Optimization of insulin-loaded nanoparticle-hydrogel delivery systems to accelerate wound healing

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Chronic wounds (CW) present a challenging problem, since current therapies are not highly effective, with extended healing time, high recurrence rates and risk of amputations [1]. Insulin is one of the cheapest growth factors available, stimulating wound healing (WH) by promoting growth of granulation tissue and reepithelialisation, improving angiogenesis and reducing healing time [2]. However, the harsh proteolytic effect in the wound bed requires a delivery system (DS) able to protect insulin from degradation [3]. Our aim was to develop an insulin-loaded multifunctional nanoparticle (Np) hydrogel DS to accelerate WH. It is foreseen this DS will accelerate WH and reduce frequency of dressing changes, improving life quality of patients and decreasing economic burden in healthcare systems. Insulin-loaded chitosan-coated PLGA Np were produced by w/o/w double emulsion technique [3] and embedded in hydrogels obtained by freeze-thawing. The DS was optimized by quality by design, varying chitosan (0.25%,0.5%,0.75%), alginate (1%,1.5%,2%), glycerine (5%, 7.5%, 10%) and number of freeze-thawing cycles (1,2,3). Np were characterized by DLS and SEM, and hydrogels by rheology. Insulin structure was evaluated by FTIR and CD. Quality by design approach revealed an optimum hydrogel formulation. Np with chitosan coating and without had a particle size of about 700 nm and 300 nm, PdI of 0.4 and 0.2 and a ZP of 60 mV and -38mV, respectively. The hydrogel had good rheologic properties for skin application and a water content of about 89%. SEM images showed that the Np incorporated into the hydrogel, maintaining its features with no relevant signs of particle aggregation. The results revealed that the insulin structure was preserved upon encapsulation and production of the hydrogel. The natural polymers used are multifunctional with chitosan promoting angiogenesis and mucoadhesion to the wound [4]. The Np showed particle size increase with the increase of chitosan concentration. The change of the ZP into positive values shows the effective chitosan coating. The developed multifunctional DS allows a sustained insulin delivery, protecting its stability and bioactivity. This prolongs residence time of insulin in wounds and may accelerate WH. In vitro results may be good indicators to further perform in vivo studies and clearly demonstrate the superior effectiveness of our approach in CW treatment.

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REFERENCES
[1] I.Garcia-Orue et al., J. Drug Deliv. Sci. Technol., 42 (2017) 2-17.
[2] A. Oryan et al., Life Sci., 147 (2017) 59-67.
[3] P. Fonte et al., Methods Mol. Biol., (2018) 255-274.
[4] H. Liu et al., RSC Adv., 8 (2018) 7533-7549.