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Reducible Disulfide-Based Non-Viral Gene Delivery Systems

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Abstract: The design and synthesis of safe efficient non-viral vectors for gene delivery has attracted significant attention in recent years due primarily to the severe side-effect profile reported with the use of their viral counterparts. Previous experiments have revealed that the strong interaction between the carriers and nucleic acid may well hinder the release of the gene from the complex in the cytosol adversely affecting transfection efficiency. However, incorporating reducible disulfide bonds within the delivery systems themselves which are then cleaved in the glutathione-rich intracellular environment may help in solving this puzzle. This review focuses on recent development of these reducible carriers. The biological rationale and approaches to the synthesis of reducible vectors are discussed in detail. The *in vitro* and *in vivo* evaluations of reducible carriers are also summarized and it is evident that they offer a promising approach in non-viral gene delivery system design.

Key Words: Disulphide bond, reducible vector, non-viral vector, gene delivery.

1. INTRODUCTION

Gene therapy is a promising and rapidly developing medical approach, which aims to cure diseases of genetic origin by correcting the over- or under-expression of genes [1]. In 1983 a bacterial gene was successfully transferred into mouse haematopoietic progenitor cells using a retroviral vector [2]. Several years later, in 1990, Drs. William French Anderson, Michael Blaese and Ken Culver at the National Institutes of Health carried out the first successful viral vector-mediated gene therapy trial on the then four-year-old Ashanthi DeSilva with the goal of treating adenosine deaminase (ADA) deficiency - a rare and severe immunodeficiency disease afflicting children [3]. In 1993 the first non-viral gene vector formulation entered clinical trials, in which a DNA-liposome formulation was injected into five HLA-B7-negative patients with stage IV melanoma. The results indicated that recombinant protein was detected in all five patients taking part in the trial [4]. On October 16, 2003, the Chinese Food and Drug Administration approved the largely controversial and first ever adenovirus-based gene therapy product Gendicine® - a treatment for head and neck squamous cell carcinoma (HNSCC). This approval remains controversial as it was granted after 5 years of trials in a mere 120 patients [5].

Although significant progress has been made in the area of gene delivery, the approach is still hampered by the lack of both safe and effective vectors for gene delivery of which non-viral vectors offer a promising and viable solution. Over the last 30 years, numerous approaches have been developed and trialed to conquer the many challenges facing gene delivery research. These approaches can be classified into three major modes of delivery: physical methods for gene therapy, viral & non-viral vector-mediated gene delivery. In recent years, although physical approaches to gene delivery have made some progress, they are still hampered by the issue of impracticality [6]. Viruses, as the products of natural evolution over millions of years, possess an excellent natural ability to traffic foreign genes to host cells. However, their inherent drawbacks, have included the stimulation of strong immune responses, oncogenesis and with fatalities also having resulted; this has greatly limited both the utility and acceptance of viral vectors in gene therapy by the US-FDA [7-12]. Hence, in recent years, attention has turned towards developing efficient non-viral vectors which are notably absent of such detrimental effects.

Many studies have shown that the ineffective release of a gene from its vector is a major intracellular hurdle and rate-limiting step in gene delivery – caused by the strong electrostatic interactions that exist between cationic-vector and anionic-gene [13, 14]. To help in addressing this issue, in recent years a number of disulfide bond-containing vectors have been designed which facilitate release of the gene from its vector, selectively in the cytosol. This was deemed possible due to the existence of a high redox potential gradient between the oxidizing extracellular space and the highly reducing cytosolic environment. Thus, the focus of this review is on the biological rationale of such vectors, approaches to the synthesis of reducible vectors and their evaluation *in vitro* and *in vivo*.

2. BIOLOGICAL RATIONALE OF DISULFIDE BOND-CONTAINING CARRIERS

2.1. The Five Major Barriers to Gene Delivery

Universally, in order for a gene to efficiently transfect a host cell population, it must first overcome five major barriers (see below) *in vivo* as illustrated in Fig. (**1**) [15-17].

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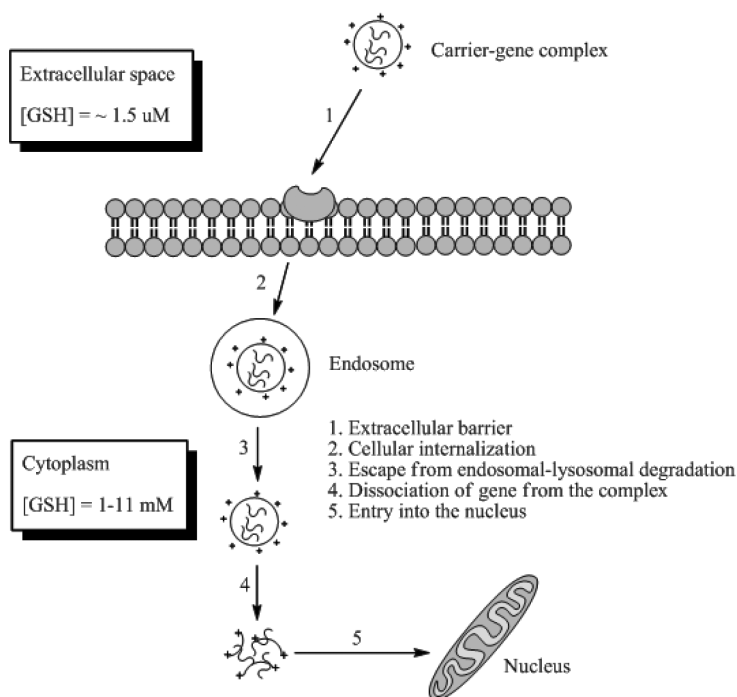

Fig. (1). Five major biological barriers to gene delivery.

- The first being that a gene (and any vector used to delivery it) must overcome extracellular physical and biochemical degradation to reach target cells intact [18]. The colloidal stability of gene delivery systems in extracellular compartments is a major obstacle due to the positively charged carrier-gene complexes readily interacting with negative-charged and highly abundant plasma proteins (e.g. serum albumin), resulting in rapid elimination of the formulation by the hosts reticuloendothelial system (RES).
- Transfer into and passing through the membrane of target cells is the next hurdle. For most non-viral vectors, cationic carrier-gene complexes interact with the negatively charged eukaryotic cell membranes *via* primarily electrostatic interactions, resulting in enhanced cellular uptake [19]. However, the overall net charge of any formulation must be moderated as too high a cationic charge can lead to cytotoxicity, resulting from cell lysis [20].
- After translocating across the cell membrane complexes generally enter the endosomal-lysosomal pathway [17]. Once inside the endosome the pH of the endosomal environment then rapidly decreases until a pH ~ 4 is reached which then triggers fusion of the now "late-endosome" with lysosomes and the release a barrage of degradative enzymes.
- If the formulation readily escapes the endosomal pathway we are a step closer to achieving transfection, although what happens between now and transfection being observed, is still very much based upon speculation. Many studies show ineffective DNA release from its vector as a major intracellular hurdle and rate-limiting step in gene delivery due to strong electrostatic interactions between cationic-vector and anionic-gene [13, 14]. So, the release of gene from its carrier is a critical step that must not be overlooked in order for effective gene delivery to take place.
- The final obstacle for gene transfer of plasmid DNA (pDNA) is migration across the nuclear membrane and entry into the nucleus. Nuclear delivery can occur by one of two approaches: the first is *via* the breakdown of the nuclear membrane during the period of mitosis and the other is through the nuclear pore complex (NPC) [21]. Several research groups have indicated that the membrane of the nucleus hinders trafficking of either free DNA or the carrier-gene complex from the cytosol and consider it a major barrier to transfection [22-25]. It is important here to also note that in the case of RNA-interference (gene silencing using short interfering RNA or "siRNA") the cytosol is the target delivery site, not the nucleus, as is the case for pDNA and oligonucleotide (ODN) based-therapeutics [26].

Given the barriers outlined above our focus was drawn towards gaining a better understanding of, and ultimately quantifying the underlying process that dictates binding of carrier and gene – a factor that has been largely overlooked to-date in non-viral vectors design, however, is paramount to achieving release of the gene once at the target site and subsequent transfection.

2.2. Biological Rationale of Disulfide Bond-Containing Carriers

The fundamental basis for employing disulphide bonds within carrier systems is to exploit the very large differences in intra- and extracellular glutathione concentrations (see Fig. (**1**)). Typically, glutathione is present within cells in both the oxidized (GSSG) and reduced (GSH) forms with the

intracellular redox state of various subcellular organelles commonly stated as the GSH:GSSG ratio [27, 28].

Although different physiological states influence the GSH level of different subcellular organelles, significant redox potential gradients are in existence between the extracellular space and various intracellular compartments. The GSH concentration in the extracellular space is ~ 1.5 μM with the ratio of GSH: GSSG being ~ 7:1 [29, 30]. However, its concentration in the cytosol is some 1000-fold higher (1-11 mM), which is the principal site of GSH biosynthesis [31-33]. Recent studies indicate that both the endosomes and lysosomes are oxidising rather than reducing, which would render them inefficient in disulfide bond reduction [34]. The GSH concentration in the nucleus is much higher (up to 20mM) [35] while that of the mitochondria is similar to that of the cytosol at 5mM [36].

Thus, marked differences in glutathione levels between the extracellular environment and the intracellular space enables disulfide bond cleavage preferentially in the reductive cytoplasmic environment. It is proposed that this leads to selective and more efficient intracellular gene release when reducible vectors are employed and so higher transfection efficiency rates.

3. REDUCIBLE CARRIERS

Recently, there has been a drive by researchers working in the field to develop disulfide bond-containing carriers for selective intracellular gene release, and these have included reducible cationic lipids, disulfide-linked poly(L-lysine) (PLL), disulfide bond-containing poly(ethylene imine) (PEI), thiol-reactive polymers, disulfide cross-linked peptides and disulfide-modified genes. Studies where these reducible carriers have been employed are summarized in Table **1** and discussed below.

3.1. Reducible Cationic Lipids for Gene Delivery

Reduction-triggered lipids were initially reported in 1998 [37-42]. Among those first studying their efficacy, Hughes J.A. *et al.* [37, 38, 43] demonstrated that reducible cationic lipids had a higher transfection efficiency and lower toxicity than non-reducible lipids. Later Wetzer *et al.* found that the position of disulfide bond within the lipid molecules influenced the reduction kinetics of disulfide bond in lipid-DNA complexes and the disulfide linker was more readily reduced in hydrophilic environments [40]. *In vitro* experiments showed that complexes formed with these optimal reducible lipids had up to 1000-fold higher transfection efficiency than their analogues absent of disulfide linkages.

3.2. Disulfide-Bond Containing Poly-L-Lysine (PLL)

In recent years, several disulfide-containing PLL-based vectors have been designed and prepared [44-48]. Miyata K. *et al.* synthesized PEG-PLL using two different thiolation reagents and formulated PEGylated polyplex micelles with disulfide crosslinked cores [46, 47]. The results show that one key factor to effectively transfecting cells is the balance between the densities of the positive charge and the degree of disulfide cross-linking of the carriers. Furthermore, studies have shown that the degree of thiolation within the carriers should not exceed 28% if high transfection rates are to be maintained [46, 47].

3.3. Disulfide-Bond Containing Polyethylenimine (PEI)

Due to the benefits of rapid escape from the endosome and selective release in the cytosol, reducible PEI has attracted added attention in the last two years with promising results obtained in the numerous studies conducted [49-54]. Transfection efficiency of reducible poly(amido ethylenimine)s was almost 20-fold higher than that of standard PEI [51]. Even the addition of 10% serum to the medium, which very often abolishes activity, did not diminish the already high transfection efficiency. In addition, confocal microscopy has highlighted that complexes formed with reducible carriers revealed greater intracellular distribution of pDNA when compared to native PEI, this being proposed due to the reductively-triggered release of pDNA in the cytosol.

3.4. Thiol-Reactive Polymers

An assortment of polymers containing disulfide bonds have been synthesized and evaluated for gene delivery, such as poly(amido amine)s [55-62], poly(β-amino ester)s [63], polyaspartamide [64], oligomerized polyamines [65], amphiphilic copolymer consisting of methacrytic acid, butyl acrylate and pyridyl disulfide acrylate [66], cationic amphihiles from α-lipoic (6,8-thioctic) acid, reducible poly(2-dimethylaminoethyl methacrylates)[67, 68], biocleavable polyrotaxane [69], chitosan [70, 71] and degradable hyaluronic acid nanogels [72]. Almost all reducible polymers showed higher transfection efficiencies and lower toxicity *in vitro* than the non-reducible systems tested.

3.5. Disulfide Cross-Linked Polypeptides

Disulfide bonds can very easily be incorporated into peptides *via* the natural amino acid cysteine, hence reducible polypeptides have been studied as a means to improving transfection efficiency of various genes [73-78]. Research undertaken by McKenzie *et al.* showed that disulfide cross-linking peptides increased gene expression without enhancing gene uptake by the cells and the observed increased gene expression depended primarily on intracellular release of a gene *via* reduction of disulfide bonds [73]. Furthermore, they introduced histidine into the disulfide cross-linking peptides to provide endosome buffering capacity [74]. These peptides comprising combinations of the residues lysine, cysteine, and histidine significantly improved gene transfer properties *in vitro*.

3.6. Disulfide-Modified Gene

Strategies whereby the gene itself is chemically tethered to another 'helper' molecule (e.g. PEG) to augment delivery and transfection has also been employed and some examples where this has been successful include bisPNA-peptide conjugates [79], DNA-cysteine conjugates [80], PEG-S-S-antisense oligodeoxynucleotide (asODN) [81] and PEG-S-S-siRNA against VEGF [82]. Micelles formed with anti-angiogenic siRNA-S-S-PEG/PEI were shown to suppress VEGF gene expression by up to 96.5% in prostate carcinoma cells, a result which proved markedly better than antiVEGF siRNA-PEG/PEI complexes (~25% suppression) with both studies being conducted in the presence of serum [82].

Table 1. Types of Reducible Carriers for Gene Delivery

Carriers	Gene	Cell Line	Reference
PEG-S-S-DOPE	Calcein, pEGFP	A431 cells	[42]
Disulfide-linker lipid	Plasmid DNA	CHO, COS-1, and MCF-7 cells	[41]
Disulfide bond within the hydrophilic moiety or hydrophobic chain	Plasmid DNA	HeLa cells	[40]
Reduction-sensitive lipopolyamine	Plasmid DNA	HepG2 and HeLa cells	[39]
1'-2' dioleoyl-sn-glycero-3'-succinyl-2-hydroxyethyl disulfide ornithine conjugate (DOGSDSO)	Plasmid DNA	Primary rat cerebral cortical astroglial and microglial cultures, SKnSH cells, HEK 293 cells, COS-1 cells	[37, 38]
Thiolated PEG-poly(L-lysine) block copolymers	Plasmid DNA	293T cells, BALB/c mice	[46, 47]
Poly[Lys-(AEDTP)]	Plasmid DNA	293T7 and HepG2 cells	[44]
Disulfide crosslinked PLL/DNA complexes	Plasmid DNA	B16F10 cells, BALB/c mice	[45]
Disulfide poly(amido ethylenediamine) (SS-PAED)	Plasmid DNA	NIH-3T3, BAEC, H9C2 and A7R5 cells, rabbit myocardial infarct model	[51, 83]
S-S-PEI	Plasmid DNA	CHO-K1, COS-7, NIH-3T3, HepG2, HCT116, HeLa, and HEK-293 cells	[84]
Crosslinked PEI	Plasmid DNA	NIH-3T3 cells, BALB/c mice	[49]
l-PEIS	Plasmid DNA	HepG2 and HeLa cells	[50]
PEI-S-S-integrin	Plasmid DNA	HeLa and MRC5 cells	[85]
Bioreducible poly(amido amine)s	Plasmid DNA	COS-7 cells	[55, 56]
Poly(β-amino ester)s with thiol-reactive side chains	Plasmid DNA	HCC cells	[63]
PESC	Plasmid DNA	B16F10 and N2A cells	[64]
Oligomerized polyamines	Plasmid DNA	B16F10, Neuro2a, HUH-7 and HT-22 neuronal cells	[65]
Amphiphilic copolymer consisting of methacrytic acid and butyl acrylate and pyridyl disulfide acrylate	Antisense oligonucleotide;	3T3 and THP-1 macrophage-like cells	[66]
Lipoic acid-derived amphiphiles	Plasmid DNA	HeLa, A549 and BHK cells	[86]
Thiolated chitosan	Plasmid DNA	HEK293, MDCK and Hep-2 cells	[70]
Chitosan-thiobutylamidine	Plasmid DNA	Caco2 cells	[71]
Multiblock reducible copolypeptides	Plasmid DNA	B16F10, HeLa, 4T1, MCF-7, COS-7 and EA.hy926 cells	[76]
Sulfhydryl cross-linked PEG-Peptide/Glycopeptide	Plasmid DNA	Hepatocytes and Kupffer cells, mice	[75]
Low molecular weight disulfide cross-linking peptides	Plasmid DNA	HepG2, COS-7 and CHO cells	[73, 74]
Disulfide cross-linked peptide	Plasmid DNA	COS-7, 293T3 and HUVE cells	[77]
siRNA-S-S-PEG	siRNA	Prostate carcinoma cells (PC-3)	[82]
PEG-S-S-asODN	Plasmid DNA	HuH-7 human cancer cells	[81]
Cysteine-DNA conjugates	DNA oligonucleotides		[80]
Bis-PNA-S-S-peptide	BisPNA		[79]
Cleavable polycation/plasmid DNA multilayers	Plasmid DNA		[87, 88]

4. INTRODUCTION OF DISULFIDE BONDS TO GENE CARRIERS *VIA* CHEMICAL APPROACHES

To-date many reports have been published describing various strategies to introduce disulfide bonds into the final structure of polyplexes [45, 46, 49-51, 55, 56, 65, 70, 71, 84, 86, 87]. These methods can be divided into three basic types: disulfide cross-linked polymers, polymers where disulfide bonds are introduced into their side-chains or where they are cross-linked *via* cysteine-containing peptides – the major contributions for each are reviewed below.

4.1. Disulfide Cross-Linked Polymers

Here disulfide bonds have been introduced into the backbone of polymers using thioglycolic acid (TGA) [70, 71], dithiobis-(succinimidylpropionate) (DSP) [49, 65, 84], dimethyl-3,3'-dithiobispropionimidate (DTBP) [45, 65], lipoic acid [86] and potassium thioacetate [50]. Among these, the most common cross-linking reagent is *N,N'*-cystaminebisacrylamide (CBA) [51, 55, 56, 87] and the typical synthetic method is highlighted in Fig. (**2**). Usually, CBA is used to synthesize bioreducible polyamidoamines (S-S-PAA) *via* addition of a primary amine [56]. The main advantage of this process is that it can be used to readily introduce a variety of functional groups into the polymer because the primary amines present on the side groups readily conjugate to the acrylamide [55].

4.2. Disulfide Linkage in Polymer Side-Chains

The introduction of disulfide bonds to the side-chains of polymers is another approach that has been explored, with reagents such as by 2-(pyridyldithio)-ethylamine (PDA) [63], *N*-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) [44, 64] and pyridyl disulfide acrylate (PDSA) [66] commonly employed. Among these, two methods employing PDA [63] and SPDP [44] are of particular interest and a schematic outlining processes for both are outlined in Figs. (**3** & **4**). In addition, these reagents have successfully been used to tether various bioactive molecules directly to their carriers *via* disulfide linkages, and examples include disulfide-containing cationic lipid [37-41], PEG-S-S-dioleoylphosphatidylethanolamine (PEG-S-S-DOPE) [42], bisPNA-peptide conjugates [79], DNA-cysteine conjugates [80], PEG-S-S-oligodeoxynucleotide [81], PEG-S-S-siRNA [82].

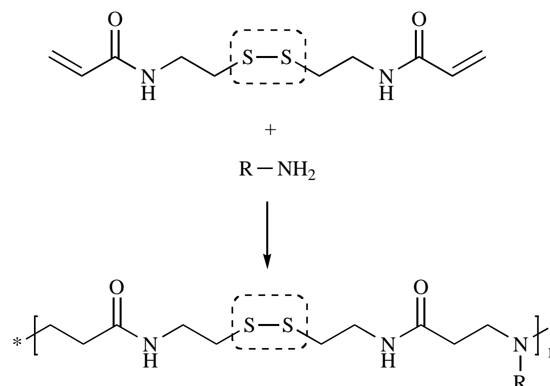

Fig. (2). Synthesis of bioreducible poly(amido amine)s by CBA (reprinted from [56], with permission).

4.3. Disulfide Cross-Linking of Peptides *Via* Cysteine

The cysteine residue is widely used to introduce disulfide bonds to various polypeptides and it has the added advantage of being compatible with many synthesis procedures and reagents commonly employed [73-75, 77]. The main benefits of this strategy are fast and efficient synthesis as well as high yields obtained as a result of well-established solid phase

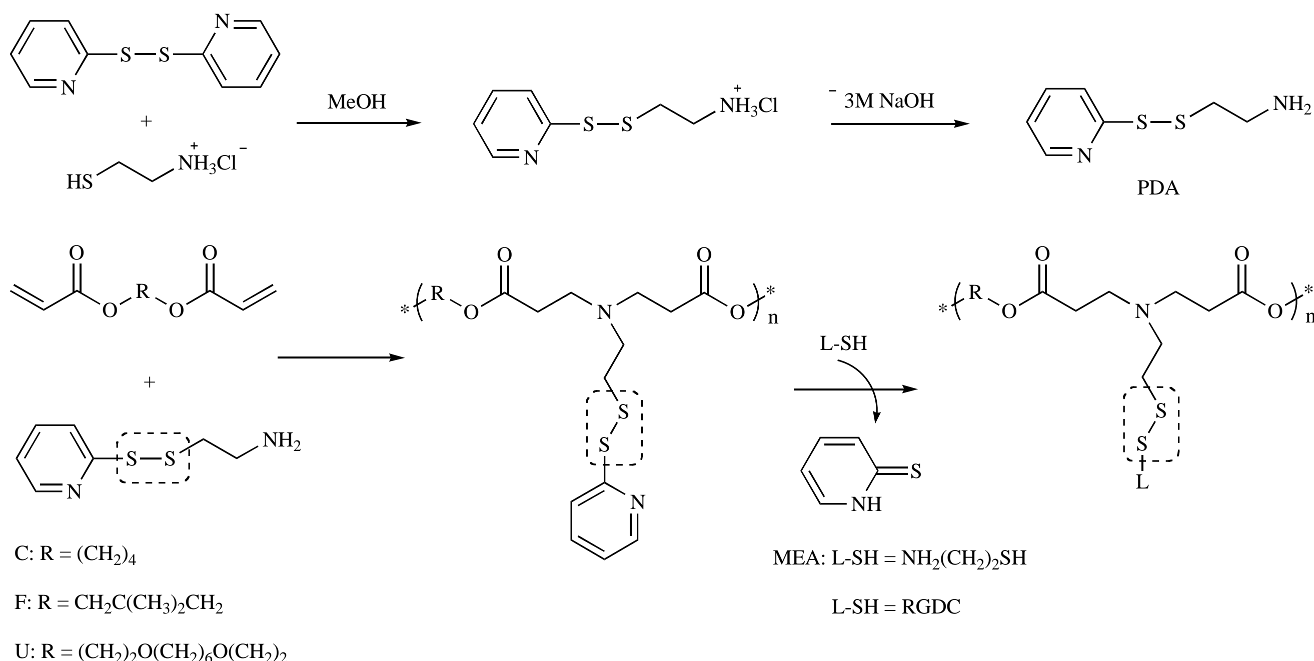

Fig. (3). Synthesis of disulfide-containing side chain by PDA. (Reprinted from [63], with permission).

peptide synthesis procedures being utilized in their preparation. In general, once synthesized, peptides are maintained in their reduced form when subjected to a low pH and an inert atmosphere (e.g. N_2 or Ar), while the cysteine residues can be made to readily form disulfide cross-linked polyplexes in the presence of a gene at neutral pH [73, 74].

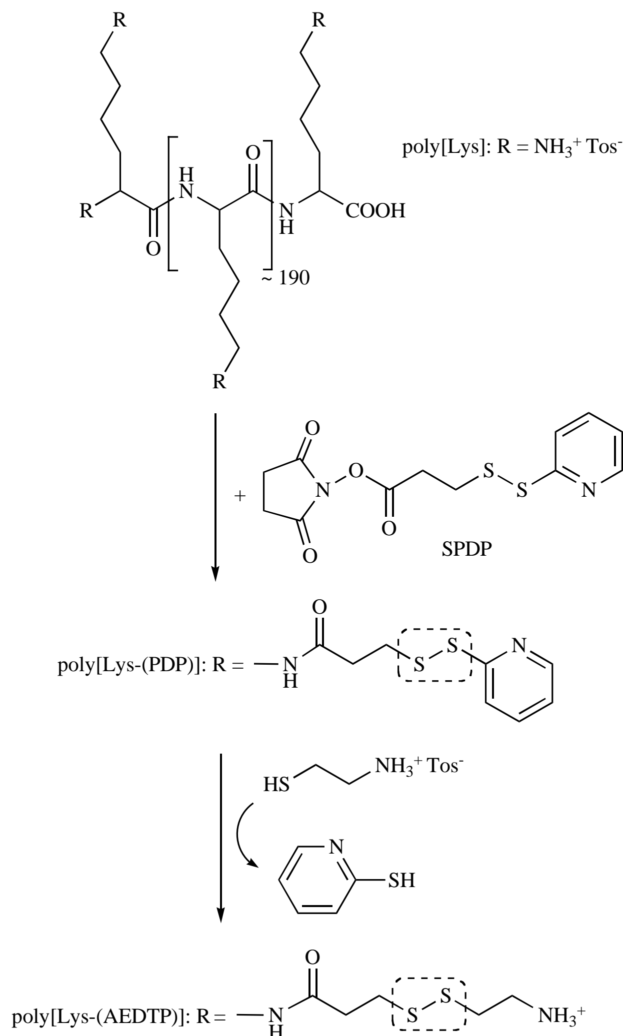

Fig. (4). Synthesis of disulfide-containing side-chain polymers *via* SPDP (reprinted from [44], with permission).

5. *IN VITRO* AND *IN VIVO* EVALUATION OF REDUCIBLE CARRIERS

Recently, most studies on reducible carriers have focused on their properties *in vitro*, with very limited research being conducted on their behaviour *in vivo*. In this section, we will discuss some of their notable physicochemical properties, which dictate their activity *in vitro*. We then conclude by briefly exploring the behaviour of these disulfide-containing vector-gene complexes *in vivo*.

5.1. Physicochemical Characterization

Typically, the diameter of carrier-gene complexes is determined by two independent approaches, dynamic light scattering (DLS) or various microscopic methods (e.g. Transmission Electron Microscopy (TEM)). The diameter of carrier-gene complexes are often measured by DLS, where the size of the particle together with the hydrodynamic boundary surrounding it is measured. Almost all complexes range in size from 100-1000nm in diameter [49, 51, 59]. As mentioned, microscopic approaches are also commonly employed, and these include atomic force microscopy (AFM), TEM, scanning electron microscopy (SEM) and scanning tunnelling microscopy (STM). Microscopy not only provides the direct size of a carrier-gene complex (in the absence of water), but also detailed morphological information with studies employing both approaches present widely in the literature [45, 47, 54, 71, 72, 78].

Another important characteristic of any given complex is its 'zeta potential'. It is the potential difference between the dispersion medium and the hydrodynamic boundary of the charged particle, and correlates to the stability of the complex. It can usually be determined at the same time as the hydrodynamic diameter using Zetasizer instrumentation [55, 61]. It should be noted however that the zeta potential of a given formulation is greatly influenced by both salt concentration and pH of the medium in which testing takes place.

To predict the dissociation of a gene from its vector in the cytosol, reduction experiments have been performed using reagents, such as dithiothreitol (DTT) [37, 44, 56, 65, 71, 87, 88] and glutathione (GSH) [50, 64, 66, 81, 89]. Lee *et al.* designed a very interesting study on GSH dependent siRNA release by determining siRNA release profiles from reducible hyaluronic acid nanogels at different GSH concentrations [72]. In the absence of GSH, only a small amount of siRNA ($< 6\%$) was released after 2 hours, while in the presence of 10 mM GSH, all the siRNA present within the nanogel was released. These results indicate that hyaluronic acid nanogels are stable in the extracellular space, whereas they can release the entrapped siRNA rapidly and quantitatively in the reductive cytosolic environment.

5.2. Transfection Efficiency and Cytotoxicity of Reducible Carriers

To-date a variety of nucleic acids including pDNA, asODN, siRNA and mRNA have been used with reducible gene delivery systems (see Table **1**). Almost all the studies show that complexes with reducible carriers resulted in much higher gene expression than their analogues where disulfide bonds were absent (see Fig. (**5**)) [44]. Wetzer and co-workers designed and synthesized various reducible cationic lipids and determined that the reduction kinetics of disulfide bonds within pDNA-lipid complexes rely on the position of the disulfide bond within the lipid molecule [40]. RNA-based therapeutics, such as siRNA and mRNA, may benefit more from the reducible delivery systems because siRNA or mRNA does not require transfer into the nucleus. As mentioned earlier Kim *et al.* developed a novel siRNA delivery system based on polyelectrolyte complex (PEC) micelles, which was formed by the interaction between siRNA-S-S-PEG and PEI [82]. The micelles showed significantly better silencing of VEGF in prostate carcinoma cells compared to siRNA-PEI complexes.

Another advantage of reducible polycations in gene delivery is that reducible carriers have been shown to be far

less cytotoxic than conventional polycation-based systems [44-54]. For instance, the PEI carrier ExGen 500® with a non-biodegradable backbone shows noticeably high cytotoxicity at concentrations of 20 µg/mL, while linear PEI derivatives with degradable disulfide linkage have negligible toxicity even at the concentration of 100 µg/mL [50]. A possible explanation for this may be that after reduction of the polycations, a decrease in their net molecular weight due to fragmentation leads to a lower charge density (due to dispersion of the fragments) and so lower cytotoxicity [27].

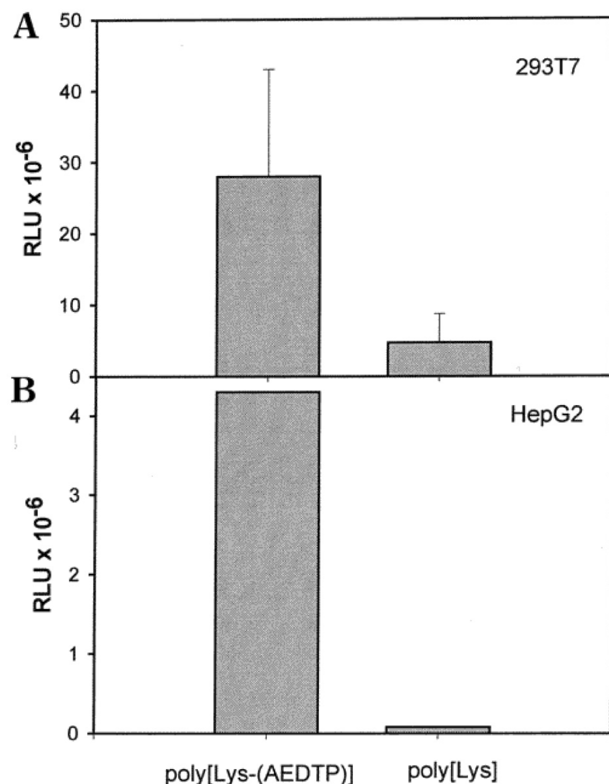

Fig. (5). Luciferase gene expression of 3-(2-aminoethyldithio) propionyl-substituted poly-L-lysine conjugate (poly[Lys-(AEDTP)]) and poly(L-lysine) in different cells in the presence of 10% fetal bovine serum and 100 µM chloroquine. (**A**) 293T7 cells; (**B**) HepG2 cells. (reprinted from [44], with permission).

5.3. *In Vivo* Behaviour of Reducible Polycations

To-date there has only been a limited number of studies describing the behaviour of reducible polycations *in vivo* [45, 47, 49, 75, 83]. The first challenge as mentioned above for reducible polyplexes *in vivo* is maintaining their stability in plasma directly after administration. In order to determine the stability of disulfide cross-linked complexes both disulfide cross-linked and non-crosslinked PLL/DNA complexes coated with PEG were injected into the tail vein of female Balb/c mice [45]. The results showed that crosslinked complexes displayed remarkably higher blood concentrations (~ 7-fold increase) 30 minutes after injection compared with non-crosslinked controls, which were rapidly cleared from the circulation.

Other studies primarily focus on the biodistribution, metabolism and gene expression of reducible carriers and gene [27, 45, 47, 75]. Most show that the reducible gene delivery systems outlined in this review accumulate mostly in the liver and are reduced in hepatocytes due to the high concentration of GSH present in liver tissue [47, 49, 75]. After intravenous administration of disulfide crosslinked PEI/DNA complexes in mice, the concentration of polyplexes in the serum was high and transfection was predominantly observed in the liver, with a significant absence of deposition in lungs which was unexpected [49]. Kwok *et al.* studied the behaviour of sulfhydryl cross-linked PEG-peptide/glycopeptide DNA co-condensates and achieved good targeting to hepatocytes [75]. The intravenous injection of the PEGylated polyplex micelles containing disulfide linkages into mice obtained similar results further suggesting that reducible cationic carriers hold many advantages over non-selectively reducible vectors *in vivo*.

6. CONCLUSIONS

Disulfide-containing gene delivery systems certainly go some way in addressing the difficulties of gene-vector release by exploiting the redox potential gradient between extra- and intra-cellular environments. Reducible carriers show excellent transfection efficiency and possess a desirable toxicity profile both *in vitro* and *in vivo*. However, this approach still requires fine-tuning and strategies whereby disulphide bridges can be more facilely and cheaply introduced into existing systems are yet to be fully developed, and these would most certainly pave the way for carriers that were both highly efficient in gene transfer and highly tolerable *in vivo*.

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