PLASMID DNA DELIVERY: Nanoparticle Topography Matters

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SUMMARY

- Non-viral delivery of DNA vaccines poses real challenges due to susceptibility to nuclease degradation of plasmid DNA (pDNA) and poor transport to the cell nucleus.
- Silica NPs (SNPs) represent an ideal candidate for delivery of DNA vaccines as they can be designed for intracellular delivery.
- Optimising design of SNPs with different nanotopographies is required to improve delivery of pDNA to the cell nucleus.
- Our results show that nanoparticles with spiky surfaces represent a superior topography for delivery of DNA vaccines with high capacity for pDNA loading, efficient transport across the cell membrane and translation of pDNA after 24 hours.
- Crucially, it is shown that the topography of these nanoparticles protects the pDNA from nuclease degradation.
- Our study demonstrates that the rational design of non-viral vectors can allow for improved delivery of pDNA into cells, leading to the enhanced production of proteins encoded by DNA vaccines.

FIGURE 1 – GENERATION OF SNPs WITH DIFFERENT NANOTOPOGRAPHIES

Silica nanoparticles (SNPs) were fabricated via assembly of resorcinol-formaldehyde resin and silica primary particles under Stöber synthesis conditions. Variation in approaches, produced SNPs with topographies of spiky (top row), hemi-sphere (middle row) or bowl (bottom row) subunits. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images show particle size and inner, hollow structure. Electron tomography further shows the hollow interior and confirms the 3D surface structure on SNPs.

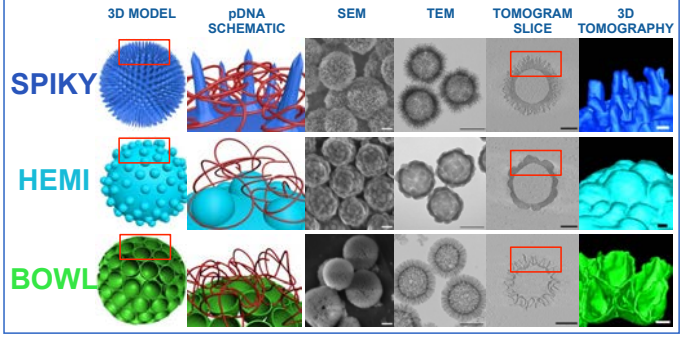


FIGURE 2 – EFFICIENT pDNA LOADING OF SNPs

(A) SNPs were analysed for pDNA loading capacity by quantifying absorption after 30 mins using Nanodrop. *(B)* Zeta potential of SNPs was measured before and after polyethylenimine (PEI) conjugation and after subsequent pDNA loading. *(C)* Gold immuno-labelling identified pDNA binding at the surface of SNPs with different topographies (red arrows). In some SNPs, pDNA was also evident at sites away from the surface of SNPs (blue arrows).

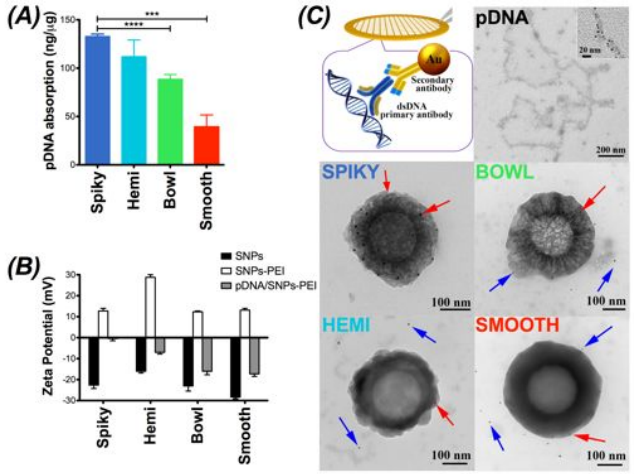


FIGURE 3 – CELLULAR UPTAKE OF pDNA-SNPs

(A) Rhodamine-labelled SNPs were compared for cellular uptake by HEK-293T cells by flow cytometry, showing that spiky SNPs are most readily internalised after 4 hours. *(B-D)* Confocal microscopy after 4, 12 or 24 hours shows that after initial transport of spiky SNPs via lysosomes, pDNA is released and shows nuclear localisation.

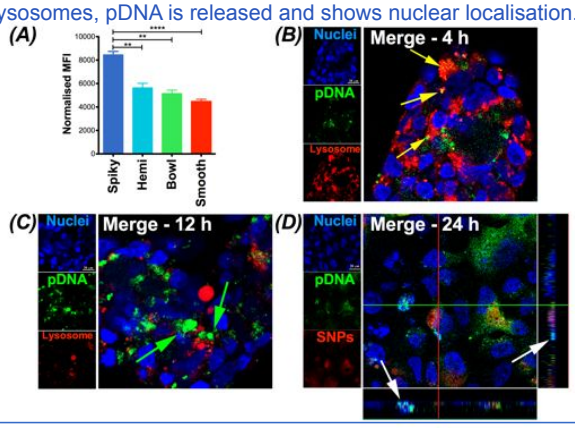
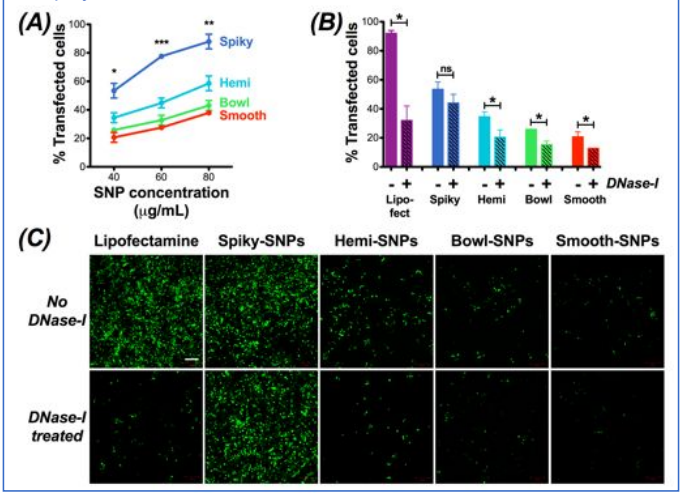


FIGURE 4 – EXPRESSION OF pDNA DELIVERED BY SNPs

Gene delivery by SNPs was assessed by transfecting pDNA encoding eGFP into cells and quantifying expression after 48 hours using flow cytometry or confocal microscopy. *(A)* Whilst all SNPs demonstrated dose-dependent transfection, optimal expression was observed with spiky SNPs. *(B & C)* pDNA delivered by three other SNPs or in Lipofectamine was susceptible to DNase-I activity, as shown by reduced frequency of GFP⁺ transfected cells. Conversely, DNase-I treatment did not reduce transfection with pDNA delivered via spiky SNPs.



CONCLUSIONS

- **We demonstrate the importance of considering nanotopography of SNPs for efficient pDNA delivery.**
- **Spiky SNPs show improved pDNA loading, protection from nuclease digestion and superior delivery into cells.**
- **This design of SNPs provide a novel strategy in the development of non-viral vectors with applications across gene therapy and vaccine delivery.**