ABSTRACT

The aim of this Ph.D. project is the development of novel topical formulations for the treatment of various wounds by innovative technologies (micro and nano spray drying). The specific objective is the production of a dry powder able to become a gel "*in situ"* in contact with wound exudates using biodegradable and biocompatible polymers. Specifically, alginate with a high content of mannuronic residues, amidated pectin with a low degree of methylation and low molecular weight chitosan are used. Such polymers active in the wound healing process, due to the formation of a hydrogel into the wound cavity, are able to control the release of encapsulated active pharmaceutical ingredients (APIs).

With this aim during the first year of this Ph.D. program, alginate-pectin (AP) submicroparticles loaded with Ac2-26 were produced through nano spray drying. Ac2-26, the N-terminal derived peptide of Annexin A1, was chosen since is able to promote cell migration and tissue repair. The FT-IR studies carried out on the formulations showed chemical interactions between Ac2-26 and polymers blend, able to improve its stability and encapsulation efficiency and control the release, till 48 h. Moreover *in vitro* wound healing assay on HaCaT cells, demonstrated the ability of Ac2-26 to accelerate closure of the wound in 24 h.

Subsequently, to enhance the properties of the powders, to the blank alginate-pectin particles, chitosan was added and a new technique was investigated: mini spray drying. To increase the gelling process, different co-solvents (ethanol and isopropanol), as well as other excipients in the form of salts (sodium bicarbonate and ammonium carbonate) were added to the alginate-pectin-chitosan (APC) formulations. Fluid uptake tests have demonstrated that the addition of sodium bicarbonate reduced the gelation time from 5 minutes to 30 seconds when the powders were in contact with wound simulated fluid. Moreover, the pro-inflammatory studies, carried out on HaCaT cells, have shown that APC powders were able to induce a higher release of IL-8 from the human keratinocytes that

could stimulate the wound healing process in difficult-healing. Having achieved good results, APC powders have been used as a vehicle for doxycycline, an antibiotic model chosen due to its inhibiting activity against matrix metalloprotease-2 (MMP-2) and metalloprotease-9 (MMP-9) hyper expressed in chronic wounds. The presence of chitosan in the powders strongly affected their size, morphology, and fluid uptake properties, as well as drug encapsulation efficiency due to chemical interactions between the polymers and the drug demonstrated by FT-IR studies. In addition, due to drug-polymer interaction, a prolonged drug release profile was observed, as well as antimicrobial activity against *Staphylococcus aureus* was enhanced (till 7 days) compared to pure doxycycline. Furthermore, doxycycline-loaded particles were able to increase drug activity against MMPs, with good activity against MMP-9 even at the lower concentration tested over 72 h.

During the third year, the polymeric blend APC has been enhanced with hyaluronic acid (H), a natural polysaccharide constituent of the extracellular matrix which plays an important role during the wound healing processes. Normally, H has a short lifecycle due to the normal turnover of the skin. However, the biodegradability test conducted *in vitro* highlighted a prolonged time degradation of H thanks to the polymeric blend thus favoring the release of the latter in the wound bed and reducing the replacement frequency of the dressings.

Lastly, during the Ph.D. course, four months were spent at the University of Lyon 1 in France, to study the feasibility of spray drying to develop nanocomposites to encapsulate lipophilic drugs. Specifically, alginate-pectin microparticles were used as a carrier to encapsulate curcumin-loaded nanoemulsions (NEs). Curcumin was used as a model drug since, exhibiting antioxidant and antimicrobial activity, can play a great role in the treatment of wounds. Stable NEs showing a droplet size of 100 nm and a neutral surface charge were obtained. NEs were efficiently encapsulated in microparticles demonstrating that the spray drying process did not alter their properties. Furthermore, microparticles allowed to sustainably release NEs in simulated wound fluid showing a release dependently to the NEs concentration.