Severe Acute Respiratory Syndrome Coronavirus 2 Virus-Like Particle and Its Application in Chinese Medical Research

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Abstract

Background: The global outbreak of coronavirus disease 2019 (COVID-19) has brought disastrous consequences to public health and medical systems, whereas no approved medications are currently available. Benefits of traditional Chinese medicine (TCM) against COVID-19 have been observed, however, the underlying mechanism actions remain unclarified. Due to high pathogenicity and infectivity of the new coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]), the lack of access to SARS-CoV-2 and biosafety level 3 (P3) facilities has impeded scientific investigations of TCM against COVID-19. Though low-pathogenic coronavirus and pseudoviral systems have been applied to substitute SARS-CoV-2 in fundamental studies, both models cannot imitate virological and clinical features associated with SARS-CoV-2. The virus-like particle (VLP) is a virological model that is safe and could be performed without biosafety protections. Aims and Objectives: To construct VLP of SARS-CoV-2 containing structural proteins of authentic viruses and resembling the morphology, partial life cycle, and immunoreactions of natural virions, and to introduce VLP into Chinese medical research. Materials and Methods: Using mammalian expression system, we have currently constructed SARS-CoV-2 VLP containing four essential structural proteins. Results: Based on this model, we propose six aspects of research that could be carried out for TCM formulas in the fight against COVID-19. Conclusion: Application of the VLP model provides a safe methodology to strengthen the response systems of Chinese medicine in preventing and controlling newly identified infectious diseases and offers collaborative opportunities for interdisciplinary deciphering of molecular and biological basis of anti-viral TCM formulas.

Keywords: Chinese medical research, coronavirus disease 2019, severe acute respiratory syndrome coronavirus 2, virus-like particles

The Lag-behind in “Chinese Solutions” against Coronavirus Disease 2019

The outbreak and pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been leading to significant loss of life, global social, and economic disruptions.[1] SARS-CoV-2 is highly pathogenic and could be easily transmitted through respiratory droplets. Since the first reported case of COVID-19, considerable research endeavors testing possible therapeutics have been intensively made, however until recently, there is still no approved drug available for COVID-19.[2] Given the current extraordinary circumstances of COVID-19 and the likelihood of re-emerging and even more frequent pandemics in the future, National Health Commission of the People's Republic of China have included Chinese medical formulas as special alternative medications in governmental guidelines for COVID-19 patients. A total of six traditional remedies have been advertised as potential COVID-19 treatments, including two prominent repurposing prescriptions being Lian-Hua-Qing-Wen, which was used in 2003 SARS pandemics, and Jin-Hua-Qing-Gan, which was developed during the 2009 H1N1 outbreaks. Although clinical investigations have proved that Chinese medical formulas are safe and efficient in mitigating symptoms and improving chest

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computed tomography images of infected patients, especially at the time when Western approaches appear to be ineffective in restricting the spread of viruses, the lack of mechanistic explanations of Chinese medical formulas substantially hampered their subsequent worldwide applications against COVID-19. Hence, the lag-behind in mechanistic proof urges molecular and biological evidence of Chinese medical formulas in order to be accepted as a “global solution” for COVID-19.

High dependency on authentic viruses and P3/P4 facilities impedes virological research

Infectious diseases have always been a threat to human life. In the recent 20 years, we have witnessed three global outbreaks of coronavirus-associated epidemics, namely SARS in 2003, Middle East respiratory syndrome (MERS) in 2012, and the current COVID-19. Despite the ongoing strengthened social-distancing measures and the continuous scientific research on coronavirus, the global medical community is still on its way struggling for therapeutic drugs against SARS-CoV-2. Indeed, a success in the research and development of a specific antiviral drug is a long and complex process. When compared to the pace and scale of drug development in cancer, the progress in virology research and antiviral development dramatically slow. The underlying obstacles are closely associated with the high pathogenicity of viruses and the stringent limitations in experimental conditions of virological research. In general, the acquisition, isolation, and evaluation of infectious pathogens, such as SARS-CoV-2 require laboratories of biosafety level 3 (P3) or 4 (P4) and professionals being specially trained. In China, it is usually the provincial and municipal Centers for Disease Control and Prevention (CDC) that is approved and supported to conduct such critical attempts. However, because the major responsibility of CDC is to control the introduction and spread of infectious diseases, and to provide consultation and assistance in promoting public health, more insightful virological and pharmacological studies are beyond their mission. In another aspect of this issue, top scientists who are competent to participate in the forefront events of novel technology, are usually employed by first-class universities or research institutes, where only limited or no P3/P4 laboratories are currently equipped. Overcoming such obstacles is in fact facing a variety of challenges. Firstly, constructing P3/P4 laboratories requires cumbersome verifications due to biosafety reasons. Secondly, even after P3/P4 laboratories being put into operation, the high maintenance costs, and insufficient space or equipment resources will also result in practical difficulties. Besides, psychological stress derived from self-protection during daily exposure to pathogenic viruses, and potential infection risks due to pathogen leakage additionally show the downside in studies with infectious viruses.

Lack of access to tools of virological studies hampered Chinese medical research in coronavirus disease 2019

The theory of Traditional Chinese Medicine (TCM) emphasizes treating disease before it develops. Therefore, characteristics of TCM, such as early interventions and combined regulation of multi-targets, greatly fit the principles of epidemic control. In the previous fight against SARS, the safety and efficacy of TCM have been recognized by the World Health Organization. Same in COVID-19 pandemics, TCM positively contributed to the prevention and control of SARS-CoV-2 infections in China. The TCM clinical participation in COVID-19 has proved its efficiency and effectivity in improving cure rate, shortening course of disease, slowing down disease progression and reducing death rate. Therefore, since the third edition of Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia, released by the National Health Commission of the People’s Republic of China, TCM has been adopted as one of the recommended therapies. However, with the success in the combat against COVID-19 using TCM and the failure of potential antiviral drug remdesivir in clinical trials, foreign scholars claimed in Nature that China was promoting coronavirus treatments based on unproven traditional medicines.[3] More specifically, they upheld that the design and conduct of TCM clinical trials failed to provide high-grade medical evidence, though a long list of TCM therapies has been applied to a variety of disorders. In addition, because the multi-component and multi-target concept of TCM is distinctive from the individual-molecule and single-target strategy in western medicine, molecular evidence indicating synergistic effects of multiple herbs remains largely unclarified.[3] In the face of global questioning, scientists supporting TCM are urgently required to reinforce virological studies for mechanistic deciphering of TCM formula.

The situation in virological research is far from being satisfactory in the TCM community. Currently, in most TCM universities or research institutes, no high-level biosafety experimental facilities for studies of anti-viral TCM are in continuous operation. In particular, the complex diversity of pathogenesis and clinical manifestations of COVID-19 suggest that investigations in TCM against SARS-CoV-2 require not only TCM researchers and virological expertise, multidisciplinary perspectives and collaborative efforts such as nanoscience should also be included. Therefore, a lack of access to high-pathogenic authentic viruses and P3/P4 facilities means we may have failed already from the starting line. In this scenario, it is of prime importance to break this bottleneck by establishing experimental models in order to shift from dependency on pathogenic viruses to a safe, feasible, and reliable practice.

Substitutitive Models for Severe Acute Respiratory Syndrome Coronavirus 2

Low-pathogenic human coronaviruses as alternative models for severe acute respiratory syndrome coronavirus 2

To date, there are seven coronaviruses that are known to affect human. Except for SARS-CoV-2, SARS-CoV, and...
MERS-CoV, which pose more serious risks, the other four coronaviruses (human coronavirus 229E [hCoV-229E], NL63, OC43, and HKU1) usually cause mild flu-like symptoms. As a rapid response to COVID-19 outbreaks, some groups have employed the low pathogenic hCoV-229E as an alternative safer model to replace SARS-CoV-2, in order to provide more pre-clinical anti-viral evidence of TCM. However, being a member of alphacoronavirus genus, hCoV-229E is dramatically distinctive from the betacoronavirus genus SARS-CoV-2, in terms of host receptor and pathogenicity. For instance, hCoV-229E invades host cells by using human aminopeptidase N as receptor, whereas SARS-CoV-2 enters host cells via angiotensin-converting enzyme 2. Besides, unlike highly contagious and virulent SARS-CoV-2, which may cause persistent fatigue, trouble in breathing and cardiac issue, people infected by hCoV-229E usually have mild illness and are able to recover with rare sequelae reported. Based on the above-mentioned viral and clinical features, it is clear that hCoV-229E and other low-pathogenic human coronaviruses are not ideal to be used as a substitute for SARS-CoV-2 research.

**Pseudo-virion system has its drawbacks**

When working with highly pathogenic and infectious viruses like SARS-CoV-2, a high degree of protection is of utmost importance and should be performed in standard P3/P4 laboratories. The pseudo-viral system, which usually utilizes adenoviral or lentiviral vectors following biosafety level 2 (BSL-2) practices have been used as experimental models to study entry events of enveloped viruses, including SARS-CoV-2. Although apparently safe, the adenoviral or lentiviral vectors maintain low risks of infection. Besides safety issues, as pseudo-viruses generally contain only one structural protein of the native virus, such as spike protein(S) in SARS-CoV-2, functions of other envelope proteins and the associated protein–protein interactions are prone to be overlooked when more than one structural protein are involved in the authentic viruses. The debate about pseudo-viruses has gained further prominence with many arguing that the immune responses induced by pseudo-viral system is more likely to be derived from the vector virus instead of the one being studied. Therefore, mechanistic evaluations associated with immune responses should be taken with cautions.

**Virus-like particles as safe and effective model for severe acute respiratory syndrome coronavirus 2**

**Molecular components of viruses**

Viruses are not organisms in the strict sense, but are small obligate intracellular parasites possessing an intimate relationship with living organisms. A complete virus particle, known as a virion, consists of nucleic acid genome, which is surrounded by a protective coat of protein called capsid. When reproducing in host cells, viruses can have an external lipid “envelope” captured from host cell membrane. Regarding each components of virions, the viral core of nucleic acid containing genetic materials is responsible for viral replication and protein synthesis after invasion. Viral envelope is made up of a lipid bilayer embedded with viral structural proteins, including viral glycoproteins. These glycoproteins bind to specific receptors and coreceptors in the membrane of host cells and play a critical role in virus-to-cell fusion when initiating invasion.

**Viral lifecycle**

Due to lack of enzyme system, which is essential for viral replication, viruses can only replicate by commandeering the reproductive apparatus of host cells. Many viruses experience several stages when infecting host cells. These stages include attachment, penetration, uncoating, biosynthesis, maturation, and release. Firstly, to initiate infection, viral structural proteins on the envelope interacts with specific cell surface receptors followed by endocytosis, which eventually ends up by uncoating and releasing viral genetic materials into host cytosol. Afterward, the virus utilizes the reproductive apparatus of the host cells to complete biosynthesis. With newly produced viral materials, progeny viruses will be packaged by self-assembly, and then be liberated from host cells into extracellular space to induce another infection cycle. To correlate virus lifecycle with its components, viral structural proteins embedded on envelope take charge of the beginning entry and the ending release stages of viral life cycle, whereas the nucleic acids carrying viral genetic information are responsible for replication.

**Virus-like particles**

Because viral genetic material is the crucial component that determines infectivity of viruses, whereas viral structural proteins which mediate both entry and release stages of viral lifecycle are irrelevant to viral pathogenicity, researchers have started to reconstitute nonpathogenic virions that are depleted of nucleic acids. In fact, genetic material-free viruses naturally exist. For example, the complete Hepatitis B virus (HBV), which is a partially double-stranded DNA virus, present in pleomorphic forms under electron microscopy, including Dane particles, filamentous particles and spherical particles. Typically, due to the absence of the HBV core, polymerase and genome, the spherical particles have a noninfectious nature, indicating that a reconstitution of VLPs derived from other viruses is highly feasible.

In virological studies, VLPs have been applied in studies of key processes of viral life cycle. Because VLPs do not contain genetic materials and regulatory proteins, that determine the pathogenicity of viruses, they are replication-deficient and noninfectious, thus can be performed in normal laboratory settings without biosafety protection. Morphologically, VLPs are hollow protein particles between 20 and 200 nm formed by self-assembly of single or multiple capsid and/or envelope proteins of the virus under certain spatiotemporal conditions. For this reason, VLPs are able to not only mimic the morphological structure of natural viruses but also preserve all viral functions mediated by natural viral structural proteins, such as recognition of receptors, endocytosis process and self-assembly. In this light, VLP constitutes a relevant model.
in molecular studies of virus entry and virion egress. In addition, as VLPs are formed by self-assembly that naturally occurs when partial or all viral structural proteins are optimally co-expressed in permissive cells, the resulting VLPs can fully represent the original morphologic and immunogenic features of the natural virus. For this angle, VLPs are currently the safest and most effective substitutions for authentic viruses, used in investigating virus–host interactions and development of antiviral drugs. In spite of virological studies, the repetitive and high-density display of epitopes officially render VLPs a promising vaccine candidate against many infectious diseases. Technically, in constructing VLPs of more than one structural protein, cell-based expression systems can be used by co-transfecting with a polycistronic vector or multiple monocistronic vectors. The advantage of the latter is to individually manipulate each envelope protein in order to understand the essential details that mediate VLP formation and release.

**Methodologies in Constructing Severe Acute Respiratory Syndrome Coronavirus 2 Virus-Like Particle**

The first VLPs were derived from HBV by expressing in either *Escherichia coli* or *Saccharomyces cerevisiae* during the 1980s. Until the beginning of this century, enveloped VLPs derived from more complexed viruses, such as influenza viruses or Ebola virus, were started to be reported. To construct VLPs, one needs to obtain gene sequences encoding viral structural proteins. Then, based on genetic sequences, molecular biology is required for the synthesis of targeted viral proteins followed by self-assembly of viral particles. Afterward, purification needs to be conducted to remove impurities from produced VLPs. To verify a successful VLP construction, morphological structure, structural protein composition and biological properties can be succeeded. Among various steps of VLP construction, selection of expression host system is one of the most important sections. A variety of expression systems have been utilized to construct VLP, including mammalian cell lines, bacteria, insect cell lines, yeast and plant cells. Although several expression systems are available for large quantities of VLP productions, each system differs with a variety of merits and drawbacks. While the complexity of construction and applications can often be problematic for mammalian systems, the correct protein glycosylation and folding patterns that greatly distinguish mammalian cells from other expression systems are actually of critical for viral infectivity.

**Bacterial expression system**

Approximately 30% of VLPs are produced in bacterial systems, using *E. coli, Pseudomonas* or *Lactobacillus*. For example, bacterial expression system has been applied for the development and production of VLP-vaccines against human papilloma virus (HPV) and cytomegalovirus. Normally, genes of viral structural proteins are codon-optimized for bacteria and cloned into commercial plasmids under the control of strong promoters to ensure high production of recombinant proteins. Advantages of bacterial expression system are high-level expression, easy operation, and low cost. However, the main drawback is its inability in posttranslational glycosylation modifications of recombinant proteins as compared to eukaryotic cells. In addition, the potential endotoxins contaminations will have great impacts on subsequent biological applications.

**Yeast expression system**

Yeast expression system is also widely used in the production of VLPs and has been applied to produce VLP-vaccines against HPV and HBV. Similar to bacterial system, yeast expression system is highly productive, efficient, feasible, and cost-effective. Especially, yeast expression system can produce posttranslational modifications without endotoxin contaminations. Nevertheless, their posttranslational modification pattern is not exactly the same as that occurs in human.

**Baculovirus/insect cell expression system**

Similarly, using baculovirus/insect cell (B/IC) system, VLPs for Chikungunya and human immunodeficiency virus have been constructed. The large size of the baculoviral genome makes it amenable for insertion of large segments of foreign DNA and simultaneous expression of several viral structural proteins. Besides, B/IC expression system is capable of performing multiple modifications of proteins to facilitate the proper self-assembly of VLPs. Moreover, baculoviruses usually have narrow host ranges, mostly limited to one or a few closely related insects’ species, which makes it relatively safe for clinical use. Even so, enveloped baculoviruses are also produced at the same time with VLPs, making purification a difficult and expensive step to follow.

**Plant expression system**

Several lines from different plant species such as *Arabidopsis thaliana* and tobacco have been successfully used for VLP production. They can be cultivated in simple media, like mammalian and other eukaryotic cells, and are able to synthesize complex multimeric proteins and perform eukaryotic post-translational process. In addition, plant-expressed orally delivered edible VLPs vaccines have been shown to induce immunogenicity efficiently.

**Mammalian cell expression system**

Several mammalian cell types are suitable for VLPs production. Although mammalian cells suffer from low productivity in producing proteins compared to other systems, mammalian cells have the capacity of producing more complex and accurate post translational modifications. For this reason, mammalian cells are typically applied to produce complex enveloped VLPs composed of multiple structural proteins, such as SARS-CoV-2. The Chinese Hamster Ovary cell line, the African green monkey kidney epithelial cell line (Vero E6), and the human embryonic kidney 293 cell line are most widely used mammalian production platforms.
The mammalian cell expression system has already been used for the generation of different types of VLPs, such as hantavirus, rabies and influenza viruses. Recently, we have successfully constructed SARS-CoV-2 VLPs using Vero E6 cells [Figure 1] by incorporating structural spike (S), small envelope (E), membrane (M), and nucleocapsid (N) protein of SARS-CoV-2.[7,41] For detailed construction protocols and characterizations, please refer to Construction of SARS-CoV-2 VLPs by Mammalian Expression System.[42]

**Application of severe acute respiratory syndrome coronavirus 2 virus-like particles in Chinese medical research**

Investigations of most highly pathogenic viruses that could potentially result in pandemics must be carried out in laboratories above biosafety level 3. For example, Ebola virus requires P4 facilities, and SARS-CoV and its closely related virus SARS-CoV and MERS-CoV need P3 laboratories. However, the Wuhan National Biosafety Laboratory is the only national P4 level laboratory in China, and totally <40 P3 laboratories are in operation. The demand for high biosafety level platforms remarkably exceeds the current supply. In the face of uncontrolled global pandemics, to complete a large number of P3/P4 facilities in a short period of time is impractical, and due to rigid operating rules, scientific experiments are often unable to be fully conducted in such laboratories. Under the circumstance that the Chinese medical community has not been fully equipped with sufficient number of P3/P4 platforms and can not operate high pathogenic pathogens, development and application of VLPs provide alternative options to rely on. Based on biological features of VLP, we propose the following six fields in which VLPs may exert its significance.

**Traditional Chinese medicine versus viral life cycle**

To elucidate anti-viral effects of TCM, insightful information
of viral lifecycle is absolutely required. As described above, viral life cycle consists of attachment, penetration, uncoating, biosynthesis, self-assembly, and release, and except for biosynthesis, VLPs are able to mimic all the other five steps of the whole lifecycle. Therefore, by using SARS-CoV-2 VLPs, the anti-viral TCM could be explored in each stage of this viral life cycle.

**Traditional Chinese medicine versus virus-induced host immune responses**

As SARS-CoV-2 VLPs naturally induce early immune-responses of host cells associated with SARS-CoV-2 structural proteins, the immunoregulation of TCM against SARS-CoV-2 is another field that supports VLP utilization.

**Traditional Chinese medicine versus individually targeted viral structural protein**

Because VLPs may be packaged by all or partial structural proteins, one may construct SARS-CoV-2 VLPs by omitting one or two protein elements to form “knockout” models of SARS-CoV-2. This approach will be beneficial in the mechanistic studies of anti-viral TCM targeting specific SARS-CoV-2 structural components. For example, in our previous study, we have successfully constructed the influenza virus VLPs (flu-VLPs) composed of different structural factors. Application of these flu-VLPs has assisted us to find out that tauroloursodeoxycholic acid, an effective component of Chinese medicine bear bile, acts to inhibit influenza A viral infection by specifically disrupting viral proton channel M2.\(^[[15]\]

**Traditional Chinese Medicine versus viral morphology**

The laboratory-prepared SARS-CoV-2 VLPs resemble morphologies of authentic viruses, thus are helpful in understanding the basic morphological properties of parental viruses. Hence, employing SARS-CoV-2 VLPs would facilitate collaboration between structural biologists with TCM researchers with the ultimate goal of developing novel anti-viral drugs or deciphering molecular mechanisms of TCM formula or compounds.

**High-throughput screening of effective compounds of Traditional Chinese Medicine**

In pioneer studies, a green fluorescent protein (GFP)-expressing transcription and replication-competent VLPs system for Ebola virus has been successfully generated, which could be used under biosafety level 1 or biosafety level 2 conditions.\(^[[43]\]

The Ebola VLPs have been used for high-throughput drug screening. For example, a repurposing screen of approved drugs had identified 53 compounds that could block Ebola VLPs entry.\(^[[44]\]

Moreover, a screening of 795 unique three-drug combinations in Ebola VLPs entry assay had found two sets of three-drug combinations, toremifene-mefloquine-posaconazole and toremifene-clarithromycin-posaconazole, that could effectively block Ebola VLPs. Thus, GFP or luciferase incorporated SARS-CoV-2 VLPs could also be generated with similar strategies for viral tracing and high-throughput screening.

**Studies for broad-spectrum anti-viral Traditional Chinese Medicine**

Complex epidemics due to a mixture of distinctive viruses may possibly occur. In fact, epidemiologists have predicted an outbreak of seasonal influenza in the fall or winter seasons, which may mix with SARS-CoV-2 and worsen the current COVID-19 epidemic to a more complexed situation. Generally, in order to prevent viral recombination, which increases biohazard risks, experimental studies using two or more viruses simultaneously are not allowed within the same biosafety platform. This means that confirmation of broad-spectrum anti-viral effects of TCM would be an impractical work. This limitation could be easily overcome by using SARS-CoV-2 VLPs and flu-VLPs, two models mimicking bioactivities of SARS-CoV-2 and influenza virus, respectively. Therefore, simultaneous study of more than two pathogenic viruses would be realized especially for TCM, which is usually used as a broad-spectrum anti-viral therapy to treat different viral infections.

**Conclusions**

VLPs are self-assembled nanostructures incorporating key viral structural proteins. Because VLPs resemble molecular and morphological features of authentic viruses but is noninfectious and nonreplicating, application of VLPs to virological studies is beneficial to accelerate anti-viral TCM evaluations when lack of access to high pathogenic viruses and P3/P4 laboratories. The establishment of technologies in constructing VLPs of different pathogenic viruses, such as SARS-CoV-2, constitutes an essential component of emergency response system of TCM in emerging and re-emerging pandemics of infectious diseases and offers collaborative opportunities for interdisciplinary deciphering of molecular and biological basis of anti-viral TCM formulas.

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**Conflicts of interest**

There are no conflicts of interest.

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