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Recent progress in gene delivery using non-viral transfer complexes

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Abstract

The delivery of genetic material into cells is a field that is expanding very rapidly. Non-viral delivery methods, especially ones that focus on the use of chemical agents complexed with genetic material, are the focus of this mini-review. More-recent uses of known transfection agents such as poly(ethylenimine), poly(L-lysine), and various liposomes are discussed, and some novel approaches (both chemical and methodical) are reviewed as well. A very brief look at how non-viral gene delivery research is being aimed at the clinic is also included. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Poly(ethylenimine); Poly(L-lysine); Liposomes; Transfection; Gene delivery

1. Introduction

The major aim of gene therapy is to deliver genetic material into cells to alter their function,

Abbreviations: DC-Chol, 3-β[*N,N'*-dimethylaminoethane]-carbamoyl cholesterol; DOGS, dioctadecylamidoglycylspermine; DOPE, dioleoylphosphatidylethanolamine; DOSPA, 2,3-dioleoyloxy-*N*-[2(spermine-carboxamido)ethyl]-*N,N*-dimethyl-1-propanaminiumtrifluoroacetate; DOTAP, *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N'*-trimethylammonium chloride; HSA, human serum albumin; LDL, low density lipoprotein; lipofectamine, a liposomal formulation of DOSPA and DOPE at 3:1 (w/w); lipofectin, a liposomal formulation of DOTMA and DOPE at 1:1 (w/w); NLS, nuclear localization signal; OLN, oleoyl-ornithinate; PEG, poly(ethylene glycol); PEI, poly(ethylenimine); PHPMA, poly[*N*-(2-hydroxypropyl)methacrylamide]; PLL, poly(L-lysine)

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usually for the benefit of an entire organism. The genetic material can be either DNA or RNA, and the altered function can be an increase or decrease in the production of a protein. The protein is not restricted to being a natural product of the host cells, and the host cells are not required to be functioning as a part of the whole organism at the time of transfection. Fundamental problems involve deciding on and isolating the appropriate gene for delivery, how to get the gene into (only) the cells of interest, and how to have the gene expressed after it has been delivered into the cells. The field of gene delivery focuses on the latter two of these goals, and has spawned aggressive research in many laboratories throughout the world. With these goals in place, it is a small step to further design applications of gene delivery for tissue engineering applications.

Through transfection, cells can be induced to produce chemicals that can be used in pharmaco-

logical settings such as hormone replacement or production of specific proteins. In this fashion, the transfected cells behave as microfactories with no labor unions and minimal property taxes. Cell transfections can be performed in vitro, with the resulting products being stored relatively easily for later use. A tissue engineering application that could utilize gene delivery involves biodegradable scaffolds for wound repair, where the scaffolds are seeded with transfected cells ex vivo. After the seeded devices are implanted, the transfected cells could recruit the appropriate host cells to migrate into the scaffold by expressing the delivered DNA — genes that perhaps code for growth factors, morphogenic proteins, or cell type-specific adhesion molecules.

Although gene delivery has great potential for the field of tissue engineering, selecting the appropriate genes to deliver as well as selecting the appropriate gene delivery vehicle are tasks that are more easily designed than implemented. Selecting and obtaining the appropriate genes to deliver is a problem that is specific to a given application and is beyond the scope of this discussion. Selecting the appropriate carrier, however, is a more universal problem that is perhaps more daunting because, unfortunately, the ideal gene delivery agent has yet to be developed or characterized. This mini-review will outline some of the more recent advances in non-viral gene delivery research to allow the reader to decide which transfection agents have characteristics that would be useful in his/her own research.

2. Standard approaches

Within the realm of non-viral gene delivery agents are liposomes and cationic polymers, which currently make up the two major classes of chemical gene delivery methods. Physicochemical aspects of many of these vehicles have been established for some time (reviewed in Refs. [1,2]), but further analyses and comparisons of agents are ongoing. A more recent example is work by Zauner et al. [3], who compared transfection aspects of liposomal complexes made with 2,3-dioleoyloxy-*N*-[2(spermine-carboxamido)ethyl]-*N,N*-dimethyl-1-propanaminium-trifluoroacetate and *N*-[1-(2,3-dioleoyloxy)propyl]-*N',N',N'*-trimethylammonium chloride (DOSPA/

DOPE, sold as Lipofectamine) against polycation-based complexes made with glycerol-enhanced transferrin-poly(L-lysine) (PLL). They found that for confluent primary human fibroblasts, the polycations yielded greater transfection. They also made the consistent finding that in non-confluent cells, the liposomes transfected cells actively engaged in the cell cycle. In an interesting set of in vivo experiments involving adult mice, Bragonzi et al. compared poly(ethylenimine) (PEI) polycations against *N*-[1-(2,3-dioleoyloxy)propyl]-*N',N',N'*-trimethylammonium chloride (DOTAP) liposomes in lung cells after double administrations of transfection complexes [4]. They found that when the first and second administrations both contained active plasmid, transfection efficiencies increased over single-transfection levels for both types of complexes. However, in similar trials that utilized a dummy plasmid for the first injection, DOTAP/DNA complexes still showed an increase in reporter expression over single-injection controls; PEI/DNA complexes did not display such an obvious increase. The PEI used (22 kDa) displayed higher transfection efficiencies than the DOTAP in single-injection experiments, although PEI of 25 kDa was less effective.

3. A closer look at an existing carrier: PEI

One of the most successful polycations used in gene delivery research today is PEI, which has been widely used for years in the paper industry. PEI is a highly polycationic synthetic polymer that is available in both linear and branched forms. It is known to condense DNA in solution, forming complexes that are readily endocytosed by many cell types. An overview of PEI chemistry, PEI/DNA characterization, and transfection issues is given in Ref. [2].

One of the advantages to using non-viral over viral gene carriers is that the non-viral vectors are not architecturally constrained to delivering plasmids at or below a given size. It has recently been shown that PEI and PLL are both able to deliver yeast artificial chromosomes (YACs) of 2.3 Mb into mammalian cells [5]. PEI's ability to compact its carried DNA appears to be an important factor in delivering large DNA constructs, as shown in the same experiments, where 67% of the successful PLL-mediated

transfections suffered damage to the delivered chromosomes. Compare this to PEI-mediated delivery, where 0% of the successful transfections involved damage to the delivered YACs [5].

The condensation of DNA by PEI is important for the delivery of smaller plasmids, as well. Whether administered *in vitro* or *in vivo*, there is potential for the PEI/DNA complexes to be exposed to degradative nucleases. In comparing PEI to PLL, it has been shown that PEI offers vastly greater protection to transported DNA against degradation by DNase 1 and DNase 2 [6]. In these experiments, PEI helped preserve DNA, intact, in all conditions tested, even when the complexes were exposed to 25–50 Units of enzyme for 24 h. Other non-viral transfection agents have been shown to offer protection below the level given by PEI. For instance, the liposome-forming agent dioctadecylamidoglycylspermine (DOGS) (also known as Transfectam), while offering resistance to DNA against nuclease digestion, offered such protection at a level significantly lower than what was observed for linear PEI [7]. Intuitively, the degradation of delivered DNA can lower the ultimate level of transgene expression, so protection of the DNA during delivery is an important consideration toward transfection.

PEI is not the ideal transfection agent, however. Delivering undamaged DNA into cells is important, but of little consequence if the carrier also acts to kill the host cells. While *in vivo* data indicate that there is no more immune response other than an acute neutrophilic reaction to aerosol delivery of PEI into rabbit lungs, even when the PEI/DNA complexes are delivered repeatedly over 21 days [7], other data show a great deal of toxicity on the cellular level. Fischer et al. demonstrated PEI's cytotoxic effects with transmission electron microscopy [8], and Putnam and Langer resorted to developing a novel vector because of the overwhelming cytotoxicity of PEI-mediated gene delivery (which yielded a reported 2% cell survival rate) [9]. While we have not seen a level of cell death as dramatic as 98%, our own work has demonstrated a significant level of PEI-induced cell death during cellular exposure to both free and DNA-complexed PEI (Fig. 1) [10].

To partially overcome PEI's toxic effects, the polymer has been conjugated with charge-neutralizing entities to yield ζ potentials closer to neutrality

for transfection complexes. Conjugation of transferrin-PEI/DNA complexes with poly(ethylene glycol) (PEG) not only yields complexes with lower ζ potentials, but is also responsible for decreased aggregation of complexes with themselves, decreased interactions with plasma proteins, and decreased cell toxicity [11]. The reduced complex aggregation and complex/blood protein interactions allow the complexes to circulate for longer periods of time *in vivo*, and improve the targeting aspects of transferrin-containing complexes perhaps due to decreased nonspecific interactions involving the highly cationic PEI. All of this has been gained with a minimal change in transfection efficiency (in *in vitro* experiments) [11].

Other recent examples of research involving conjugated PEI include the attachment of sugar moieties to PEI for targeting purposes. Mannosylated PEI has been used to target conjugate/DNA complexes to antigen-presenting cells with limited success [12]. Although targeting was achieved through interaction of the complexes with mannose receptors, the number of cells expressing the delivered reporter gene was very low. In further experiments of the same investigation, adenoviral particles were attached to the mannose-PEI/DNA complexes to improve transfection efficiency, but it was found that the complexes no longer entered the tested cells via pathways utilizing mannose receptors [12]. (Although the mannose conjugation did not produce good transfection results, the value of conjugation of PEI with adenoviral particles was demonstrated.) Another sugar that has been conjugated with PEI for transfection is galactose, in this case for the purpose of hepatocyte targeting [13]. While the results of such conjugation were successful, it has been shown that the transfection efficiency of galactose-PEI/DNA complexes can be improved without a loss in cell specificity by manipulating the length of the conjugated galactose chains [14]. These experiments showed that transfection efficiency was increased when the conjugated complexes had a reduced overall size.

Other PEI research has been published along lines that are more practically oriented. For instance, while one of the benefits of polycationic gene delivery methods is that the delivery vehicles are relatively easy to store, the storage issue was taken a step

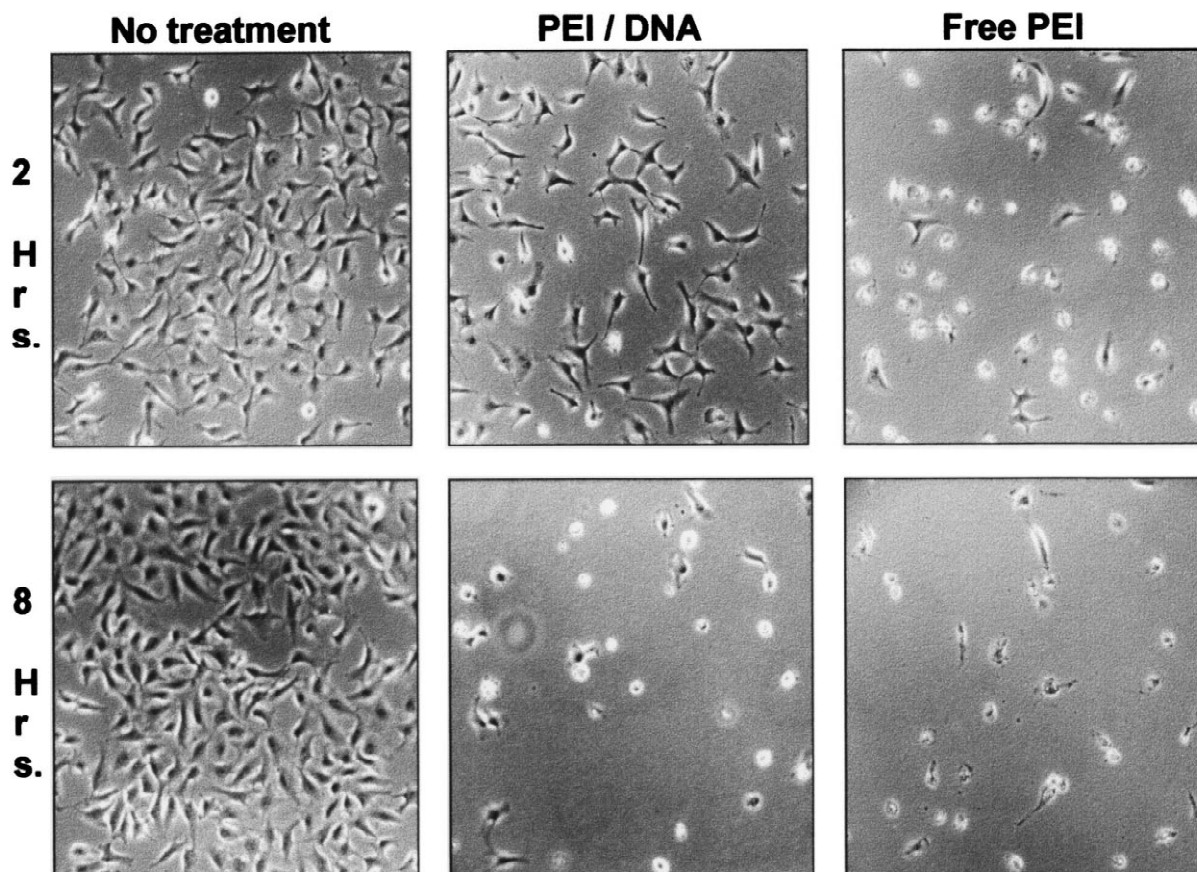


Fig. 1. Phase contrast images of cells at various time points following exposure to complexed or free PEI (High MW-S); 50 000 cells/well were seeded. (A) Untreated cells, 2 h post-transfection; (B) cells treated with PEI/DNA complexes, 2 h post-transfection; (C) cells treated with free PEI, 2 h post-transfection; (D) untreated cells, 8 h post-transfection; (E) cells treated with PEI/DNA complexes, 8 h post-transfection; and (F) cells treated with free PEI, 8 h post-transfection.

further when it was shown that transferrin-PEI/DNA (along with transferrin-polylysine/DNA and a version of adenovirus) could be successfully freeze dried and rehydrated for use weeks later [15]. This has the potential to make gene delivery accessible in a wider array of clinical settings, including rural communities and underdeveloped countries. Another development in PEI research, which used an engineering approach to aid gene delivery, utilized micropumps to slowly (20 $\mu\text{l}/\text{min}$) deliver PEI/DNA complexes to a specific solid mass tumor type *in vivo* [16]. It was found that there are instances where slow delivery of PEI/DNA complexes yields transfection results that are improved over rapid syringe injection or systemic delivery of complexes.

4. New carriers, new techniques

While PEI is rapidly becoming a classic among gene delivery methods, other molecules are continuously being examined in an attempt to find a completely suitable vector for various gene therapy applications. One example is chitosan, a cationic aminopolysaccharide that is both biocompatible and resorbable. Chitosan has been used to construct microspheres for the delivery of urease [17] and glycolic acid [18], among others. PEGylation of chitosan has also been performed as part of charcoal encapsulation for research into an artificial liver [19]. Chitosan PEGylation, in conjunction with alginate, has also been used for cell encapsulation [20]. These

experiments, which boil down to using a cationic molecule for biological use, can be viewed to imply that chitosan is a candidate molecule for the encapsulation of oligonucleotides for cellular delivery. Such work has already begun, with investigations indicating that chitosan-mediated gene delivery has low toxicity and little liver accumulation when low molecular weights are used [21]. It has also been shown that chitosan/DNA complexes can transfect a variety of cell types with varying results [22,23], with in vivo administration also a possibility [24]. Comparisons with CaPO₄-, Lipofectamine-, and PEI-mediated transfection have been made, also with varying results [22,23].

Other innovative approaches to non-viral gene delivery include copolymers containing β -cyclodextrin [25], lactose-PEG-PLL graft copolymers [26], and liposomes utilizing the single-tailed fatty acid oleoyl-ornithinate (OLON) in conjunction with DOPE [27]. Additional graft copolymers examined for gene delivery include PLL-arabinogalactan [28], the polyether Pluronic 123 grafted with PEI [29], and poly[*N*-(2-hydroxypropyl)methacrylamide] (pHPM-A) grafted with 2-(trimethylamino)ethyl methacrylate [30]. Additionally, pHPMA, bearing pendant chains of the amino acids GFLG, have been used to coat PLL/DNA complexes with some success, especially in terms of preventing serum proteins from binding to the DNA carrier [31]. (The relevance of serum protein binding is discussed later.) Naturally occurring lipids are also being used in fresh transfection methodologies. For instance, low-density lipoprotein (LDL) has been used with stearyl PLL to create transfection complexes [32,33]. In addition, cholesterol has been utilized for gene delivery in the form of 3- β [*N*-(*N*',*N*'-dimethylaminoethane)-carbonyl] cholesterol (DC-Chol). Liposomes made from DC-Chol/DOPE have been successfully used for neuron cell transfection in vitro [34]. While many non-viral transfection agents show promise for eventual clinical applications, the search for the ideal transfection agent is still far from over.

In addition to new carrier molecules for transfection, novel methods of nonviral gene delivery are also appearing in the literature. Vectors termed solvoplexes, which use organic solvents such as di-*N*-propylsulfoxide to aid the delivery of DNA, have been investigated for transfection of airway

epithelia via intratracheal injection and microspray delivery [35]. The solvoplexes are stable enough for microspraying and can be easily stored at room temperature. In an attempt to enhance post-endosomal transfection, a peptide fragment patterned from the influenza virus haemagglutinin (INF-SGSC) has been used to enhance nuclear localization and transcription of delivered DNA [36]. Finally, the concept of layering components onto DNA has been used to manufacture transfection complexes, such as in experiments using PLL and succinylated PLL [37]. Such technology is also available through the use of certain commercially available products.

5. Mechanism

Published hypotheses as to the mechanisms of gene delivery have existed nearly as long as gene delivery itself. Hypotheses, by their very nature, contain speculations that are based on a set of chosen facts. However, problems can arise when the hypotheses are accepted as fact without rigorous proof. There have been significant recent advances that partially elucidate the mechanisms behind nonviral gene delivery. One point that has become clearer with each published report is that not all gene delivery vehicles operate in the same fashion. Even a simple modification of a delivery molecule can alter the cellular processing of the agent dramatically. As a result, one should exercise caution when interpreting the results of any given study before applying the results to his or her own chosen delivery system. This does not mean that what is shown for one gene transfer methodology is not applicable to others; one should merely be aware that there is not a single explanation of cellular processing that covers all non-viral gene delivery vehicles.

An illustration of this exists in lipofection, where it is widely known that not all liposomes are the same. On the surface this is obvious in that transfection of a given cell type with different liposomal agents will yield different transfection efficiencies. The reason behind the varying transfection efficiencies lies in the mechanism for each type of gene transfer, whether it is a difference in cellular entry routes or disparate DNA release kinetics. For instance, it has been demonstrated that the single-tailed

cationic lipid oleoyl-ornithate (OLON), when used with dioleoylphosphatidylethanolamine (DOPE), has a higher transfection efficiency than other liposomes [27]. This improvement is attributed to improved DNA release from the carrier complexes within cells. In a similar fashion, not all polycations yield identical transfection results. In the cases of PLL and PEI, it has been shown in the human endothelial cell line (EA hy926) that polymer/DNA complexes made with the respective polycations are routed differently within the cells [6]. This time, instead of altered release kinetics, it is a difference in cellular processing routes that is the cause of unequal transfection efficiencies between the gene delivery methods in this cell line. With PLL/DNA, the complexes are routed to and degraded within lysosomes, while PEI/DNA complexes are trafficked through lysosome beds and into cell nuclei without degradation (Fig. 2).

Having pointed out the pitfall of extrapolating mechanistic results to various modes of gene delivery or cell types, it is time to discuss the more recent facts in the literature so readers can consider how the information might relate to their own transfection methods. One of the first steps toward successful transfection is achieving DNA entry into cells. The successful use of receptor targeting — for instance neuronal cell targeting using tetanus toxin fragment C [38], or T-lymphoid cell targeting via anti-CD4 [39] — is a good implication that some complexes enter cells via endocytosis. Additionally, vinblastulin

has been used to depolymerize microtubules and thereby indicate endocytotic DNA entry for CaPO_4 precipitation and lipofection transfection methods [40]. It is possible that not all molecules used in the past for targeting necessarily act via specific receptor-mediated endocytosis routes, though. In work involving the targeting ligand transferrin (versus apotransferrin), attached to liposome/DNA complexes, Simoes et al. have indicated that the internalization of such complexes occurs independently of transferrin receptors [41]. The group further backs up its claim with competitive inhibition data, although their results seem to contradict conventional wisdom and intuition. However, receptor-mediated endocytosis is not the only possible route for cellular entry, and a given vector may enter cells in more than one fashion. Examples of this include liposomes made with the cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) mixed with phosphatidylethanolamine, which have been shown to enter cells via endocytosis or, to a lesser extent, via fusion with plasma membranes [42]. For the polycation PLL, cellular entry of PLL/DNA complexes is accomplished by the complexes first binding to sulfated, membrane associated proteoglycans [43]. The variable expression of proteoglycans on cell surfaces might help explain the variation of transfection efficiencies between cell types.

Keeping in mind that if a complex is to be endocytosed it must first bind to a component of the cell exterior, the prospect of improved transfection through cell-surface modification arises. Wojda et al. used biotinylation of cells to aid transfection via avidin-PEI/DNA complexes, achieving transfection efficiencies similar to those obtained through endocytic uptake via transferrin receptor targeting [44]. Apparently, endocytosis of polycation/DNA complexes is the common route of cellular entry for polycation-mediated gene delivery. However, endocytosis is a broad term that encompasses specific receptor binding, generalized receptor attachment, or attachment to a cell membrane component (proteoglycan or otherwise) prior to internalization. The specific type of endocytosis used by a given delivery system is therefore dependent upon the surface characteristics of the transfected cell, the ligands attached to the transfection complexes, and the properties of the base carrier used.

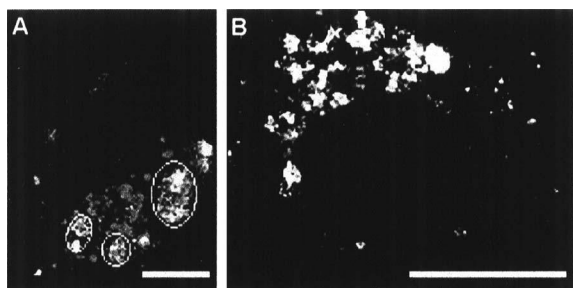


Fig. 2. Confocal images of transfected cells containing labeled vectors and lysosomes. Each panel shows one cell, each bar represents 10 μm . (A) PEI-mediated transfection. Lysosomes and transfecting complexes do not merge. PEI/DNA is shown within drawn circles, lysosomes surround the regions (darker grey). (B) PLL-mediated transfection. PLL/DNA and lysosomes merge, shown by bright white.

Backing up one more step from endocytosis or membrane fusion, there is another factor that must be considered even before attachment of transfection complexes to cell surfaces — the complexes must first reach the cells they are to transfect. For *in vitro* work using serum-free transfection conditions this is generally not an issue. However, in living organisms there are many additional concerns that must be addressed in the process of getting the transfection complexes into the area of the desired cells. For systemic delivery, these concerns include host immune responses, undesired transfection of upstream cells (which removes the complexes from the blood and prevents transfection of the desired cells), and attachment of transfection complexes to blood proteins (thus marking them for clearance by the liver). This latter consideration, although the least severe in terms of adverse effects to the organism, is one of the larger barriers to non-viral gene delivery. Investigations involving cationic, anionic, and neutral liposomes have revealed that cationic liposomes bind the greatest amount of serum proteins, and show greater accumulation in the liver [45]. This finding becomes more important for gene therapists in light of the fact that positively charged moieties are the logical choice for binding to and condensing polyanionic DNA for cellular delivery. What binds DNA very well might also bind serum proteins very well, which could mark the complexes for degradation in the liver. For any non-viral transfection method, the carrier/DNA complexes' overall ζ potential should therefore be considered. As with the PEI research already mentioned, PEGylation has been used to alter the final surface charge concentration of several types of complexes. Such 'steric stabilization' of the carrier with poly(ethylene glycol) has been used by Kwok et al. (although this group actually desired to get their complexes to the liver during *in vivo* transfection) [46].

An ambiguous situation exists, however, with regard to the blood protein human serum albumin (HSA). It has been shown that this protein will bind to PLL/DNA complexes *in vitro*, thus lowering the complexes' ζ potentials as expected [47]. In fact, HSA has been implicated as the major protein to associate with PLL/DNA complexes in serum, and is suspected of being the major marker for clearance of these complexes from the blood [47]. Interest-

ly, *in vitro* investigations involving the intentional binding of HSA to liposome/DNA complexes prior to gene delivery yielded improved transfection results over investigations using the same complexes without HSA [48]. Here, improved transfection refers both to the amount of reporter expressed overall and the percentage of cells that expressed the luciferase reporter. The HSA-modified lipoplexes also yielded higher reporter expression *in vivo* in mouse spleen and lungs (although lung tissue is the location of the first capillary beds that the complexes are exposed to following venous injection). With the accumulation of protein-bound complexes in the major organ of the immune system, the organ responsible for removal of blood toxins, or in the very first capillary bed they travel through, it appears that binding of HSA (and other serum proteins) is something that is best avoided for efficient systemic gene delivery.

After cellular entry of the transfection complexes, at some point there must be a separation of the delivered genetic material from its carrier. The separation could occur within or outside of cytoplasmic vesicles, and could theoretically also take place within the nucleus. As already mentioned, OLON-DOPE yields increased transfection efficiency presumably because of thermodynamically more favorable separation of the carried DNA after cellular entry [27]. Further demonstration of this concept comes from work with modified oligonucleotides used for antisense activity against mRNA. It has been shown that PEI can efficiently deliver both phosphodiester and phosphorothioate oligonucleotides into cells, but the phosphodiester oligonucleotides displayed more antisense activity partially because of more favorable release kinetics [49].

Another transfection consideration involving the genetic material itself concerns the length of the delivered DNA, as was demonstrated to have an effect upon its translocation into the nucleus via simple diffusion or active transport [50]. The report of these findings also showed that the addition of a nuclear localization signal (NLS) increased both the size limit and amount of DNA nuclear transport, and that the use of an expression cassette enhanced results even more. The use of nuclear localization signals to enhance non-viral transfections has been used in many laboratories with encouraging results.

A recent example is a signal peptide derived from SV40 which was used to enhance lipofection 3-fold [51]. The signal PKKKRKVEDPYC was also used to increase PEI- and Transfectam-mediated transfection significantly in terms of the amount of DNA required for *in vitro* transfection [52]. In addition, the yeast transcriptional activator, GAL4, has been shown to both bind DNA and separately act as a nuclear localization signal, binding to the β -subunit of the NLS-receptor importin complex [53]. (Note that conventional signals, such as the SV40-derived signal just mentioned, bind to the α -subunit of the complex.)

It is also the case that exposure to a gene carrier can cause stimulation of non-delivered genes inside host cells. Lipofectin has been shown to stimulate the production of interferon- γ in spleen cells from two mouse types [54], and other liposomes (Lipofectamine and DOSPER) have been shown to induce interferon- β expression (plus other endogenous interferon-stimulated genes) [55]. Elevation of secreted levels of tissue plasminogen activator, plasminogen activator-inhibitor type-1, and von Willebrand factor has also been seen in a human endothelial cell line following transfection with Lipofectin and Lipofectamine, as well as following transfection using the polycationic PEI [10].

6. A step toward the clinic

While many groups are researching the basic science behind non-viral transfection, other teams are pushing forward with experiments that have more direct clinical implications. These experiments range from intended treatments on the molecular level to aides intended to act at the organism level. For instance, by using chimeric RNA/DNA oligonucleotides, a specific base pair of the rat factor IX gene has been targeted somewhat successfully for A to C nucleotide conversion in hepatocytes [56]. The method described in this work shows promise for the permanent repair of chromosomal mutations by chimeric oligonucleotides. On a more general scale, linear PEI, while often employed to deliver DNA by some, has also been used for the delivery of nitric oxide in an attempt to speed healing in full thickness wounds in rats [57]. (It must be noted that the attempts at wound healing were relatively fruitless

because of the toxicity of the PEI used.) In transplantation experiments involving the left lung of F344 rats, proximal pulmonary artery segments were transfected with liposome/DNA complexes *ex vivo* prior to the completion of the transplantation procedure. The effects of transfection conditions (time, temperature, pressure) [58] and storage conditions post-transfection [59] were noted, and provide promise for the eventual use of gene therapy as a standard part of transplantation procedures.

Additional clinically oriented investigations include the use of antisense oligodeoxynucleotides to bind to and essentially inactivate mRNA. One specific application of this technique was performed in hypertensive rats by targeting $\beta(1)$ -adrenergic receptor mRNA, which significantly reduced the number of $\beta(1)$ -adrenergic receptors in kidney and produced antihypertensive effects up to 33 days post-treatment [60]. An example of a clinical trial of non-viral gene delivery involves liposomal delivery of plasmids encoding vascular endothelial growth factor [61]. The trials were performed on humans undergoing percutaneous transluminal coronary angioplasty, and it is touted that the treatments were feasible and well tolerated. However, in these treatments there was no detectable VEGF transfection product in the systemic circulation following the procedure, which is likely due to ineffective or nonexistent gene transfer.

The potential benefits of gene delivery vary throughout a wide array of clinical settings. Approaches to eventual therapies range from the permanent repair of a single-base chromosomal mutation to the transient suppression of a given gene product through delivery of antisense oligodeoxynucleotides. Recent events in clinical gene delivery trials have raised valid concerns regarding the safety of using gene delivery as medical treatment, which has moved the eventual realization of such treatments to a more distant point in the future. Although still far off, non-viral components used for gene delivery still hold great promise for improving the human condition, and in several differing ways.

7. Final remark

In this brief report we have offered some of the recent developments in nonviral gene transfer. The

field continues to advance, and expand to address issues pertinent to any non-viral method of gene delivery. While improvements to transfection efficiencies have been made, and steps toward understanding the underlying mechanism behind several gene delivery vehicles have been completed, the issues of toxicity, transfection efficiency, and host response remain problems for some gene delivery applications. There is much work that has yet to be performed before any non-viral gene delivery vehicle can be a viable option for the treatment of certain human conditions. However, every passing month seems to yield important advances in gene delivery technology that are helping to reach that end.

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