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Design of magnetic gene complexes as effective and serum resistant gene delivery systems for mesenchymal stem cells

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ABSTRACT

Gene engineered mesenchymal stem cells (MSCs) have been proposed as promising tools for their various applications in biomedicine. Nevertheless, the lack of an effective and safe way to genetically modify these stem cells is still a major obstacle in the current studies. Herein, we designed novel magnetic complexes by assembling cationized pullulan derivatives with magnetic iron oxide nanoparticles for delivering target genes to MSCs. Results showed that this complexes achieved effective gene expression with the assistance of external magnetic field, and resisted the adverse effect induced by serum proteins on the gene delivery. Moreover, neither significant cytotoxicity nor the interference on the osteogenic differentiation to MSCs were observed after magnetofection. Further studies revealed that this effective and serum resistant gene transfection was partly due to the accelerated and enhanced intracellular uptake process driven by external magnetic field. To conclude, the current study presented a novel option for genetic modification of MSCs in an effective, relatively safe and serum compatible way.

1. Introduction

During the past decades, bone marrow mesenchymal stem cells (MSCs) have elicited considerable attention in biomedical applications owing to their several unique capabilities, such as self-renew, relative ease of isolation and expansion *in vitro*, and differentiate into a variety of cell types (Pittenger et al., 1999). Especially, the low immunogenicity (Griffin et al., 2010) and free ethical concerns of MSCs (Qi et al., 2016; Rodriguez-Menocal et al., 2015) have made them been widely studied in regenerative medicine, tissue engineering, gene therapy, etc. Our recent studies further demonstrated that MSCs had potentials for targeting tumor-sites, which made them be promising candidates for delivering therapeutic genes to tumor tissues (Zhang et al., 2015, 2014a). In most cases, MSCs were required further genetic modifications to enhance their therapeutic effects, such as using brain-derived neurotrophic factor (BDNF) modified MSCs to enhance their regenerative ability for a middle cerebral artery occlusion (MCAO) (Kurozumi et al., 2004) and using stromal cell-derived factor-1 (SDF-1) modified MSCs to promote their wound healing activity for a skin defect (Nakamura et al., 2013). Nevertheless, the application of gene engineered MSC in stem-cell-based therapy is still severely hampered by our current inability to genetically modify these cells effectively and safely (Shah et al., 2013; Thakor et al., 2011).

In our previous studies, we had developed a cationized pullulan derivative by using spermine (a polyamine) to conjugate pullulan, as a novel vector for delivering genes (Jo et al., 2007). Pullulan is a natural water-soluble polysaccharide with repeated unit of maltotriose condensed through α-1,6 linkage (Jo et al., 2007). This polysaccharide could interact well with the asialoglycoprotein receptor on MSCs, resulting in an efficient internalization of the cationized pullulan (Jo et al., 2010b). Thus, this spermine-pullulan (SP-pul) was extremely suitable for the gene delivery to MSCs as demonstrated in our previous studies (He et al., 2011; Hu et al., 2014; Zhang et al., 2014a). However, like most of other cationic polymers, the transfection efficiency of SP-pul on MSCs decreased sharply in the presence of serum due to the negatively charged serum proteins which can competitively combine with polycation

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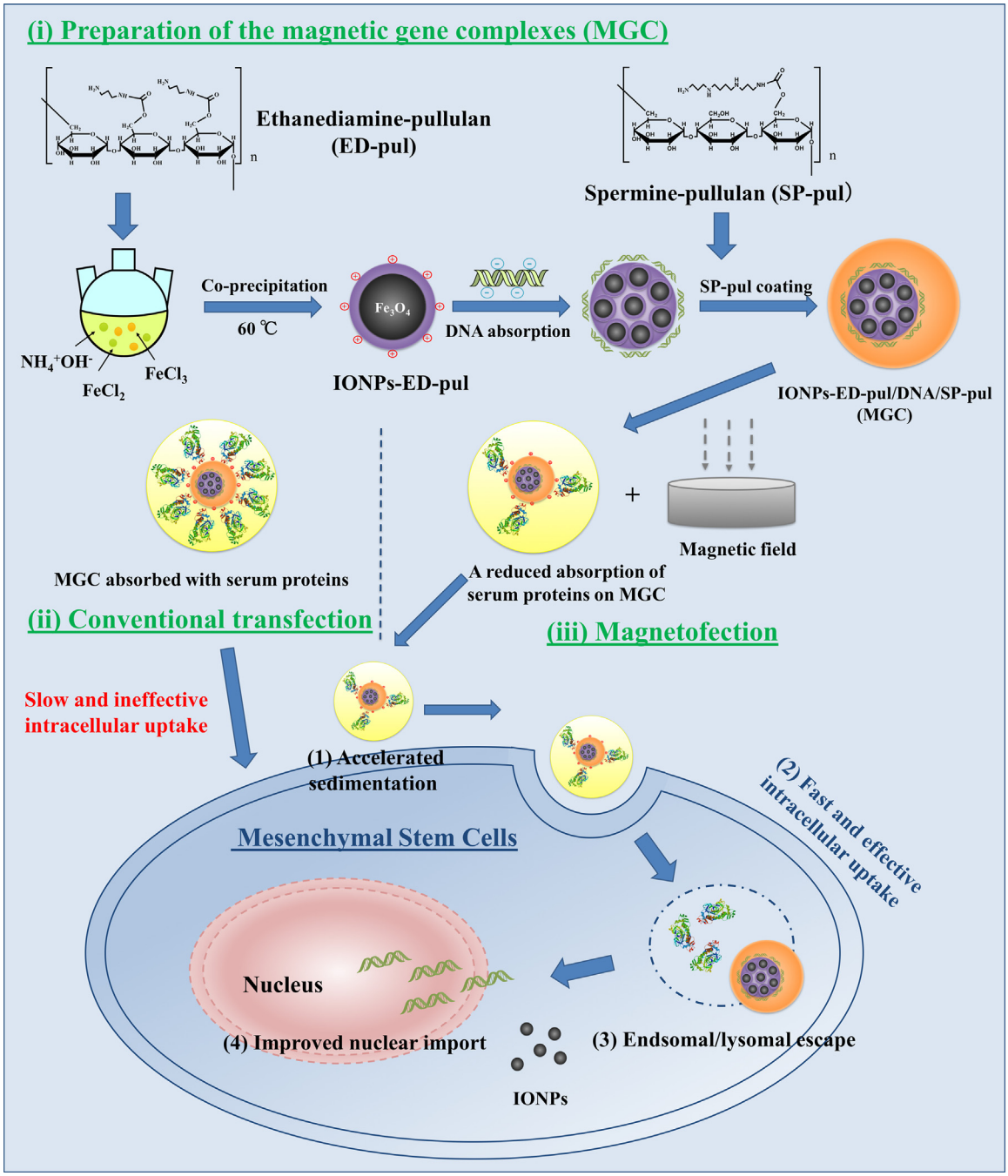
to reduce the combination of polycation with DNA (Wu et al., 2011). Thereby, this transfection usually conducted in the absence of serum to avoid such negative influence. But MSCs are very sensitive to the *in vitro* culture conditions (Wagner and Ho, 2007), which means the absence of serum may bring several safety risks such as increased cytotoxicity or loss of vital properties as stem cells (Alakpa et al., 2016; Ettayebi et al., 2016).

Therefore, with the aim to create effective, less risky and serum resistant transfection agents for MSCs, we introduced magnetic nanoparticles (MNPs) to SP-pul-based transfection system. Several studies have proved that MNPs are promising adjuvants for improving gene transfection efficiency with the assistance of external magnetic field (Kami et al., 2014; Ma et al., 2011; Namgung et al., 2010; Plank et al., 2003; Shah et al., 2013). And the gene transfection using magnetic materials, termed magnetofection, has significant effect on the level of gene expression (Arsianti et al., 2010a). Moreover, this physical technique has been demonstrated to promisingly reserve the transfection efficiency under serum conditions (Sun et al., 2012; Xie et al., 2015). Thereby, magnetofection is regarded as a promising approach for effective and serum compatible gene transfection.

The objective of this study is to design a novel magnetic complex with pullulan derivatives for effective, relatively safe and serum resistant gene delivery to MSCs. Iron oxide nanoparticles



Scheme 1. Schematic representation for the preparation of the magnetic gene complexes (MGC) and studies of their application as effective and serum resistant gene delivery vectors to MSCs.