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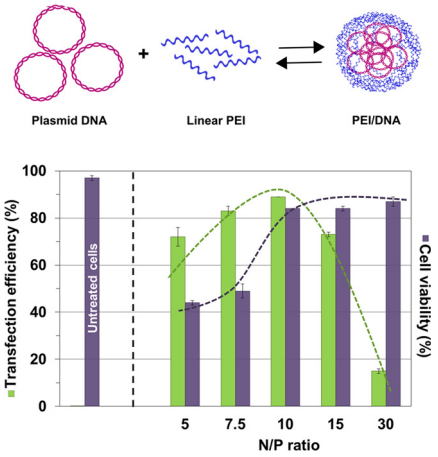
Control of the transfection efficiency of human dermal fibroblasts by adjusting the characteristics of jetPEI®/plasmid complexes/polyplexes through the cation/anion ratio

Yulia N. Sergeeva[a], Laura Jung[b], Claire Weill[c], Patrick Erbacher[c], Philippe Tropel[b,1], Olivier Felix[a], Stéphane Viville[b,d,2], Gero Decher[a,e,f,g,*]

[a] Institut Charles Sadron (CNRS, UPR 22), Université de Strasbourg, 23 rue de Loess, 67034, Strasbourg Cedex 2, France
[b] Institut de Génétique et de Biologie Moléculaire et Cellulaire (INSERM, U964; CNRS, UMR1704), Université de Strasbourg, 1 rue Laurent Fries, 67400, Illkirch, France
[c] Polyplus Transfection, BIOPARC, 850 Boulevard Sébastien Brant, 67400, Illkirch, France
[d] Centre Hospitalier Universitaire, Université de Strasbourg, 1 place de l'hôpital, 67000, Strasbourg, France
[e] International Center for Frontier Research in Chemistry, 8 allée Gaspard Monge, 67083, Strasbourg, France
[f] Faculté de Chimie, Université de Strasbourg, 1 rue Blaise Pascal, 67008, Strasbourg, France
[g] Institut Universitaire de France, 103 Boulevard Saint-Michel, 75005, Paris, France

GRAPHICAL ABSTRACT



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ABSTRACT

Primary cells are in general more difficult to transfect than most animal cells. Here we report on the efficient transfection of human dermal fibroblasts with jetPEI®/DNA complexes (polyplexes). The polycation/DNA ratio was varied with respect to both transfection efficiency and cell viability and the different DNA-polyplexes were characterized by different physicochemical methods, namely ζ-potential measurement, light scattering and scanning electron microscopy. Interestingly these data point to the fact that our DNA-polyplexes possess a core-

Abbreviation: DLS, dynamic light scattering; TEM, transmission electron microscopy; GFP, green fluorescent protein; PEI, poly(ethylene imine); DNA, Deoxyribonucleic acid

* Corresponding author at: Institut Charles Sadron (CNRS, UPR 22), Université de Strasbourg, 23 rue de Loess, 67034, Strasbourg Cedex 2, France.
E-mail address: decher@unistra.fr (G. Decher).
[1] Present address: Bluestem Engineering SAS, 4 Avenue Valmont, 13009, Marseille, France.
[2] Present address: Institut de Parasitologie et Pathologie Tropicale, EA 7292, Fédération de Médecine Translationelle, Université de Strasbourg, 3 rue Koeberlé, 67000, Strasbourg, France and Laboratoire de diagnostic génétique, UF3472-génétique de l'infertilité, Hôpitaux Universitaires de Strasbourg, Université de Strasbourg, 67000, Strasbourg, France.

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shell structure in the wet state. We find that the range of jetPEI®/DNA ratios, expressed as N/P (nitrogen-to-phosphate) ratio, at which high transfection and high cell viability is observed is quite narrow. Transfection efficiency is high for N/P-ratios below 15 and cell viability is high at high N/P-ratios, the optimum of both occurs in the range of 10 to 15. The transfection maximum is observed at over 80% of transfected cells in the range of N/P-ratio of 7.5 to 10, whereas the generally recommended N/P-ratio for jetPEI®/DNA transfection experiments is 5. At a N/P-ratio of 15, transfection is still as high as at a N/P-ratio of 5, but viability is almost twice as high. We found that the cytotoxicity of the polyplexes is related to their size, and is very small for particles of small size. Our data suggest that the morphologies of the polyplex-particles discussed here are better described by a core-shell structure rather than a conventional homogeneous “scrambled egg” structure.

1. Introduction

Gene therapy has attracted much interest as an alternative approach for the treatment of severe human diseases. The therapeutic effect is achieved by delivering exogenous nucleic acids into the target cells that induce the production of therapeutic proteins or modulate damaged cellular functions [1–3]. Due to large size, negative charge, and hydrophilicity, nucleic acids cannot easily cross the cellular membrane and need to be incorporated into a delivering carrier [4]. This carrier condenses DNA into small particles and allows DNA transfer through the cell membrane and protects genes from degradation in the cellular environment [1–4]. Depending on the carrier, gene delivery can be achieved using viral or non-viral methods. The viral techniques are based on the use of viruses which genetic material has been replaced with therapeutic genes [5]. Although these systems were found highly effective, their therapeutic applications are however limited due to the possible side effects such as immunogenicity and oncogenic effects [6,7]. In this context, non-viral carriers such as positively charged polymers have attracted much interest due to their high accessibility and the low adverse effects level. In this case, gene delivery performed by polyplexes, soluble interpolyelectrolyte complexes of the nucleic acid and the positively charged polyelectrolyte also called transfection agent [8–12]. The transfection yield and the cytotoxicity depend on the chemistry transfection agent and polyplex properties such as size, charge, and composition. These characteristics are controlled by the parameters of the flocculation process such as the concentration, the molecular weight and the chemical nature of the reagents, their stoichiometric balance (N/P ratio), the mixing order, the ionic strength, pH and the temperature of the reaction mixture [13]. In addition, the transfection ability of the complexes prepared under similar conditions may vary for different cell lines. Thus, for the fabrication of an efficient carrier with low toxicity, it is indispensable to optimize its preparation for a specific cell line.

A general approach that allows adjusting the transfection rate of a particular type of cells is to control the stoichiometric balance between the number of positive and negative charges by employing an increased concentration of positively charged macromolecules in the reaction mixture (increasing *N* at constant P in the N/P ratio). Typically, this leads to the formation of the smaller size complexes with an increased ζ-potential. The latter is assumed to play an important role in the floc stability in a buffer by preventing particle fouling and sedimentation and their interactions with biological media such as blood [13,14]. The positively charged vectors are known to enhance vector cell binding and their internalization [15–20] through the ionic interactions with proteoglycans present on the cell surface [21–23]. The main drawback of this approach is that with an increase of N/P ratio the polyplexes with a high charge density induce undesirable cytotoxic effects due to the aggregation of the complexes on the cell surface leading to membrane rupture [24,25]. In this context, particularly challenging remains the transfection of primary cells, where a low efficiency of the complexes is typically coupled with high cytotoxicity [26–29].

Here, we report on how the DNA concentration controls the cytotoxicity and the ability of the polyplexes to transfect CPRE2 cell line, human adult dermal fibroblasts, established from the abdominoplasty of a 38-year-old woman suffering from obesity [30]. Linear poly(ethylene imine) (jetPEI®, Polyplus Transfection™) was selected as a transfection agent. Due to its high positive charge density jetPEI® effectively condenses DNA into small particles and protects them from the degradation in endosomes [16,31]. The buffering capacity of amine groups or “proton sponge” effect results in the osmotic swelling of endosomes leading to the endosomal escape of the particles into the cytosol [32]. Although PEI-based vectors were proved to be highly efficient in the transfection of various cell lines [7,9,11,12,33], the possible toxic side effects of PEI limit its application in systematic transfection experiments [34–36]. In present work, polyplexes with different N/P ratios were prepared using jetPEI® and plasmid DNA coding green fluorescent protein (GFP) and were characterized by ζ-potential measurement, light scattering, and scanning electron microscopy. The transfection yield was calculated based on GFP expression detected by flow cytometry and fluorescent microscopy. We found that the cytotoxicity and the transfection properties of the polyplexes are related to their size (N/P ratio) and not to their ζ-potential. Our data suggest also that the morphologies of the studied polyplex-particles are better described by a core-shell structure rather than a conventional homogeneous “scrambled egg” structure.

2. Experimental section and materials

2.1. Materials

Linear poly(ethylene imine) (jetPEI®, $\overline{M_w} \approx 22\,000$ g/mol, Polyplus Transfection™, Illkirch-Graffenstaden, France) was used as received. Plasmid DNA (gWiz GFP, 5757 bp) was obtained from Aldevron (Fargo, ND). Sodium chloride (≥99.5% pure, suitable for cell culture) was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). All chemicals were used without further purification. Water used for the preparation of all solutions was purified with a Milli-Q Gradient water purification system (Millipore, Molsheim, France). The solution of sodium chloride was filtered through a 0.22 µm filter before use. All experiments were performed with freshly prepared solutions.

2.2. Cell culture

CPRE2 cell line, primary adult human dermal fibroblasts, was established from an abdominoplasty of a 38-year-old woman suffering from obesity. Briefly, the tissue was treated mechanically to remove hypoderm (especially adipose tissue) and then treated with enzymes to remove the epidermal part. Then, dermal fibroblasts were selected upon culture.

The cells were maintained at 37 °C under 5% CO_2 atmosphere in Opti-MEM® medium supplemented with 10% (v/v) fetal bovine serum (FBS; Sigma-Aldrich Co., St. Louis, MO). The complete procedure for establishing the cell line is described elsewhere [30].

2.3. Complex preparation

The solutions of PEI/DNA complexes were prepared by mixing jetPEI® and DNA solutions in 0.15 M NaCl at corresponding