Edible Vaccines

A new approach to oral immunization

Mikko Mutti, Sara Tran & Sarah Zepf-Freitag

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1. Preface

Vaccines are the leading treatment against infectious diseases and have accomplished incredible changes in the field of healthcare. Unfortunately, there are still millions dying in many developing countries since the required vaccines are either not reliable enough or unaffordable for the locals.

Most of us associate the word vaccine with "*healthcare*", "*protection*" but unfortunately also with "*uncomfortable injections*". Science now makes it possible for us to replace the negative association with the words "*food*", "*good taste*" and even with "*enjoyable"* due to new inventions in the industry of biotechnology. Biotechnologists have created the concept of edible and enjoyable vaccines and could so give the proverb "*an apple a day keeps the doctor away*" a whole new meaning.

In this paper we treat the subject of edible vaccines which is a vaccine produced by a genetically modified plant. The fruit or vegetables (e.g. banana, potato, tomato) of this plant are able to treat infectious diseases like Hepatitis B, Cholera and many more.

> *"One day children may get immunized by munching on foods instead of enduring shots. More important, food vaccines might save millions who now die for lack of access to traditional inoculants." – William H. R. Langridge (September 2000)*
>
> *"Let food be thy medicine and medicine thy food" – Hippocrates (460 BC – 370 BC)*

We chose this topic because we think everybody should have access to inexpensive vaccines and be able to live a healthy life. We were also fascinated by the idea behind edible vaccines and its enormous consequences for developing countries. The production and use of edible vaccines are still in its early stages but we believe they will be an important part of our future society.

But how is that possible? Are there any risks? Are edible vaccines going to replace conventional vaccines? What are the advantages and disadvantages of edible vaccines? All these questions and many more are going to be answered in the following paper.

2. Introduction

Vaccines are primary tools in programmes for health intervention for both, humans and animals. Despite the worldwide immunisation of children against diseases, there are still 20% of infants left un-immunised causing approximately two million deaths each year, particularly in the remote and poor areas of the world. In the last decade, there have been significant improvements in the field of medicine and healthcare due to the development of newer, safer and highly effective vaccines. Although, these new developed vaccines have an advantage over common conventional vaccines, there is also a downside to them.

The highly expensive costs, storage and transportation issues pose a disadvantage to these vaccines, which makes the access of developing countries to vaccines underprivileged. It would be helpful for human society if cost of production could be reduced and if they could be distributed without refrigeration. In addition, vaccines could not be very popular because of unavailability of electricity for its storage in remotest area in developing countries. So as an alternative, biotechnologists have come up with a concept in the recent years. It is the new approach to oral immunization by using plants as an efficient production system for vaccines. Expression of antigens and antibodies as vaccines against antigens of pathogens[1] in transgenic plants is a convenient and inexpensive source for these immunotherapeutic[2] molecules. In order to solve the limitations, researchers found a way to produce oral vaccines in transgenic plants. Therefore the concept of edible vaccines was born.

Edible vaccines are cost-effective, easy-to-administer, easy-to-store (no problem of refrigeration) and fail-safe. Therefore they create a very effective vaccine delivery system, which is being accepted in a sociocultural way, especially for developing countries.

All section of people can afford to buy plant-derived edible vaccines to prevent a large number of diseases like Hepatitis B virus (HBV) infection, which is probably the single most important cause of persistent viremia[3] in humans.

2.1 Definition

> *"Edible vaccines are nothing but genetically manipulated food containing organisms or related antigens that may provide active immunity against infection. Simplified edible vaccines are pharmaceuticals made of plant or animal." – Medical dictionary (2009)*

Edible vaccines against many microorganisms are being developed, with the goal of using them to vaccinate children in non-industrialised countries, where there are complications with the use of traditional injectable vaccines.

[1] Anything that can produce a disease.
[2] The treatment of a disease by inducing an immune response.
[3] The presence of viruses in the blood.

2.2 History

Dr. Charles Arntzen first developed the concept of edible vaccines in the 1990s. Soon after learning of a World Health Organization (WHO) call for inexpensive, oral vaccines that needed no refrigeration, Dr. Arntzen of the Arizona State University visited Thailand in 1992 unaware of how this would redirect his career. However, after observing a young Thai mother using a banana to calm her crying infant, he was struck with a startling and life changing idea. Plant biologists had already developed ways of introducing selected genes into plants and inducing the transgenic plants to manufacture the encoded proteins. Perhaps, he mused, food could be genetically engineered to produce vaccines in their edible parts, which could then be eaten when inoculations were needed.

> "*Edible vaccines were first tested on human beings in 1997, when scientists asked volunteers to eat anti diarrheal transgenic potatoes produced by the Boyce Thompson institute at Cornell University. After eating the potatoes almost all the volunteers produced antigens in their bodies just as if they had received as traditional anti-diarrheal vaccination. And they experienced no adverse side effects.*" *- Chetan D. M., Dinesh K. P. (2006). Health and Pharmaceutical Biotechnology (p. 71)*

Dr. Arntzen's vision on edible vaccines would have enormous advantages. The plants could be grown locally at low costs, using the usual growing processes of a given region. In addition the crops could potentially be grown indefinitely without farmers having to buy more seeds or plants each year. Locally grown vaccines would also solve the transportation and storage problems. Difficulties in transporting traditional preparations over long distances, keeping them cold on route and at their destination can be avoided. And at last, being edible, the vaccine would replace syringes. Due to a combination of dedication and applied biotechnology, Dr. Arntzen's inspired vision was on its way to reality. The success lies in building a link between plants, foreign DNA, and vaccines.

Today, most new vaccines contain a specific protein or set of proteins from a pathogen of interest and not the pathogen itself. A protective immune response can result from this more limited (and inherently less risky) exposure. Dr. Arntzen's invention, like most of the modern vaccines, is still build upon the same fundamental principle: If the immune system is trained to recognize a pathogen prior to infection, the disease can be prevented when the actual pathogen is confronted.

The concept of edible vaccines to prevent diseases is great news in the field of biotechnology and for people around the world. Nevertheless, the actual delivering of edible vaccines or any other vaccination protocols into the world is not that simple. What succeeds in the developed world does not easily succeed in the developing world.

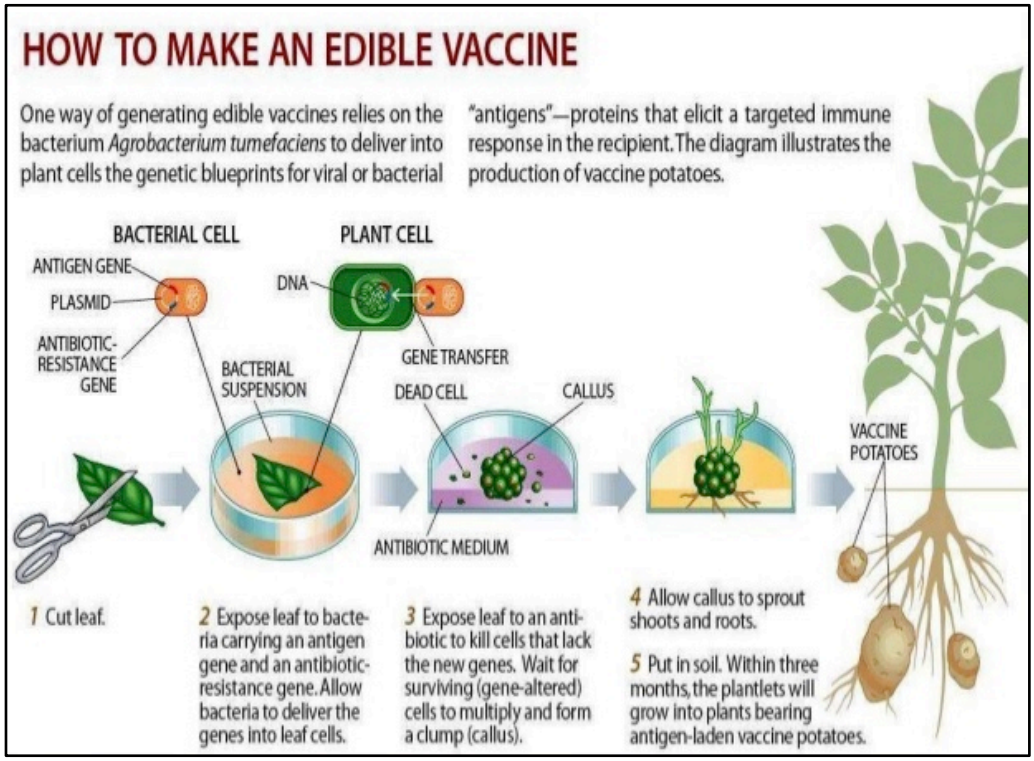
3. Description of engineering technique

There are various different techniques to create edible vaccines. One of the most commonly used method to generate edible vaccine is called Agrobacterium mediated gene transfer. It is based on the bacterium *Agrobacterium tumefaciens.*

Overall, the production of edible vaccines is based on introducing the gene of interest in the selected plant to create proteins encoding from the same gene. After accomplishing the transformation, the plant is now called a transgenic plant.

The appropriate gene construct is inserted into the T-region of a disarmed Ti plasmid of *Agrobacterium tumefaciens*. The gene of the Ti plasmid allows the bacterium transfer the DNA into plant cells and thus manipulates it genetically. With it, they trigger tumour growth and therefore plant diseases. The DNA, which has been recombined, is now placed into the *Agrobacterium tumefaciens*, which is co-cultured with the plant cells.

Products, which can be consumed raw, are the ones who are preferred to be used as edible vaccines to avoid degradation during cooking. Thus, plants like tomato, banana and cucumbers are often the plants of choice. This specific method works especially well for dicotelydenous plants like potato or tomato but there is also a downside causing low yield and the process is quite slow.



Picture 1: Description of engineering technique

4. Documentation

4.1 Interview with William H. R. Langridge

Our interview partner is William H. R. Langridge. He is a professor in the department of biochemistry and at the Centre for Molecular Biology and Gene Therapy at the Loma Linda University School of Medicine. The interview took place via E-Mail on the 5th of April 2016. We were not able to take any pictures of the lab because he lives in America. We did contact him and asked if it would be possible to send us some picture but he has not answered yet.

1. **When did you start your researches about edible vaccines? Could you tell us about your success in career in general?**

Started plant vaccine research in 1990. Success is a relative concept. There are ups and downs, but in my case generally ups. Our lab is now studying how the vaccines work as we have moved them from plants as a delivery system to vaccinia virus (DNA vaccine) and finally to bacteria (E. coli) as the vaccines can be generated rapidly in large amounts in bacteria.

2. **What was your motivation or reason that led to your research?**

Wanted to help people overcome disease in a simpler fashion that was ecologically friendly and not costly.

3. **What are the different steps of producing edible vaccines?**

Plants can be transformed using Agrobacterium in vivo transformation systems in which the bacterium does the transformation for you.

4. **What are the advantages and disadvantages of edible vaccines?**

The advantages are simple, vaccine-containing plants can be grown almost anywhere a plant can grow. The disadvantages are that you must test your plant tissues for vaccine efficacy and safety.

5. **Are there any side effects or risks with edible vaccines?**

They have not been tested in humans so the side effects remain generally unknown. However, they have been tested in animals (mice) and no unusual effects were noted.

6. **Which are the most valuable fruits/vegetables if it comes to edible vaccines?**

I am not certain whether there is a difference. However, it is much easier to generate plant-based vaccines in edible plants, especially in the tobacco family (Solanaceae) of which many species are food plants.

7. **When did you experience your first success and what was it like?**

The first time that I saw a protein band on a separation gel that I could recognize as the vaccine protein was a thrilling moment and has set the stage for all other vaccines being produced in plant tissues for me.

8. **How do see the future of edible vaccines?**

When people become less afraid to use recombinant plants (which in all these years have never hurt anyone) these vaccine generating plants will begin to find their place in the vaccine field, especially in developing countries where expensive vaccine preparations are less or unavailable.

9. **Do you think edible vaccines are going to be normal medical products?**

Eventually perhaps in 10-20 years.

10. **Do you think edible vaccines are going to replace conventional methods?**

Unlikely, but they will add to the existing methods and may replace some of the more conventional vaccines in vaccine regimes that involve immunization with many vaccines at one time. This may help to avoid dangerous conditions that may contribute to abnormalities such as autism

11. **What is the most recent news of your research?**

We are preparing to introduce our vaccines into "humanized mice" to test vaccine efficacy and safety in the human immune system in a non-human patient. This will be a step closer to introducing these vaccine into human patients. We are currently testing a vaccine that can prevent type 1 diabetes. This is a new type of vaccine that instead of stimulating an inflammatory response suppresses it and we hope it will work to suppress autoimmunity!

5. Discussion

The use of edible vaccines has different advantages but also disadvantages. Because the genetically modified plants can be produced in local area and the created vaccine does not have to be refrigerated, there are no expensive transportation and power costs. Therefore, the edible vaccine in form of a fruit or vegetable can be sold to an affordable price. The banana vaccine for Hepatitis B is expected to cost about 2 cents a dose whereas the currently available injectable vaccine for Hepatitis B costs $125 for a dose. Bananas are in general a very convenient fruit for producing edible vaccines. Bananas are grown in the tropic and subtropics, therefore in countries where cholera vaccine is so greatly needed. In addition to that, children love to eat bananas, which small babies can also digest.

However, the cost for injectable vaccines against Diphtheria, Tetanus etc. are so cheap now that there would be no reason to develop edible vaccines against these diseases. But on the other hand the use of syringes for vaccination can cause infections due to possible contamination of needles that have been used before. This risk may seem small but it is an upcoming problem in developing countries and could be minimized by using edible vaccines.

One of the disadvantages is the difficulty to control the dosage of the vaccine. The question is whether a high dosage will provoke oral tolerance of an invading bacteria or virus instead of an immune response. The dosage, which is needed for adults is also very different from the dosage needed for children. To determine the correct dosage, one needs to know the persons weight, age, the fruits/plants size, ripeness and protein content. In addition to that, the oral intake of vaccines is not convenient for infants and therefore it is probably better to concentrate the vaccine dosage in one teaspoon of baby food rather than in a whole fruit.

One of the main advantages of edible vaccines is, that they will trigger the immunity at the mucosal[4] surfaces such as those that line the mouth. It is a prevention of diseases instead of a cure. The oral mucosa is the body's first line of defence against different bacteria. Unfortunately before the oral intake, you first have to test the produced plant tissues for vaccine efficacy and safety. Scientists do not know yet, whether the consumed antigens will be able to survive the acid conditions in the stomach and even if they did, whether it will be possible for them to trigger the immune system in the right way. Experiments with humans have only shown promising results but it is still unknown what will happen if the subject comes in contact with the actual virus.

If you cross two plants, which produce different vaccines, you will be able to get a plant, which produces more than only one antigenic protein. This can lead to combination effects of vaccines and would be highly useful in developing countries like in Africa.

In the process of developing edible vaccines are many hurdles, as well. For example many of the first edible vaccines were successfully produced in potato plants, however raw potatoes are not very appetising and even poisonous. Unfortunately scientists discovered that during the cooking process of the potato almost 50% of the proteins in the vaccines broke down and are not working anymore. Therefore, they either have to increase the amount of vaccine molecules in the potato or search a new plant that can be eaten raw.

[4] Skin inside the nose, mouth etc. that produces mucus to protect itself.

5.1 Current status and future

Currently, small technology companies mostly do the researches, as edible vaccines are mainly designed to markets of developing countries. However the support of the researches are very small, since only few international aid organizations and national governments are rendering support, leaving the effort for edible vaccines under-funded. A further obstacle for the researches to continue is the lack of investors because of confidential issues. There has been several clinical trials with candidate vaccines. Antigen expressions in plants like tobacco, potatoes and tomatoes have turned out successful in the past. It is today not only used as a great prevention of infectious diseases but has also found its application in the human spectrum where it is used as a prevention of autoimmune diseases and others like cancer therapy and birth control. Plant-based vaccines have also been proven as quite effective in the animal industry, since expensive injections pose a significant burden in the livestock industry.

The future of edible vaccines may be affected by resistance to GM (genetically modified) foods but there is slowly growing acceptance of transgenic crops in both industrial and developing nations. Studies show that there has been a significant increase in transgenic[5] crops from 1.7 million to 44.2 million hectares from 1996 to 2000 as well as the growing number of countries using transgenic crops (from 6 to 13).

In further researches, it was shown that there is a possibility for genetically modified plants to produce vaccines against diseases, which do not have a vaccine yet. This could be a possible cure for life-threatening infections like AIDS, Diarrhoea or dental caries, which is a big problem in developing countries.

Although, edible vaccines are GM plants, it is hoped that these vaccines will avoid serious controversy since their purpose is to save lives. Even though no commercial plant-based edible vaccine is currently available, many scientists are anticipating that this vaccine, which is the best solution for so many people facing the risk of death from infectious disease, will be available in the near future.
In order for its success, science must pass the obstacles surrounding the production of an edible vaccine and the fears related to GM foods must be solved.

Closer understanding to edible vaccines is the most important link to the future. If we can build a sustainable and protected future with resources, which are already available in a very simple form, then the human adults and children will incorporate the vaccination system into their daily life by simply buying edible vaccines in food stores.

[5] Plant that contains genes from a different species

6. Summary

Edible vaccines are pharmaceuticals derived from the expression of antigens in transgenic plants. It is a concept, which has been introduced in the 1990s and developed by Dr. Charles Arntzen. It is a convenient vaccine delivery system for developing countries since the oral immunization has several advantages. The high costs, storage and transportation issues of conventional vaccines would vanish by using edible vaccines, however the dosage of the edible vaccine is difficult to control if you do not have any personal information like weight or ripeness/size of the fruit.

The plant-based edible vaccines are currently developed for a variety of human and animal diseases. For example trials of edible vaccines against hepatitis B have reported encouraging results but it will still take time until this concept finds its way to the market because further research is needed. A time period of 10 – 20 years is estimated until edible vaccines can be used as daily medical products. The future of the research depends on the people being less afraid to use recombinant plants.

This new concept of oral immunization could save millions of lives, especially in the poorer countries, where vaccination against infectious diseases is needed. Edible vaccines are used as a protection against diseases rather than a cure.

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