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The polyplex, protein corona, cell interplay: Tips and drawbacks

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ABSTRACT

Polyplexes (PX) are soft materials, obtained by blending polycations and nucleic acids, designed for gene delivery applications. While much is known about the transfection properties of PX, their protein corona, the biomolecules interacting with colloids once in a biological environment, represents an underlooked parameter in gene transfection. In this study, linear and branched polyethylenimines (*l*PEI and *b*PEI), the golden standard among non-viral vectors, were selected and used throughout the work: their physicochemical properties and protein corona when complexed to DNA were studied and linked to the toxicity and transfection efficiency arisen upon their delivery to cells. Interestingly, *l*PEIDNA and *b*PEIDNA complexes were characterized by similar physicochemical features, but different biological behavior. In fact, the biological milieu where cells and PX interact greatly influences their size, stability and transfection abilities. Using PX as a soft material model system, we spotlighted structure-activity relationships and methodologies that can help interpret their biological behavior and guide future studies in the field.

1. Introduction

Polyplexes (PX) belong to a class of soft materials composed of cationic polymer (Pol)-nucleic acid (NA) complexes assembled by electrostatic interactions, mostly used for the delivery of genetic materials to cells or organisms avoiding the typical side-effects of viral gene delivery [1,2].

Over the last two decades an increasingly number of polymers have been designed and PX shaped and tested: different supramolecular strategies have been devised for maximizing both association of the two counterparts and enabling an intracellular NA release from the complex [3]. Indeed, an ideal delivery vector should be able to protect the NA against degradation [4,5], promote internalization of the genetic material into target cells [6,7] and avoid detrimental effects on cells [8]. Among cationic polymers, polyethylenimines (PEI) are widely used in this field due to their easiness of preparation in different configurations (linear or branched, *l*PEI and *b*PEI, respectively) and their high transfection ability [9–11]. Cationic Pol like PEI had, historically, attracted scientific interest for their positive charge, matching the NA negative charge, allowing the formation of stable Pol/NA complexes. A given PX formulation is described by the N/P that is the ratio of cationic nitrogen atoms (N) of the Pol used to complex a given quantity of NA that bear the anionic phosphate groups (P). PX with an N/P > 1 should ideally have a net positive charge thus facilitating cell uptake and endosomal escape.

Furthermore, uncomplexed Pol amino groups have an intrinsic buffer ability endeavoring lysosomal proton sequestering, which eventually provokes lysosomal swelling, rupture and NA release in the cytoplasm [12].

It is known that the behavior of nano-delivery systems in the biological environment is unpredictable [13] due to their interactions with the surrounding biomolecules [14].

Spontaneous adsorption of proteins on bulk solids and colloids was demonstrated to alter the characteristics of the sorbent surfaces [15–18]. These durable adsorbing layers of biomolecules, known as PC [19–21], are responsible for the interactions between artificial objects and cells. Representing the biological identity of the material, PC modulates the physiological and pathological response of the host organism to the guest material [4,22,23]. Historically, most of the studies regarding protein adsorption on surfaces and colloids were focused on hard materials, whereas the study of soft materials, except some exceptions [24,25], remains

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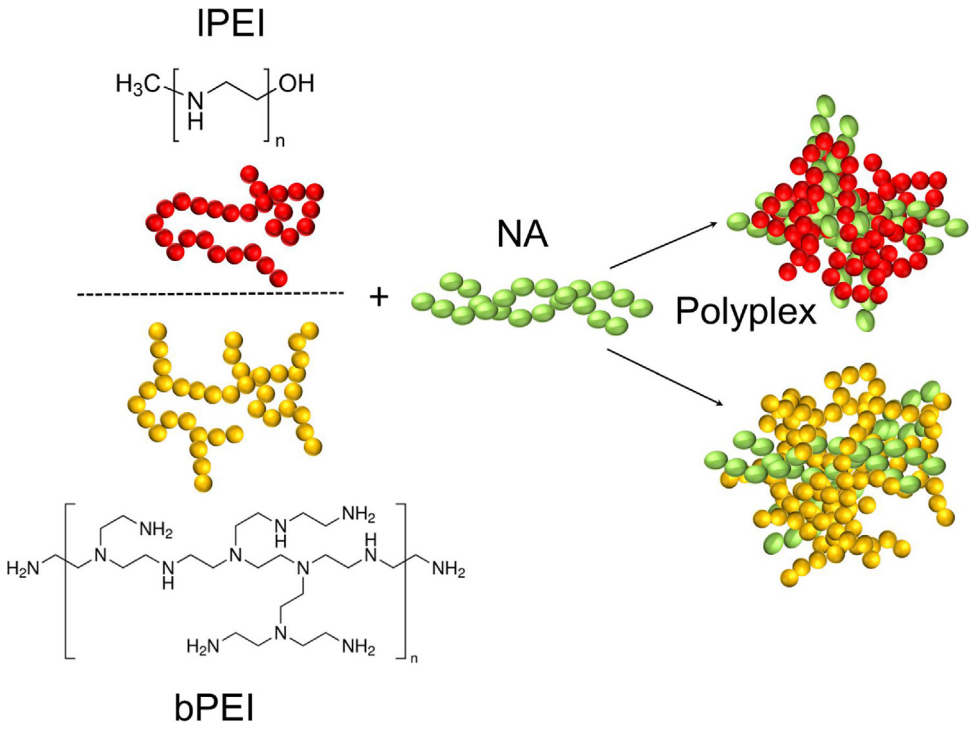

Fig. 1. Schematic of PEI-DNA assemblies: polyethylenimines (*l*PEI and *b*PEI) assembled through electrostatic interactions with nucleic acids (NA) to form Polymer/NA complexes called Polyplexes (PX): (red) linear (*l*PEI) and (yellow) branched polyethylenimine (*b*PEI), and (green) NA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

an unmet need, due to the lack of ad hoc characterization and purification methodologies [26,27].

Here, we studied how PXs formed with two prototypical PEIs, namely *l*PEI and *b*PEI, interact with the environmental proteins present in cell culture media (Fig. 1). In particular, we focused on the structure-function relationship correlating the colloidal stability of complexes with their final cellular fate. Overall, we performed a physical-chemical characterization of pristine PXs and the protein corona (PC)-coated PXs (PXPC) in different cellular culture conditions (medium with increasing percentage of serum) together with a study of their cytotoxicity and transfection efficiency.

2. Material and methods

2.1. Materials

The list of the materials and suppliers used follows: 25000 Da *l*PEI (Polysciences, Germany); 25000 Da *b*PEI (Sigma Aldrich, Italy); 10 μg/μL Salmon Sperm DNA (ssDNA, Invitrogen, Italy); pGL3-Control Vector, 5.2 kbp, and Luciferase Assay System (Promega, Italy); Dulbecco's Modified Eagle's Medium (DMEM), 1 M HEPES solution, 100 mM Sodium pyruvate, 100× Penicillin-streptomycin, Dulbecco's Phosphate Buffered Saline (PBS), 10× Trypsin solution, 0.4% Trypan Blue solution, Sodium chloride (NaCl), and Fetal Bovine Serum (FBS) (all from Sigma Aldrich, Milan, Italy); 100× L-Glutamine solution (Euroclone, Italy); polystyrene sterile 96-well cell culture plates (Corning Costar, Italy).

All the solution were filtered through a 0.20 μm syringe filters (Corning Costar) to guarantee the colloidal purity of the preparations.

2.2. Preparation of polymer stock solutions

Polymer stock solution was prepared mixing PEI stock in the selected solvent [11].

The dose of stock polymer reagent was added to NaCl solution in a test tube and then mixed. After the complete dissolution, the pH of the polymer stock solution was checked and adjusted with NaOH or HCl to pH 7. The solutions (one for *l*PEI and the other for bPEI) were filtered under biological hood (Olympia 1.8 D7492–Celbio, Italy) with a 0.20 μm filter. Polymer stock solutions were stored at 4 °C.

2.3. Preparation of PXs

The PX suspension was obtained (Fig. S1) diluting the Pol stock with 150 mM NaCl to the desired concentration, and adding DNA to this preparation.

A concentration of 0.32 μg DNA/cm^2 per well was used for transfection experiments. In all experiments, the volumes of dispersing solution were calculated in order to maintain the concentration of DNA constant to 0.02 μg/μL in every PX suspension as well as the N/P (that was kept equal to 30). Pol stock solutions (20 mM) were diluted in 150 mM NaCl prior to use in order to achieve the desired N/P. The incubation time was adjusted as a function of the specific purpose of the experiment. Subsequently, the PX dispersion was mixed with DMEM typically supplemented with 10% FBS to obtain the final transfection suspension. The PX dispersion was characterized as previously described [11,28]. Transfection suspensions were incubated for one hour at 37 °C in DMEM supplemented with FBS. After that, the suspensions were either analyzed or processed or used in transfection experiments. In this study DMEM at different concentration of FBS (hereafter referred to as cDMEM) was used, to give the following final FBS concentration of 1%, 2.5%, 5%, 10% and 50% (v/v of FBS in DMEM).

2.4. Physical-chemical characterization of PXs

DLS experiments were carried out using a Zetasizer Nano ZS (Malvern Instrument, UK), equipped with a 633 nm red laser and