

Supporting Information

Nano-hybrid electrospun non-wovens made of wool keratin and hydrotalcites as potential bio-active wound dressing.

*Demetra Giuri^a, Marianna Barbalinardo^b, Giovanna Sotgiu^a, Roberto Zamboni^a, Morena Nocchetti^c, Anna Donnadio^c, Franco Corticelli^d, Francesco Valle^b, Chiara G.M. Gennari^e, Francesca Selmin^e, Tamara Posati^{*a} and Annalisa Aluigi^{*a}.*

^a *Institute of Organic Synthesis and Photoreactivity – National Research Council, via P. Gobetti 101 40129 Bologna, Italy.*

^b *Institute of Nanostructured Materials – National Research Council, via P. Gobetti 101 40129 Bologna, Italy.*

^c *Dipartimento di Scienze Farmaceutiche – Università degli Studi di Perugia, via del Liceo, 1, 06123, Perugia, Italy;*

^d *Institute for Microelectronics and Microsystems – National Research Council, via P. Gobetti 101 40129 Bologna, Italy.*

^e *Department of Pharmaceutical Sciences, Università degli Studi di Milano, via G. Colombo, 71 – 20133 Milano (Italy)*

Section I. Preparation of cystein-S-sulphonated keratin

Merino wool fibres (21 μm fineness) were cleaned by Soxhlet extraction with petroleum ether to remove fatty matters and dried at 21°C and 60% relative humidity overnight. Subsequently, keratin was extracted from wool by sulphitolysis reaction. Briefly, 5g of cleaned fibres were cut into snippets and dispersed in 100 ml of aqueous solution containing urea (8M), sodium metabisulphite (0.5M) and sodium dodecyl sulphate (SDS, 0.1M), under mechanical shaking at 65°C overnight. The mixture was sieved (120 μm cut-off), filtered with vacuum filter (10-16 μm cut off), dialyzed against distilled water using a cellulose tube (molecular weight cut-off 12-14 kDa) for 3 days at room temperature, changing the distilled water four times a day, until the conductivity of external water reached the value of 5 $\mu\text{S}/\text{cm}$. The resulting aqueous solution was freeze-dried in order to obtain pristine keratin powder.

During the sulphitolysis, cystine disulphide bonds are cleaved by sulphite ions to give reduced keratin (WS^-) and cysteine-S-sulphonate keratin (WSSO_3^-).

Section II. Synthesis of ZnAl-HT nanoparticles intercalated with anionic diclofenac (HTD)

Colloidal aqueous dispersion of ZnAl-HT nanoparticles having formula $[\text{Zn}_{0.72}\text{Al}_{0.28}(\text{OH})_2]\text{Br}_{0.28} \cdot 0.69 \text{H}_2\text{O}$ were prepared by double-microemulsion technique as described in previous works [reference 21 in the main manuscript]

The intercalation of Diclofenac (D) into the ZnAl-HT nanoparticles was obtained as previously reported [reference 21 in the main manuscript]. Briefly, 1g of ZnAl-HT in bromide form was equilibrated with 46 mL of a hydroalcoholic (50% vol/vol) solution 0.1M of DNa (Br-/D- molar ratio 1:2) under a nitrogen atmosphere at 60 °C for 2 days. After cooling, the mixture was centrifuged with an Allegra™ 64R Centrifuge (Beckman Coulter) at 12000 rpm for 10 minutes. The residue was washed 3 times with carbon dioxide-free water and finally dried at 50 °C or properly dispersed in water.

The composition of the hybrid compound was obtained by combining ICP analysis and TG data and resulted: $[\text{Zn}_{0.68} \text{Al}_{0.32} (\text{OH})_2] (\text{D})_{0.16} (\text{CO}_3)_{0.08} \cdot 0.67\text{H}_2\text{O}$ (D content 35% w/w) [*reference 20 in the main manuscript*].