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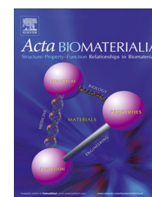

Full length article

Mesenchymal stem cell fate following non-viral gene transfection strongly depends on the choice of delivery vector

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ABSTRACT

Controlling the phenotype of mesenchymal stem cells (MSCs) through the delivery of regulatory genes is a promising strategy in tissue engineering (TE). Essential to effective gene delivery is the choice of gene carrier. Non-viral delivery vectors have been extensively used in TE, however their intrinsic effects on MSC differentiation remain poorly understood. The objective of this study was to investigate the influence of three different classes of non-viral gene delivery vectors: (1) cationic polymers (polyethylenimine, PEI), (2) inorganic nanoparticles (nanohydroxyapatite, nHA) and (3) amphipathic peptides (RALA peptide) on modulating stem cell fate after reporter and therapeutic gene delivery. Despite facilitating similar reporter gene transfection efficiencies, these nanoparticle-based vectors had dramatically different effects on MSC viability, cytoskeletal morphology and differentiation. After reporter gene delivery (pGFP or pLUC), the nHA and RALA vectors supported an elongated MSC morphology, actin stress fibre formation and the development of mature focal adhesions, while cells appeared rounded and less tense following PEI transfection. These changes in MSC morphology correlated with enhanced osteogenesis following nHA and RALA transfection and adipogenesis following PEI transfection. When therapeutic genes encoding for transforming growth factor beta 3 (TGF-β3) and/or bone morphogenic protein 2 (BMP2) were delivered to MSCs, nHA promoted osteogenesis in 2D culture and the development of an endochondral phenotype in 3D culture, while RALA was less osteogenic and appeared to promote a more stable hyaline cartilage-like phenotype. In contrast, PEI failed to induce robust osteogenesis or chondrogenesis of MSCs, despite effective therapeutic protein production. Taken together, these results demonstrate that the differentiation of MSCs through the application of non-viral gene delivery strategies depends not only on the gene delivered, but also on the gene carrier itself.

Statement of Significance

Nanoparticle-based non-viral gene delivery vectors have been extensively used in regenerative medicine, however their intrinsic effects on mesenchymal stem cell (MSC) differentiation remain poorly understood. This paper demonstrates that different classes of commonly used non-viral vectors are not inert and they have a strong effect on cell morphology, stress fiber formation and gene transcription in MSCs, which in turn modulates their capacity to differentiate towards osteogenic, adipogenic and chondrogenic lineages. These results also point to the need for careful and tissue-specific selection of nanoparticle-based delivery vectors to prevent undesired phenotypic changes and off-target effects when delivering therapeutic genes to damaged or diseased tissues.

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1. Introduction

Adult mesenchymal stem cells (MSCs) are a promising cell source for regenerative medicine due to their multipotent differentiation capacity [1] and immunomodulatory properties [2]. Controlling the phenotype of MSCs is a central challenge in tissue engineering and regenerative medicine. The fate of progenitor cells can potentially be modulated through the introduction of exogenous genes for the cell-mediated synthesis of specific proteins. This approach may be preferable over the delivery of recombinant cytokines and growth factors which involves the administration of non-physiological concentrations, due to the short half-life, fast body clearance and a lower therapeutic effect in comparison to natural proteins [3–5]. A variety of genes have been explored to this end, including members of the transforming growth factor-beta (TGF-β) superfamily of proteins such as bone morphogenic protein 2 (BMP2) and transforming growth factor-beta 3 (TGF-β3), whose overexpression has been previously reported to enhance bone and cartilage regeneration *in vivo* [6–8]. But the success of gene therapy ultimately depends on the gene delivery mechanism to maximise nucleic acid uptake and, consequently, downstream protein production [3,4,9–11].

Traditionally, viral vectors such as retrovirus, lentivirus and adenovirus, have been used for the delivery of genes into cells via a process known as transduction [10,12,13]. Although they offer high transduction efficiencies and stable gene expression, many limitations remain associated with viral vectors [14–16] such as insertional mutagenesis [17], immunogenicity [11], limited DNA packaging capacity [18] and cumbersome large-scale production [19]. Non-viral gene carriers are promising alternatives for gene delivery and have the potential to address these limitations [9]. Moreover, the transient expression associated with these systems can be more compatible with the natural wound healing processes [20]. Several non-viral vectors are commonly used for gene delivery, including lipids, polymers, cell penetrating peptides (CPPs) and inorganic nanoparticles [13]. While such systems can be used to efficiently transfect cells with specific genes, the effects that these non-viral vectors have on stem cell fate remain relatively unknown [21,22].

Cationic lipid-based and polymeric DNA vectors such as lipofectamine and polyethylenimine (PEI) are amongst the most widely used non-viral gene delivery methods [13,22,23], and are often used as a gold standard for non-viral gene transfection [3]. However their potential cytotoxicity [13,24] and sensitivity to media supplementation with serum and antibiotics [25] limits their use in tissue engineering applications. Among alternative options, inorganic nanoparticles made of calcium phosphate, gold or silica, have been drawing attention for their use in tissue engineering due to their biocompatibility, wider availability, long-term stability, ease of preparation and low toxicity [3,26–28]. More recently, different classes of peptides [29,30], such as the RALA amphipathic peptide (RALA) comprised of repeating arginine/alanine/leucine/alanine units [30], have been developed as novel nucleic acid carriers [31,32], showing excellent cytocompatibility and moderate transfection efficiencies *in vivo* and *in vitro* [30,33]. These non-viral delivery vehicles are promising in terms of compatibility and transfection efficiency, however the suitability of a gene delivery vector for stem cell-mediated tissue engineering is not only determined by its transfection efficiency, cytocompatibility and levels of expression of the gene product, but also by its chemical composition and how the intracellular delivery of such nanomaterials may influence stem cell fate [22].

Therefore, the objective of this study was to first compare the capacity of three different classes of non-viral gene delivery vectors (PEI, nanohydroxyapatite (nHA) and RALA) to transfect bone marrow-derived MSCs. The impact of intracellular delivery of such nanomaterials on the viability, cytoskeletal structure and multi-lineage differentiation potential of MSCs was assessed. We then used these vectors to deliver BMP2 and TGF-β3 genes to MSCs as a means to promote either osteogenesis or chondrogenesis in a 2D or 3D environment, and investigated the influence of different gene carriers on MSC lineage commitment. Collectively the results of this study demonstrate that gene vectors with comparable capacities to transfect MSCs with reporter and therapeutic genes can have dramatically different effects on MSC differentiation.

2. Materials and methods

2.1. Plasmid propagation

Four different plasmids were used in the current study: two plasmids encoding for the reporter genes green fluorescent protein (pGFP, Amaxa, Lonza Cologne AG, Germany) and luciferase (pLUC, pGaussia Luciferase; New England Biolabs, Massachusetts, USA), and another two encoding for the therapeutic genes BMP2 (donation from Prof. Kazihusa Bessho, Kyoto University, Japan) and TGF-β3 (InvivoGen, Ireland). Plasmid amplification was performed by transforming chemically competent *Escherichia coli* bacterial cells (One Shot TOP10; Biosciences, Ireland) according to the manufacturer's protocol. The transformed bacteria were cultured on LB plates with 50 mg/L kanamycin (Sigma-Aldrich, Ireland) as the selective antibiotic for pGFP and 100 mg/L ampicillin (Sigma-Aldrich, ireland) as the selective antibiotic for pLUC, pTGF-β3, and pBMP2. Bacterial colonies were harvested and inoculated in LB broth (Sigma-Aldrich, Ireland) and incubated overnight for further amplification. The harvested bacterial cells were then lysed, and the respective pDNA samples were purified using qiagen plasmid kit (MaxiPrep Kit; Qiagen, Ireland). Nucleic acid concentration (ng/μL) was determined by analyzing the 260:280 ratio and 230 nm measurement using NanoDrop spectrophotometer (Labtech International, Uckfield, UK). Plasmids in this study were used at a concentration of 0.5 μg plasmid in 1 μL Tris–EDTA (TE) buffer.

2.2. Preparation of delivery vectors and vector-pDNA complexes

The synthesis of the nHA particles was performed as previously described [34]. Briefly, a solution of 12 mM sodium phosphate (Sigma-Aldrich, Ireland), containing 0.017% DARVAN821A (RTVanderbilt, Norwalk, USA) was added to an equal volume of a 20 mM calcium chloride solution (Sigma-Aldrich, Ireland) and filtered through a 0.2 mm filter (Fisher, Ireland). nHA-pDNA complexes were prepared by adding 150 μL of the nHA solution to 2 μg of pDNA pretreated with 0.25 M CaCl2 (Sigma-Aldrich, Ireland) as previously optimized [26,27]. The nHA-pDNA solutions were not incubated prior to transfection in order to avoid particle aggregation that could impair cellular uptake as previously optimized [26].

PEI-pDNA complexes were prepared using branched PEI with a molecular weight of 25 kDA (Sigma-Aldrich, Ireland). PEI was condensed with pDNA in an N:P ratio (the molar ratio of positively charged nitrogen atoms in the PEI to negatively charged phosphates in the pDNA backbone) of 7, a ratio previously optimized for MSC transfection [35]. The PEI-pDNA solution was then incubated for 30 min for complex formation.

The RALA peptide was synthesised as previously described [30]. Briefly, the peptide was produced by 9-fluorenylmethyloxycarbonyl (Fmoc) solid-state peptide synthesis (Biomatik, USA) and supplied as a desalted, lyophilised powder. The product was purified and validated by reversed-phase high-performance liquid chromatography (RPHPLC); molecular mass was confirmed

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