Acta Biomaterialia 8 (2012) 1156–1163

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

A new tool for the transfection of corneal endothelial cells: Calcium phosphate nanoparticles

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ARTICLE INFO

Article history:
Received 17 June 2011
Received in revised form 5 September 2011
Accepted 12 September 2011
Available online 17 September 2011

Keywords:
Calcium phosphate
Nanoparticles
Cornea
Endothelial cell
Gene transfer

ABSTRACT

Calcium phosphate nanoparticles (CaP-NP) are ideal tools for transfection due to their high biocompatibility and easy biodegradability. After transfection these particles dissociate into calcium and phosphate ions, i.e. physiological components found in every cell, and it has been shown that the small increase in intracellular calcium level does not affect cell viability. CaP-NP functionalized with pcDNA3-EGFP (CaP/DNA/CaP/DNA) and stabilized using different amounts of poly(ethylenimine) (PEI) were prepared. Polyfect®-pcDNA3-EGFP polyplexes served as a positive control. The transfection of human and murine corneal endothelial cells (suspensions and donor tissue) was optimized by varying the concentration of CaP-NP and the duration of transfection. The transfection efficiency was determined as EGFP expression detected by flow cytometry and fluorescence microscopy. To evaluate the toxicity of the system the cell viability was detected by TUNEL staining. Coating with PEI significantly increased the transfection efficiency of CaP-NP but decreased cell viability, due to the cytotoxic nature of PEI. The aim of this study was to develop CaP-NP with the highest possible transfection efficiency accompanied by the least apoptosis in corneal endothelial cells. EGFP expression in the tissues remained stable as corneal endothelial cells exhibit minimal proliferative capacity and very low apoptosis after transfection with CaP-NP. In summary, CaP-NP are suitable tools for the transfection of corneal endothelial cells. As CaP-NP induce little apoptosis these nanoparticles offer a safe alternative to viral transfection agents.

1. Introduction

Transplantation of the cornea is the most common and successful form of tissue grafting [1], thanks mainly to the immune privilege of the eye, i.e. normal immunity elements are absent. Factors contributing to ocular immune privilege include immunological isolation, absence of a vascular supply, presence of a vascular barrier, the role of the lymphatic system, the role of antigen-presenting cells and major histocompatibility complex antigen expression [2,3]. As such, grafting represents the ultimate hope for millions of people world wide who are blind due to corneal pathologies to successfully restore vision. While uncomplicated corneal transplantations are successful in about 90% of first time recipients [4], the failure rates in vascularized or inflamed beds or in patients with a previously failed graft often reach 50–90% [5]. It has long been established that corneal cell death occurs in donor corneas during storage prior to transplantation [6]. 30% of all donated corneas have to be discarded due to apoptosis (premature programmed cell death) of the innermost corneal layer, i.e. the endothelium [7–9]. At the same time there is a shortage of human donor corneas available for transplantation [10–12].

As corneal endothelial cells are considered to have very low proliferative capacity, [13] and transplant failure is accompanied by apoptosis of the grafted cells, we assumed that preventing apoptosis, especially of endothelial cells within the graft, is a way to prevent transplant failure. Our group and others have already shown that corneal graft rejection can be prevented by gene therapy with Bcl-xL [13] and p35 using an HIV-based lentiviral vector [14,15]. Although many studies have shown that gene therapy with viral vectors may be safe and effective in animal models of some eye diseases, the use of viral vectors can be problematical in clinic applications because of the viral backbone of the plasmids, especially in the case of a non-life-threatening disease like

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doi:10.1016/j.actbio.2011.09.013