

# **Influence of chitosan on a polysaccharide blend *in situ* gelling powders for wound dressing**

*Amante C.<sup>ab</sup>, Russo P.<sup>a</sup>, Aquino R.P.<sup>a</sup>, Del Gaudio P.<sup>a</sup>*

*<sup>a</sup>Department of Pharmacy, University of Salerno, Fisciano (SA), Italy*

*<sup>b</sup>PhD Program in Drug Discovery and Development, University of Salerno, Fisciano, (SA), Italy*

The aim of this work is the production of innovative formulations, based on natural polymer blend, loaded with doxycycline, intended for wound healing applications. Powders able to become gel in contact with wound fluid, have been manufactured by mini spray drying, an easy to scale-up technology widely used in the pharmaceutical field.

After a series of experiments three formulations were selected (APC\_111, 113 and 117) keeping the concentration of alginate and pectin constant and increasing the chitosan concentration in order to evaluate the influence of chitosan on the powder. All formulations showed good yield of the process and as reported by SEM images the increase of chitosan leads to a higher surface roughness and a reduction of the particle size. Moreover, all formulations reached maximum swelling in about 5 minutes. However, APC\_117 is able to absorb a greater quantity of SWF, increasing its weight by 12 times.

In order to verify that the combination of the polymers was not cytotoxic on the wound some tests on human keratinocytes were conducted. MTT test did not show any significant cytotoxic activity for none concentration and combination tested. Based on these results, proinflammatory activity on different cytokines was evaluated. All formulations, but only at the maximum concentration tested, increased IL-8 release from keratinocytes.

After assessing that powders have good properties and they were not toxic formulations loaded with doxycycline were prepared. Doxycycline was chosen, not only for its wide antibacterial spectrum but, also, for the possible inhibition of host matrix metalloproteinases involved in cell migration essential for wound healing. Microparticles with 2% of drug turned out to be the best in terms of process yield, size distribution and encapsulation efficiency. As in the blank formulations also here chitosan has a strong influence on the process and on the particle properties. On the contrary doxycycline did not influence the morphology of the particles but influenced the swelling of powders. In fact, while APCD 111 and 113 have a similar trend to the blank, APCD\_117 with 2% of doxycycline reached lower swelling than blank formulation. This may be due to different interaction between chitosan and doxycycline.

Drug release studies reported that all formulations showed a burst effect in the first hours (5 h) followed by a prolonged release of doxycycline until 24 h. However, while APCD\_111 released 60% of the drug in 6 hours, formulation with lower concentration of chitosan released 30% of the drug in the same time.

Diffusion test against *Staphylococcus aureus* showed an antimicrobial effect not only for doxycycline, but, also for the synergic effect between doxycycline and chitosan chosen.

In order to evaluate the activity of doxycycline against metalloproteinases gelatinase zymography assay was carried out. Gel showed that doxycycline released from hydrogel inhibited the activity of metalloproteinases more than only doxycycline even at 0.5 µg and its effect was stable until 72h.

In conclusion mini spray drying technology allows to produce powder formulations able to move rapidly into a gel when in contact with wound fluid with prolonged antimicrobial activity, that could be used as wound dressing.