Pectin–Coated Polyethylenimine Polyplexes as Effective Gene Delivery Systems

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Abstract—Gene therapy is believed to be the therapy of the future because it aims to eradicate causes rather than symptoms of diseases. The generic aim of gene therapy is to deliver DNA inside cells in order to achieve a stable and regulated expression of transgenes in the target cells or tissues without side effects. Spontaneous entry of naked DNA inside cells is an inefficient process, consequently, a major hurdle to the therapeutic application of gene therapy is the lack of suitable vectors for the delivery of nucleic acids. The principal strategies for gene delivery involve viral-mediated and non-viral methods. Among non-viral vectors, polycationic polymer branched polyethylenimine (b-PEI) has proved to be an efficient gene delivery vector but features high cytotoxicity and instability in presence of serum proteins and extracellular matrix components. PEI polyplexes suffer from high cytotoxicity and instability in presence of serum or extracellular matrix (ECM) components. PEI polyplex positive charge is the main reason of cytotoxicity and interaction with serum proteins (Koyama et al., 2003), coagulation system (Maruyama et al., 2004) and complement system (Plank et al., 1996), major problems particularly for in vivo gene therapy.

To overcome these limitations, in this study, b-PEI polyplexes were coated with pectin (Fig. 1b) as anionic polysaccharide to shield the positive charge on polyplexes and to obtain stable dispersions of Pectin Coated Polyplexes (PCP). Compared to other typical anionic polysaccharides as alginites, pectins show a complex structure, with regions rich in galactose residues which can promote targeted gene delivery. Accounting this peculiar characteristic, modified pectins were also studied as non-viral gene delivery systems (Katav et al.).

I. INTRODUCTION

Gene therapy can be broadly defined as the introduction of genetic material into cells to transiently or permanently alter the cellular phenotype. Gene therapy has become one of the most intensively developing fields for current clinical research since it offers new treatment possibilities for many common inherited and acquired human diseases that conventional clinical procedures fail to cure. These diseases include monogenic disorders, such as cystic fibrosis, but also more complex disorders, such as cardiovascular diseases, nervous system diseases, autoimmune diseases, and cancer.

It is well-known that direct administration of naked DNA to tissues or cells is rather ineffective (Pezzoli et al., 2007; Giordano et al., 2006, Candiani et al., 2010). Indeed, naked plasmid DNA is unable to cross biological membranes since its negative surface charge creates repulsion with the negatively charged cell surface. Hence a carrier called vector must be used to increase the delivery rate of genes into cells. A vector can be defined as a system that provides protection from gene degradation ensuring gene transcription within cells. The main vectors for gene delivery are viral and non-viral carriers. One of the major hurdles to the continued development and therapeutic application of nucleic acids is the lack of suitable vectors for their delivery.

Among non viral vectors, polycationic polymer branched polyethylenimine (b-PEI, Fig. 1a) has shown high transfection efficiency both in vitro and in vivo (Ito et al., 2006). However, b-PEI polyplexes suffer from high cytotoxicity and instability in presence of serum or extracellular matrix (ECM) components. PEI polyplexes suffer from high cytotoxicity and instability in presence of serum or extracellular matrix (ECM) components. PEI polyplex positive charge is the main reason of cytotoxicity and interaction with serum proteins (Koyama et al., 2003), coagulation system (Maruyama et al., 2004) and complement system (Plank et al., 1996), major problems particularly for in vivo gene therapy.

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![Fig. 1. Chemical structures of b-PEI (a) and pectin (b).](image)

The effect of pectin coating on condensation behavior, size, ζ-potential, polyplex stability in presence of heparin and on transfection ability in Cos-7 cell line was evaluated.

II. MATERIALS AND METHODS

Plasmid DNA encoding for the secreted Gaussia Luciferase (pCMV-GLuc) was purchased from Clontech Laboratories (Mountain View, CA, USA); pectin (low
methoxyl, DE 42%, MW 76 KDa), was kindly provided by Herbstreit & Fox Neuenbuer (Germany); 25 kDa branched polyethylenimine (b-PEI), heparin and all chemicals were from Sigma-Aldrich (Milan, Italy) if not differently specified.

DNA/b-PEI polyplexes were prepared in dH₂O adding pCMV-GLuc DNA or calf thymus DNA to a 25 kDa b-PEI solution at different concentrations, yielding the desired charge ratio (CR, +/-).

The binding ability of b-PEI to DNA was assessed fluorimetrically by monitoring the displacement of SYBR-Green I from DNA at increasing CR (λex = 485 nm; λem = 535 nm).

Pectin Coated Polyplexes (PCP) were prepared by adding different amounts of pectin to CR 8 b-PEI polyplex, obtaining different pectin/DNA (w/w) ratios (0, 0.75, 1.5, 3 and 7); the resulting solutions were incubated for 20 min at r.t. (Fig. 2).

![Fig. 2. Preparation of pectin coated polyplexes (PCP).](image)

The effect of pectin coating on DNA condensation was also studied: PCPs were prepared in presence of SYBR-Green I and the fluorescence was monitored over time. The stability of polyplex and PCP in presence of anionic polysaccharides was examined at CR 8 and at different pectin/DNA (w/w) ratios by measuring the ability of different amounts of heparin to restore the fluorescence of DNA/SYBR Green I.

Particle size and ζ-potential of polyplexes and PCP were measured by Malvern Zeta Nanosizer.

For transfection, Cos-7 cell lines were seeded in 48-well plates at a density of 1.5 x 10⁴ cells/cm²; 24 hours later, medium was replaced with fresh complete medium (DMEM containing 10% Fetal Bovine serum, FBS) containing polyplexes or CPC (0.08 µg of plasmid/cm²) with different pectin/pDNA ratios (0, 0.75, 1.5, 3, and 7). Forty-eight hours later, cytotoxicity was evaluated by AlamarBlue cell viability assay (Invitrogen Life Technologies, San Giuliano Milanese, Italy) according to manufacturer’s guidelines. Viability of untreated control cells was assigned as 100%. Luciferase expression was assessed by assaying the culture medium with Gaussia Luciferase Assay Kit (New England BioLabs, Hitchin, UK).

Statistical analysis was performed by ANOVA test. Significance was retained when p<0.05.

### III. RESULTS AND DISCUSSION

By monitoring the displacement of SYBR-Green I from DNA, we noticed that 25 kDa b-PEI effectively condensed DNA at CR ≥ 3 (data not shown). Polyplexes formed at CR 8 had a mean diameter of 125 ± 4 nm and an overall charge of +41 ± 2 mV. Because of the high cytotoxicity of b-PEI polyplexes and of their instability in presence of ECM negatively charged molecules, we decided to reduce polyplex positive charge by coating them with pectin, an anionic polysaccharide, obtaining PCPs (Fig. 2) at different pectin/DNA w/w ratios.

Firstly we investigated the stability of polyplexes after pectin coating: at pectin/DNA w/w 7, pectin could partially disassemble CR 8 polyplexes, inducing DNA release, although at lower ratios polyplex disassembly was negligible (Fig. 3).

![Fig. 3. DNA release from b-PEI CR8 polyplexes after coating with different amounts of pectin.](image)
Since extracellular polyanionic glycosaminoglycans (GAGs) can interact with and disassemble positively charged b-PEI polyplexes, we evaluated the stability of PCP in presence of heparin. Heparin effect was concentration and time dependent. At heparin/DNA (w/w) ratio 5 or higher, heparin could completely disrupt b-PEI polyplexes prepared at charge ratio 8. As expected, instead, the DNA release was significantly delayed and reduced after pectin coating of b-PEI polyplexes as represented in Fig. 5, demonstrating the increased stability of polyplexes in presence of negatively charged ECM components.

CONCLUSIONS

Concluding, we here demonstrated that pectin was suitable for coating b-PEI polyplexes to form PCP in order to decrease their surface charge. This, in turn, led to decreased transfection with a concomitant lowering in cytotoxicity and higher stability. We assessed that the best pectin/DNA (w/w) ratio for optimum viability and transfection efficiency was 1.5. Moreover, we demonstrated that the stability of PCP in presence of polyanionic glycosaminoglycans such as heparin was increased. Altogether, high transfection effectiveness in vitro and stability in ECM-like environment indicate that PCP vectors are potential tools for in vivo gene delivery applications.

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