Novel physical chitosan gel: *in vivo* evidence of its potential in wound healing

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In?ammation, new tissue formation, and remodelling are the critical step of a wound healing process which is triggered by a series of cellular and biochemical activities. In particular, the growth of ?broblasts signi?cantly contributes to ?ll a wound by producing collagen and ?bronectin that form a new extracellular matrix (ECM), resulting in contracting and healing of the wound [1]. The use of hydrogel as medication for the wound healing is very promising since they are moist and hydrated, and able not only to absorb exudate but also prevent the loss of evaporative water, and so the wound dehydration, so providing an ideal environment for the wound healing process [2]. Chitosan (CHI), is a linear polysaccharide composed of glucosamine and N-acetyl glucosamine units linked by β-(1-4) glycosidic bonds, whose beneficial effects in wound healing are well documented [3]. In particular, it has been shown that platelets are activated by chitin so that an immediate haemostasis is obtained after CHI-based dressing application to traumatic and surgical wounds. Moreover, angiogenesis and production of vascular endothelial growth factor is strongly up-regulated in wound healing when macrophages are activated by CHI [2,3]. Furthermore, CHI has also been suggested in wound healing due to its properties to inhibit microbial growth [4]. On the basis of these premises, the aim of this work was to formulate novel sterile biocompatible physical CHI gels, able to fill the irregular cavity of a wound and accelerate the healing process. The gel was prepared starting from a sterile powder and the following steps carried out under aseptic conditions. In particular, a thermal treatment with autoclave was imposed to the starting CHI powder to assure the sterility of the formulations, necessary for wound medication. This caused a change of CHI molecular weight, as revealed by GPC analysis. Moreover, thermal analysis, performed by a Differential Scanning Calorimetry, indicated that the macromolecules, before and after the thermal treatment, differ in their strength of water-polymer interaction leading to a different macroscopic/rheological behaviour. Rheological analysis revealed, indeed, that the thermal treatment leads to a change of CHI formulation viscoelastic and flow properties. Then, we evaluated the effects of CHI on human foreskin foetal fibroblasts (HFFF-2). CHI gel was able to significantly induce fibroblast proliferation as well as migration in a scratch assay model. Importantly, the formulation did not affect cell viability. In order to confirm our results, we investigated the effect of CHI gel at 3 and 10 days on a mouse model of pressure ulcers. CHI gel induced a faster healing of pressure ulcers, as compared with untreated mice, by increasing in a marked and significant manner the collagen, as well as the haemoglobin content and the expression of Wnt, a transcription factor strictly related to proliferation and angiogenenesis, of ulcers after 3 and 10 days of treatment. In contrast, CHI gel prevented, in a marked and significant manner, TNF-a expression as well as MPO activity at both time points considered. Taken together, these results suggest the potential of this novel developed system in wound healing also considering that they can be loaded with drugs to be delivered in the inflammatory environment of tissue ulcers thus achieving a further improvement of the healing process.

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