Human Vaccines & Immunotherapeutics 10:5, 1306–1308; May 2014; © 2014 Landes Bioscience

Safety and efficacy of DNA vaccines

Plasmids vs. minicircles

Sofia Stenler[1], Pontus Blomberg[1,2], CI Edvard Smith[1*]

[1]Department of Laboratory Medicine; Clinical Research Center; Karolinska Institutet; Stockholm, Sweden; [2]Vecura; Clinical Research Center, Karolinska University Hospital; Stockholm, Sweden

While DNA vaccination using plasmid vectors is highly attractive, there is a need for further vector optimization regarding safety, stability, and efficiency. In this commentary, we review the minicircle vector (MC), which is an entity devoid of plasmid bacterial sequences, as an alternative to the traditional plasmid construct. The commentary highlights the recent discovery by Stenler et al. (2014) that the small size of an MC enables improved resistance to the shearing forces associated with e.g. pneumatic delivery methods. This observation may have implications for the regulatory agencies' requirement of plasmid integrity and quality.

Keywords: DNA vaccination, non-viral gene therapy, pneumatic delivery, supercoil, topoisoforms, vaccine, vector safety

*Correspondence to: CI Edvard Smith; Email: edvard.smith@ki.se

Submitted: 01/23/2014;

Accepted: 02/01/2014

http://dx.doi.org/10.4161/hv.28077

Introduction

Plasmid-based vectors (pDNA) for gene delivery have received great attention as possible agents for vaccination, so called DNA vaccination. Among the advantages of using pDNA are the ease of both development and production as compared with conventional vaccine manufacturing. Moreover, DNA vaccines are known to be very stable at room temperature, which is of significance for both transport and storage.[1] Since the antigen is expressed from the pDNA the target cell, the resulting peptide is more likely to resemble the native form of e.g., a viral protein, with all the necessary post-translational modifications. However, also for pDNA, there are many hurdles to overcome. Safety, efficiency, and stability are key features for any vaccine agent.

MC and Plasmid—Comparing the Vector Types

A pDNA vector consists of circular DNA containing an expression cassette with the gene of interest and regulatory sequences as promoter and polyadenylation signals, as well as sequences needed for propagation in the bacteria, such as origin of replication and selection markers. The selection marker is often a gene conferring antibiotics resistance, despite regulatory agencies recommending avoiding these in production of plasmids for therapeutic use.[2] The MC is a plasmid-based vector for gene delivery containing only the expression cassette and thus devoid of bacterial sequences. There are a number of published systems for MC production.[3-8] In principle, a parental plasmid is constructed consisting of the eukaryotic expression cassette flanked by recombination sites. Outside these sites are the sequences needed for plasmid propagation in bacteria. Induction of recombination produces an MC, containing only the gene of interest with suitable regulatory sequences. A comparison of the advantages of MC vs. plasmids and the corresponding selected references are provided in **Table 1**.

A Safer and More Robust Construct

A reason for considering the MC as a safer alternative to the conventional plasmid is the avoidance of spreading antibiotic resistance. The recombination event

©2014 Landes Bioscience. Do not distribute.

BioNTech-Pfizer Exhibit 1130
BioNTech SE and Pfizer Inc. v. ModernaTX, Inc. IPR2023-01358
Page 1 of 3

Table 1. Minicircle (MC) vs. plasmid in biological assays

Advantages of MC	Assay	Selected references
Prolonged expression	Mouse liver gene therapy for human factor IX and alpha1-antitrypsin	Chen et al., 2003
Enhanced serum stability	Incubation in human serum	Zhao et al., 2010
Improved shearing resistance	Sonication and nebulization	Cantanese et al., 2012
Stronger CD8+ T-cell response	Vaccine challenge in mouse model of listeriosis	Dietz et al., 2013
More robust supercoiled fraction	Biojection through mouse hide	Stenler et al., 2014

Comparison of different properties of the MC and plasmid, with selected references.

removes all bacterial sequences from the vector, including any antibiotic resistance genes used as selection markers; these will not be transferred into the patient when using an MC construct.

A less intuitive but important difference between a plasmid and an MC vector is the differential tolerance against shearing forces now reported by Stenler et al.[9] While mechanical shearing of DNA forms the basis of many techniques in molecular biology, including next-generation sequencing, fragmentation of DNA is conversely the enemy of therapeutic approaches, including the use of DNA vaccines. Among the risks that WHO,[10] FDA,[11] and EMA[12] lists for the use of DNA vaccines is the hazard of integration into recipient's chromosomal DNA with the resulting risk of insertional mutagenesis or spreading of antibiotics resistance genes. The probability of chromosomal integration increases if the introduced pDNA has been linearized.[13] Any plasmid preparation will contain pDNA with different topologies: Super-coiled material, open circular, and linear. This is the reasons that the regulatory authorities require the plasmid preparation intended for vaccination or gene therapy to contain a high percentage of supercoiled material. In their "Considerations for Plasmid DNA Vaccines for Infectious Disease Indications," the FDA recommends a minimum specification for supercoiled plasmid content of >80% whereas the Swedish Medical Products Agency only accepts a higher level of >85% and normal industry expectation for supercoiled plasmid levels are >90 or >95%.[14]

This is all well and good, but what about the quality of the construct after delivery, when it reaches the cell? In a recent study, Stenler et al. have investigated the fate of MC and pDNA after delivery trough mouse hide using pneumatics. Pneumatics is a promising method for vaccine delivery,[15-17] although it is also associated with the shearing of DNA.[15] The MC, however, seems to be able to withstand these shearing forces to a much greater extent than the plasmid. In the study, the topology of a plasmid and an MC was compared after pneumatic delivery through mouse skin. What is perhaps most noteworthy is that the plasmid DNA is partially destroyed and does no longer met the FDA requirements of an 80% super-coiled fraction. The MC construct fared much better, with the nicked fraction beings ten times lower than for the full-length plasmid construct. This shearing of the plasmid not only increases the risk of insertional mutagenesis but also results in a lower effective dose, since linearized and open circular topologies have been shown to have lower transfection efficiencies and expression.[18,19] The Biojector results in the study by Stenler et al. corroborate with a study of the ability of MCs to resist shearing forces caused by sonication.[20] In another report, the MC was dramatically more stable in serum as compared with full-length plasmids.[21] This of course has implications for injection based delivery of the DNA vaccine.

Enabling a Higher Effective Dose

The MC's smaller size also enables a higher effective dose. On a weight basis, an MC batch will contain more moles of vector, and thus a higher amount of expression cassettes, than the corresponding amount of a plasmid, simply due to the removal of the bacterial plasmid backbone. This is an important feature for vaccine vector because, as Cai et al. note in their expert review,[22] the amount of DNA required per vaccine dose is large, and this in a limited volume of a few milliliters or less.

Enhanced T-Cell Response

As previously reported by Dietz et al., the MC shows promise as a DNA vaccine vector.[23] The MC showed a higher and prolonged expression in vitro and in vivo in mice and an enhanced immunogenicity in vivo. In a challenge experiment, the MC vector conferred better protection and elicited a stronger antigen specific $CD8^+$ T-cell response in a mouse model of listeriosis.

A prolonged gene expression has also been shown for other MC constructs in a gene therapy setting.[5,9] This is thought to be due to less heterochromatin formation in the MC DNA as compared with plasmid, where the transcriptionally inactive plasmid backbone promotes heterochromatin formation and subsequent vector silencing.[24,25]

Conclusion

The MC construct shows great promise as a vector for gene delivery including DNA vaccination. In this commentary, we have discussed how its smaller size affects both the safety and the efficiency of the vector. Especially, we have pinpointed the increased resistance to shearing that the MC has shown. It is likely that the shearing forces developed during pneumatic delivery are stronger than for most other DNA transfer technologies. However, irrespective of the mode of delivery, fragmentation is never completely absent. This suggests that whenever chromosomal

©2014 Landes Bioscience. Do not distribute.

BioNTech-Pfizer Exhibit 1130
BioNTech SE and Pfizer Inc. v. ModernaTX, Inc. IPR2023-01358
Page 2 of 3

integrations are deemed undesirable, MCs would be preferable. Thus, in addition to the other advantages with therapeutic DNA preparations being devoid of prokaryotic sequences, the improved resistance to shearing forces identified by Stenler et al.,[9] should now also be added to the list. It will be interesting to see whether this new finding will influence regulatory bodies in their continuous strive for the generation of safer therapeutic DNA, including vaccines.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Quaak SG, Haanen JB, Beijnen JH, Nuijen B. Naked plasmid DNA formulation: effect of different disaccharides on stability after lyophilisation. AAPS PharmSciTech 2010; 11:344-50; PMID:20204715; http://dx.doi.org/10.1208/s12249-010-9391-2
2. FDA. U.S. Department of Health and Human Services, Food and Drug Administration: Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy. 1998.
3. Darquet AM, Cameron B, Wils P, Scherman D, Crouzet J. A new DNA vehicle for nonviral gene delivery: supercoiled minicircle. Gene Ther 1997; 4:1341-9; PMID:9472558; http://dx.doi.org/10.1038/sj.gt.3300540
4. Bigger BW, Tolmachov O, Collombet JM, Fragkos M, Palaszewski I, Coutelle C. An araC-controlled bacterial cre expression system to produce DNA minicircle vectors for nuclear and mitochondrial gene therapy. J Biol Chem 2001; 276:23018-27; PMID:11304530; http://dx.doi.org/10.1074/jbc.M010873200
5. Chen ZY, He CY, Ehrhardt A, Kay MA. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression in vivo. Mol Ther 2003; 8:495-500; PMID:12946323; http://dx.doi.org/10.1016/S1525-0016(03)00168-0
6. Chen ZY, He CY, Kay MA. Improved production and purification of minicircle DNA vector free of plasmid bacterial sequences and capable of persistent transgene expression in vivo. Hum Gene Ther 2005; 16:126-31; PMID:15703495; http://dx.doi.org/10.1089/hum.2005.16.126
7. Kay MAHC, He CY, Chen ZY. A robust system for production of minicircle DNA vectors. Nat Biotechnol 2010; 28:1287-9; PMID:21102455; http://dx.doi.org/10.1038/nbt.1708
8. Mayrhofer P, Blaesen M, Schleef M, Jechlinger W. Minicircle-DNA production by site specific recombination and protein-DNA interaction chromatography. J Gene Med 2008; 10:1253-69; PMID:18767031; http://dx.doi.org/10.1002/jgm.1243
9. Stenler S, Wiklander OP, Badal-Tejedor M, Turunen J, Nordin JZ, Hallengärd D, Wahren B, Andaloussi SE, Rutland MW, Smith CE, et al. Micro-minicircle Gene Therapy: Implications of Size on Fermentation, Complexation, Shearing Resistance, and Expression. Mol Ther Nucleic Acids 2014; 2:e140; PMID:24399204; http://dx.doi.org/10.1038/mtna.2013.67
10. WHO. The WHO Expert Committee on Biological Standardization 56th report: Number 941. Geneva: World Health Organization, 2007.
11. FDA. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research: Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications. 2007.
12. EMEA. Comittee for Veterinary medicinal products: Notes for Guidance: DNA-vaccines non-amplifiable in eukaryotic cells for veterinary use. In: Products TEAftEfM, ed., 2000.
13. Brinster RL, Chen HY, Trumbauer ME, Yagle MK, Palmiter RD. Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. Proc Natl Acad Sci U S A 1985; 82:4438-42; PMID:3892534; http://dx.doi.org/10.1073/pnas.82.13.4438
14. Hitchcock AG, Sergeant JA, Rahman SF, Tharia HA, Blom H. Scale-Up of a Plasmid DNA Purification Process. BioProcess International 2010; 8:46-54
15. Vahlsing HL, Yankauckas MA, Sawdey M, Gromkowski SH, Manthorpe M. Immunization with plasmid DNA using a pneumatic gun. J Immunol Methods 1994; 175:11-22; PMID:7930633; http://dx.doi.org/10.1016/0022-1759(94)90327-1
16. Wang R, Epstein J, Baraceros FM, Gorak EJ, Charoenvit Y, Carucci DJ, Hedstrom RC, Rahardjo N, Gay T, Hobart P, et al. Induction of CD4(+) T cell-dependent CD8(+) type 1 responses in humans by a malaria DNA vaccine. Proc Natl Acad Sci U S A 2001; 98:10817-22; PMID:11526203; http://dx.doi.org/10.1073/pnas.181123498
17. Hallengärd D, Bråve A, Isaguliants M, Blomberg P, Enger J, Stout R, King A, Wahren B. A combination of intradermal jet-injection and electroporation overcomes in vivo dose restriction of DNA vaccines. Genet Vaccines Ther 2012; 10:5; PMID:22873174; http://dx.doi.org/10.1186/1479-0556-10-5
18. Kano Y, Miyashita T, Nakamura H, Kuroki K, Nagata A, Imamoto F. In vivo correlation between DNA supercoiling and transcription. Gene 1981; 13:173-84; PMID:6263758; http://dx.doi.org/10.1016/0378-1119(81)90006-8
19. Cupillard L, Juillard V, Latour S, Colombet G, Cachet N, Richard S, Blanchard S, Fischer L. Impact of plasmid supercoiling on the efficacy of a rabies DNA vaccine to protect cats. Vaccine 2005; 23:1910-6; PMID:15734063; http://dx.doi.org/10.1016/j.vaccine.2004.10.018
20. Catanese DJ Jr., Fogg JM, Schrock DE 2nd, Gilbert BE, Zechiedrich L. Supercoiled Minivector DNA resists shear forces associated with gene therapy delivery. Gene Ther 2012; 19:94-100; PMID:21633394; http://dx.doi.org/10.1038/gt.2011.77
21. Zhao N, Fogg JM, Zechiedrich L, Zu Y. Transfection of shRNA-encoding Minivector DNA of a few hundred base pairs to regulate gene expression in lymphoma cells. Gene Ther 2011; 18:220-4; PMID:20962872; http://dx.doi.org/10.1038/gt.2010.123
22. Cai Y, Rodriguez S, Hebel H. DNA vaccine manufacture: scale and quality. Expert Rev Vaccines 2009; 8:1277-91; PMID:19722898; http://dx.doi.org/10.1586/erv.09.84
23. Dietz WM, Skinner NE, Hamilton SE, Jund MD, Heitfeld SM, Litterman AJ, Hwu P, Chen ZY, Salazar AM, Ohlfest JR, et al. Minicircle DNA is superior to plasmid DNA in eliciting antigen-specific CD8+ T-cell responses. Mol Ther 2013; 21:1526-35; PMID:23689601; http://dx.doi.org/10.1038/mt.2013.85
24. Chen ZY, Riu E, He CY, Xu H, Kay MA. Silencing of episomal transgene expression in liver by plasmid bacterial backbone DNA is independent of CpG methylation. Mol Ther 2008; 16:548-56; PMID:18253155; http://dx.doi.org/10.1038/sj.mt.6300399
25. Riu E, Chen ZY, Xu H, He CY, Kay MA. Histone modifications are associated with the persistence or silencing of vector-mediated transgene expression in vivo. Mol Ther 2007; 15:1348-55; PMID:17457320; http://dx.doi.org/10.1038/sj.mt.6300177

©2014 Landes Bioscience. Do not distribute.

BioNTech-Pfizer Exhibit 1130
BioNTech SE and Pfizer Inc. v. ModernaTX, Inc. IPR2023-01358
Page 3 of 3