CHITOSAN NANOPARTICLES FOR THE DELIVERY OF GENES TOWARD TISSUE REGENERATION

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Chitosan is a natural polysaccharide that due to its biocompability and low toxicity has been studied for numerous biomedical applications. Chitosan has positive charge in low acidity solutions, being able to produce nanocomplexes with DNA upon electrostatic interaction. Consequently, chitosan has been proposed as a gene delivery vector.

Growth factors play a key role in tissue regeneration. The delivery of these proteins *in loci* has been proposed as a mean to promote or modulate regeneration. Due to the above-mentioned properties, chitosan could be a promising candidate as a vector to mediate the deliver of genes encoding for growth factors, however these vectors were found to have limited gene delivery efficiency in comparison to other polycationic systems.

One of the major barriers described for gene delivery mediated by these systems is the particle escape from the endosomal pathway upon endocytosis. In this work, the incorporation of imidazole units in the chitosan backbone was performed in order to increase the buffering capacity of the polymer in the physiological pH range (7.4-5) and ultimately improve particles escape from the endosome, leading to an efficient gene expression. Chitosan with three different degrees of substitution were prepared. The modified polymers (CHimi) maintain the ability to condense DNA in particles of mean size of approximately 200nm (Zetasizer nano ZS, Malvern). The transfection activity mediated by CHimi polymers was investigated in 293T cells, using pCMV-Sport βGal as reporter gene. The results show that the introduction of imidazole moieties into chitosan backbone leads to a higher transfection activity, in comparison with the starting material. Transfection was found to be dependent on the degree of substitution of the polymer and on the N/P (amino groups of the polymer / phosphate groups of DNA) molar ratio of the complexes.

A gene delivery vector to be applied in a regenerative medicine scenario should guarantee non-toxicity, as well as controlled gene expression during the healing time period. Prospecting this application, the gene expression kinetics and the transfected cell viability were assessed up to 7 days after the transfection. CHimi-based vectors promote a sustained gene expression during this period without altering the viability of the cells.

The present results show that CHimi-based vectors are promising candidates as gene vehicles for regenerative medicine applications, what is currently under investigation.

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