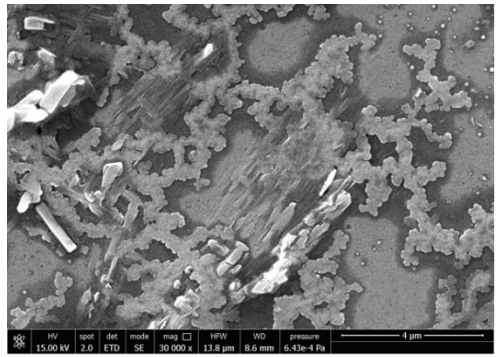
Different chitin sources

ChNF/Celabor (Glentham, Shrimp) (1.5%)

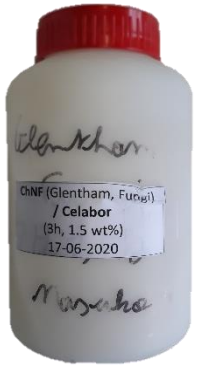


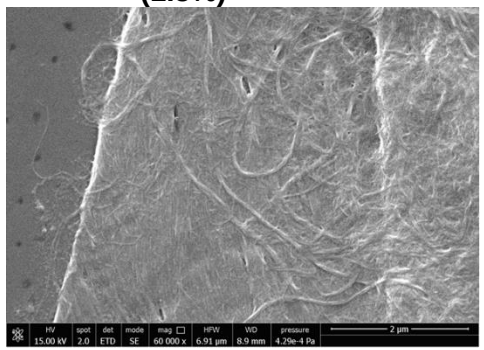
- Distilled water

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

- Distilled water: hexafluoroisopropanol ((60:40) w/w)

ChNF/Celabor (Glentham, Mushroom) (1.5%)





- Distilled water

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

The chitin became soluble in dilute acetic acid at the degrees of deacetylation (DD) of ca. 28% or over and soluble in water at the DD of ca. 49%.
The solubility of the partially deacetylated chitins had a close relationship with their crystal structure, crystallinity, and crystal imperfection as well as the glucosamine content.

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Electrospray of CN on the surface of AL foil and cellulose substrate

Sample #	Chitin type	Solvents	Concentration (w/w)	Voltage (kV)	Flow rate (mL/h)	Distance (cm)
1	ChNF/Celabor (Shrimp-based) (1.5%)	Distilled water	0.52	17	0.136	7
2	ChNF/Celabor (Shrimp-based) (1.5%)	Distilled water: Acetic acid ((50:50) w/w)	0.52	17	0.136	7
3	ChNF/Celabor (Shrimp-based) (1.5%)	Distilled water: HFIP ((60:40) w/w)	0.52	17	0.136	7
4	ChNF/Celabor (Glentham, Mushroom-based) (1.5%)	Distilled water	0.52	17	0.136	7
5	ChNF/Celabor (Glentham, Mushroom-based) (1.5%)	Distilled water: Acetic acid ((50:50) w/w)	0.52	17	0.136	7

Electrospray of CN on the surface of AL foil and cellulose substrate

(Linari Engineering s.r.l., Pisa, Italy)

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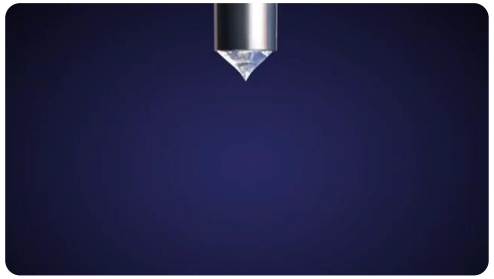
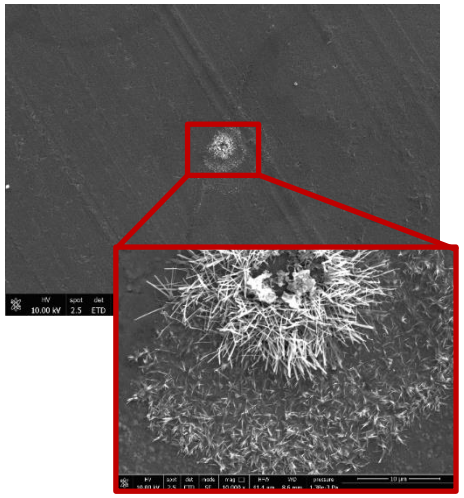
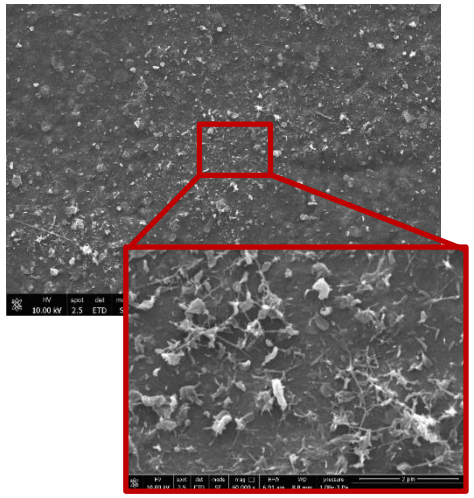
Electrospray of CN (shrimp-based) on the surface of AL foil



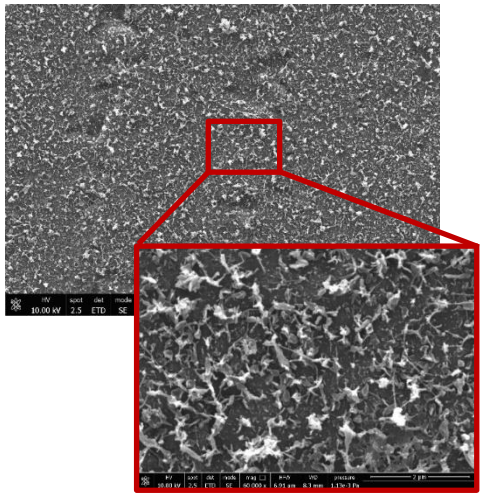
Distilled water



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



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



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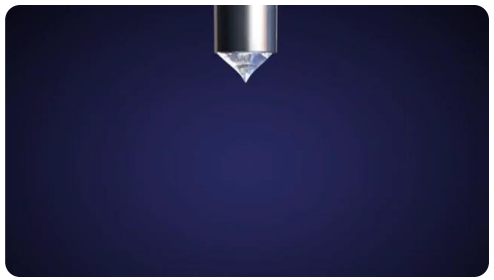
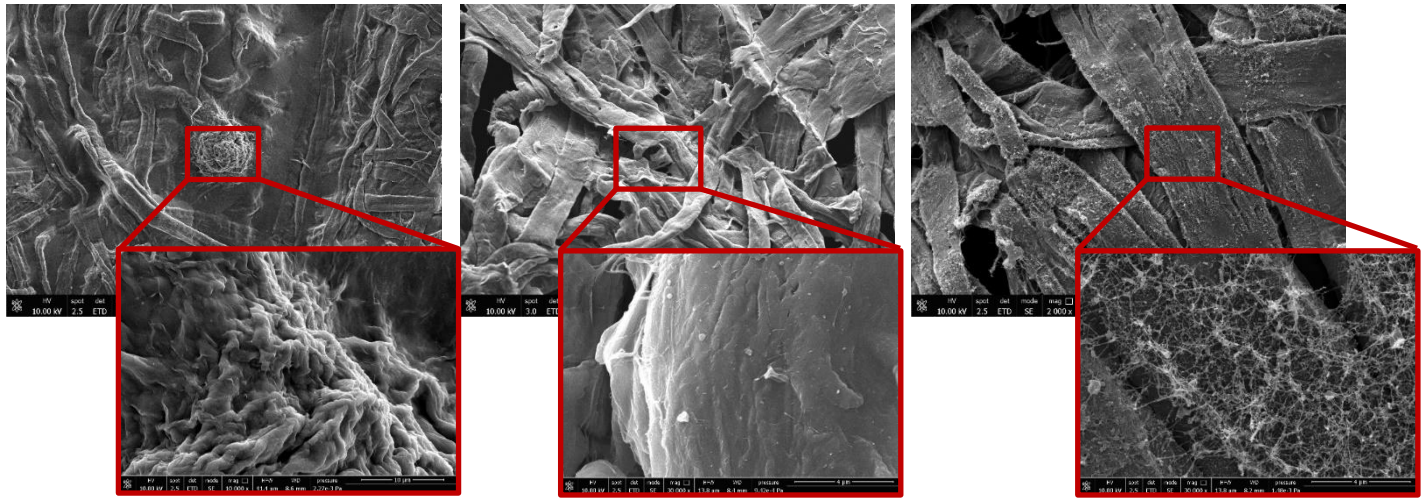
Electrospray of CN (shrimp-based) on the surface of cellulose substrate



Solvent: Distilled water

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

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



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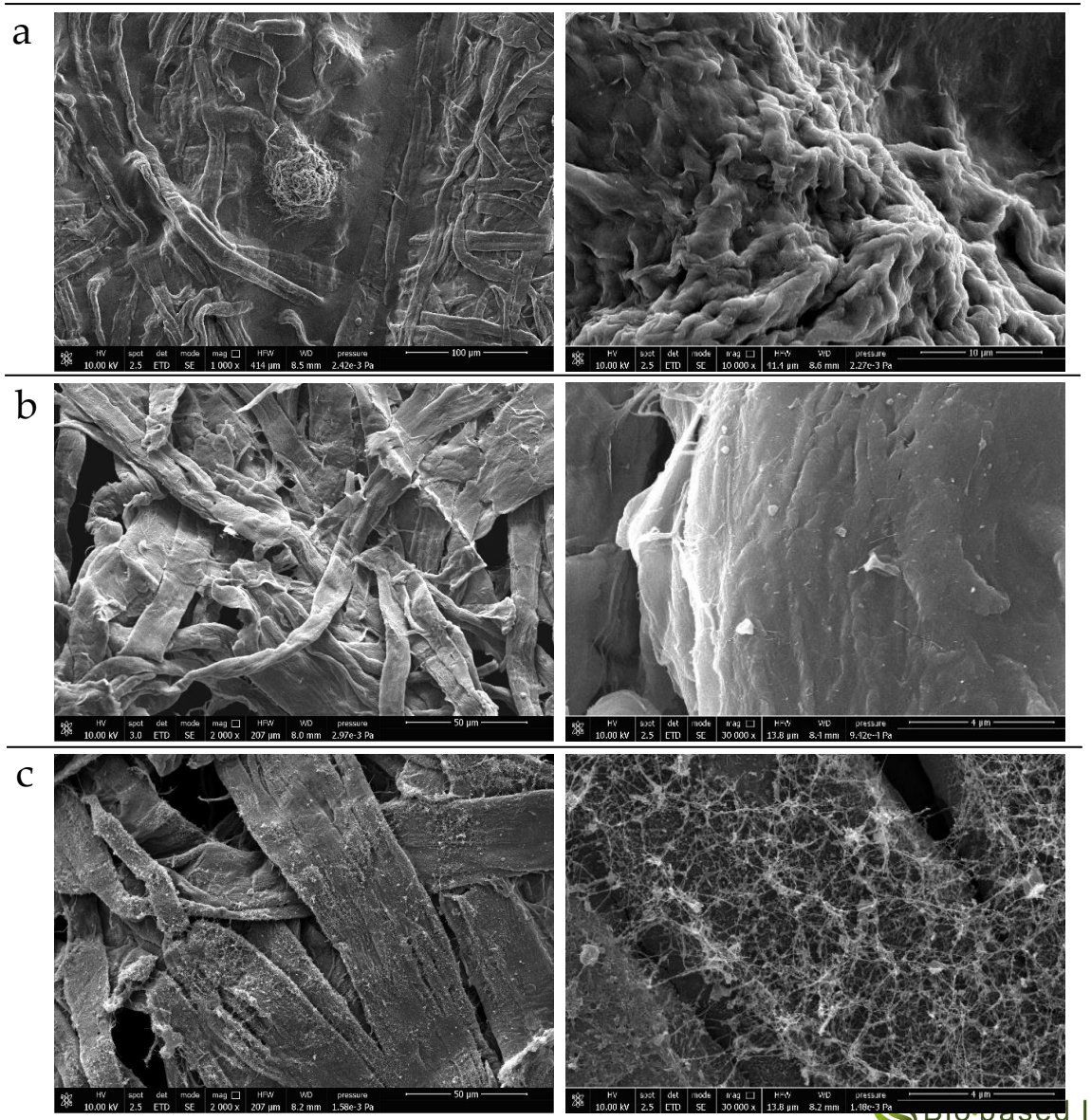
Morphology by SEM



SEM analysis of cellulose tissue decorated with electrosprayed sCNs using different solvent systems:

a) distilled water,

b) distilled water/acetic acid (50/50 w/w), and

c) distilled water/HFIP (60:40) w/w).

Left column shows zoomed-out (2000×), while right column shows zoomed-in (30,000×) magnifications.

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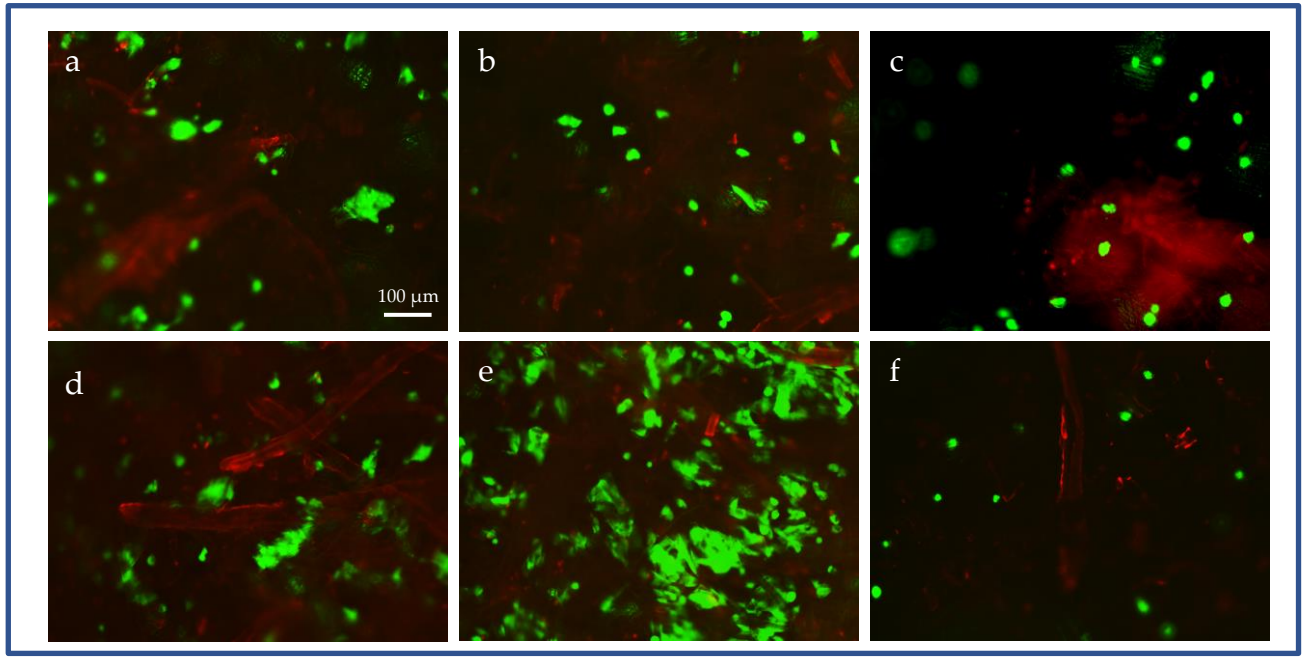
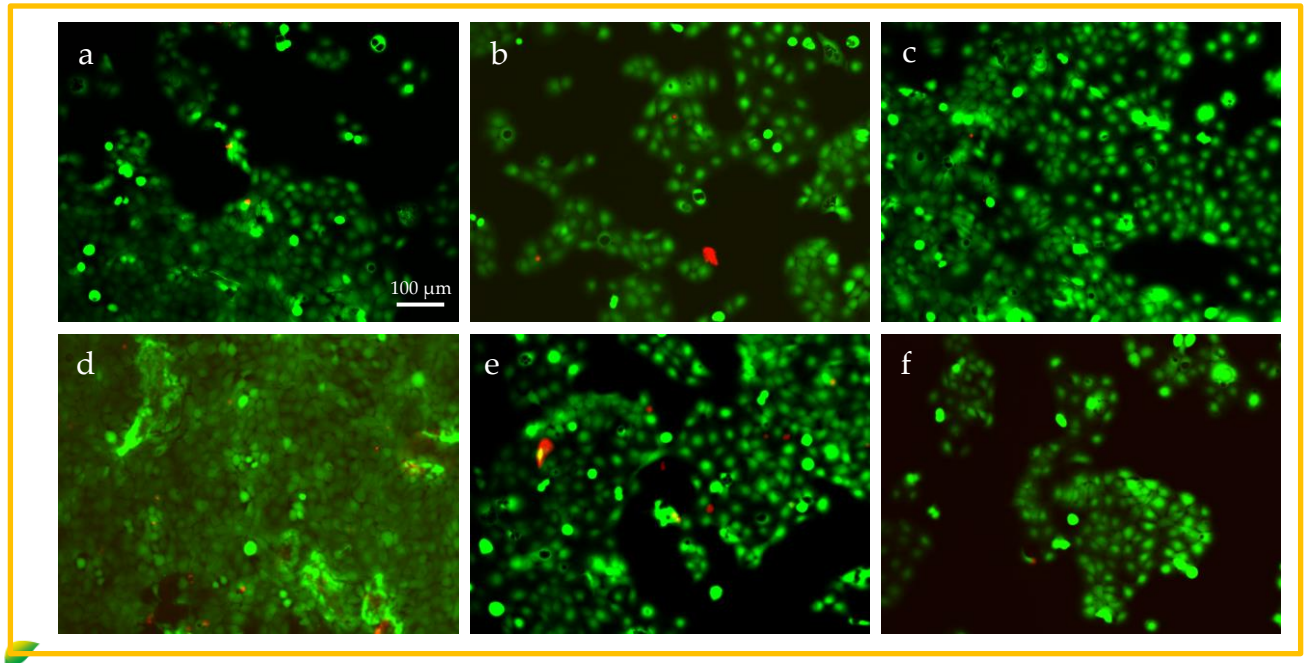
Cytotoxicity Tests





Direct & indirect cytotoxicity tests: Live/Dead viability test performed on HaCaT cell line seeded on cellulose tissues electrosprayed with:

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

Viable cells are stained in green, dead cells are stained in red, the cellulosic tissue shows autofluorescence mainly in the red channel.

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Cell colonization by SEM

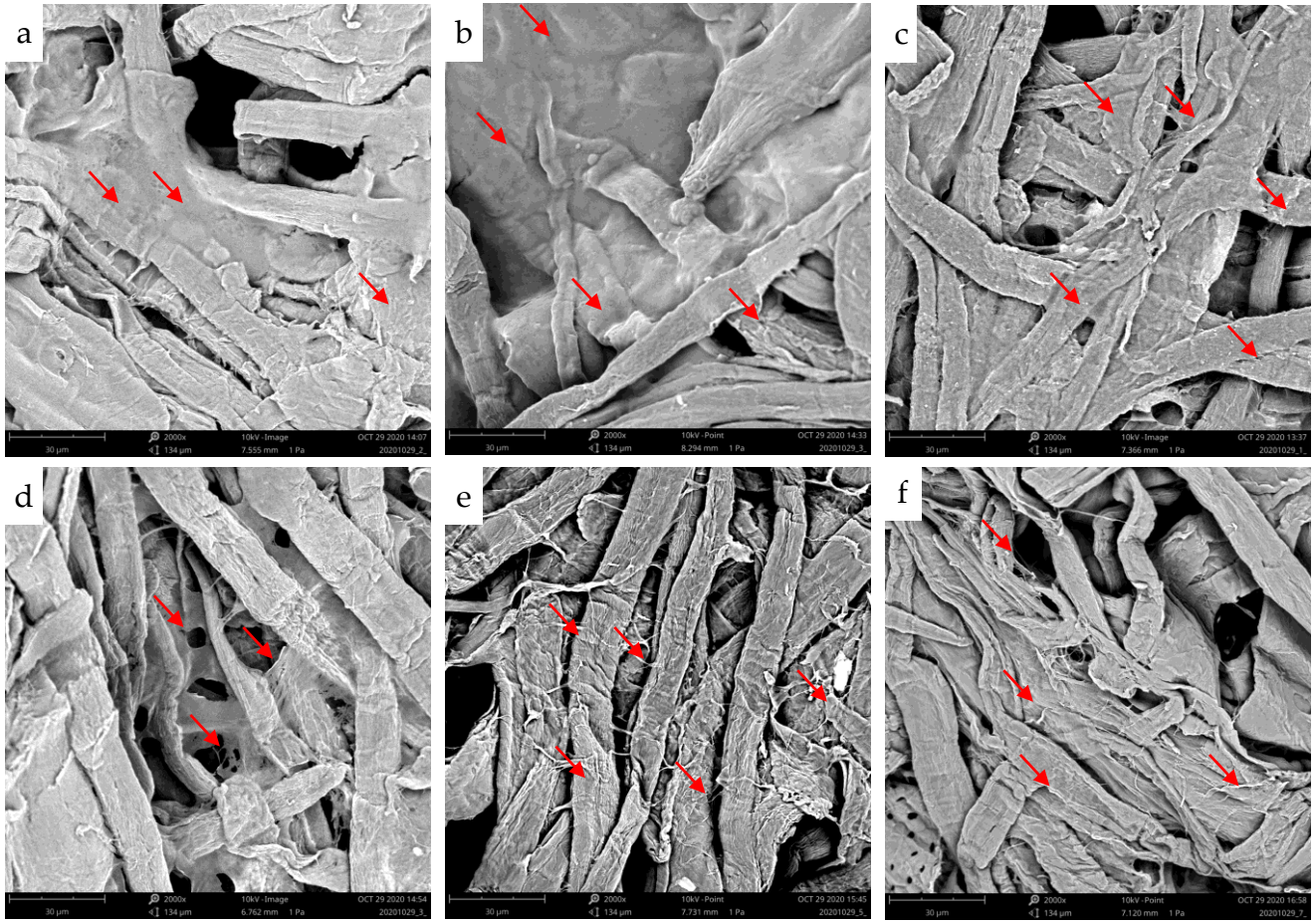

Table. Average metabolic activity given as AlamarBlue reduction percentage (%AB_{red}) performed at 24 h performed on HaCaT cells in presence of cellulose tissue, coated via different CN/solvent suspensions (n = 2).

Cellulose tissue sample	Electrospray solvent(s)	% AB_{RED}
sCN-coated	distilled water	73%
sCN-coated	distilled water/acetic acid	79%
sCN-coated	distilled water/HFIP	98%
mCN-coated	distilled water	95%
mCN-coated	distilled water/acetic acid	88%
Pristine	none	71%

Direct cytotoxicity test: SEM analysis performed on HaCaT cells cultured on cellulose tissues electrosprayed with:

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

Some densely cell populated areas are pointed by red arrows. Original magnification 2000×.

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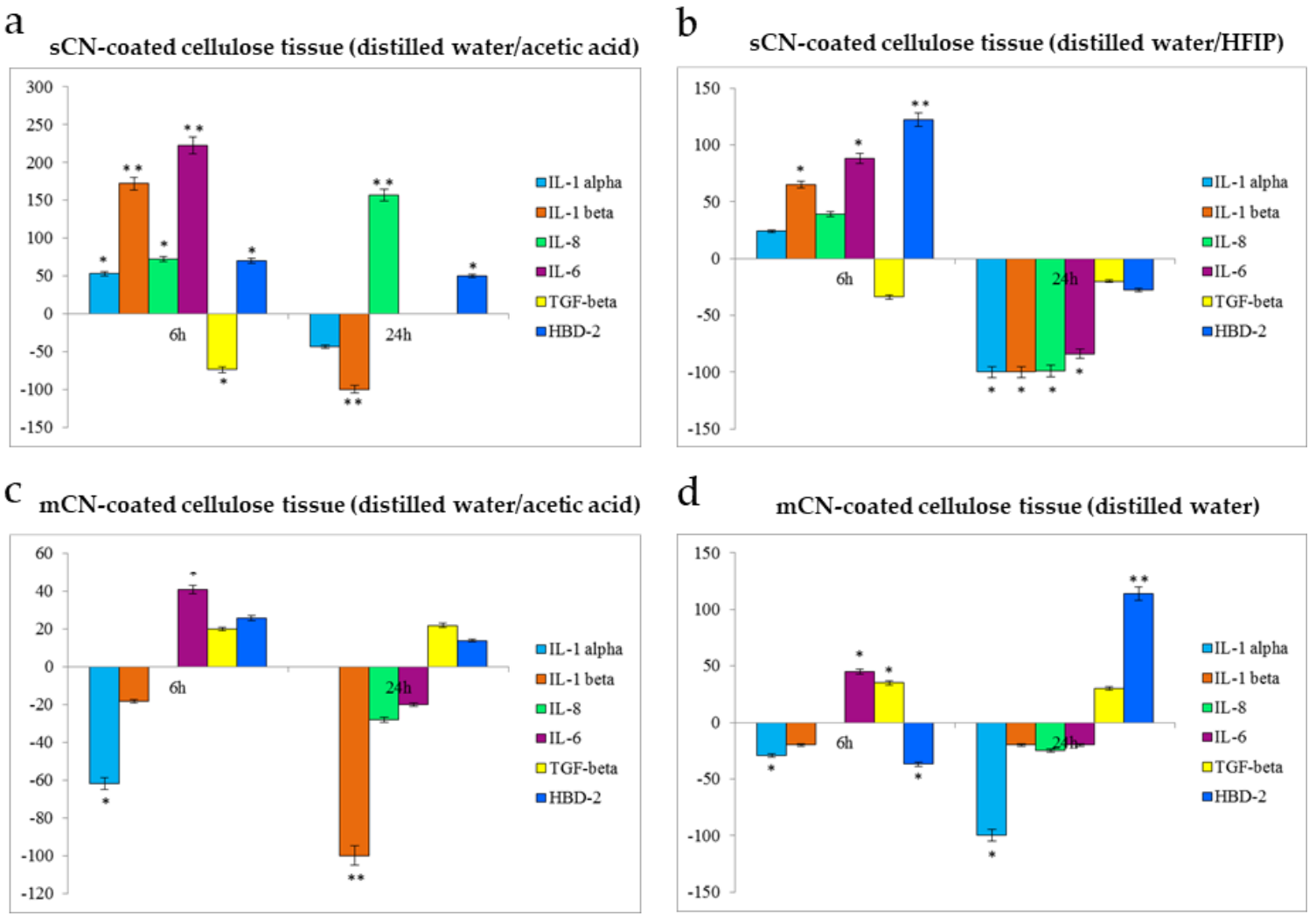
Immunomodulation



Bar graphs showing the results of quantitative RT-PCR related to different cytokines involved in the inflammatory response of HaCaT cells and HBD-2 produced by HaCaT cells after being exposed to the different CN-coated cellulose tissues for 6 h and 24 h. The results were normalized by the expression in cells treated with pristine cellulosic tissue as a control and thus are given as mRNA expression percentage. Statistically significant differences determined using student *t*-test are indicated by * $p < 0.05$ and ** $p < 0.001$.

The outcomes obtained showed a different profile based on the CN sources applied onto the cellulosic substrate.
In fact, while the sCNs showed marked early pro-inflammatory and indirect antimicrobial activity (a,b), the mCNs displayed a weak pro-inflammatory behavior and a predominant anti-inflammatory activity, with insignificant induction of IL-6 and a delayed induction of HBD-2 (c,d). Interestingly, sCN-coated cellulose tissues electrosprayed with water/HFIP mixture, showed a well-defined downregulation of all the pro-inflammatory cytokines in 24 h (b). sCN-coated cellulose tissue using only distilled water, did not show any difference with respect to pristine cellulose tissue, which confirms the insufficient and inhomogenueus coating.
TNF-α, a powerful pro-inflammatory cytokine, was not modulated in any samples.

Conclusions

- An easy and efficient method was set up to uniformly decorate the surface of cellulose tissue via electrospray of CNs extracted from different sources, i.e., shrimp and mushroom derived. Among different water-based solvent systems tested for CN dispersion, a mixture of water and acetic acid (50/50%) was the most effective to have the cellulose tissue decorated with low aggregated CNs.
- We performed direct and indirect cytotoxicity tests to evaluate the compatibility in vitro with HaCaT cells, which successfully adhered to the tissue and were highly viable in culture media conditioned with the material supernatants.
- The use of solvents did not affect the final cytocompatibility as a result of their effective evaporation during the electrospray process.
- Such completely bio-based functional tissues possessed promising anti-inflammatory and indirect antimicrobial activity, even though slight differences could be observed according to the diverse CN source/solvent system.

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Thank you!