Keratin based nanofibres by electrospinning: fabrication and applications

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**INTRODUCTION OF MYSELF**

**Research activities:**
- Design of new polymeric fibres;
- Set-up of new textile processes;
- Study of handle, wear properties and comfort of textiles;
- Development of analytical methods for the quality assessment of fibres and fabrics.

**High Textile Technology laboratory (LATT):**
- Low Temperature Plasma Electrospinning

**My main research topic:**
- Processing of biopolymers from textile materials
a non food protein at high sulphur content

Wool

 Feather

Horns & Nails

Hair and stratum corneum of the skin
Keratin is distinguished from other structural proteins (fibroin or collagen) by the number of cysteine amino acid residues in the protein molecules (7-20% of the total amino acid residues).

The disulphide covalent bonds confer to keratin a high resistance to chemical and enzymatic attacks.
Millions of tons/year of keratin wastes from

1. Poor quality raw wool not fit for spinning
2. Hair and feathers from butchery
3. By-products of the textile industry

Disposal of keratin wastes → environmental problem!!

Moreira et al., Mycopathologia (2007) 163, 153-160
KERATIN WASTES → Industrial applications

- Animal Feedstuff
- Fire Fighting Foams
- Cosmetics
- Fertilizer

Hydrolyzed Keratin Powder
- Keratins (84%)
- Other proteins, lipids (16%)

**Matrix**
- High-sulphur content keratin (8% wt) (HS)
- MW: 28-11 kDa
- Disordered structure

**Cuticle**
- High-sulphur content keratin (8% wt) (HS)
- MW: 28-11 kDa
- Disordered structure s/β sheet

**Intermediate filaments**
- Low-sulphur content keratin (2-3 %wt) (LS)
- MW: 60, 45 kDa
- α-helix structure

**WOOL**

- Protopilars
- Nuclear remnants
- Microfibril
- Macrofibril
- Cortical cell
- Nuclear remnants
- Paracortex
- Orthocortex
- Endocuticle
- Exocuticle
- Epicuticle
- Cuticular scale
- Cortex

- Cutting pattern
- Textural modifications
**KERATIN EXTRACTION**

- **Reducing agents**
  - Thioglycolic acid
  - DTT (dithiothreitil)
  - 2-Mercaptoethanol
- **Oxidizing agents**
  - Peracetic acid
  - Performic acid
- **Sulphitolysis** \((\text{SO}_3^{2-}, \text{HSO}_3^-, \text{S}_2\text{O}_5^{2-})\)

\[
\text{Wool-S-S-Wool} + \text{SO}_3^{2-} \rightarrow \text{Wool-S}^- + \text{Wool-S-SO}_3^-
\]

- Protein denaturing agent: UREA
Freeze-dried Keratin

Isoelectric Point

(pI) = 4-4.5

Molecular weight distribution

Low sulphur content keratin

High sulphur content keratin

Amino acid composition

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mol (%)</th>
</tr>
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<tbody>
<tr>
<td>CYA</td>
<td>0.36</td>
</tr>
<tr>
<td>ASP</td>
<td>8.31</td>
</tr>
<tr>
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</tr>
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<tr>
<td>PRO</td>
<td>6.71</td>
</tr>
<tr>
<td>LANT</td>
<td>1.02</td>
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<tr>
<td>CYS + CYS-SO_3^-</td>
<td>8.45</td>
</tr>
<tr>
<td>TYR</td>
<td>3.41</td>
</tr>
<tr>
<td>VAL</td>
<td>6.30</td>
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<td>MET</td>
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Hydrophilic Amino Acids

Hydrophobic Amino Acids

**Amino acid composition**

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**Functional groups able to bind cations**

> Adsorbent for heavy-metals or cationic dyes

**Keratin can fix formaldehyde**

1. Reaction step

```
Protein-NH₂ + HOCH₂-CH₂-OH → R-N-CH₂OH
```

Aminomethylol Derivative

2. Reaction step

```
R-N-CH₂OH + NH₂ → R-N-N=C=-C-R' + H₂O
```

Acide Amide

e.g. asparagine, glutamine

**Applications**

- Keratin for wastewater treatment and air cleaning
- Keratin for fixing formaldehyde

**References**


WOOL KERATIN FOR BIOMEDICAL APPLICATIONS


**Cytotoxicity test**

Concentration-dependent and time-dependent effect of RK-based microparticles on LDH release by THP-1 monocytic cell line

![Microparticles](image)

Spry-drying process

Keratin/Ceramides Film

Scaffold sponges


Biodegradable Vessel

F. Selmin, F. Cilurzo, A. Aluigi, S. Franzé, P. Minghetti, Results in Pharma Science 2 (2012) 72-78

KERATIN PROCESSING

Wool

Keratin powder

Nanofibrous Membrane

Microspheres

Hydrogels


Research project funded by Pfizer International Operations

C. Vineis, A. Aluigi, A. Varesano, C. Tonetti, G. Mazzucchetti, Alimenta XXI 3 2013

NANOFIBROUS MEMBRANES

- very high surface to volume ratio
- highly porous, interstitial pore size within nano range
- design flexibility for chemical/physical functionalization
- unique physical, chemical, electrical, optical properties

PP microfibres (5 micron) and electrospun nylon nanofibers (200 nm)
NANOFIBRES

Tissue Engineering Scaffolds
- adjustable biodegradation rate
- better cell attachment
- controllable cell directional growth

Hemostatic Devices
- higher efficiency in fluid absorption

Cosmetics
- higher transfer rate

Protective Clothing
- chemical barrier
- lightness
- comfort (transpiration properties)

Wound Dressing
- prevent scars
- bacterial shielding

Drug delivery
- increased dissolution rate
- drug nanofibre interface

Electrical conductors
- ultra small devices

Filter media
- higher efficiency filtration

Sensor devices
- higher sensitivity

Technical Textiles
- acoustic insulation
- thermal insulation
KERATIN NANOFIBRES: WHY?

Keratin Properties
- Biodegradable \textit{(in vitro and in vivo)} [1]
- Fibroblast and osteoblast cell growth [2]
- Heavy-metals and formaldeyde absorption [3]

Nanofibrous Membrane Properties
- High surface to volume ratio
- High porosity
- High surface functionality

Biomedical Applications
- Scaffolds for cell growth
- Medical Textiles
- Drug delivery systems

Filtration Systems
- Air Filters
- Water depuration systems from heavy-metals or cationic dyes

ELECTROSPINNING

NANOFIBERS BY ELECTROSPINNING PROCESS

Basic setup for an electrospinning apparatus
High voltage supplier [10 ÷ 30 KV DC or AC]
Syringe with a small diameter needle [0.2 ÷ 1.5 mm]
Collecting screen

Electrospinning Process

Surface tension
Electrostatic force

Syringe
Polymer Fluid
Capillary
Generator
Collecting Screen

Taylor cone
Whipping
Nanofibers
CONTROL OF THE NANOFIBRE MORPHOLOGY AND STRUCTURE

Intrinsic properties of the polymer fluid
- Viscosity
- Conductivity
- Surface tension

Operational parameters
- Applied voltage
- Tip to collector distance
- Fluid flow rate

Environmental conditions
- Temperature
- Humidity
<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solvent</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin/PEO [1-2]</td>
<td>Water</td>
<td>Tissue Engineering</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Air filters</td>
</tr>
<tr>
<td>Keratin/Fibroin  [3]</td>
<td>Formic Acid</td>
<td>Tissue Engineering</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water depuration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Air filters</td>
</tr>
<tr>
<td>Keratin/PA 6 [4]</td>
<td>Formic Acid</td>
<td>Water depuration</td>
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<tr>
<td></td>
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<td>Air filters</td>
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<tr>
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<td></td>
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</tr>
</tbody>
</table>

**Keratin Nanofibres**

**Keratin/Fibroin Blend Nanofibres**

Keratin (84% wt)

(60-18) kDa

α-helix

**Wool**

**Bombix Mori Silk**

Fibroin (75% wt)

(~300) kDa

β-sheet

**Blend Solutions**

<table>
<thead>
<tr>
<th>Keratin in Formic Acid 15% wt</th>
<th>Fibroin in Formic Acid 15% wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Electrospinning Conditions**

<table>
<thead>
<tr>
<th>Applied Voltage (kV)</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate (ml/min)</td>
<td>0.005</td>
</tr>
<tr>
<td>Work Distance (cm)</td>
<td>10</td>
</tr>
<tr>
<td>Capillary Diameter (mm)</td>
<td>0.2</td>
</tr>
<tr>
<td>Deposition Time (min)</td>
<td>20</td>
</tr>
</tbody>
</table>
Additivity Rule

\[ \ln \eta_T = \sum_i w_i \ln \eta_i \]

- \( w_i \) = weight fraction of the \( i^{th} \) component
- \( \eta_T \) = theoretical viscosity
- \( \eta_i \) = solution viscosity of \( i^{th} \) component

Viscosity

- Enhanced interactions between the two proteins

Conductivity

- Experimental data; - theoretical viscosity
MORPHOLOGY OF KERATIN/FIBROIN BLEND NANOFIBRES

Keratin/Fibroin 0/100
Diameter: 945 ± 483 nm

Keratin/Fibroin 10/90
Diameter: 518 ± 205 nm

Keratin/Fibroin 30/70
Diameter: 372 ± 125 nm

Keratin/Fibroin 50/50
Diameter: 207 ± 66

Keratin/Fibroin 70/30
Diameter: 344 ± 137 nm

Keratin/Fibroin 90/10
Diameter: 233 ± 57 nm

Keratin/Fibroin 100/0
Diameter: 169 ± 49 nm
“Bottom-up” configuration of electrospinning apparatus


**Solution Properties**

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solute</td>
<td>Keratin powder</td>
</tr>
<tr>
<td>Solvent</td>
<td>Formic Acid (98%)</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>15% wt</td>
</tr>
<tr>
<td>Viscosity</td>
<td>0.18 ± 0.01 Pa s</td>
</tr>
<tr>
<td>Conductibility</td>
<td>2.1 mS/cm</td>
</tr>
</tbody>
</table>

**Electrospinning Conditions**

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied voltage</td>
<td>25 kV</td>
</tr>
<tr>
<td>Flow - rate</td>
<td>3 µl/min</td>
</tr>
<tr>
<td>Tip to target distance</td>
<td>20 cm</td>
</tr>
<tr>
<td>Deposition time</td>
<td>60 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>21-25 °C</td>
</tr>
<tr>
<td>Relative Humidity</td>
<td>45-50%</td>
</tr>
</tbody>
</table>
**PROPERTIES OF KERATIN NANOFIBRE MEMBRANES**

- **Fibre diameter (nm)**: 223 ± 74
- **Thickness (µm)**: 50
- **Porosity (%)**: 90
- **Specific surface area (m²/g)**: 13.59

![Flexible!!]
KERATIN NANOFIBRE MEMBRANES AS ADSORBENTS FOR...

Formaldehyde \[^1\]

Cationic Dyes \[^2\]
(Methylene Blue)

Heavy-metals \[^3\]
(Cu\(^{2+}\))

ACTIVE FILTER MEDIA

Adsorption

Filtration

Decrease of formaldehyde concentration with an initial concentration of 0.6 ppm (100%) during time in the presence of filters

Tests performed at 20°C and 65% r.h.

**Methylene Blue and Cu(II) Adsorption**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Keratin Nanofibres</th>
<th>Wool Fabric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre diameter (nm)</td>
<td>223 ± 74</td>
<td>19 µm</td>
</tr>
<tr>
<td>Thickness (µm)</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>90</td>
<td>69.7</td>
</tr>
<tr>
<td>Specific surface area (m²/g³)</td>
<td>13.59</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Adsorption Capacity**

\[
q(mg/g) = \frac{q_0 - q_i}{m}
\]

- \(q_0\) = \(MB/Cu(II)\) amount in the stock solution before adsorption
- \(q_i\) = \(MB/Cu(II)\) amount in the stock solution after adsorption

The higher adsorption capacities of keratin nanofibrous membranes compared to wool fabrics are due to their higher porosity and higher surface area per unit mass.
# COMPARISON WITH OTHER NANOSTRUCTURED MEMBRANES

## Cu(II) adsorption

<table>
<thead>
<tr>
<th>Membrane</th>
<th>$C_0$(mg/L)</th>
<th>Adsorption Capacity (mg/g)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin nanofibre membrane</td>
<td>50</td>
<td>11.3</td>
<td>A. Aluigi et al. Text. Research J. 83 (2013) 1574-1586</td>
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<tr>
<td>Keratin nanofibre membrane</td>
<td>4</td>
<td>5.8</td>
<td>A. Aluigi et al. Text. Research J. 83 (2013) 1574-1586</td>
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</table>
Thank you for your attention!!