DNA Vaccines

A research paper analysing the genetic engineering technique for DNA-vaccines in relation to the West Nile virus

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1. Preface

The outbreak of the COVID-19 pandemic in spring 2020 showed us how important vaccines can be. The issue was omnipresent and different types of vaccines were developed in a hurry such as viral vector vaccines, protein subunit vaccines, mRNA vaccines, and in India a DNA vaccine. These vaccines were much discussed in the media and in our daily life. Many people felt insecure not only due to the virus itself, but also due to the diversity of vaccines and the unknown effects these new products might have. Due to this experience, we had a general interest in vaccines. The task to write a term paper on genetic engineering gave us the opportunity to take a closer look at one type of vaccine, the DNA vaccine. We think that DNA vaccines are especially interesting because they are a rather new type of vaccine. They are said to be very promising for the future because they have certain advantages over the other vaccine technologies.
In this paper we would like to answer the questions in which domains DNA vaccines are used, how they are engineered and how they work. Besides, we want to look at the advantages that DNA vaccines offer compared to mRNA vaccines, but also what makes it difficult to use them and what threats they pose.

2. Introduction

We live in a globalized world in which infectious diseases spread quickly and cause problems not only for individuals but for the whole health system. This was recently shown by the COVID-19 pandemic. Within a short time, a pandemic creates the need to develop new vaccines. But the idea of vaccines is not new. For centuries, people tried not only to cure, but also to prevent diseases. The finding and inventing of vaccines were an important advancement in medicine, as they stimulate the immune system in a way that it can later recognize and fight a pathogen before a disease can occur (prophylactic vaccine) or as they help to fight a disease that has already developed (therapeutic vaccine). Due to vaccines, the number of deaths from infectious, but also non-infectious diseases could be considerably decreased.
Today, there are many different types of vaccines available or in development. Three main groups can be distinguished. The first group of vaccines uses the whole pathogen, as for example the yellow fever vaccine containing live, attenuated viruses, or the polio vaccine containing inactivated, destroyed viruses.(1) The second group of vaccines uses only subunits of the pathogen, as for example the vaccine against hepatitis B containing only the surface protein of the virus. The vaccines of the third group use the genetic material of the pathogen.(2) This group includes viral vector vaccines, mRNA vaccines and DNA vaccines. DNA vaccines are the youngest generation of vaccines and were created to fight against viruses, bacteria, parasites, and cancer.
In the 1980s, the idea of producing vaccines by DNA engineering was born. In 1986, the first DNA vaccine was developed against hepatitis B virus. In the early 1990s, the concept of DNA vaccines was further developed. It was demonstrated that a gene sequence encoding for an antigen and inserted into a plasmid was able to provoke an immune response when injected into the skin or muscles of mice. After a long period of research, development and tests, some DNA vaccines were approved for veterinary use in the USA, for example in 2005 a vaccine for horses against the West Nile virus or a melanoma vaccine for dogs.(3) For further use in humans it took much longer, although several clinical trials were made, for example with vaccines against HIV, cancer, influenza, human papillomavirus, and malaria. But all these vaccines showed only a low immune response. One of the main problems was that the plasmids into which the DNA sequences of a pathogen were integrated and thus served as a vector could not be taken up well enough by the cells due to an inefficient delivery. In the following years, delivery methods were improved, for example with dermal patches instead of injections with needles. Finally, in August 2021 another DNA vaccine was

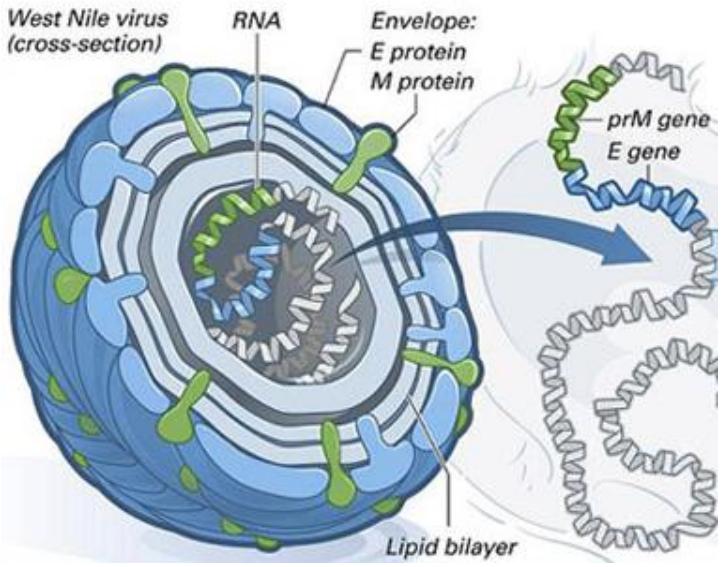
authorized for use in humans. It was a vaccine against COVID-19 approved for emergency use in India.(3) The vaccine contains plasmids with a DNA sequence that encodes the spike protein of the virus. The immune system makes an immune response against the spike protein and produces immune cells that during future attacks of the virus can fight it.

3. Description of the engineering technique to produce a DNA vaccine (illustrated by the West Nile virus vaccine)

The DNA vaccine is a type of gene-based vaccine. Instead of using the “traditional” mechanism of injecting an either weakened or inactivated pathogen or fragments of it, genetic vaccines contain a form of nucleic acid. Once injected, the nucleic acid acts as a template to produce antigens through protein biosynthesis. Other types of gene-based vaccines besides DNA vaccines include viral vector vaccines, as well as the more common type, mRNA vaccines.(4) The differences between the technology of DNA vs. mRNA vaccines will be discussed further on.
We will be illustrating the necessary steps for the engineering technique used to produce a DNA vaccine with help of the DNA vaccine against the West Nile virus. The West Nile virus is an enveloped, single-stranded RNA virus. It can infect birds (they are the primary host), but also mammals such as humans and horses. The approved DNA vaccine is only used in horses.

3.1 Isolation of the genes of interest

The aim is to isolate a certain gene sequence of the West Nile virus, which encodes two of its proteins: the prM gene and the E gene. The prM gene encodes the transmembrane protein M of the virus, the E gene encodes the envelope protein E. Later, in the vaccinated individual, these genes will be expressed and lead to an immune response of the body.(5)



In a first step, the single-stranded RNA is extracted from the West Nile virus (fig. 1). A sample with cells of an infected individual is used. The sample is added to a lysis buffer in a silica membrane column. The buffer destroys the cells of the individual and the shell (capsid) of the virus by denaturing the proteins. Besides, it creates binding conditions that favour the absorption of RNA on the silica membrane of the column. Ethanol is added to adjust RNA binding conditions. Then the RNA is washed out of the membrane by elution.

Fig. 1: Gaining of the single-stranded RNA of the West Nile virus

During the next process, the reverse transcription (fig.2), the enzyme reverse transcriptase forms a cDNA strand, which is complementary to the previously gained single-stranded RNA. For this, the enzyme transcribes the RNA strand into a double-strand by adding complementary nucleotides to the primer. A hybrid is formed, consisting of one RNA and one cDNA strand. Then the RNA strand is released. The remaining DNA strand gets a complementary DNA strand to form a double-stranded DNA.

Fig. 2: During reverse transcription the single-stranded RNA of the virus becomes a double-stranded DNA.

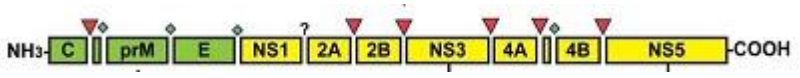


Fig. 3: The genome of the West Nile virus

To gain the target DNA sequence of the West Nile virus, i.e. the above-mentioned prM and E genes (fig. 3), the polymerase chain reaction (PCR) is used (fig. 4). For this, the double-stranded DNA of the virus is taken, as well as a heat-resistant DNA polymerase and many single nucleotides of all 4 types. Besides, two types of primers are needed: One primer must be complementary to one of the ends of the target DNA sequence on one strand, the other primer must be complementary to the second end of the target DNA sequence on the other strand. The double-stranded DNA is heated and the strands separate. Then they are cooled and the primers can form hydrogen-bonds with the single strands. They are heated again and the DNA polymerase adds the complimentary nucleotides to the 3' ends of the primers. The PCR goes through different cycles. After 3 cycles, ¼ of the DNA strands produced correspond to the target DNA sequence. With every additional cycle, the number of target DNA sequences grows exponentially and finally is much bigger than the number of the other strands.

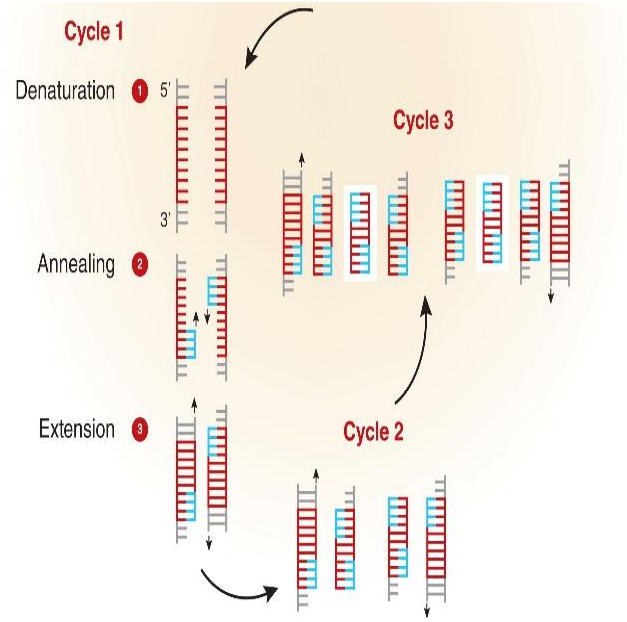


Fig. 4: Polymerase chain reaction (PCR)

3.2 Insertion of the target DNA sequence into a transfer vector

The primers used during PCR are not only complementary to the ends of the target DNA sequence, but they also contain a special base sequence. This sequence or restriction site serves as a distinctive mark for restriction enzymes (KasI and NotI). These restriction enzymes recognize the restriction sites before and after the target DNA sequence. At these sites, they cut the DNA double strand in a way that "sticky ends" are formed: The two strands of the DNA don't have the same length, but some unpaired nucleotides. They are "sticky" because they can easily bind with another "sticky end" with complementary nucleotides by base pairing.
To create a "vehicle" or transfer vector to get the target DNA sequence into host cells, in this case into bacteria, the target DNA sequence is inserted into a plasmid from *E. coli*. Plasmids are very important for genetic engineering. A plasmid is a small, circular, double-stranded DNA molecule found in bacteria, but also in some yeast and fungi. It is separate from the chromosome, reproduces independently and is not necessary for cell life. A free plasmid can be taken up by a cell through its cell wall (transformation). Due to its characteristics, a plasmid can be used to integrate new DNA and to bring it into a cell.
The plasmid is cut with the same restriction enzyme as the DNA of the West Nile virus. By doing so, it is guaranteed, that the DNA fragment and the cut plasmid have "sticky ends" that are complementary to each other.(6)
The DNA sequences of the West Nile virus containing prM and E genes are mixed with the plasmids. Their "sticky ends" join by base pairing. Then the two DNA parts are sealed with the help of the enzyme DNA ligase. They form a circular molecule, the recombinant plasmid that can serve as a transfer vector.
The plasmid used for the West Nile virus derives from another engineered plasmid which contains a gene promoter.

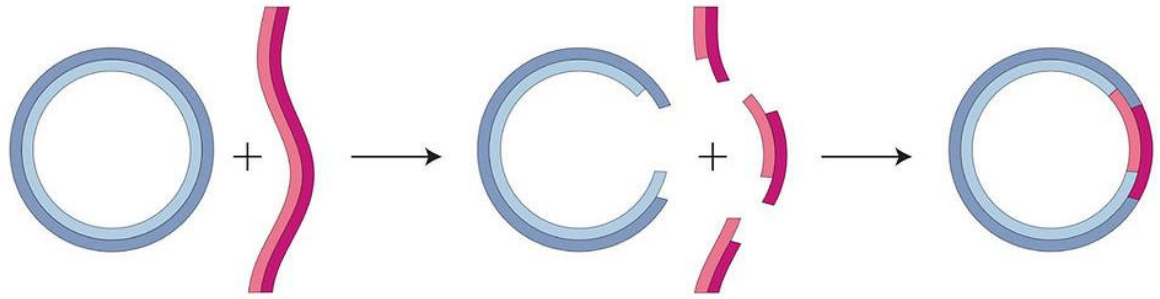


Fig. 5: Engineering of the transfer vector

3.3 Transfer of the vectors into bacteria

The engineered vectors are introduced into bacteria. To make the cell membrane of the bacteria porous or "competent", the bacteria are treated chemically, for example by exposing them to saline solutions such as $CaCl_2$. They can also be treated by thermal shock, electroporation, etc.(7) Then the plasmids are added to the bacteria and heated to 37°C. This makes the cell walls of the bacteria more permeable and also increases the chance for the vectors to enter the bacteria. If the procedure is successful, recombinant bacteria are formed.

3.4 Selection of the genetically modified bacteria[1]

As the transfer of vectors into bacteria is difficult, only some of the bacteria take up the vectors and are, thus, genetically modified. To distinguish the genetically modified bacteria from the others, marker genes are inserted into the plasmid, for example an antibiotic resistance gene. Bacteria that have taken up the recombinant plasmid will multiply when left on an antibiotic plate for several hours, the others will not show any signs of reproduction.

3.5 Multiplying the transformed bacteria

Bacteria which show antibiotic resistance and which are thus genetically modified are multiplied. By warming the bacteria to 37°C and adding LB-medium[2] ideal conditions are created. When a mother cell splits, not only its own DNA is passed to the daughter cells, but also the recombinant plasmids.

3.6 Purification

The bacteria must be freed from the LB-medium. They are then put into a lysis buffer, to break up the bacteria. The plasmid DNA must be separated from the chromosomal DNA of the bacteria. This is done by binding it to a silica membrane column. The plasmids are eluted and can be used for the vaccine.

3.7 Injection

DNA vaccines are injected intramuscularly (injection into skeletal muscle), dissolved in saline solution. Cases of intradermal injection (injection into dermis) take place with the advantage of the dermis containing more immune cells and thus leading to faster uptake and stronger immune response.
Another variant is the use of a gene gun, where helium particles aid plasmid DNA absorbed onto gold micro particles to enter the cells.(9)
Another possibility is a delivery via a mucosal surface, for example the nasal mucosa.

3.8 Immune response

The plasmid DNA is taken up by some of the cells around the injection site. The plasmid enters the nucleus. The promoter in the plasmid initiates the transcription of the DNA sequence into mRNA, which then goes back to the cytoplasm. In the cytoplasm the mRNA is translated, and the proteins of the virus are produced: the transmembrane protein and the envelope protein. They are expressed on the surface of muscle cells and on antigen presenting cells (APCs). The immune system doesn't recognise these antigens and thus raises a warning signal. This stimulates the production of antibodies that fight the antigens. Memory immune cells are formed. When a vaccinated individual is exposed to the virus later in its life, its immune system recognises the antigens and provides protection.

3.9 Special treatments of the plasmids

For a successful transcription and translation of the genes, it is important to design plasmids with a strong promoter. Besides, the insertion of a special intron can improve the stability of mRNA and therefore lead to a better protein expression.(10) Furthermore, it is crucial to avoid instability of plasmids. As this is often due to their noncoding backbone sequences, they should be avoided if possible.(11)

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[1] As we were not able to find any information on this point, we describe here one of the methods of selection used in other domains.
[2] Lysogeny broth: a commonly used growth medium containing NaCl, yeast extract and bacto-tryptone.(8)

4. Documentation and pictures of the contacted research institute

We were able to interview Dr. Philip V'kovski of RocketVax AG, Basel. The company is advised by Professor Volker Thiel and his team of the University of Berne. Dr. Annika Kratzel, member of the team, sent us some pictures of their lab. The interview can be found in the appendix of this paper.

Fig. 6: Dr. Philip V'kovski, RocketVax, Basel

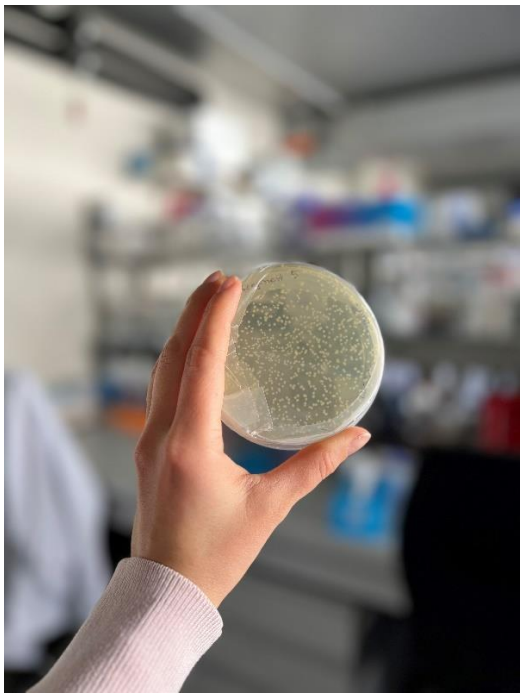

Fig. 7: E. coli colonies with integrated plasmids after selection

Fig. 8: Purification of the plasmid DNA in a silica membrane column (when put into a centrifuge)

5. Discussion

5.1 What progress was made with the application of DNA vaccines?

The first ever DNA vaccine was developed in 1986 and was used against the hepatitis B virus. But this was not the first vaccine against hepatitis B. In 1981, researchers developed a blood-derived vaccine against the virus, called “Heptavax”. The production technique used for “Heptavax” included collecting blood from an infected donor and inactivating the virus. This method was seen as risky, which is why it was removed from the marketplace in 1986. With the development of the new DNA vaccine method, the first low risk vaccine against hepatitis B was brought to the marketplace. It replaced the previous vaccine and is still of great importance until this day.(12)
DNA vaccines have been under development ever since. The focus for improvement was mainly concentrated on enhancing the immunogenicity of the vaccine. The first generations of DNA vaccines brought to the marketplace showed low immunogenicity. This meant that they did not provoke a high immune response against the pathogen and therefore didn’t provide much protection from future exposure.
A lot of progress regarding the enhancement of the immunogenicity was made through adjuvants. Adjuvants are added to the vaccine and enable a considerably higher immune response against the pathogen, making them a lot more effective than without.
Also, the development of an adaptive electroporation system (EP) has led to higher immunogenicity results. The EP technique is able to increase the permeability of cells by creating transient pores within the membrane. This allows the plasmid DNA to enter the cell a lot easier, therefore also increasing the effectiveness of the DNA vaccine.(13)

5.2 What future research steps will be made for DNA vaccines?

Vaccine technologies have been rapidly improving, especially since the outbreak of COVID-19. Even though DNA vaccines have made a lot of progress within the past few decades, there are still certain aspects of its immune response that are not quite understood by scientists. In comparison to other types of vaccines (for e.g. mRNA vaccines), the usage of DNA vaccines is not very common. In fact, DNA vaccines have only been approved by the FDA (U.S. Food and Drug Administration) for the use in certain animal diseases. There are only few DNA vaccines purposed for human use, as there is comparatively little research on DNA vaccines and not much scientific analysis.(14)
However, it is safe to say that in the future DNA vaccines will undergo further development. Not only has the outbreak of COVID-19 lead to a rapid increase in research being done on DNA vaccines, but it is also presumed that climate change will lead to an outbreak of potentially thousands of new viruses and infectious diseases. Therefore, one can assume that despite the challenges DNA vaccination faces, research and progress will definitely continue in the future.(15)

5.3 DNA vs. mRNA vaccines, pros and cons

Both DNA as well as mRNA vaccines are types of gene-based vaccines. The most fundamental difference between the two is that one uses DNA, the other uses mRNA as a template to produce antigens through protein biosynthesis. Both vaccine-technologies have various advantages, as well as disadvantages. DNA vaccines are considered a lot more stable than mRNA vaccines. They don’t have special storage requirements, such as to be stored at considerably low temperatures like the more fragile mRNA. This also makes transport a lot easier and saves costs. The development and production are also considered less complicated than with mRNA vaccines, as the purification of large amounts of DNA from the viruses is possible. This also adds on its inexpensiveness.(4) (16) (Interview V’kovski)
On the other hand, DNA vaccines have significant disadvantages. Before the viral proteins needed to provide immunity can be synthesised, the plasmid DNA must go through one

more step than with the mRNA. The plasmid DNA must cross through the cytoplasm and the nucleus membrane in order to reach the nucleus, in which it is transcribed into mRNA. Only after this extra step does the transcribed mRNA go back into the cytoplasm to be translated into proteins. With an mRNA vaccine, the process of protein biosynthesis is shortened considerably, because the mRNA only needs to reach the cytoplasm. Once it has entered through the cell membrane into the cytoplasm it is directly synthesized into the necessary proteins.
Because the protein synthesis for DNA vaccine is so much more extravagant than with the mRNA vaccine, it leads to noticeably lower immunogenicity. This is why DNA vaccines are generally reliant on an adjuvant or an EP.(17) (Interview V'kovski)

5.4 Threats and opportunities

For the production of DNA vaccines, safety plays a vital role. It is extremely important to avoid the risk of integration of the plasmid DNA into the host DNA. Besides, it must be avoided that the host forms anti-DNA antibodies, because this would lead to auto-immune diseases. It is also essential that genetic material is not spread to the environment, because just a few engineered plasmids or fragments of plasmids could lead to a transformation of the microflora. If vaccines are used for food producing animals, it is important to respect food safety. Another risk of DNA vaccines is that the use of plasmids may spread antibiotic resistance.
Despite these risks, the opportunities DNA vaccines offer must be kept in mind. They will hopefully be successful and prevent many people from diseases and death. Furthermore, research, production and sale of DNA vaccines lead to the founding of new companies and create new jobs and income. And because DNA vaccines are easy and quick to produce and don't require a special transport or a storage at low temperature, they will hopefully be affordable and available to people living in poor countries with hot climate and thus lead to a fair distribution of vaccines.

6. Summary

The rather new technology of DNA vaccines, first researched in the 1980s, is a branch of immunology with a promising future. New approaches to diseases with this pathogen tailored technique make it essential for the medical industry. Genetic material is extracted from pathogens, inserted into engineered transfer vectors, replicated and finally injected into the body of an individual where, when having entered the cell nucleus, it produces mRNA. The mRNA is translated in the cytoplasm into proteins which trigger an immune response. Later in its life, the vaccinated individual should be protected against the pathogen, because the immune system recognizes the antigens. Although some DNA vaccines were approved for use, such as some products for animals and, for human use, the vaccine against hepatitis B and the COVID-19 vaccine released in India in 2021, not many DNA vaccines have successfully passed clinical trials.(18) Despite many positive aspects of DNA vaccines, such as their low costs and easy transport and storage, the comparatively low immunogenicity provided accounts for the fact that its usage is still not very common. However, the lately increased research may bring new insights and lead to the breakthrough of DNA vaccines.

7. References

7.1 Bibliography

1. Polio vaccine. In: Wikipedia [Internet]. 2023 [zitiert 16. Februar 2023]. Verfügbar unter: https://en.wikipedia.org/w/index.php?title=Polio_vaccine&oldid=1139545026

2. Vaccine. In: Wikipedia [Internet]. 2023 [zitiert 16. Februar 2023]. Verfügbar unter: https://en.wikipedia.org/w/index.php?title=Vaccine&oldid=1139627314

3. DNA vaccine. In: Wikipedia [Internet]. 2023 [zitiert 17. Februar 2023]. Verfügbar unter: https://en.wikipedia.org/w/index.php?title=DNA_vaccine&oldid=1132735216

4. Genetic vaccine. In: Wikipedia [Internet]. 2022 [zitiert 16. Februar 2023]. Verfügbar unter: https://en.wikipedia.org/w/index.php?title=Genetic_vaccine&oldid=1118579108

5. author U authorUnknown. English: DNA vaccines are composed of a small, circular piece of DNA — called a DNA plasmid — that contains genes that code for proteins of a pathogen. When the vaccine is injected into the host, the inner machinery of the host cells “reads” the DNA and converts it into proteins from the pathogen. Recognizing that the proteins are foreign, the cells display them on their surface to alert the body’s immune system — both helper T cells, which spur the production of antibodies, and killer T cells, which kill infected cells outright. [Internet]. 2005 [zitiert 16. Februar 2023]. Verfügbar unter: https://commons.wikimedia.org/wiki/File:Making_of_a_DNA_vaccine.jpg

6. Recombinant Plasmid - an overview | ScienceDirect Topics [Internet]. [zitiert 16. Februar 2023]. Verfügbar unter: https://www.sciencedirect.com/topics/neuroscience/recombinant-plasmid

7. Gómez LA, Oñate AA, Gómez LA, Oñate AA. Plasmid-Based DNA Vaccines [Internet]. Plasmid. IntechOpen; 2018 [zitiert 16. Februar 2023]. Verfügbar unter: https://www.intechopen.com/chapters/61091

8. admin. LB (Lysogeny Broth) Medium [Internet]. Laboratory Notes. 2021 [zitiert 16. Februar 2023]. Verfügbar unter: https://www.laboratorynotes.com/lb-lysogeny-broth-medium/

9. DNA-Impfstoff. In: Wikipedia [Internet]. 2022 [zitiert 16. Februar 2023]. Verfügbar unter: https://de.wikipedia.org/w/index.php?title=DNA-Impfstoff&oldid=227211333#cite_note-Neuer_WHO-Leitfaden_zu_modernen_DNA-Impfstoffen-1

10. Alarcon JB, Waine GW, McManus DP. DNA Vaccines: Technology and Application as Anti-parasite and Anti-microbial Agents. In: Baker JR, Muller R, Rollinson D, Herausgeber. Advances in Parasitology [Internet]. Academic Press; 1999 [zitiert 16. Februar 2023]. S. 343–410. Verfügbar unter: https://www.sciencedirect.com/science/article/pii/S0065308X08601529

11. Oliveira PH, Mairhofer J. Marker-free plasmids for biotechnological applications – implications and perspectives. Trends Biotechnol. 1. September 2013;31(9):539–47.

12. Hepatitis B Foundation: History of Hepatitis B Vaccine [Internet]. [zitiert 16. Februar 2023]. Verfügbar unter: https://www.hepb.org/prevention-and-diagnosis/vaccination/history-of-hepatitis-b-vaccine/

13. The Development of DNA Vaccines [Internet]. News-Medical.net. 2021 [zitiert 16. Februar 2023]. Verfügbar unter: https://www.news-medical.net/life-sciences/The-Development-of-DNA-Vaccines.aspx

14. Sreenivas S. What to Know About DNA Vaccines [Internet]. WebMD. [zitiert 16. Februar 2023]. Verfügbar unter: https://www.webmd.com/children/vaccines/what-to-know-dna-vaccines

15. BBVA. Viruses and climate change: how the two threats converge | BBVA [Internet]. NEWS BBVA. [zitiert 16. Februar 2023]. Verfügbar unter: https://www.bbva.com/en/sustainability/viruses-and-climate-change-how-the-two-threats-converge/

16. Engineering DNA vaccines against infectious diseases | Elsevier Enhanced Reader [Internet]. [zitiert 16. Februar 2023]. Verfügbar unter: https://reader.elsevier.com/reader/sd/pii/S1742706118305002?token=70C2D063791093C7A87BC2F28D0CE5ACE4F2B60F7FD51744C354463ABCB359F2167862605387D2AD44583DBBE803377D&originRegion=eu-west-1&originCreation=20230216202225

17. How do DNA vaccines work? [Internet]. 2021 [zitiert 16. Februar 2023]. Verfügbar unter: https://www.medicalnewstoday.com/articles/dna-vs-mrna-vaccines-similarities-and-differences

18. Reuters. India gives emergency approval for world's first COVID-19 DNA vaccine. Reuters [Internet]. 20. August 2021 [zitiert 16. Februar 2023]; Verfügbar unter: https://www.reuters.com/business/healthcare-pharmaceuticals/india-approves-zydus-cadilas-covid-19-vaccine-emergency-use-2021-08-20/

7.2 Table of figures

Fig. 1: DNA vaccine https://en.wikipedia.org/wiki/DNA_vaccine

Fig. 2: Reverse transcription https://www.thermofisher.com/ch/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/rt-education/reverse-transcription-applications.html

Fig. 3: Genome of the West Nile virus https://www.bing.com/images/search?view=detailV2&ccid=EXvveMjp&id=9FEB1D58FBA177193A6682A854D2253ADE8D933A&thid=OIP.EXv

Fig. 4: Polymerase chain reaction (PCR) https://www.jidonline.org/article/S0022-202X(15)36139-X/fulltext

Fig. 5: Transfer vector https://www.byarcadia.org/post/genome-editing-101-gene-editing-techniques

Fig. 6: https://rocketvax.com/team/project-management

Fig. 7 and 8: Provided by Dr. Annika Kratzel, Group Volker Thiel, Federal Department of Home Affairs FDHA, Institute of Virology and Immunology IVI, in cooperation with the Vetsuisse Faculty of the University of Berne

7.3 Further reading

https://d2jx2rerrg6sh3.cloudfront.net/image-handler/ts/20210422091204/ri/673/picture/2021/4/shutterstock_1881778573.jpg

https://www.news-medical.net/life-sciences/The-Development-of-DNA-Vaccines.aspx

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7105045/
https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccines-quality/dna

https://cdn.who.int/media/docs/default-source/biologicals/vaccine-quality/guidelines-for-assuring-the-quality-and-non-clinical-safety-evaluation-of-dna-vaccines70ee1b3e-88a6-40af-8989-fbff8304a377.pdf?sfvrsn=521ee591_1&download=true

https://www.news-medical.net/health/What-is-a-DNA-based-vaccine.aspxct

https://de.wikipedia.org/wiki/DNA-Impfstoff

https://www.gelbe-liste.de/nachrichten/unterschiede-corona-impfstoffe

https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccines-quality/dna

https://www.intechopen.com/chapters/61091

https://pubmed.ncbi.nlm.nih.gov/21765081/

https://en.wikipedia.org/wiki/Vaccine

https://en.wikipedia.org/wiki/DNA_vaccinehttps://pubmed.ncbi.nlm.nih.gov/24432284/

https://www.news-medical.net/health/What-is-a-DNA-based-vaccine.aspx

https://pubmed.ncbi.nlm.nih.gov/17058502/

8. Appendix: Interview with Dr. Philip V'kovski, RocketVax AG, Basel

Dr. Philip V'kovski is a biologist and virologist. He studied infection biology and after his studies he went into research, primarily basic research for corona viruses.
He is currently working for RocketVax in Basel and supports the development of live-attenuated vaccines for SARS-CoV-2. Although DNA vaccines are not his current domain, he kindly offered to answer some of our questions regarding DNA vaccines.

Herr V'kovski, Sie arbeiten eben nicht mit DNA-Impfstoffen. Wieso würde bei Ihnen kein DNA-Impfstoff in Frage kommen?

Bei Impfstoffen ist es so, dass es viele verschiedene Strategien gibt. Es geht immer darum, das Immunsystem adäquat mit etwas zu stimulieren, das Sinn macht und sicher ist, also nicht gesundheitsgefährdend. Man möchte ja nicht die Leute krank machen oder ihnen etwas Schädliches geben. Man möchte sie im Gegenteil vor etwas schützen. Dabei gibt es verschiedene Methoden, um das Immunsystem anzuregen und dadurch eine gute Immunantwort zu erzielen. Bei Corona Viren hilft die mucosale Immunität sehr gut. Das kommt vor allem im Atembereich vor. Da möchte man eine gute Immunantwort erzielen, damit das Virus sofort blockiert ist.
Bei DNA Impfstoffen ist es so, dass man DNA nutzt. Die DNA soll in die Zelle hineingelangen, die dann ein Protein translatiert, sodass das Immunsystem ein fremdes Protein kennenlernt. Mit Coronaviren könnte man dies probieren, ein DNA Impfstoff wäre aber nicht meine erste Wahl, da er einfach nicht die passende Immunantwort abrufen würde.

Ist die Immunantwort denn zu langsam? Zu umständlich? Oder einfach allgemein nicht passend?

Es gibt verschiedene Immunantworten, z.B. machen DNA-Impfstoffe eine systemische Immunantwort, also Antikörper, die im Blut und in der Lymphe zirkulieren und dort schützen. Diese Antikörper gehen nicht genug in die Schleimhaut. Das sieht man an den jetzigen Impfstoffen, bei SARS-CoV-2, die schützen sehr gut gegen eine schwere Krankheit, aber nicht gegen eine Infektion. Für andere Krankheiten würde das vielleicht super passen.
Es geht somit immer um eine adäquate Immunantwort für ein adäquates Pathogen.

Welche Arbeitsschritte sind allgemein bei der Herstellung einer Impfung nötig und welche speziell bei Ihrer SARS-Impfung? Der Vergleich von der Herstellung einer DNA-Impfung zu Ihrer Vektor Impfung würde uns interessieren.

Plasmide oder Gene zu klonieren, bzw. DNA in grosser Menge herzustellen, ist ein allgemeiner Prozess. Und das macht man auch, wenn man Viren abschwächen möchte. Da suchen wir uns ganz genau aus, wie wir diese Viren abschwächen möchten. Es gibt verschiedene Mutationen, die man einbringen kann. Hier arbeiten wir tatsächlich mit Plasmiden. Und da entstand vermutlich auch das Missverständnis, dass wir mit DNA-Impfungen arbeiten, das wäre einfach insofern, dass wir DNA so modifizieren, wie es uns passt.

Was sind konkrete Vor- und Nachteile einer DNA-Impfung?

Der Vorteil ist, dass DNA sehr stabil ist. Das habt ihr vielleicht gelesen, DNA ist allgemein sehr stabil. Im Labor, unter Forschungsbedingungen, kann man sie für eine gewisse Zeit im Kühlschrank aufbewahren. Im Gefrierschrank ist sie noch länger stabil. Die Stabilität ist ein grosser Vorteil der DNA - im Vergleich zur RNA, die sehr fragil ist und viel schneller dekadiert als DNA. Ausserdem ist die Herstellung von DNA günstig im Vergleich zu Proteinen oder Viren. Da ist DNA eigentlich der erste Schritt [in der Entwicklung anderer Vakzine] und wenn man da aufhört, ist es billiger.

Lässt sich ein Kostenpunkt festlegen, wieviel eine Produktion einer DNA-Impfung in etwa kostet?

Sehr schwierig.

Bei Ihrer Impfung für SARS, was sind denn da so die Ausmasse?

Das kann stark variieren, es gibt sehr teure Schritte, es gibt einfachere Schritte. Es kommt immer darauf an, was für Quantitäten man möchte. Und dann ist es natürlich auch so, dass es sich dabei nicht um einen Stoff handelt, der aus irgendeinem Labor rauskommt. Das muss unter ganz präzisen Bedingungen gemacht werden, in einer Firma, die spezialisiert ist und wo alles extrem kontrolliert wird. Für Tiere, und für Menschen natürlich auch, ist sehr wichtig, dass jeder Schritt sehr gut charakterisiert und alles perfekt dokumentiert wird und dass nichts daneben gehen kann. Das ist ein grosser Aufwand und kostet Zeit und Geld.

Warum gibt es so wenige DNA-Impfstoffe?

Das ist eine gute Frage, denn das ist mir auch aufgefallen. Was ich denke, ist, dass eine Impfung angepasst sein muss an das Pathogen. Wenn die DNA nicht die richtige Immunantwort erregt, dann ist der Wirkstoff vielleicht einfach nicht passend. Ausserdem, bei der Injektion der DNA in den Muskel mit einer Spritze, ist es nicht garantiert, dass das Immunsystem reagiert. Und dann gibt es keine Immunantwort und somit auch keinen Schutz.

Ist die Immunantwort wahrscheinlicher bei RNA-Impfstoffen?

Ja. Bei den RNA-Impfstoffen gibt es Lipide, die sich um die RNA wickeln. Das hilft der RNA, um in die Zelle hineinzugelangen. Und sobald sie in der Zelle drin ist, wird sie translatiert und im Beispiel mit den SARS-2 Impfungen wird das Spike [Protein] von der Zelle translatiert. Das funktioniert ziemlich gut, da die RNA im Cytoplasma translatiert wird. DNA dagegen muss in einem ersten Schritt in die Zelle hineingelangen. Es ist auch besser, wenn man etwas dazugibt, damit sie besser in die Zelle hineinkommt. Wenn die DNA dann in der Zelle ist, muss sie in den Kern der Zelle, damit etwas passiert. Wie sehr die DNA das Immunsystem anregen kann, bezeichnet man als Immunogenität. Dafür braucht es diese vielen Schritte, in die Zelle hinein, in den Kern und erst dann translatieren. Diese vielen Schritte sind eher ein Nachteil.