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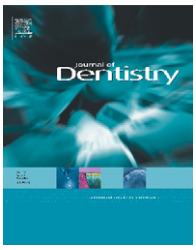

Efficient gene transfer to periodontal ligament cells and human gingival fibroblasts by adeno-associated virus vectors☆

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ABSTRACT

Objectives: We explored for the first time the possibility to deliver a reporter gene (Green Fluorescence Protein) to human primary periodontal ligament (PDL) cells and human gingival fibroblasts (HGF) using shuttle vectors derived from adeno-associated virus (AAV). Since AAV transduction rates on other human primary fibroblasts have been previously shown to depend on the particular cell lineage and on the employed viral serotype, we determined the most effective AAV variant for periodontal cells comparing different vector types.

Methods: AAV serotypes 1–5 encoding GFP in single stranded (ss) and self-complementary (sc) vector genome conformations were used to infect primary HGF and PDL cells. Two days post-infection, the percentage of GFP expressing cells was determined by flow cytometry.

Results: Highest transduction rates for both cell types were achieved with self-complementary vectors derived from AAV-2, resulting in GFP expression in up to 86% of PDL cells and 50% of HGF. Transgene expression could be observed by optical microscopy for 2 months after infection. Lower but detectable rates were obtained with serotypes 1, 3 and 5.

Conclusions: The efficacy demonstrated here and the safety and versatility of AAV technology indicated in previous studies clearly suggest the potential of AAV vectors as tools for gene transfer to periodontal tissues.

1. Introduction

Increasing knowledge of molecular pathways characterizing periodontal disease has opened the way for novel therapeutic concepts. Among these, the possibility to modulate the local expression of genes (gene therapy)[1] holds the potential to address simultaneously several aspects of the pathology by (a) restoring normal gene expression levels in affected tissues; (b) combating bacterial colonization of gingival pockets by expression of genes that interfere with bacterial metabolism or help create a bacteria-hostile environment in infected tissues; (c) releasing anti-inflammatory molecules in affected tissue; (d) expressing genes that promote differentiation of specific cell types in order to reconstitute degenerated tissues.

Viruses have been successfully employed to introduce and express genes in numerous target tissues over the last two decades.[2] Besides offering a powerful and versatile tool to dissect molecular pathways and investigate the role of different

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proteins and cellular factors by over-expressing desired genes or by blocking their expression, this technique is currently employed in a number of clinical trials where therapeutic genes are introduced in patient tissues. In the field of periodontology, pioneering studies have been conducted using adenovirus to deliver platelet-derived growth factor (PDGF) genes to osteoblasts, cementoblasts, PDL cells and gingival fibroblasts[3–7] demonstrating robust expression of the transgene in targeted tissues and stimulation of tissue regeneration in large periodontal defects.[8] In other studies, adenovirus vectors were used to deliver bone morphogenetic protein (BMP) genes, resulting in enhanced alveolar bone repair, stimulated cementogenesis and PDL fiber formation in rats.[9,10] Hemagglutinating virus of Japan (HVJ) was used to mediate OPG gene transfer in periodontal tissue, resulting in inhibition of RANKL-mediated osteoclastogenesis and significant reduction of orthodontic tooth movement[11] and inhibition of lipopolysaccharide-induced alveolar bone resorption in rats.[12]

Since the availability of gene transfer technologies that offer a robust safety profile is crucial for the success of these approaches, we aimed to assess the efficiency of gene transfer to human primary PDL cells and gingival fibroblasts by vectors derived from adeno-associated virus (AAV). AAV is a member of the parvovirus family, has a non-enveloped capsid of 25 nm in diameter that contains a single stranded DNA genome encoding two open reading frames (rep and cap) that can be excided and replaced by a desired transgene to produce infectious gene transfer vectors (Fig. 1). To date, several AAV serotypes have been isolated, differing in their capsid amino acid sequence and tropism.[13,14] These vectors can be easily produced at high titers and efficiently transduce a wide spectrum of cell types.[15–17] Importantly, no human pathologies have been related to AAV infection and no severe side effects have ever been elicited upon AAV vectors injection.[18] Because of these favourable characteristics, AAV has become one of the most popular gene therapy vectors, is intensively studied and currently employed worldwide in numerous clinical trials.[19,20]

Since some fibroblasts types are refractory to AAV infection[21,22] and transduction efficiencies depend on the used serotype,[23,24] we explored the potential of AAV vectors for the study and the cure of periodontitis comparing the ability of vectors derived from AAV of types 1–5 to deliver and express a foreign gene in PDL cells and HGF.

In addition, refinement of AAV vector technology has led to the generation of viral particles containing a self-complementary genome that circumvents the intracellular double strand synthesis step required by conventional vectors. This results in higher and faster transgene expression in several cell types.[25] Therefore, we compared transduction efficiencies of single stranded and self-complementary genome vectors.

2. Materials and methods

2.1. *Cell culture*

PDL cells and HGF were isolated from third molars extracted because of dysfunctional position in the jaw from 18 to 35

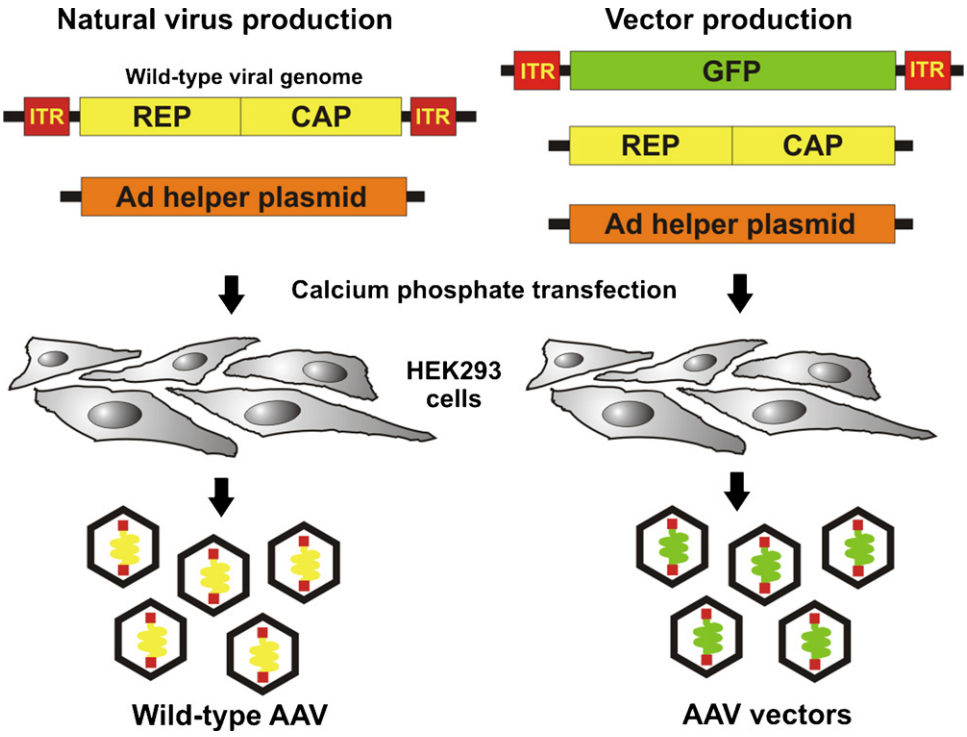

Fig. 1 – Production of wtAAV compared to production of AAV vectors. For vector production, viral genes (rep and cap) encoded between packaging signals (ITRs) are replaced by a transgene (e.g. GFP). The so obtained construct is transfected in HEK293 cells together with a plasmid carrying the viral genes devoid of packaging signals and a plasmid carrying adenoviral genes that promote virus replication. Only DNA sequences comprised by two ITRs will be encapsidated in progeny viral particles.