ABSTRACT De Cicco Felicetta, Dottorato Scienze Farmaceutiche, XIV ciclo NS, 2015/2016

The Ph.D project titled "New Encapsulation technologies for the development of in situ gelling micro-nano particulate systems for the wound healing" aimed to develop novel topical formulations by innovative technologies, based on supercritical CO_2 (SC-CO_2) and nanospray drying. The specific goal of the project was the design and development of *in situ* gelling formulation in form of powders or aerogels for wound healing using biodegradable and biocompatible polymers, active in wound healing process, as carrier for the encapsulated drugs. Uniform filling of the wound bed, good transpiration properties and controlled drug release profiles have been used to evaluate the quality of the different devices.

During the first year, prilling/supercritical antisolvent extraction (SAE) tandem technique and supercritical assisted atomization (SAA) were studied.

Prilling/SAE was tuned for the first time for the production of aerogel beads. Alginate based aerogels with narrow size distribution and high fluid uptake values were successfully obtained after an accurate study of the process parameters and solvent exchange conditions needed to use SAE as curing process.

SAA was applied for the production of an *in situ* gelling dry powder formulation, loaded with gentamicin. High mannuronic content alginate and low methoxyl degree pectin were used as carrier. Very high process yields were obtained (up to 71%), and the powder obtained by SAA showed good technological properties and were able to overcome gentamicin hygroscopicity. When in contact with simulated wound fluids powders moved rapidly to gel enabling a prolonged release of the drug (between 6 and 10 days). Furthermore, formulations were able to increase antimicrobial activity of GS on both *S. aureus* and *P. aeruginosa* strain.

The second year was focused on nano-spray drying technique. It was applied for the production of an *in situ* gelling nanoparticulate powder using an alginate and pectin blend as excipient for gentamicin formulations. Operative parameters were set to produce particles in nanometric size (~350 nm) with very high process yield (92%) and good encapsulation efficiency (83%). Powder formulations were able to gel rapidly when in contact with simulated wound fluids, had good transpiration and adhesive properties and were able to prolong drug release *in vitro* (till 6 days). Antimicrobial activity of GS loaded formualtions on both *S. aureus* and *P. aeruginosa* strain were also prolonged.

In the same year, a hydrogel based on alginate high mannuronic content or chitosan low molecular weight loaded with Ac2-26 Annexin 1 derived peptide was developed to test the ability of the peptide healing promoting activity. Stability of Ac2-26 was increased due to the formation of ionic interactions between peptide and polymers. Ac2-26 hydrogel formulations were tested on wounded

mice. *In vivo* experiments pointed out the efficacy of the alginate loaded hydrogel to increase wound closure, about 60% faster compared to control.

During third year, in order to enhance technological properties of the *in situ* nanoparticulate powder produced during the second year, a three components polymer blend (alginate, chitosan and amidated pectin) was used to test nano spray drying technology. Doxycycline was used as antimicrobial drug due to its well-known inhibiting activity against MMP9 that could enhance wound healing process. Formulations produced by nano spray drying presented very high process yields (up to 99%) and encapsulation efficiency (up to 98%) while powder gelation time was also reduced (5 minutes) enabling fast and complete covering of a wound. A prolonged drug release profile was observed due to drug/polymer interaction, as well as antimicrobial activity against *S. aureus* strain was enhanced (till 7 days) compared to pure doxycycline.

Doxycycline was also used as model drug to test the feasibility of prilling/SAE tandem technique for the production of core/shell aerogel particles. Aerogels were obtained by prilling in co-axial configuration, using amidated pectin as core and alginate high mannuronic content as shell. Particles were able to easily gel when in contact with exhudates due to the wide aerogel exposed surface area, absorbing large amount of fluid. Core and shell were well separated with doxycycline distributed homogeneously only into the pectin core. As a result, aerogels presented high encapsulation efficiency (up to 87%) and prolonged drug release, till 48 hours.

Moreover, durting the Ph.D course, three months were spent at University of Santiago de Compostela, to study the feasibility of supercritical impregnation method coupled with prilling and supercritical drying to manufacture novel wound healing aerogel devices. Ketoprofen and norfloxacin, were used as model drugs due to their solubility in SC-CO_2. As expected, aerogels presented high porosity, very useful to absorb large quantities of exhudates. Impregnation process was successful for ketoprofen whereas norfloxacin loaded aerogels exhibited poor drug content.