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Short communication

Polyethylenimine-polyacrylic acid nanocomposites: Type of bonding does influence the gene transfer efficacy and cytotoxicity

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ABSTRACT

The main aim of the current study is to compare the physicochemical properties, cytotoxicity and gene-transfer ability of electrostatically and covalently linked nanocomposites of polyethylenimine (PEI) and polyacrylic acid (PAA) on mammalian cells. Two series of nanocomposites, ionic PEI-PAA (iPP) and covalent PEI-PAA (cPP), were synthesized by varying the amounts of polyacrylic acid (PAA). Physicochemical characterization revealed that iPP nanopcomposites were of bigger sized than cPP nanocomposites with zeta potential almost comparable. Nucleic acid binding assay displayed that iPP and cPP nanocomposites, having sufficient cationic charge, efficiently interacted with plasmid DNA and completely retarded its electrophoretic mobility on agarose gel. In vitro MTT assay showed slightly higher cell viability of cPP/pDNA complexes over their ionic counterparts. Both the series of nanocomposite/pDNA complexes exhibited considerably higher transfection efficacy compared to pDNA complexes of native bPEI and the standard transfection reagent, Lipofectamine, with cPP/pDNA complexes performed much better than iPP/pDNA complexes. Flow cytometry further confirmed these findings where cPP-4/pDNA complex showed transfection in ~85% HEK293 cells, while iPP-2/pDNA complex transfected ~67% HEK293 cells. Lipofectamine/pDNA and bPEI/pDNA complexes could transfect just ~35% and ~26% HEK293 cells. All these results demonstrate the superiority of covalently linked nanocomposites (cPP) which could be used as efficient carriers for nucleic acids in future gene delivery applications.

1. Introduction

Branched polyethylenimine (bPEI, 25 kDA) has been the most widely used cationic polymer for gene delivery applications and is considered as 'gold standard' [1–3]. The inherent cationic charge and proton-sponge property with amine functions at every third position, that can be protonated, make it a unique polymer for in vitro studies. However, high cytotoxicity and low hemocompatibility due to high density of cationic charge have significantly affected its clinical efficacy. In order to address these concerns, extensive modifications have been incorporated to develop efficient and safe chemical vectors to deliver nucleic acids in vitro and in vivo [4,5]. Among these modifications, one of the strategies is to prepare ternary complexes i.e., coating pre-formed PEI-pDNA complexes with anionic polymers [6–10]. However, addition of such anionic species to PEI-pDNA complexes sometimes causes flocculation and formation of bigger sized complexes [11–14]. Hence, in this approach, large variations in the size of the ultimate ternary complexes have been observed. Due to such variations, low degree of cellular uptake and internalization significantly affect the efficacy of the pDNA complexes. In this regard, Zhang et al [15]. and Shang et al. [16] have reported that small-sized particles are uptaken and internalized two-times more as compared to large-sized particles. Therefore, we hypothesized that if we could control the size of the particles by first allowing interaction of bPEI with varying amounts of oppositely charged polyacrylic acid, further interaction with pDNA would not result in the formation of heterogeneous population of nanoparticles. The projected strategy would not only help in reducing the charge-associated toxicity of bPEI but uptake and internalization would also be efficient resulting in higher transfection efficiency. In another study, Sun et al. have used bPEI decorated polyacrylic acid stabilized magnetic nanoparticles for gene delivery applications [17]. Cationic chains of bPEI have been held together by electrostatic interactions. These bPEI-based magnetic nanoparticles showed low transfection efficiency in HEK293 and U87 cells

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but in presence of serum and external magnetic field, the particles showed high gene transfer ability.

Polyacrylic acid (PAA), a water soluble biodegradable polyelectrolyte, has been finding numerous biomedical applications [18] and copolymers of PAA with polyethylene oxide and polypropylene oxide are being used as pharmaceutically safe components for ocular, oral, mucosal and topical delivery of drugs [19]. Keeping these facts in mind, we have attempted to synthesize size-controlled nanocomposites by allowing interactions between cationic PEI and anionic polyacrylic acid (PAA) in two ways, viz., electrostatically and covalently. The covalently linked nanostructures were synthesized by using a water soluble condensing reagent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) [20]. The resulting PEI-PAA (PP) nanocomposites, ionic (iPP) and covalent (cPP), were characterized for their size and zeta potential measurements and then evaluated for their transfection efficiency and cytotoxicity in mammalian cell lines.

2. Results and discussion

Branched polyethylenimine has been considered as off-the-shelf 'gold standard' in gene delivery, however, charge-associated toxicity and non-specific interactions with the blood constituents have limited its widespread use in in vivo applications. Here, we have not only attempted to address the cytotoxicity issue as well as improve the gene carrying capacity of the PEI-PAA (PP) nanocomposites but also investigated how the type of linkages influences the properties (physicochemical and biological) of the resulting nanocomposites. The interactions between two polymers resulted in the formation of ionic (iPP) and covalent (cPP) nanocomposites. ^{1}H NMR of cPP-4 nanocomposites showed peaks at δ1.05–2.3 and 2.6–3.5 corresponding to acrylic acid and ethylenimine protons, respectively. Peak at δ8.07 due to amide (CO—NH) protons confirmed the covalent inclusion of polyacrylic acid in the cPP nanocomposites, while the same was not detected in the NMR spectrum of iPP-2 nanocomposites. Extent of primary amine groups blocked in cPP polymers was determined by the standard TNBS assay [21]. It was found to be cPP-10% (6.6%), cPP-15% (8.2%), cPP-20% (10.5%), cPP-25% (12.7%), cPP-30% (14.5%). The values in the parenthesis represent the actual percent of primary amines blocked in cPP polymers. It was observed that covalent attachment of polyacrylic acid onto bPEI occurred in the range of ~48–66%, which showed a decrease on increasing the attempted percent of conjugation. It was also inferred from the quantification results that higher percentage of primary amines was blocked during the reaction as these functional groups were located at the periphery of the bPEI polymer.

The particle size and surface charge on the nanocomposites and their pDNA complexes were measured by Zetasizer Nano-ZS (Malvern Intsruments, UK). The data revealed that iPP nanocomposites were found to be bigger (~349–181 nm) in size as compared to their covalent counterparts (~284–163 nm, Table S1), which ensured that covalently bound nanocomposites were more compact than ionic nanocomposites. As the concentration of polyacrylic acid increased, size of the nanocomposites decreased suggesting more compactness in the structures of nanocomposities at higher concentration of polyacrylic acid due to multipoint contacts. On complexation with pDNA, the size of the complexes in both the series displayed a marginal increase (Table S1). Likewise, surface charge measurements showed a decrease as we increased the concentration of polyacrylic acid down the series which confirmed that more and more amount of polyacrylic acid was bound to cationic bPEI (Table S1). On complexation with negatively charged pDNA, both iPP and cPP series of complexes showed a further decrease in the surface charge, however, the total charge remained positive.

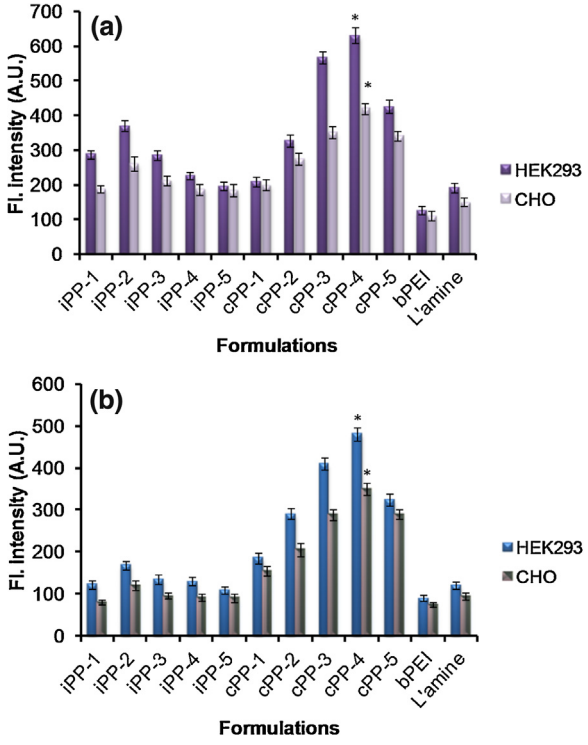

Fig. 1. Transfection assay of iPP, cPP, bPEI and Lipofectamine/pDNA complexes at their best *w*/*w* ratios in the absence (a) and presence (b) of serum in HEK293 and CHO cells.

To find out the amount of nanocomposites required to retard the mobility of a fixed amount of pDNA on an agarose gel, pDNA complexes of iPP and cPP nanocomposites were prepared at different weight ratios and run on the gel. The results showed that both iPP and cPP nanocomposites retarded the mobility of pDNA at weight ratios 0.7–1.33, which were higher than the weight ratio at which native bPEI retarded the mobility of pDNA (i.e., 0.5) (Fig. S1). This could be attributed to partial charge blockage on bPEI by anionic polyacrylic acid. The results were also in complete agreement with the zeta potential values, where it was observed that as we increased the amount of polyacrylic acid, surface charge on the nanocomposites decreased i.e., more cationic charge on bPEI was blocked. Here too, as the amount of polyacrylic acid increased from iPP-1 to iPP-5 and cPP-1 to cPP-5, there was a gradual increase in the amount of nanocomposites required to retard the mobility of pDNA (300 ng).

Gene transfer capability of iPP and cPP nanocomposites was assessed in absence and presence of serum on HEK293 and CHO cells. The assay was performed at various *w*/*w* ratios. Primarily, the expression of green fluorescent protein was observed under a fluorescence microscope and then subjected to quantitative analysis. The results showed that nanocomposite/pDNA complexes exhibited considerably enhanced transfection efficiency as compared to bPEI and Lipofecatmine/pDNA complexes. Among both the series of complexes, cPP/pDNA complexes showed higher efficacy than their ionic counterparts (iPP/pDNA complexes) in absence and presence of serum. In absence of serum, iPP-2/pDNA and cPP-4/pDNA complexes showed ~1.7–2.9 and 2.8–5.0 folds higher transfection as compared to bPEI and Lipofectamine/pDNA complexes in both the cells (Fig. 1). In presence of 10% FBS, the overall transfection efficiency of all the complexes decreased, still, cPP-4/pDNA complex exhibited much higher gene transfer capacity (~3.7–5.3 folds) than iPP-2/pDNA complex (~1.3–1.9 folds) establishing clear cut superiority of covalently formed nanocomposites over their ionic counterparts (Fig. 1). Transfection efficiency of both the series of