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Co-delivery of SOX9 genes and anti-Cbfa-1 siRNA coated onto PLGA nanoparticles for chondrogenesis of human MSCs

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ABSTRACT

Some genes expressed in stem cells interrupt and/or enhance differentiation. Therefore, the aim of this study was to inhibit the expression of unnecessary genes and enhance the expression of specific genes involved in stem cell differentiation by using small interfering RNA (siRNA) and plasmid DNA (pDNA) incorporated into cationic polymers as co-delivery factors. To achieve co-delivery of siRNA and pDNA to human mesenchymal stem cells (hMSCs), two different genes were complexed with poly(ethyleneimine) (PEI) and then coated onto poly(lactide-co-glycolic acid) (PLGA) nanoparticles (NP). To evaluate co-delivery of siRNA and pDNA into hMSCs, cells were transfected with green fluorescence protein (GFP) pDNA (GFP pDNA) and GFP siRNA (GFP siRNA). The percentage of GFP-expressing hMSCs decreased from 25.35 to 3.7% after transfection with GFP-DNA/PLGA NP (NPs) or GFP siRNA/PLGA NPs, whereas GFP-DNA/PLGA NPs and scramble siRNA (MOCK)/PLGA NPs had no effect on GFP expression. hMSCs cotransfected with coSOX9-pDNA/NPs and Cbfa-1-siRNA/NPs were tested both *in vitro* and *in vivo* using gel retardation, dynamic light scattering (DLS), and scanning electron microscope (SEM). The expression of genes and proteins associated with chondrogenesis was evaluated by FACS, RT-PCR, real time-qPCR, Western blotting, immunohistochemistry, and immunofluorescence imaging.

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1. Introduction

During the process of tissue regeneration, several molecules are involved in stem cell differentiation into specific tissues [1]. During neo-tissue formation by stem cells, many genes are translated into proteins that induce or inhibit tissue regeneration and formation [2–5]. Of these, SOX family genes are known to be potent inducers of neo-cartilage formation.

Transfection of the SOX9 gene into human mesenchymal stem cells (hMSCs) plays a pivotal role in the expression of collagen type IIa1 (COL IIa1) genes, which are then involved in inducing aggrecan gene expression. SOX9, an important transcription factor involved in chondrogenesis by hMSCs, belongs to the SRY-type family of high mobility group (HMG) box proteins [6–8]. The highly expressed COL IIa1 gene encodes the a2-chain of type XI collagen, a minor component of cartilage collagen fibrils [9,10], while the Agc gene encodes the core protein of aggrecan, the major cartilage-specific proteoglycan [11].

The Cbfa-1 gene (which opposes the function of the SOX9 gene), is a high mobility group domain transcription factor expressed during osteogenesis. Cbfa-1 is expressed as a consequence of bone morphogenic protein expression (BMP); the protein products of both these genes control the formation of bone tissue and osteogenic differentiation [12]. Overexpression of the Cbfa-1 gene in hMSCs up-regulates osteogenic gene expression in a wide range of cell types, whereas overexpression of the Cbfa-1 gene induces the formation of extracellular matrix to support bone growth [13,14]. Therefore, overexpression of Cbfa-1 can retard neo-cartilage formation.

In an attempt to solve this problem, small interfering RNA (siRNA) silencing of specific target genes has been used to block the expression of genes to treat various diseases and stem cell differentiation [15,16]. Therefore, the safe and effective delivery of siRNA using non-toxic materials is important for gene and cell therapies. Thus, siRNA targeting Cfba-1 was used to abrogate the expression of Cbfa-1 genes in hMSCs during cartilage neogenesis.

To enable successful transfection of plasmid DNA (pDNA) and siRNA into cells for gene therapy, adequate vehicles are necessary for efficient delivery of the target genes to the target cells [17–20]. Both negatively-charged SOX9 and Cbfa-1 siRNA and positively-charged polymers can be easily complexed via ionic–ionic interactions. Polyethylenimine (PEI) can form polyplexes with both

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