Review article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Current updates and research on plant-based vaccines for coronavirus disease 2019

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The primary outbreak of severe acute respiratory syndrome coronavirus 2, causing pneumonia-like symptoms in patients named coronavirus disease 2019 (COVID-19) had evolved into a global pandemic. COVID-19 has surpassed Middle East respiratory syndrome and severe acute respiratory syndrome in terms of rate and scale causing more than one million deaths. Development of an effective vaccine to fight against the spread of COVID-19 is the main goal of many countries around the world and plant-based vaccines are one of the available methods in vaccine developments. Plant-based vaccine has gained its reputation among researchers for its known effective manufacturing process and cost effectiveness. Many companies around the world are participating in the race to develop an effective vaccine by using the plant system. This review discusses different approaches used as well as highlights the challenges faced by various companies and research groups in developing the plant-based COVID-19 vaccine.

Keywords: COVID-19, Pandemic, Plant system, SARS-Cov-2, Vaccine

Introduction

The primary outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China in December 2019, causing pneumonia-like symptoms in patients named coronavirus disease 2019 (COVID-19) had since evolved into a global pandemic, threatening public health after infecting more than 50 million individuals and causing more than one million deaths across the globe [1,2]. Similar to the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) outbreak back in 2002 and 2012, COVID-19 is caused by a pathogenic coronavirus that targets the bronchial epithelial cells in the human respiratory system [3,4]. However, the rate and scale in which COVID-19 is spreading global far surpasses those of SARS or MERS. The absence of specific antiviral drugs or therapies against human coronaviruses stresses the importance of preventative measures in managing the spread of the virus [5]. Despite various countermeasures in place to curb the spread of the disease in many countries, including social distancing and mandatory face masks, the number of positive cases are still on the rise. Hence, the development of an effective vaccine to combat the spread of COVID-19 infections is paramount in containing the disease and suppressing the emergence of new infection cases. In addition, the vaccines are required to be manufactured at low cost with high outputs to meet the global population demand.

Among the many types of vaccine development methods available, plant-based vaccines had been gaining attention among scientists to be an effective way of producing large scale vaccines [6,7]. Plant systems are advantageous in the production and delivery of vaccines due to the introduction of the oral route of vaccine delivery through edible vaccines, in addition to the cost efficiency in manufacturing plant-based vaccines [7,8]. Due to the rapid global infectivity of COVID-19, the rapid formation of target products that is offered by plant bioreactors is imperative in swiftly curbing the pandemic. The significance of edible plant-based vaccines in tackling diseases, especially COVID-19, was reviewed in detail by Sohrab [8]. Following that, this review aims to discuss the recent developments in producing plant-based vaccines for COVID-19.

Technologies Used in Plant-Based Vaccines Production

Plant-based vaccine productions involve various stages, such as introduction of COVID-19 antigen to the plants, selection of suitable antigen as the possible candidate for vaccine production and screening process. All these stages could be carried out by using various approaches such as *Agrobacterium*-mediated transformation, virus-like particle (VLP) technologies and others. The following topic in this article would discuss briefly about the various technologies used in plant-based vaccine production and applicable in COVID-19 vaccine production.

Virus like particles

VLPs are nanomaterials that are able to resemble the original natural virus, without being infectious and harmless to other organisms [9-11]. Due to this characteristic, VLPs are believed to be a promising candidate for antigen delivery to induce the immune system in humans [9,10]. Studies had shown that VLPs are capable in inducing host immune response, when there is lack of adjuvant, lesser adverse effect and develop polydisperse system [9]. Nevertheless, using the VLP approach in vaccine production has its own drawback, where VLPs are not capable to self-replicate as the actual virus, thus it needs to be produced at a larger scale, to provide adequate amount of dose to induce immune response [12]. Besides that, during the downstream process involving VLPs, it requires a lot of time and is not cost effective, as it involves density gradient technique to purify the VLPs [13]. Through the molecular biology approach, it is possible to design the VLP to accommodate more than one antigen to exhibit a better immune response at minimum scale of dose and this will help to cut down the cost of vaccine manufacturing. The prominent aspect of using VLPs in vaccine manufacturing is that not all VLP design has potential to be a vaccine candidate [9]. Some of the successfully developed plant-based VLPs are for hepatitis B that has been expressed in tobacco and potato plant, hepatitis E in potato plant and human papillomavirus in potato and tobacco plant [9]. Studies were also conducted to design VLPs encoding for COVID-19 mRNA and the effectiveness of the vaccine in promoting immune response of the vaccine has been studied in animal models. The result obtained from this study seems promising that using VLP approach will lead to a new era of plant-based vaccine [14]. At the time of writing, some companies are working on using VLPs to produce plant-based vaccine for COVID-19, which will be discussed further in this review.

Agrobacterium-mediated nucleus transformation

Agrobacterium-mediated nucleus transformation is one of the modern gene introduction method widely used in transgenic plants. *Agrobacterium tumefaciens* is a bacterium that is widely used as a vector in plant biotechnology to infect and incorporate the desired foreign gene into the plants tissue cell [15]. The basic mechanism of transformation occur in this approach involves *A. tumefaciens* carrying the desired gene that need to be transformed into the host and the nature of *Agrobacterium* in infecting plant cells. After infecting, the genetic material of *A. tumefaciens* would be transferred into the host cell, which enables transformation process to be easily performed in plants [6,15,16]. In vaccine production, the desired gene will be introduced into the *A. tumefaciens* by removing the tumorigenesis causing genes in the plasmid to obtain disarmed Ti-plasmid and the viral genes will be incorporated into the plasmid [6,16]. The plasmid will be integrated into *A. tumefaciens* by using *vir* gene and the plasmid will be transformed into the host by the bacterium infecting the host cells [6]. *Agrobacterium*-mediated transformation is widely accepted by the researchers due to its benefits where, it possesses incorporation of well-defined DNA fragments, efficient production rate of the transgenic plants and cost efficient [15,17]. Some of the successfully expressed antigen in transient plants via *Agrobacterium*-mediated nucleus transformation are cholera toxin B subunit protein, hepatitis B surface antigen Norwalk virus capsid protein [6]. At the time of writing, some companies are working on using *Agrobacterium*-mediated transformation to produce plant-based vaccine for COVID-19, which will be discussed further in this article.

Bioinformatics-assisted vaccine designing

Bioinformatics is one of the multidisciplinary approaches where it focuses on computer science and biology. One of the focuses of this field involves the structural and functional studies of genetic materials and to perform in silico analysis [18,19]. This scope of bioinformatics will be helpful in vaccine production, as bioinformatic tools will help to reduce the time in the antigen and the epitope selection process [18,20]. It has to be highlighted that bioinformatic tools used in this technology are only used in vaccine designing and selection process. The rest of the vaccine production does involve the conventional methods in the manufacturing. One of the prominent roles of bioinformatics tool in vaccine design is predicting the reactivity of the antibody epitope in the immune response and led to an efficient vaccine manufacturing process. This technology could be applied in plantibody and revolutionized the manufacturing process of plantibodies [20].

Reverse vaccinology is one of the widely utilized approach in the vaccine designing. Reverse vaccinology is a technique where bioinformatic tools would be used to design the vaccine by selecting the specific antigen or peptides required to induce immune response [18,21,22]. These selected peptide or antigen would be tested in mice to validate and further analyze the efficiency of the peptide or antigen [21]. Once the peptide or antigen is finalized, it can be incorporated into plant genome to produce plant-based vaccines. It is crucial that the genome sequence of the studied virus to be available to perform this technique. Reverse vaccinology saves a lot of time in vaccine production and allows to screen all multiple peptides at once to ease the selection process [18,21].

Overview of Current Efforts

Various companies and research group around the world are aiming to develop an effective plant-based COVID-19 vaccine to fight against this pandemic. Each of this companies and research group demonstrated their innovations in developing the vaccine based on their previous studies. Their ideas in developing the vaccine will be further discussed in the following section.

Medicago

Medicago, a Canadian biopharmaceutical company with headquarters in Quebec City, Canada has taken its spot among many other biopharma companies working hard to produce a viable and effective vaccine to combat the COVID-19 infection [23]. Medicago has been employing plants as a bioreactor for the production of its vaccine mainly due to the rapidity of the process, ability to complement the target strains accurately, ability to scale up and its versatility for the production of antibodies aside from vaccine using the plant-based technology [23]. The Quebec City-headquartered biopharma company is employing VLPs which are grown in *Nicotiana benthamiana*, a tobacco related plant species [24-26]. *N. benthamiana* is commonly used in biopharmaceutical production due to its ability to express gene sequences that are heterologous in nature [27]. The biotech company is utilizing genetically modified *A. tumefaciens* which are able to infect plants by integrating their DNA into the plant cells to produce the VLP [28,29]. These VLPs are structurally similar to viruses but differ in the sense that its viral DNA or RNA are absent making it replication-deficient [13], hence making it relatively safer compared to traditional vaccines. The vaccine will be able to stimulate an immune response towards the targeted virus without replicating within the patient.

The primary benefit of using *N. benthamiana* is the ease of the manipulation and agroinfiltration process where the genetically modified *A. tumefaciens* are infiltrated into the leaf tissue of *N. benthamiana*, assisting a high rate of transgene expression [16,30]. Thus, enabling VLP production at a higher rate. This technique is commonly utilized as genetic modification of the entire plant itself is not required. Medicago is partnering with GlaxoSmithKline (GSK) by using GSK's vaccine adjuvant technology for the purpose of boosting immune responses which allows lower doses of vaccine to be administered [25]. It was reported that promising results were obtained from Medicago's phase 1 clinical trials where its vaccine candidate developed antibody responses in the clinical trial volunteers after two doses of the adjuvanted candidate vaccine in a short-time period with relatively mild side effects [31,32]. Based on the promising phase 1 clinical trials data, with subject to the regulatory approvals, Medicago plans to progress to a phase 2/3 clinical trials [32,33]. The efficiency of the vaccine highly depends on the results obtained from phase 2/3 of the clinical trials to ensure it does not harm individuals whom are administered with the vaccine. Aside from the current efforts to produce a vaccine for the novel COVID-19, Medicago has successfully produced a plant-based Quadrivalent VLP Influenza Vaccine using its Proficia plant-based technology [16,23]. A previous success of producing vaccine via a plant-based approach provides confidence of a potential development of a plant-based vaccine for COVID-19.

Kentucky Bioprocessing

Kentucky BioProcessing Inc. (KBP) is primarily known for utilizing tobacco plants for the production of vaccines along with other biopharmaceutical products [34]. It is a Reynolds American Inc. subsidiary American biotech company operating in Owensboro, Kentucky, United States and is also one of the members of the British American Tobacco Group [35]. In the efforts of developing a COVID-19 potential vaccine, similar to Medicago, KBP has utilized *N. benthamiana* as a bioreactor for the production of the target protein [36]. The widely used agroinfiltration technique is also employed by KBP to infiltrate the tobacco leaves with the genetically modified *A. tumefaciens* to enable the production of the target protein [37]. This indirect gene transfer technique increases the rate of transgene expression. This technique is similar to the method used by Medicago as both the companies utilize tobacco-like plants for the development of their candidate COVID-19 vaccines. The general principle is to introduce particles that are structurally similar to the virus but are incapable of replication making it non-infectious to stimulate an immune response.

Apart from the current COVID-19 vaccine development, KBP has successfully developed ZMapp for treatment of Ebola [38]. Thus, providing a higher confidence in the potential success in COVID-19 vaccine development. Soon after decoding the genome of SARS-CoV-2 virus early in 2020, pre-clinical works began. KBP reported that its candidate vaccine showed a positive result with stimulation of immune responses in its pre-clinical trials [39]. Proceeding from its pre-clinical trials, KBP has registered for phase 1 clinical trial in July 2020 and has yet to begin its recruitment process as of the time this article is written as it awaits approval from the U.S. Food and Drug Administration [40].

iBio

A Texas based biotherapeutics company, iBio is developing its potential COVID-19 vaccine, IBIO-200 and IBO-201 [41]. iBio is known for its FastGlycaneering and FastPharming systems which gives it a major advantage in both expandability and speed in producing therapeutics [41]. The company is using its FastPharming system to rapidly produce the VLPs by agroinfiltration of genetically modified *Agrobacterium* into *Nicotinia benthamiana* very much similar to the approaches used by Medicago and Kentucky BioProcessing [42]. However, iBio's FastPharming in place merges a few different technologies and approaches to enable the rapid production of high-quality proteins by combining automated hydroponic system, vertical farming technique to optimize the growth and the use of glycan engineering technologies [42]. These combinations make the system a very systematic and effective system to successfully produce vaccines. With this system in place, iBio has the ability to produce its SARS-CoV-2 VLPs in only a few weeks [43]. In its pre-clinical results, the candidate vaccine IBIO-201 was reported to possess the ability to stimulate an immune response against the SARS-CoV-2 virus, neutralizing it [44]. The company's LicKM technology, involving the use of a booster would potentially aid in the development of the vaccine as it could enhance the immune response against the virus [44]. This enables the vaccine to be administered at a lower dose to achieve similar expected immune responses. The IBIO-201 also showed a noticeably higher titers of the anti-spike neutralizing antibodies compared to its other candidate vaccine, IBIO-200 leading to its decision to select the IBIO-201 as its leading candidate vaccine [45,46].

Currently, the IBIO-200 and IBIO-201 is still in its pre-clinical stage of development. Apart from the two vaccines being developed, in August of 2020, iBio announced its worldwide license agreement with another biotech company, Planet Biotechnology Inc. in its efforts to develop a therapeutic for COVID-19 [47]. The therapeutic being developed is an antibody-based vaccine. The candidate vaccine utilizes angiotensin converting enzyme 2 (ACE2)-Fc, which is a recombinant protein made up from human ACE2 and the Fc fragment of human immunoglobulin G (IgG) antibody [47,48]. The principle behind the usage of ACE2-Fc is to neutralize the virus via the binding of ACE2-Fc to the receptors on the SARS-CoV-2 virus hindering it from binding to the ACE2 receptors found on the surface of healthy cells [49-52].

Zyus

The Canadian based biotechnology company, Zyus Life Sciences also participated in the efforts of developing a vaccine for the novel COVID-19 [53]. Zyus is known for its cannabinoid-based therapeutics [53]. Zyus is working with University of Saskatchewan's Vaccine and Infectious Disease Organization-International Vaccine Centre (VIDO-InterVac) to develop a vaccine for the novel COVID-19 using a species of plant which is closely related to tobacco, *N. benthamiana* [54,55]. VIDO-InterVac is a world leader in research and development of vaccine against infectious disease in both humans and animals [56]. In the efforts of exploring various possible techniques to produce antigen that could possibly be used to develop a vaccine, VIDO-InterVac has partnered with Zyus [57]. In its preliminary research, ZYus proved that vaccines can be developed using plant-based approaches by expressing antigens that could potentially stimulate anti-COVID-19 antibodies, providing protection to vaccinated individuals [55]. As of July 2020, Zyus reported its positive breakthrough in the vaccine development process. A potential antigen for the development of the vaccine was successfully expressed, isolated and purified [55]. Serum obtained from patients that have recovered from the COVID-19 infection was also used to test the antigen expressed. It was reported that their plant-based SARS-CoV-2 antigen was recognized by the antibodies found in the serum indicating the potential plant-based vaccine could provide protection against COVID-19 [55]. Although the development of plant-based vaccines is comparatively uncommon to other approaches of vaccine development, a plant-based approach is advantageous when it comes to scaling up [58].

Beijing CC-Pharming

Beijing CC Pharming is a China based biomedical technology research and biopharma product development company that has been established since 2017. It has been actively researching and focus on developing products by utilizing plant bioreactor technology. Plant bioreactors involves utilizing genetically modified plants to produce desired bioproducts such as peptides and viral antigens, which can be used for treatments and other causes [59]. CC Pharming has joined the race in developing a vaccine to COVID-19 during this pandemic and decided to partner up with iBio in February 2020. CC-Pharming and iBio had been working together since 2018, which has led them to this new project in this current pandemic [60,61]. This collaboration is to study and produce COVID-19 vaccine that is purely based on plant bioreactor technology and uses VLPs technology [61]. VLP are nanomaterials that are synthesized to be a non-harmful material to humans, that has capacity to induce human immune system by producing proteins and antigens that resemble the actual viral peptides [11]. This approach will ensure the vaccine developed has high efficiency rate and a very low toxicity rate towards. Asides that, the major production of COVID-19 vaccine from this collaboration will be based on, iBio's Fast-Pharming technology, which was designed to provide medical assistances in a very short time during a pandemic [62]. If the vaccine has been successfully developed, CC-Pharming has agreed to aid iBio in testing the vaccine in China [60]. At the moment this article is written there are no information regarding the progress of the vaccine development by these two companies where, their current status or stage of research is still unknown but further updates regarding iBio other COVID-19 projects has been discussed earlier [63].

Centre for Research in Agricultural Genomics

Maria Coca and Juan Jose Lopez-Moya from Centre for Research in Agricultural Genomics (CRAG), a Spain based research center which emphasize on studying genomic components of plants and farm animals has planned to utilize their facility and proficiency in the field of plant biotechnology to develop COVID-19 antigens for vaccine production. The research plan that has been proposed will enhance the production efficiency, isolation and purification process. These researchers from CRAG have decided to work with other research centers which are The National Centre for Biotechnology, The Institute for Plant Molecular and Cellular Biology, and Center for Edaphology and Applied Biology of Segura to carry out validation test on animals and cultures [64]. The proposed approach on this COVID-19 vaccine by these two researchers from CRAG involves the genetic modification of a plant virus to produce the COVID-19 antigens. These researchers have successfully developed antifungal proteins in tobacco plant in their previous studies, where the exact similar approach will be used upon this COVID-19 antigen production. This study is believed to not only be limited to tobacco plant but also includes lettuce [64]. This development could lead to an oral vaccine for COVID-19 where it is easily accessible around the world and could reduce the cost involve in the manufacturing process [65,66].

Newcotiana

Newcotiana is a project that has been fund by the European Union, that targets to substitute the nicotine present in tobacco plants with desired bio-compounds by applying New Plant Breeding Techniques (NPBT) to benefit the field of medicine and cosmetics [67-69]. Few commonly used NPBT by the researchers in Newcotiana are CRISPR/Cas9, agroinfiltration, grafting, and intra-genesis [67]. NPBT are techniques that are been developed to be applied in breeding genetically modified plants, where the utmost well-known is CRISPR/Cas9, where introduction of desired gene into the genomes is possible [70]. Due to the current pandemic situation, Newcotiana has also involved in the efforts of providing an improved COVID-19 vaccine manufacturing process and efficient plan in developing the antigens. Newcotiana has released the plant genome that they have been working on to the public with reasoning that it will help to counter any deficiency in the production capacity of the proteins needed to fight the COVID-19 [69]. In the research on developing vaccine for COVID-19, Newcotiana has chosen to work with *N. benthamiana* which is widely used tobacco plant in bio-pharmaceutical production. Newcotiana has modified the genomic sequence of the tobacco plants to boost its capability in producing bio-pharmaceutical compounds at a higher rate. This process requires, for the researchers to understand the *N. benthamiana*'s genomic sequencing, therefore led to Newcotiana to share it to public as mentioned earlier [71]. Researchers are confident in utilizing *N. benthamiana* to produce COVID-19 vaccine, as it also has contributed in other vaccine production previously such as Ebola [72]. Other than that, *N. benthamiana* is preferred by the researches for its rapid growth rate and capable of heterologous gene sequence expression, which is some of the few prominent factors to be selected as a bio-factory for vaccine production [27]. Newcotiana's plan would be involving the introduction of the desired genes into the plants leaves and the sap which will lead the plant to produce the antigens required for the COVID-19 vaccine and by this, the chances of producing huge amounts of protein would be higher in comparison to other methods [72].

Daniel Garza

Daniel Garza, a biotechnologist from Institute of Biotechnology of the Autonomous University of Nuevo Leon at Mexico has participated in the race of developing the COVID-19 vaccine by proposing to develop edible vaccine using genetically modified tomato plants [73,74]. The approach that is used to develop the vaccine will involve bioinformatic and computational genetic engineering tools to determine the possible antigens that could be expressed in the genetically modified crops [74]. The bioinformatic tool used will allow Garza and his team to identify the antigens that could lead to the activation of human immune response towards the antigen by utilizing the 'in silico' process. In silico approach allows the researchers to identify, design the antigens that is suitable to be expressed into the modified crops and to obtain better view of the antigen's properties. This approach will reduce the time spent on the selection process of the antigen in the lab before actually testing the antigen on test animals or cell cultures [75]. A specific method used by Garza and his team to identify the list of possible antigens that could be used for edible vaccine production involves reverse vaccination strategy. Reverse vaccination strategy comprises of sequencing COVID-19 genome and to search for genes that can contribute as a vaccine candidate [76]. These antigens can undergo in silico process for the selection process and can undergo isolation and further studies in cell culture and animal testing [75,76]. The successfully identified antigen gene sequence will be introduced to the tomato plant by performing *Agrobacterium*-mediated transformation. At the time of writing, Garza and his team are currently at the analysis and selection stage [74].

Main Challenges of Plant-Based Vaccines

The potential of plant-based vaccines against the COVID-19 pandemic is undeniable. However, the process of producing human vaccines through plants is easier said than done. The efforts of producing plant-based vaccines began as early as 1989, however to date, there are still no plant-based vaccines that have been approved for human use [6,77]. Clearly there are significant trials and tribulations in making plant-based vaccines available for public consumption. This effort is made significantly more difficult due to the novelty of the virus in question.

In general, the main difficulties in producing plant-based vaccines lie behind antigen and host plant selection, dosage consistency and Good Manufacturing Practice procedures, as reviewed by Laere et al. [6]. Of these, dosage consistency is a big concern when dealing with a pandemic where a large distribution of the vaccine is involved. It is intrinsically challenging to standardize the conditions and amount of products in a plant bioreactor, due to the numerous factors present tied to the usage of plants as a bioreactor, such as plant maturity, climate, and size. However, it is paramount that there is a degree of consistency in the vaccine produced that immunity can be conferred through consumption of the product, especially when public health is at stake.

The conference of immunity toward SARS-CoV-2 is another important concern to be raised in vaccine development. While convalescent COVID-19 patients may retain antibodies against the virus, studies show that the levels or neutralizing antibodies are not maintained in the long term, and are

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found to start waning around 3 months after infection [78]. In addition, reinfection cases had been reported several months after a first infection [78-80]. However, in a study conducted by Dan et al. [81] following 185 COVID-19 patients for several months post-infection showed that immunological memory to SARS-CoV-2 is able to persist for an extended period of time. There is still much to be uncovered regarding the correlates of immunity to COVID-19. This gap in data can impede efforts in determining the long-term efficacy of vaccines produced, in addition to creating false impressions among recovered patients that they are 'safe' from re-infections. Further research conducted on convalescent patients would yield beneficial data to the development of vaccines for COVID-19.

Conclusion

Researchers have conducted many studies in their work of developing an effective vaccine for the novel coronavirus. With the increasing number of COVID-19 cases, vaccines could efficiently contain the outbreak by reducing the occurrence of new infection cases. A number of different approaches including a plant-based approach have been used in the time consuming and complex process of vaccine development. Method of developing vaccines using a plant-based system has been gaining the attention of scientists especially due to its ability to produce vaccines in a larger scale. Several technologies are used in plant-based vaccine production including VLPs that resemble the SARS-CoV-2 virus, Agrobacterium mediated nucleus transformation to generate transgenic plants and bioinformatics to perform in silico analysis. This plant-based system has been used by many biotech companies and research institutes for the research and development of COVID-19 vaccines. As with every approach utilized, plant-based vaccines have its own challenges namely dosage consistencies, selection of antigen and plant host, manufacturing challenges and unknown immune correlates of COVID-19 patient. Despite these challenges, plant systems are advantageous in terms of its cost effectiveness and ability to produce high outputs. Several plant-based COVID-19 vaccines that are currently being developed have shown promising results in its preclinical and clinical phases indicating potential for a successful development of an effective vaccine.

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