SILICA RE-EMERGES AS POTENTIAL NUCLEIC ACID DELIVERY VECTOR

Nigel Theobald, Chief Executive Officer of N4 Pharma, looks at the challenges of oligonucleotide delivery and explores approaches to overcome them.

Rapid progress in molecular biology over the last three decades has led to nucleic acids, such as plasmid DNA (pDNA), messenger RNA (mRNA) and small interfering/silencing RNA (siRNA) being proposed for use as therapeutic agents. The market has seen significant research and development investment in this field by pharma and biopharma companies, with the pDNA market alone expected to see a growth rate of approximately 23% to 2024.[1]

It was reported that, at the end of 2018, the number of clinical trials in which oligonucleotides had been either tested as vaccines or used to inhibit specific cellular processes or replace faulty genes was close to 600 (in the period 2016–2018). These developments in the market highlight the increasing enthusiasm for the potential of DNA/RNA-based therapies.

However, while promising as potential prophylactic vaccines and treatments for cancer and other diseases, nucleic acids are difficult to formulate as drugs. It is widely accepted that an effective nanoparticle delivery system would be key in enabling them to be used successfully in a therapeutic setting. As such, research efforts have been focused on the significant challenge of nucleic acid delivery.

EARLY RESEARCH

When considering cancer therapeutics specifically, initial research focused on the delivery of small-molecule drugs, with encapsulation into a variety of liposome structures and pegylation among the favoured approaches. The objectives for successful drug delivery are today, as they were then, to protect the drug substance from early or rapid degradation in the body; to deliver it preferentially to the target site of action; and to offer a combination of high loading capacity, controlled release with extended half-life, no leakage and no interference with the stability of the encapsulated product.

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In addition, good biocompatibility, low toxicity and biodegradability, as well as a clear understanding of the mode of action of the delivery system are critical factors. Nonetheless, multiple barriers need to be overcome in order to achieve successful delivery of nucleic acids – such as protecting nucleic acids against digestion by nucleases in extracellular and intracellular space; transporting a negatively charged, hydrophilic molecule across the negatively charged, hydrophobic cell and nuclear membrane; and ensuring immunogenicity of vaccine products.

CURRENT DELIVERY SYSTEM HURDLES

Initial attempts to deliver nucleic acids to target cells were focused on viral systems, which have high delivery efficacy – but their widespread use is limited by immunogenicity and toxicity concerns. Lipid nanoparticles (LNPs) then emerged

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as a popular alternative and are considered to be the current standard for nucleic acid delivery. They can protect nucleic acid from digestion and can be produced with a catatonic outer membrane to facilitate cell entry.

However, there are several limitations to lipid systems. As a result of the liposome interaction with the lipid components of the cell membrane, issues such as cell toxicity – which leads to the release of systemic inflammatory cytokines – is a serious disadvantage. Liposomes can also accumulate in the liver and spleen, with the resulting possibility of hepatotoxicity.

Research has since been directed at silica. However, while inert and safe, most silica systems tested to date have been smooth mesoporous particles – meaning the nucleic acid is attached to the side of the particle, limiting the amount that would be successfully delivered into the cell. As a result, researchers are searching for an alternative, effective, non-lipid delivery solution that protects the nucleic acid, delivers enough of it into the cell for the required immune response and ensures safety and immunogenicity.

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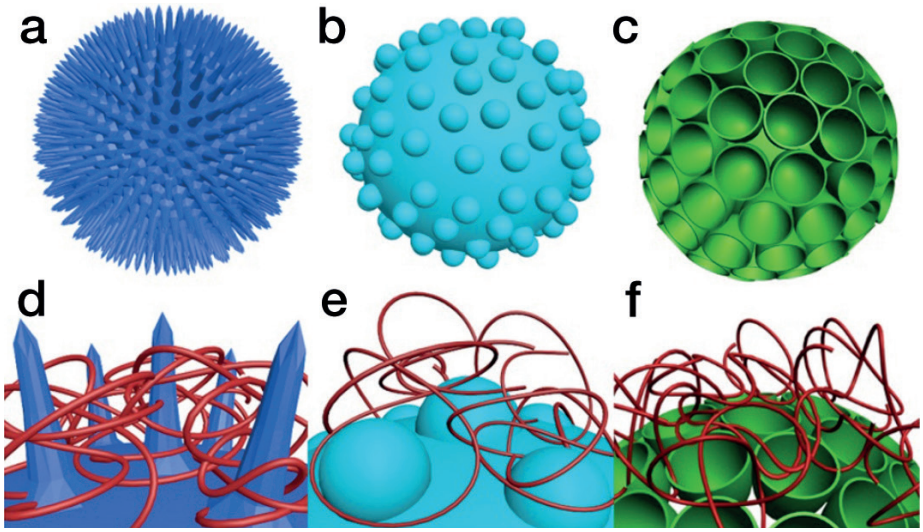


Figure 1: 3D model images displaying silica nanoparticles featured with spiky (a), raspberry (b) and flower-like (c) morphologies and spike (d), hemisphere (e) and bowl (f) type subunit nanotopographies conjugated with plasmid DNA at the interface.

Approaches using 're-engineered' silica nanoparticles (SNPs) that have been adapted to have a high surface area and high capacity are being considered as suitable alternatives to LNPs.

NOVEL SNPS SHOW PROMISE

By functionalising silica to alter its topography, researchers are now demonstrating how it can be considered a viable delivery system for nucleic acids. In a recent comparative study, scientists at the University of Queensland (UQ, Australia) investigated how the structure of SNPs impacts their performance as a nucleic acid delivery system.[2] SNPs with spiky, raspberry and flower-like morphologies were constructed with spike, hemisphere and bowl type subunits, respectively (Figure 1). To facilitate successful binding of each particle type with pDNA, negatively charged bare SNPs were modified with branched polyethylenimine (PEI) with a molecular weight of around 10 kDa, and plasmid DNA expressing enhanced green fluorescent protein (pDNA-EGFP) was loaded on the PEI modified SNPs (SNPs-PEI).

Scientists at UQ found that the spiky type subunits exhibited stronger binding affinity towards pDNA molecules and allowed effective protection against nuclease degradation when compared with the other morphologies and a commercial transfection agent. Out of the three, the spiky nanoparticles were shown to facilitate efficient cellular uptake, endosomal escape and delivery of pDNA to the nucleus most effectively, leading to successful intracellular gene expression and the highest transfection rate. The spiky SNPs also achieved high pDNA loading capacity up to 133 ng/µg. In comparison, flower-like SNPs-PEI (Flw-SNPs-PEI) showed a loading capacity of 114 ng/µg, slightly lower than Spiky-SNPs-PEI, and the raspberry-like SNPs-PEI (Ras-SNPs-PEI) and smooth-surfaced SNPs (S-SNPs-PEI) displayed a significantly lower loading capability (89 and 38 ng/µg respectively) (Figure 2).

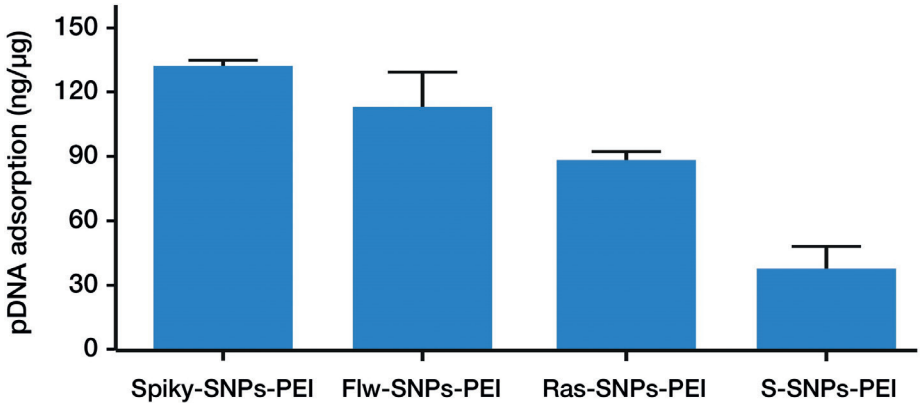


Figure 2: Loading capacity of pDNA-EGFP on Spiky-SNPs-PEI, Flw-SNPs-PEI, Ras-SNPs-PEI and S-SNPs-PEI.

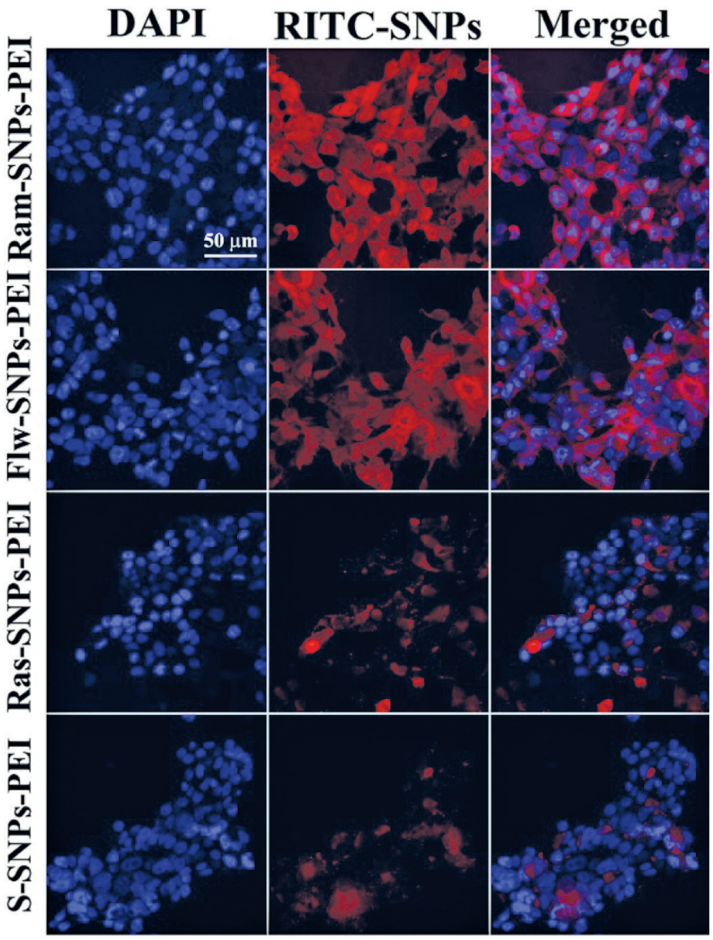


Figure 3: Cellular uptake analysis of pDNA/SNPs-PEI formulations in HEK-293T cells at a nanoparticle concentration of 40 μg/mL. Confocal images of cells incubated with pDNA loaded RITC-labelled SNPs-PEI (red fluorescent) for 4h.

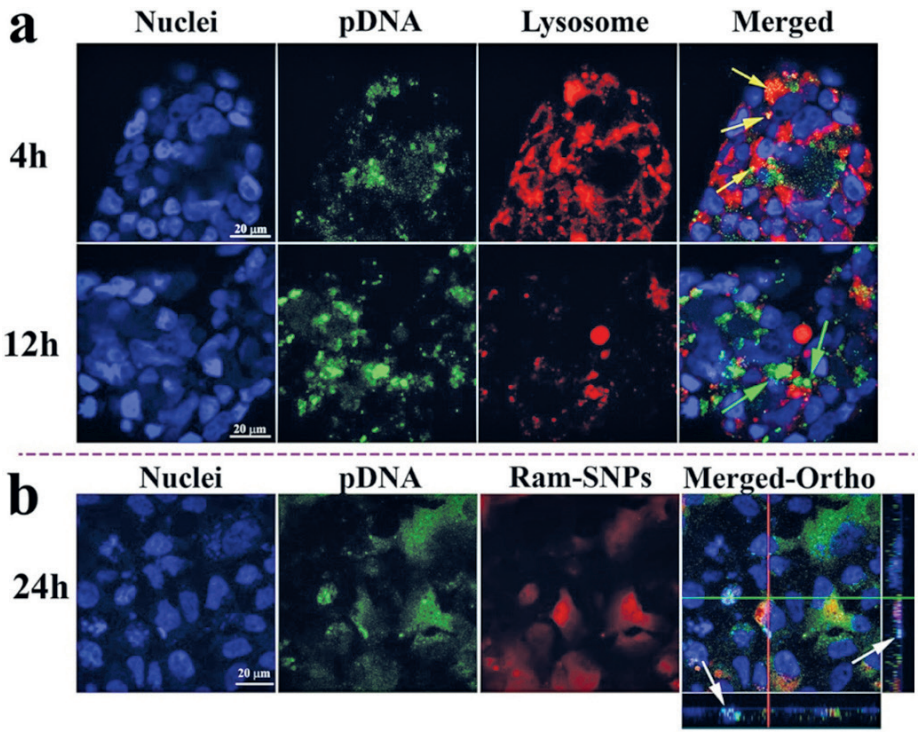


Figure 4: Intracellular tracking of fluorescein labelled-pDNA (green) in HEK-293T cells delivered by Spiky-SNPs-PEI at a nanoparticle concentration of 80 μg/mL. (a) Confocal images of cells incubated with pDNA/Spiky-SNPs-PEI for 4 and 12 h (a) and 24 h (b).

CELLULAR UPTAKE

The goal of an ideal gene vector is to deliver pDNA intracellularly and achieve transfection efficacy. Each of the pDNA/SNPs-PEI formulations were compared for their cellular uptake ability in human embryonic kidney cells 293T (HEK-293T). SNPs-PEI were firstly labelled with rhodamine B isothiocyanate (RITC) and then loaded with pDNA. Formulations were incubated with cells for four hours, followed by nuclei staining using 4',6-diamidino-2-phenylindole (DAPI). The cellular uptake was evaluated by confocal microscopy and flow cytometry. As shown in Figure 3, the nuclei show in blue fluorescence, while silica nano-formulations taken up by the cells are red. Judged by the intensity of red fluorescence, the Spiky-SNPs-PEI formulation exhibits the highest cellular uptake, followed by Flw-SNPs-PEI, Ras-SNPs-PEI and S-SNPs-PEI formulations. Quantitative analysis by flow cytometry revealed the same trend judged from the median fluorescent intensity (MFI).

TRANSFECTION EFFICIENCY

Early experiments visualised the intracellular transportation of only the pDNA/Spiky-SNPs-PEI formulation. This was tracked by labelling pDNA with fluorescein (green) and Spiky-SNPs-PEI with RITC (red) and the results are highlighted in Figure 4. The yellow arrows indicate that when pDNA and Spiky-SNPs-PEI were conjugated at four- and 12-hour (a) time points, pDNA was entrapped in endo/lysosomes (stained by lysotracker, red), while green arrows indicate successful endo/lysosomal escape of pDNA. At 24 hours (b), orthogonal side views from z-stack confocal images reveal the successful delivery of pDNA into nuclei, as indicated by the white arrows (b).

Subsequent experiments evaluated the gene delivery efficacy of all variants of the SNP complexes by transfecting pDNA-EGFP into HEK-293T cells. Spiky-SNPs-PEI demonstrated a significantly higher transfection efficacy compared with the other three complexes at all dosages. The transfection efficacy of Spiky-SNPs-PEI was 88% at a nanoparticle concentration of 80 μg/mL.

FURTHER TESTING ADDS WEIGHT

A range of further studies and experiments has been conducted to help characterise

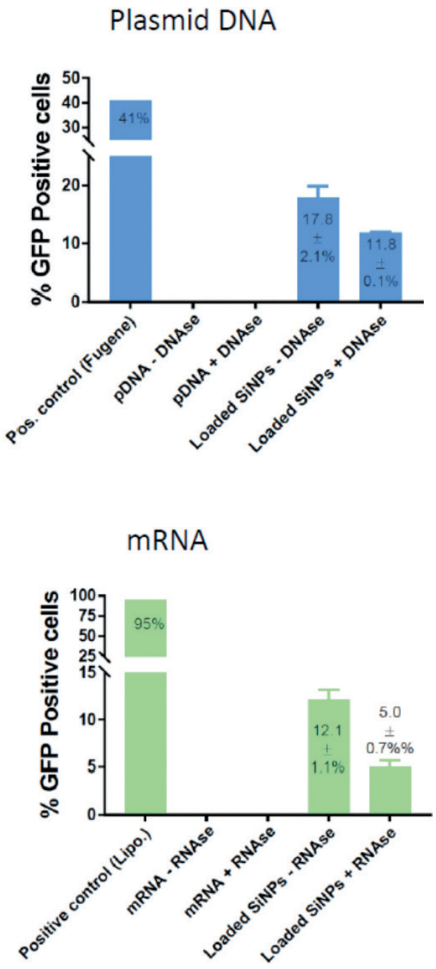
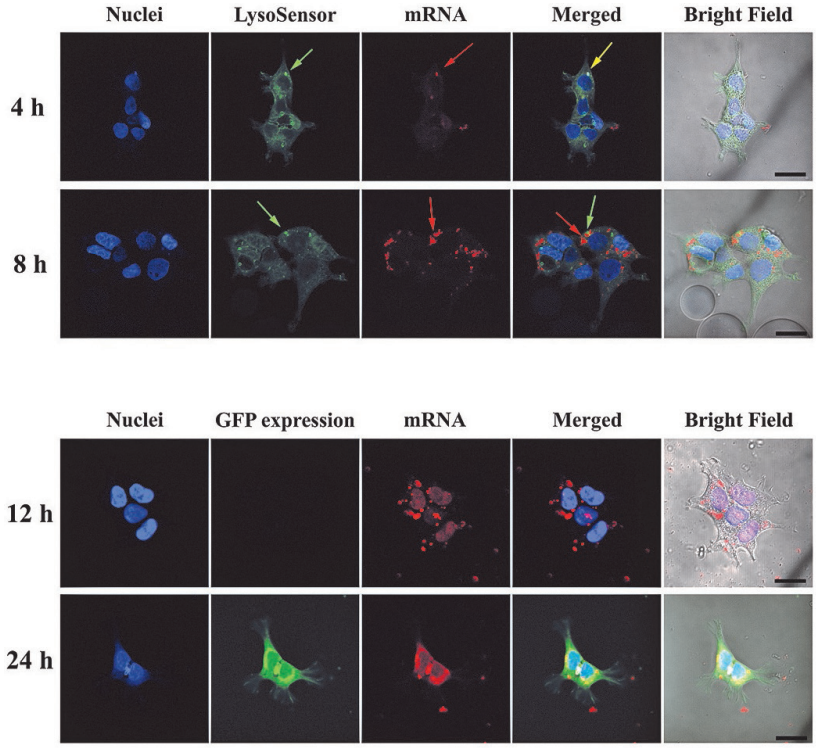


Figure 5: Spiky SNP affords both DNA and RNA protection from nuclease.



- At 4 hours, mRNA (red) is associated with lysosomes (green)
- At 8 hours, some mRNA has escaped the lysosomes
- At 12 hours, mRNA is within the cytoplasm, but no GFP expression is seen
- GFP expression is visible after 24 hours

Figure 6: Confocal microscope images of spiky SNP-mediated delivery of mRNA into the nucleus and gene expression.

the spiky SNP and its performance. For example, a study was conducted to assess SNP protection against endonucleases using HEK293 cells. The spiky structure was shown to afford both DNA and RNA significant protection from nuclease digestion, at around 67% for DNA and 41% for RNA (Figure 5).

The study, done in parallel with RNA, has provided important insights into the delivery of SNPs. For example, confocal microscopy studies have shown mediated delivery of mRNA into the nucleus and gene expression. Figure 6 summarises these findings, showing that at four hours, mRNA is associated with liposomes, at eight hours, some mRNA has escaped the liposomes and at 12 hours, mRNA is within the cytoplasm but no gene expression is seen. At 24 hours, gene expression is visible.

DEVELOPMENTS IN SILICA

The specific properties of a new functionalised SNP, such as increased surface area, have refocused attention on to silica as a potential drug delivery vector. The unique surface of the particle traps and protects the looped structure of nucleic acids and is designed to deliver the cargo directly into the cells. Compared with other topographies, the spiky structure of the SNP has proven to be best at facilitating efficient cellular uptake, endosomal escape and delivery of the payload to the nucleus. The safety profile of silica is well documented, with it being converted into silica acid in the body and naturally passing out, with no accumulation in the liver.

COVID-19 DEVELOPMENTS

N4 Pharma is currently undertaking a proof of concept research project using a COVID-19 spike DNA plasmid to explore the ability of Nuvec® to be used as an alternative delivery system by those developing COVID-19 DNA or RNA vaccines.

The proof-of-concept work will show how Nuvec® is capable of loading the COVID-19 plasmid and transfecting cells with the plasmid *in vitro* and *in vivo*. The research work is looking to demonstrate to those developing nucleic acid COVID-19 vaccines how Nuvec® could be a beneficial, alternative and safe delivery system for subsequent vaccines they may be looking to develop for COVID-19 or other viruses that may well surface in the future.

> "Silica is now being seriously considered as a drug delivery vehicle to improve efficacy and overcome the significant drawbacks of current nucleic acid delivery systems such as LNPs."

Although cancer therapy has improved and survival rates increased,[3] innovative approaches such as gene therapy and RNA/DNA vaccines are emerging with great excitement about how they could transform cancer treatment. Silica is now being seriously considered as a drug delivery vehicle to improve efficacy and overcome the significant drawbacks of current nucleic acid delivery systems such as LNPs.

ABOUT THE COMPANY

Established in 2014, N4 Pharma is a specialist pharmaceutical company developing a novel silica nanoparticle (SiNP) delivery system that is initially being directed towards pDNA/mRNA delivery in oncology. The business is built around a strong intellectual property portfolio that is licensed from the University of Queensland (Australia). N4 Pharma listed on the AIM (London, UK) in 2016 and is managed by an experienced team of scientists and business executives with significant know-how gained both in big pharma and other smaller, specialist pharma/biopharma discovery and development enterprises.

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ABOUT THE AUTHOR

Nigel Theobald has more than 25 years' experience in healthcare and in building businesses, strategy development and its implementation – and a strong network covering all aspects of pharmaceutical product development and commercialisation. He was the head of healthcare brands at Boots Group in 2002 before leaving to set up a series of successful businesses, including Oxford Pharmascience Group, which he grew over five years into an AIM-quoted company with a market capitalisation of £40 million upon departure. Mr Theobald formed N4 Pharma in 2014.