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# **Innovative antibacterial biocomposite films based on biomacromolecules and nanostructured zinc oxide**

By

**Paolo Pino**

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**Supervisor(s):**

Prof.ssa F. Bosco

Prof.ssa B. Onida

**Doctoral Examination Committee:**

Prof.ssa B. Vigani, Referee, University of Pavia

Prof.ssa E. Ghedini, Referee, University of Venezia

Prof.ssa E. Verne', Referee, Polytechnic of Turin

Prof. M. Banchemo, Referee, Polytechnic of Turin

Prof.ssa P. Santi, Referee, University of Parma

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## Abstract

Chronic wounds are a large global health issue affecting 2% of the population in western countries, with mortality rates becoming as high as 52% in some cases. Chronic wounds can be caused by – or cause – infections, as pathogens do not encounter the natural barrier represented by the skin and can interfere with the normal healing process. It has been estimated that these infections are responsible for 75% of post-operative deaths. In parallel, treating infections is becoming more difficult, due to antimicrobial resistance (AMR), i.e., the tendency of microorganism to adapt and develop resistance against the drugs that are used to eradicate them. Without further action, AMR is predicted to cause around 10 million deaths per year by 2050. This demands for urgent solutions, including new types of wound dressings. Many dressings have been used and developed over time, from traditional cotton gauzes to modern films, foams, hydrogels, and hydrocolloids. In recent years, a new class of dressing materials has emerged: bionanocomposites. These are composites made by biomacromolecule-based matrixes filled with nanostructured materials. The former primarily bring high biocompatibility and biodegradability, while the latter have been found to have antimicrobial properties. Bionanocomposites can also possess many other beneficial features, such as anti-inflammatory, hemostatic, cellular proliferation and tissue regeneration properties. Common biomacromolecules include polysaccharides like chitosan, cellulose, alginate and starch, to proteins like collagen, gelatin and chitin. Many nanostructured materials have been combined with these polymers. The most common are metal and metal oxide nanoparticles. Among all these, nanostructured zinc oxide (nZnO) is one of the most effective in terms of antibacterial properties, and showed lower toxicity compared to metal nanoparticles. Plus, zinc is an essential element for the human body, and is involved in wound healing and the immune system. For all these reasons, this work concerns the development, characterization and testing of nZnO-containing bionanocomposites using biomacromolecules that have yet been poorly explored but that possibly hold good potential, in an effort to develop new solutions.

The research therefore started from the synthesis of a nanostructured zinc oxide following a simple wet chemical precipitation technique. The nZnO particles are spherical with an average size of 30 nm, a specific surface area of 49.5 m<sup>2</sup>/g, a wurtzite-type crystal structure, and a 3.49 eV bandgap. The oxide has positive electric charge in neutral pH water and shows the presence of some carbonate species on the surface. Its antibacterial efficacy has been tested against Gram-positive *Staphylococcus epidermidis* and Gram-negative *Escherichia coli*. The nZnO revealed far more effective than a commercial, micron-sized ZnO. The minimum inhibitory concentration of nZnO resulted equal to 120 and 480 µg/mL against *S. epidermidis* and *E. coli*, respectively, in line with other values reported in the literature.

At this point, several bionanocomposites with this nZnO were formulated. In a first instance, whey proteins were investigated. These are a byproduct of the dairy industry with uses as food additives and for encapsulation and delivery of nutraceuticals. Composite films were made with Whey Protein Isolate (WPI). These showed high transparency in the visible range (around 70%), which is important to ensure good wound inspection. Their swelling properties are comparable with those of some commercial alginate-based wound dressings. nZnO improved UV-barrier properties, swelling in phosphate buffered saline, elastic modulus and tensile strength, and decreased optical transmittance, mass degradation in artificial sweat, and elongation at break. The mechanical properties were comparable with those of the human skin, which is good to ensure dressing compliance with the underlying tissue. A nZnO concentration-dependent antibacterial activity was observed against *S. epidermidis* and *E. coli*. The values were higher for the first strain and in line with scientific literature.

After WPI,  $\beta$ -glucans were explored. They are known for their immune-stimulatory properties. Yeasts  $\beta$ -glucans/nZnO films were prepared via solvent casting. The films were amorphous and had lower transparency and swelling abilities compared to WPI, but had very good antimicrobial properties. Composites were also made with poly(3-hydroxybutyrate-co-3-hydroxyvalerate), which is often used in scaffolds for tissue engineering due to its high biocompatibility. However, the films were inhomogeneous and had the lowest water affinity and antibacterial activity.

Finally, cellulose-based bionanocomposites were investigated at the Pulp & Paper Research Center of McGill University, Montreal, Canada. To this end, a recently developed mildly-carboxylated cellulose was used, denominated carboxymethyl fiber (CMF), and incorporated with nZnO for the first time. Films had good transparency and mechanical properties. The oxide increased water contact angle and surface roughness, as well as the swelling ability, which was the highest ever reported in this work. The antibacterial activity was slightly lower than that of WPI-based composites and decreases if a CMF matrix with a higher degree of substitution of carboxyl groups was used, due to the antibacterial  $Zn^{2+}$  being sequestered by the negative carboxyl groups.

Of all the bionanocomposites considered, WPI-nZnO offered the best compromise among high transparency, swelling, antibacterial action, ease of production, low cost and environmental impact, homogeneous nZnO dispersion, and mechanical properties comparable with the human skin. For this reason, WPI-nZnO composites underwent water contact angle measurements, hydration tests, viscoelastic properties measurements after hydration, and degradation tests in liquid oxidative environment. The presence of nZnO increased water contact angle while maintaining its values within the range of common wound dressings. Zinc oxide also improved hydration and allowed to better retain good viscoelastic properties. This indicates that the composite dressing is well suited to remain compliant with skin strains even after absorbing wound exudates upon use. The films were also stable in oxidative environments and degraded less when nZnO was present. WPI-nZnO composites were also tested against pathogen *S. aureus* and *E. coli*. This time, the samples were more effective against the *E. coli*, showing that the films might also work well against some Gram-negatives. Subsequently, *in vitro* cytotoxicity and proliferation tests on human dermal fibroblasts were carried using culture media previously conditioned with WPI and WPI-nZnO samples (also called extracts). In the first test, cytotoxicity was observed when cells were exposed to non-diluted extracts, but disappeared at 1:2 dilution. This same dilution enhanced proliferation in the second test, compared with control samples. Finally, *in vivo* wound healing tests were carried on rats. Wounds treated with WPI or WPI-nZnO samples healed correctly and without any delay or sign of toxicity compared to non-treated wounds, as revealed by histological analyses, where clear evidence of re-epithelialization and tissue restoration could be found.

In conclusion, this work contributed to the progress in the field of advanced wound dressing materials through the design, preparation and study of new potential candidates. To this end, the systematic assessment of the materials explored allowed to outline a standard framework and set of tools to compare and mature different bionanocomposites, so that these new technologies can be developed more successfully. In doing so, a novel material – WPI-nZnO composites – was identified and its promising properties demonstrated, towards a potentially better future healthcare.

