PhD in Ingegneria Chimica

Research Title: "*Innovative antibacterial biocomposite films based on biomacromolecules and nanostructured zinc oxide*"

Funded by	MIUR – Dipartimento di Eccellenza

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Context of the research activity	The research context is the development of antimicrobial films based on innovative biocomposite materials obtained by the combination of whey proteins concentrate (WPC) and nanostructured zinc oxide (NsZnO). The proposed multidisciplinary project is fully part of the strategic research sectors on innovative materials and technologies for health and food preservation. In recent years, several methods for the preparation of *in situ* forming hydrogels have been developed: the use of chemical crosslinking agents and the photopolymerization of precursor monomers can be an example (Y. Li *et al*., Chem. Soc. Rev., 41, 2012, 2193-2221). These approaches present several drawbacks concerning the toxicity issue that limits their applications. Innovative approaches, that avoid the use of toxic crosslinking agents, are under investigation to overcome the limitations associated with the preparation of *in situ* forming biodegradable hydrogels. Biomacromolecules (e.g. proteins and polysaccharides) are of significant interest as biomaterials for human healthcare systems (B. Gupta *et al*., Carbohydr. Polym., 98, 2013, 1160-1165). Whey proteins, in particular WPC, have been applied in the biomedical and food field because they represent a cheap and abundant food industry by-product. They are characterized by excellent biocompatibility and biodegradability as well as nutritional and

functional properties (e.g. high solubility, water absorption, gelation and emulsifying capability).
Bionanocomposites represent an emerging group of advanced materials resulting from the combination of biopolymers with inorganic components (R. Zafar *et al*., Int. J. Biol. Macromol., 92, 2016, 1012-1024). As an example, the addition of NsZnO in the biopolymeric matrix aims to impart novel functionalities, such as the antibacterial activity (A. Sirelkhatim *et al*., Nano-Micro Lett., 7, 2015, 219-242)
ZnO is known as antimicrobial agent, its activity is based on three distinct mechanisms: 1-photocatalytic antibacterial mechanism, 2-intrinsic antimicrobial properties of Zn^{2+} ions, 3-destabilization of microbial membranes upon direct contact of ZnO particles with the cell walls (A. Sirelkhatim *et al*., 2015). The antibacterial activity is significantly affected by various parameters, among which the morphology, size and concentration of ZnO nanostructures can be enumerated (L.C. Ann *et al*., J. Environ. Chem. Eng., 3, 2015, 436-444). The shape-dependent activity is correlated with the active facets percentage in the nanoparticles. ZnO with different morphologies possesses different active facets percentage, which strongly affects the antibacterial activity (M. Ramani *et al*., Colloid Surf B Biointerfaces, 117, 2014, 233-239).
The characterization of the morphology and structure of NsZnO by means of high-resolution techniques (scanning electron microscopy-SEM and transmission electron microscopy-TEM) plays a key role in the understanding of the correlations between physico-chemical properties and antibacterial activity. The knowledge and the tailoring of NsZnO features represents a challenge in the control and the optimization of innovative antibacterial biocomposites.
Still in the context of gaining knowledge on the biocomposite properties, the characterization of the organic matrix based on biomacromolecules (e.g. whey proteins), both in terms of polymerization and interaction with NsZnO, is crucial and could be achieved by means of vibrational spectroscopic techniques working at the nanoscale.
The antimicrobial activity of biocomposite films is usually evaluated *in vitro,* against different microbial classes (bacteria and fungi), by means of viable tests, on solid media (Agar diffusion test and Parallel streak method -AATCC-147).
The study of the interaction between the microorganisms and the biocomposite by means of electron microscopy is challenging and it represents an advancement in the knowledge of the antibacterial mechanism of the system in relation to the different growth modalities (i.e. planktonic growth or biofilm formation).

The project aims to obtain a detailed description of the biocomposite material and to understand the correlations between

	microscopic and chemical-structural properties and the antibacterial activity; this knowledge will allow to engineer innovative films with possible application in wound dressing and food packaging. The proposal is part of the collaboration between the biotechnological group (BEAR) and the material chemistry group (SMAC) and it will be the opportunity to reinforce the synergy between biology and material science in DISAT. The facilities of the "Dipartimento di Eccellenza" and the "Interdepartmental Center PolitoBIOMed Lab - Biomedical Engineering Lab" ensure the feasibility of the project.

Objectives	The main object of the project is the development of innovative antibacterial biocomposite films based on WPC obtained from renewable sources (i.e. recovered from cheese whey by means of an ultrafiltration process) and NsZnO. A simple method (based on protein denaturation and film casting) for the protein film production will be applied; further protein films will be modified with NsZnO in order to confer antimicrobial properties. NsZnO will be prepared in controlled conditions in order to obtain the desired features in terms of morphology, specific surface area and particle size. The antibacterial properties of the biocomposites will be evaluated against Gram + and Gram- bacteria. The experimental activity will develop as follows: 1. The prepared WPC films will be characterized by means of infrared spectroscopy and SEM/TEM. Moreover, in view of the suggested application of the films in wound dressing, the film wettability (contact angle measure) and the oxygen permeability will be investigated. 2. NsZnO will be prepared by means of established methods (F. Leone *et al.*, J. Cleaner Prod., 172, 2018, 1433-1439) and new ones aimed to reduce the use of

	toxic solvents and reagents in view of a more sustainable procedure. The structure and the physical-chemical properties of NsZnO will be fully characterized. In particular, TEM and SEM will be used to gain information on morphology and reactive exposed facets. 3. The dispersion of NsZnO in the protein films will be controlled and optimized. The use of spectroscopy and electron microscopies will be crucial in this task. 4. The antibacterial properties of the single components (WPC and NsZnO) and of the final biocomposites will be studied by means of traditional techniques (Agar diffusion test and Parallel streak method -AATCC-147). Nevertheless, one challenging objective of the proposal will be the characterization of the interaction between materials and tested microorganisms by means of electron microscopy. Gram + (*Staphylococcus epidermidis* LMG 10474) and a Gram – (*Escherichia coli* LMG 08063) bacterial strains will be tested.

Skills and competencies for the development of the activity	The preferred profile of the candidate is Chemical engineer, Biomaterial engineer, Chemist or graduate in Material Science, and Biotechnologist. In particular, a curriculum including the following knowledge is appropriate: Fundamental of molecular biology and microbiology Fundamental of material science Fundamental in physical chemistry and material characterization techniques. Moreover, the ability to work in a multidisciplinary team together with the autonomy in planning and conducting the research are requested. The research activity involves the ability to face and manage complex and interdisciplinary problems along with a certain degree of creativity and flexibility.