Deposition of Chitin Nanofibrils on different substrates by Electrospray Technique

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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.

- **A** Plasma treatment or wet chemical method
  - Plasma or wet chemical treatment
  - Immobilization of protein, enzyme, growth factor, drug
  - Biocompatible nanofibers
  - Functionalized surface

- **B** Surface graft polymerization
  - Electrosyn nanofibers
  - Induced by plasma or radiation
  - Monomer
  - Surface graft polymerization
  - Immobilization of protein, enzyme, growth factor, drug
  - Biologically or therapeutically functionalized nanofibers

- **C** Co-electrospinning
  - Blend solution
  - Electrospinning
  - Surface orientation
  - Biologically or therapeutically functionalized nanofibers

- **D** Deep coating
  - a) dipping
  - b) withdrawal
  - c) evaporation

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

- Poor control of adhesion and composition
- Rigorous processing conditions
- Long reaction times
The Basic Principle of the Electrospinning

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

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.

The Basic Principles of Electrospray

1. Solvent evaporation
2. Polymer diffusion
3. Chain entanglement (the number of entanglements per chain in solution)

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.

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

A. Electrospinning

B. Electrospaying

C. Simultaneous electrospinning & electrospaying

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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.
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

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)

Azimi et al, Electrospayed Shrimp and Mushroom Nanochitins on Cellulose Tissue for Skin Contact Application, Molecules, 26, 4374
Electrospray of CN (Mushroom-based) on AL foil and cellulose substrate

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

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

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.

(a) sCNs (water); (b) sCNs (water/acetic acid); (c) sCNs (water/HFlP); (d) mCNs (water); (e) mCNs (water/acetic acid). (f) Pristine cellulose tissue.
Increasing the knowledge and understanding of smart nanomaterials and nanotechnologies as applied to biopolymers and validating them in the market.
Developing a biodegradable and at least 90% bio-based nanostructured biocompatible non-woven tissue for use in wound dressings.
P(3HO-co-3HD/ PHB) fiber

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

Solution properties: concentration (11 w/w%), PHB/PHOHD (1:10), solvents: (chloroform/2-butanol)(70:30 (v/v)), additive: 0.002 g/mL LiBr.

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.
Electrosprayed CNs on the surface of PHOHD/PHB fibers

Electrosprayed CLAs on the surface of PHOHD/PHB fibers

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

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<th>SAMPLE</th>
<th>%AB&lt;sub&gt;RED&lt;/sub&gt;</th>
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<tr>
<td>CN-coated</td>
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**Reduction in %mRNA/Ctrl**

- **IL-1**: 
  - PHB/PHOHD fibers
  - CN-coated PHB/PHOHD fibers
  - CLA-coated PHB/PHOHD fibers

- **IL-6**: 
  - PHB/PHOHD fibers
  - CN-coated PHB/PHOHD fibers
  - CLA-coated PHB/PHOHD fibers

- **IL-8**: 
  - PHB/PHOHD fibers
  - CN-coated PHB/PHOHD fibers
  - CLA-coated PHB/PHOHD fibers

- **HBD-2**: 
  - PHB/PHOHD fibers
  - CN-coated PHB/PHOHD fibers
  - CLA-coated PHB/PHOHD fibers

- **TNF-α**: 
  - PHB/PHOHD fibers
  - CN-coated PHB/PHOHD fibers
  - CLA-coated PHB/PHOHD fibers
(PEOT/PBT) electrospun fibers

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.
Electrospinning of (PEOT/PBT)/CN composites fibers

Electrospaying of CN nanoparticles

\[\text{CNs} \quad \text{sCNs} \quad \text{mCNs}\]

**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

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%

(particle diameter: 500-900 nm)
Electrospray of PLGA NPs on the surface of (PEOT/PBT)/CN composite fibers

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
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).
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

electrospinning of cellulose nanofibrils

NanoCell
50 rpm fiber diameter: 375 ± 118
electrospinning of cellulose nanofibrils
In addition, the fiber coated with chitin nanofibril 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-inflammatory properties.
THANK YOU