Maria P et al., Pharmaceut Reg Affairs 2017, 6:1(Suppl)
DOI: 10.4172/2167-7689-C1-025

Expression of antimicrobial peptide Hcap18/LL-37 following non-viral delivery of plasmid DNA encoded by CAMP gene in human fibroblasts and keratinocytes

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Skin wounds caused by burns have high global incidence. Autologous tissue recovery in these lesions is ineffective, due to affected area low vascularization and the susceptibility of the patients to infections caused by multi-resistant microorganisms, resulting in a high mortality rate. Non-viral vectors continue to be an attractive alternative to viral vectors due to their safety, ease of preparation and scale-up. These systems could represent a strategy to treat or improve skin wounds by genetically modifying own patients cells. In this work, we have developed a system of non-viral transfection of human keratinocytes and fibroblasts, consisting in a polymer/plasmid DNA complex (polyethyleneimine/CAMP modified Lenti-IRES bicistronic vector with tRFP), known as polyplexes in order to overexpress the antimicrobial peptide hCAP 18-LL37, which has been shown to exhibit a broad spectrum of antimicrobial activity as well as additional defensive roles such as regulating the inflammatory response and promoting re-epthelialization and wound closure. By measuring the amount of free pDNA, the formation and stability of the complexes were determined. Transfection efficiency in 2D cultures was evaluated by flow cytometry. Quantification of mRNA by RT-qPCR demonstrated the expression of the CAMP gene in transfected keratinocytes and fibroblasts; this suggests that the antimicrobial peptide hCAP18 / LL-37 are being expressed at higher levels than those of the same non-transfected cells. These are promising results for the use of polyplexes in the transfection of different cell types and stimulation of a gene of interest overexpression as the CAMP gene, with important antimicrobial and angiogenic effects on cutaneous wound healing.

Biography

Maria P is a Microbiologist and Bioanalist from the University of Antioquia. Her experience has been focused on scientific research, and, currently, she is a member of the Tissue Engineering and Cell Therapy Group. As a PhD student, with a student loan from COLCIECIAS (scholarship Program No.727 of 2015), she has been working on the development of a non-viral transfection system of human fibroblasts and keratinocytes, incorporated in an 3D skin model in order to over-express the antimicrobial peptide hCAP 18-LL 37 as a strategy for the treatment of skin wounds.

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