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Vaccine development against coronavirus (2003 to present): An overview, recent advances, current scenario, opportunities and challenges

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ABSTRACT

Background and aim: The pandemic COVID-19 occurring due to novel emerging coronavirus-2019 (SARS-CoV-2) is severely affecting the worldwide public health, culture, economy and human social behaviour. Till date, there is no approved medicine/treatment to cure COVID-19, whereas, vaccine development efforts are going on high priority. This review aimed to provide an overview of prior art, recent advances, vaccine designing strategies, current scenario, opportunities and challenges related to development of coronavirus vaccine.
Method: A literature survey was conducted using Scopus, PubMed and Google Scholar with the search key as: coronavirus vaccine, SARS vaccine, MERS vaccine and COVID-19 vaccine. Articles related to above search query were retrieved, sorted, analyzed and developed into an easy-to-understand review.
Results: The genome phylogenetic analysis suggested that genomic sequence of SARS-CoV-2 is almost 80% similar to that of SARS-CoV, further both these viruses bind to same host cell receptor ACE-2. Hence it is expected that, previously available literature data about coronavirus vaccine designing may play crucial role in development of rapid vaccine against COVID-19. In view of this, the present review discuss (i) existing information (from 2003 to present) about the type of vaccine, antigen, immunogenic response, animal model, route of administration, adjuvants and current scenario for designing of coronavirus vaccine (ii) potential factors and challenges related to rapid development of COVID-19 vaccine.
Conclusion: In conclusion, we discuss possible clues/ target sites for designing of vaccine against SARS-CoV-2 virus based on prior-art.

1. Introduction

The novel coronavirus infection has been frequently emerging periodically in various countries around the globe which are of zoonotic origin and belongs to the family Coronaviridae [1–3]. These coronaviruses are specifically enveloped positive-sense single-stranded RNA virus which are particularly segregated into four various genera namely, α-coronavirus, β-coronavirus, γ-coronavirus and δ-coronavirus [4–6]. The endemic coronavirus infection was first identified around 1960, while till date various seven coronavirus infections are identified [4,5]. Four coronavirus infections (HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1) were endemic which causes mild illness involving immune-compromised systems, common colds and flu like symptoms [4,6]. Two coronaviruses infection SARS-CoV and MERS-CoV emerged in 2002-03 and 2012-13 respectively were epidemic which causes the lethal acute respiratory infections in humans and flue like illness [2].

More recently from December 2019, the novel coronavirus disease-2019 (COVID-19) is the current pandemic caused by SARS-CoV-2 virus which showing the symptoms like severe pneumonia, mylegia, headache, high fever, fatigue, dry-cough and dyspnea [7,8]. The isolation of this mystifying virus and phylogenetic examination demonstrated close similarity with SARS-CoV virus that appeared in year 2002-03 and hence refereed as “severe acute respiratory

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syndrome coronavirus-2" (SARS-CoV-2) [9]. As of now (May 31, 2020) almost 61,83,559 cases have been confirmed with COVID-19 with almost 3,71,364 fatalities around the world (in 212 countries) [10]. Till date no approved treatment is available for curing COVID-19, based on the drug repurposing and in-vitro inhibition strategy various drugs such as acyclovir, chloroquine, ganciclovir, hydroxychloroquine, remdesivir, ribavirin, lopinavir, ganciclovir and ritonavir are used to treat COVID-19, however none of the drug is approved by the FDA for the COVID-19 treatment [2,3]. Further, infectivity of SARS-CoV-2 virus is much stronger compared to SARS-CoV virus with the basic reproductive number 3.0 to 5.7 which indicate the spreading of infection of COVID-19 (from infected person) to next another 3.0 to 5.7 persons [11].

Thus, at present there is no effective drug candidate or specific treatment available for COVID-19 [2,3,7]. Further, high mortality rates, higher reproductive number, uncontrollable contagious nature and its potency to cause pandemic have grabbed a very serious attention of molecular biologist around the world towards development of rapid vaccine in order to control transmission and infection of SARS-CoV-2 virus. However, vaccine development involves several important steps such as antigen study, selection of effective antigen, antigen stability, screening study (animal model, route of vaccination, adjuvant selection), clinical trials on human, clinical trials data analysis, quality control, technology transfer, easy scale-up, universal approval, and high cost investment ($200–1000 Millions) which take at least 1.5–3 years (or more) to develop the vaccine [12–14]. In case of COVID-19 vaccine, the initial observations about full length genome phylogenetic analysis suggest that genetic structure of SARS-CoV-2 is almost 80% similar to that of SARS-CoV [9,15]. Hence it is expected that, previously available related literature data/experience and existing knowledge about vaccine designing attempts against the coronavirus (SARS/MERS) disease may be helpful to design rapid vaccine against COVID-19 [9,12–15].

In the present scenario, the prior art/experience of SARS-CoV vaccine development regarding to antigen, immunogenic response, use of animal model, challenge to animal model, route of administration and use of adjuvants may be have crucial and great importance in designing of rapid vaccine against COVID-19. In view of this, the present, review article (i) sorts and summarizes the existing information (from 2003 to 2019) about type of vaccine, antigen, immunogenic response, animal model, route of administration, and adjuvants for designing of coronavirus vaccine, (ii) proposes possible target clues for COVID-19 vaccine design (iii) discusses the present scenario of vaccine development against COVID-19 (iv) elucidate the potential factors for COVID-19 vaccine development and finally (v) presents challenges and opportunities for rapid vaccine development of COVID-19.

2. Vaccine development against COVID-19: The Universal high priority problem

The mission for designing of vaccine against COVID-19 is on high priority and considered as an essential global problem for a molecular biologist [16]. The vaccine designing attracted serious attention of the whole world with a generous anticipation in order to overcome from this pandemic outbreak [11]. Various reasons are attributed to develop the potential rapid vaccine on high priority.

Infection control: The fast globalization, increased international travel, immigration and drastic environmental changes led to increase appearance and spreading of novel viruses which may causes the chronic infectivity [15–17]. Vaccination is one of the important part of public health concern to combat various kinds of infectious diseases, that saved several lives in the medical history [17]. Effective vaccination is always important to break off the chain of virus infection as well as community virus transfer/transmission [2,3,16,17]. Further, vaccination can be used as a prophylaxis for the anti-viral treatment which boosts immune response against pathogen infection and offers protection from possible epidemic [17]. Moreover, public vaccination campaign also postpones various preventive measurement events such as social distancing, quarantine, lock-down, contact history and tracing etc [11,13,16,17]. Thus the fundamental objective of the vaccination is to acquire the innate immunity and to get protected against highly contagious pathogens like SARS-CoV-2 [16,17].

Future emergence: In the last two decades coronavirus outbreak seriously affected the human culture, life-style, natural human behaviour and economy throughout the world [2,3]. The Universal determinant for the coronavirus vaccine development has faded up as the SARS and MERS are no longer (extremely rare) seen after 2004 and 2013 epidemic outbreaks respectively (Fig. 1) [13,18]. However, at present there is an urgent need to develop the vaccine in order to curb the present pandemic [15–18]. Till date various six coronaviruses are known to infect human, while no vaccine is approved against coronavirus disease [6]. SARS-CoV-2 is the newly emerging seventh coronavirus and possibility for the further remerging mutated (eighth) novel coronavirus in near future cannot be rule out [2,3,6,13,15–17]. Hence there is an extremely urgent need to develop the coronavirus vaccine, in order to contain present pandemic as well as the possible future emergence of coronavirus outbreak [15–17].

Repurposing of the drug: There is no approved treatment as well as no approved drug is available for the COVID-19 till date [2,17]. Repositioning of the drug is the strategy that is applied for the treatment of COVID-19 based on in-vitro inhibition analysis and antiviral mechanism [2,3]. However, use of random drugs for COVID-19 treatment may develop resistance power of pathogen, may have lethal and detrimental side effects which restricts the direct use of anti-viral drugs and knock the door for designing of vaccine (as a better and safer option) [3,17].

Homology: In the initial period (January 2019) of the novel SARS-CoV-2 outbreak, much more was not known about the SARS-CoV-2 virus and it was the biggest challenge to know about actual viral structure, molecular biology, genome sequencing and phylogenetic relationship [2,9,14]. However, at present it is confirmed that, SARS-CoV-2 virus has similar kind of properties like SARS-CoV virus [9,16]. The phylogenictic study also showed almost 80% gene sequence homology of SARS-CoV-2 virus with SARS-CoV virus [3,9]. This may open another door of the hope regarding to rapid development of vaccine since, previous efforts of the vaccine

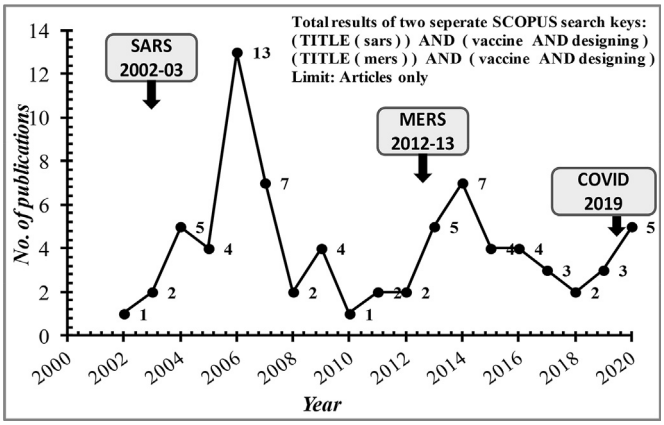

Fig. 1. Scopus related literature survey regarding to vaccine development against coronavirus (by date May 29, 2020).

developments are available in literature which may play a crucial role in rapid vaccine designing [13,15,16].

Mutation of virus: In general RNA viruses showed fast mutation rate, this may be responsible for uncertain immune response during re-emergence of mutated viral strain [19]. However, in case of the SARS-CoV-2 virus, mutation rate was observed to be slower and hence it may be hopefully possible that SARS-CoV-2 virus infection can be control by vaccination [19,20]. Recently, Guo et al. [20], reported long term perseverance of neutralizing antibodies of SARS-CoV infected health worker (year 2003) which increases the anticipation regarding to rapid vaccine development against SARS-CoV-2 virus [20].

Thus, considering all the above reasons, an efficient vaccine against COVID-19 may play a noteworthy role in controlling the spread of SARS-CoV-2 virus and hence the whole world is looking to get the successful vaccine as early as possible. However, vaccine designing is the challenging task which involves study of various factors such as determination of viral gene/protein/amino-acid, identification of effective antigen, route of immunization, animal model study, immune-response study, clinical trials, and safety concern etc. The specific efforts to design the effective vaccine have already been attempted/started while review of some previously reported literatures may play a crucial role in development of vaccine.

3. Vaccine strategy against coronavirus disease

Vaccines that mimic the natural infection are the most extraordinary achievement in the medical history of human beings which save several millions lives every year [21]. In public health sector, these vaccines have worldwide impact in improving the human and animal health and standard of living [22]. In vaccination, various antigen peptides in recombinant form or in derived form or inactivated pathogenic form are employed to induce cell-mediated immunity [21–25]. Thus, the vaccination is ideal platform to develop defence mechanism against infectious diseases considering its higher selectivity as compared to antimicrobial agents [21–25]. Further, effective and safe vaccination is very essential in playing a chief role to break off the chain of disease transmission from zoonotic (wild-life) reservoirs or infected person to vulnerable hosts [11,13,16,17]. Considering the zoonotic viruses disease such as coronaviruses diseases 2019, the in-vivo efficiency of developed SARS-CoV vaccine candidate may be helpful in looking at homologous gene sequence [9]. Nevertheless, the research related to development of SARS vaccine did not get its exclusive momentum, since, no new case has been reported in last 17 years [12,13].

In mean time, some research groups have developed some vaccine strategies against coronavirus diseases which include the live-attenuated, inactivated vaccine, protein subunits, viral vector vaccine platforms [26–79]. It is always a skilful, critical and challenging task to develop the vaccine within short period of time, which may take generally an average 1.5–3.0 years for possible successful designing of vaccine against newly emerging pathogen [8,15–17]. The hurry/rush/race in development of fast-track vaccine (under any influence) may be dangerous [15,16]. Looking at this urgent need, several previous coronavirus vaccine designing attempts/literature cannot be ignored which may offers a possible significant clue to deliver a successful vaccine against COVID-19 within a short period of time. The antiviral vaccines development strategies include first generation vaccine (live-attenuated and inactivated vaccine), second generation vaccine (protein subunit and vector base vaccine), and third generation vaccine (nucleic acid and nano-material based vaccine).

3.1. First generation vaccine

3.1.1. Live attenuated vaccine

Historically, live attenuated vaccines have always received great importance because of its quickly available high immunogenic response due to presence of natural antigenic material [21]. It is successfully used against various infectious diseases such as polio, rubella, chicken pox, and mumps etc [21,26]. Further live attenuated vaccine possesses the great capacity to deliver/present different kinds of antigens across the virus life-cycle in their parent conformations [26–30]. This is the first generation vaccine, various efforts have been reported to develop the live attenuated vaccine in the past against coronaviruses [26–34] (Table 1, entries 1–9). Bukreyev et al. [26], developed an experimental live-attenuated SARS vaccine for direct immunization which was showed good immune response (production of neutralizing serum antibodies) in immunized eight African green monkeys [26] (Table 1, entry 1). Kapadia and co-workers [27] designed recombinant attenuated vesicular stomatitis virus vaccine expressing SARS-CoV spike protein which displaying the passive antibody transfer induced by the vaccine to prevent SARS-CoV infection [27] (Table 1, entry 2). Netland et al. [28], designed live attenuated vaccine by deletion of accessory protein and E gene which showed full and partial protection in BALB/c mice and hACE2 Tg mice respectively from SARS-CoV infection [28]. Further, they observed induction of anti-virus T cell and antibody responses [28] (Table 1, entry 3). Graham et al. [29], demonstrated live-vaccine formulation against SARS-CoV virus in mice [29]. (Table 1, entry 4). Escriou lab-mates [30] designed live attenuated recombinant measles vaccine which displaying production of high-titre neutralizing antibodies and Th-1 based immune response in mice [30] (Table 1, entry 5). Jimenez-Guardeño et al. [31], proposed mechanism of the reversion to virulence in live attenuated vaccine which can be avoided by deletion of E-gene. This clue in vaccine designing was offered protection in mice against SARS-CoV [31] (Table 1, entry 6). Menachery et al. [32], investigated the combination of various strain's attenuated vaccines which may work as a better option to protect against coronaviruses related diseases [32] (Table 1, entry 7). Regla-Nava et al. [33], proposed attenuated vaccine designing against SARS-CoV virus by using mutant E-protein which offered complete protection in mice [33] (Table 1, entry 8). However, attenuated virus showing the lung injury, pro-inflamatory cytokine and neutrophil influx with higher $CD4^+$ and $CD8^+$ T Cell count [33]. DeDiego and co-workers [34] designed attenuated SARS-CoV vaccine candidate having absence of E gene, which displaying in-vitro as well as in-vivo inhibition of SARS infection [34]. However, they reported the inflammation to the lung of hamster [34] (Table 1, entry 9).

In conclusion, a live-attenuated vaccine is characterized to develop protective immune response without producing actual disease related symptoms in host. Several reports were showing the production of humoral and cellular immune response against SARS-CoV live attenuated vaccine [26–34]. However, these vaccines possess the safety issues such as live-attenuated strain virus may return back to its original pathogenic form or development of more potent and mutant virulent strain [30–34]. Further, some reports showed inflammation to liver and lung, neutrophil influx, and pro-inflammatory cytokine after getting challenge in animal model [33,34]. Besides this, it has drawbacks such as unsuitability of vaccination to immunologically sensitive population, requirement of multiple, frequent or high dosages of vaccination, reversing to virulence and appearance of low response in immune-compressed hosts which having comorbidities [21,30,32,34].

3.1.2. Inactivated vaccine

Virus inactivation is carried out by using radiation technique

Table 1
First generation vaccines against coronavirus disease.

Entry	Virus, Vaccine, year	Animal model	Antigen	Study/finding	Author [Ref.]
1	SARS-CoV, Live attenuated, 2004	African green monkeys	SARS-CoV envelope spike protein-recom-binant attenuated influenza virus.	Investigated experimental live-attenuated SARS vaccine for direct immunization which showed good immune response (production of neutralizing serum antibodies) in immunized eight African green monkeys.	Bukreyev et al. [26]
2	SARS-CoV, Live attenuated, 2005	Mice	Attenuated vesicular stomatitis virus (VSV) expressing SARS-CoV spike protein	Designed recombinant attenuated VSV vaccine expressing SARS-CoV spike protein which displaying the passive antibody transfer to prevent SARS-CoV infection.	Kapadia et al. [27]
3	SARS-CoV, Live attenuated, 2010	BALB/c mice, and hACE2 Tg mice	Live attenuated with deletion of the E protein and accessory proteins.	Studied live attenuated vaccine by deletion of accessory protein and E gene which showed full and partial protection in BALB/c mice and hACE2 Tg mice respectively from SARS-CoV infection. Further, they observed induction of T cell and antibody responses.	Netland et al. [28]
4	SARS-CoV, Attenuated, 2012	Mice	Engineered inactivated of SARS-CoV-2 virus	Investigated live-vaccine formulation against SARS-CoV virus in mice.	Graham et al. [29]
5	SARS-CoV, Attenuated, 2014	Mice	Live attenuated recombinant measles vaccine	Live attenuated recombinant measles vaccine which displaying production of high-titre neutralizing antibodies and Th-1 based immune response in mice.	Escriou et al. [30]
6	SARS-CoV, Live attenuated, 2015	Mice	Attenuated SARS-CoV lacking of full-length E gene	Proposed mechanism of the reversion to virulence in live attenuated vaccine which can be avoided by deletion of E-gene. This clue in vaccine designing offered protection in mice against SARS-CoV.	Jimenez-Guardeño [31]
7	SARS-CoV, Attenuated, 2018	Mice	Live attenuated mutant SARS-CoV strains	Investigated combination of various strain's attenuated vaccines which may work as a better option to protect against coronavirus diseases.	Menachery etb al. [32]
8	SARS-CoV, Live attenuated, 2015	BALB/c mice	Live attenuated SARS-CoV with lack of E protein	Attenuated vaccine designing against SARS-CoV virus by using mutant E-protein which offered complete protection in mice. However, attenuated virus showing the lung injury, pro-inflamatory cytokine and neutrophil influx with higher $CD4^+$ and $CD8^+$ T Cell count.	Regla-Nava [33]
9	SARS-CoV, Live attenuated, 2007	Hamster	Recombinant SARS-CoV virus with lack of E gene	Designed attenuated SARS-CoV vaccine candidate having absence of E gene, which displaying in-vitro sa well as in-vivo inhibition of SARS infection. However, this vaccine displayed lesser inflammation to the lung of hamster.	DeDiego et al. [34]
10	SARS-CoV, Inactivated, 2003	BALB/c mice	SARS-CoV virus inactivated by use of β-propiolactone	Preparation of the inactivated SARS-CoV vaccine by using β-propiolactone in presence of aluminum hydroxide adjuvants which boosting strong antibody levels against SARS-CoV.	Tang et al. [35]
11	SARS-CoV, Inactivated, 2004	Mice	SARS-CoV virus inactivated by use of β-propiolactone	Tested proficiency of inactivated SARS-CoV vaccine which induced neutralizing antibodies in mice with high dose of antigen.	Tang et al. [36]
12	SARS-CoV, Inactivated, 2004	BALB/c mice	SARS-CoV inactivated by formaldehyde and mixed with $Al(OH)_3$	Inactivated SARS-CoV vaccine by use of formaldehyde with aluminium hydroxide which preserved antigenicity and showed stimulation of neutralizing antibodies production in mice. Further they proposed that, polypeptides protein N or S could be the possible target to generate SARS-CoV vaccine in future.	Xiong et al. [37]
13	SARS-CoV, Inactivated, 2004	Mice	SARS-CoV UV- inactivated with or without an adjuvant	Developed UV-inactivated SARS-CoV vaccine which induces the humoral immunogenic response in mice with aluminium hydroxide gel used as adjuvants. Further they proposed generation of lymph node T-cell proliferation and cytokine production such as IFN-γ, TNF-α.IL-5, IL-4, IL-2.	Takasuka et al. [38]
14	SARS-CoV, Inactivated, 2004	NM	SARS-CoV virus inactivated by the β-propiolactone	Inactivated vaccine by β-propiolactone which stimulates neutralizing antibodies to obstruct virus entry. Moreover, S protein of receptor binding domain is a major component to induce potential neutralizing antibodies with need of appropriate caution to avoid the harmful immune responses.	He et al. [39]
15	SARS-CoV, Inactivated, 2005	Rhesus monkey	SARS-CoV inactivated	Investigated of an inactivated SARS-CoV vaccine potency in rhesus monkey which indicated humoral and mucosal immunity.	Zhong et al. [40]
16	SARS-CoV, Inactivated, 2005	Rhesus monkey	SARS-CoV inactivated	Studied inactivated SARS-CoV vaccine potency in rhesus monkey which stimulated humoral and mucosal immunity.	Zhou et al. [41]
17	SARS-CoV, Inactivated, 2005	Balb/c mice	SARS-CoV inactivated	Production of immune response (specific antibodies) after 15 days of immunization by inactivated vaccine combined with various 3 kinds of adjuvants like $Al(OH)_3$, Freund's, and CpG).	Zhang et al. [42]
18	SARS-CoV, Inactivated, 2006	Mice	SARS-CoV inactivated by UV and formaldehyde	Proposed two step inactivation by formaldehyde and UV ray to design inactivated vaccine which produces high levels of neutralizing antibodies and stimulates interferon-γ as well as interleukin-4 production in mice.	Spruth et al. [43]
19	SARS-CoV, Inactivated, 2006	Rhesus Monkey	SARS-CoV inactivated by β-propiolactone	Studied inactivation of SARS-CoV by use of β-propiolactone and tested in monkeys which displaying prevention of replication of virus with sufficient induction of antibodies.	Qin et al. [44]
20	SARS-CoV, Inactivated, 2007	Mice	SARS-CoV inactivated by UV and formalin	Investigated inactivation of SARS-CoV vaccine designing by UV-ray and formalin treatment which showed strong immune response (IgG and interleukin-4 generation) in mice.	Tsunetsugu-Yokota et al. [45]
21		NM	SARS-CoV inactivated by UV	Developed vaccine by UV-inactivation of SARS coronavirus which can be used against corona-virus disease.	

Table 1 (*continued*)

Entry	Virus, Vaccine, year	Animal model	Antigen	Study/finding	Author [Ref.]
	SARS-CoV, Inactivated, 2008				Tsunetsugu-Yokota et al. [46]
22	SARS-CoV, Inactivated, 2008	NM	Inactivated SARS-CoV	Investigated the influence of the various immunization protocol for inactivated SARS virus which indicating significant production of IgG and IgA antibodies by an intraperitoneal immunization than intranasal immunization.	Gai et al. [47]
23	SARS-CoV, Inactivated, 2010	BALB/c mice and golden Syrian hamsters	SARS-CoV virus inactivated by the β-propiolactone	Studied efficiency of β-propiolactone inactivated SARS-CoV virus vaccine in the mice and golden Syrian hamsters which displayed boosting of antibodies after multiple-dosages	Roberts et al. [48]
24	MERS-CoV, Inactivated, 2016	Mice	Inactivated MERS-CoV	Injected an inactivated MERS-CoV vaccine to mice, which indicating the production of neutralizing antibodies in mice. However, inactivated MERS-CoV vaccine displayed hypersensitive-type lung pathology risk.	Agarwal et al. [49]

(UV-ray, X-ray or γ-radiation) or by using chemicals (such as formaline, methanol or β-propiolactone) which preserves the antigenic character of virus particles and demolishing actual infectivity [22]. The induction of immune responses through the inactivated pathogens is measured as a standard and successful vaccination pattern from many years [22,35]. Various inactivated vaccine formulations are successfully available against influenza, polio, hepatitis A, and rabies pathogen etc [22,35–38]. Several efforts have been attempted to design the inactivated vaccine formulations in order to get effective protection from SARS or MERS coronavirus as listed in Table 1 (Table 1, entries 10–24) [35–49]. Tang et al. [35,36] carried out preparation of the inactivated SARS-CoV vaccine by using β-propiolactone in presence of aluminum hydroxide adjuvants which boosting strong immune response (neutralizing antibody) against SARS-CoV [35,36] (Table 1, entries 10,11). Xiong et al. [37], designed inactivated SARS-CoV vaccine by use of formaldehyde with aluminium hydroxide which preserved antigenicity and showed stimulation of neutralizing antibodies production in mice. Further they proposed that, polypeptides protein N or S could be the possible target to generate SARS-CoV vaccine in future [37] (Table 1, entry 12). Takasuka and group [38] evaluated the performance of UV-inactivated SARS-CoV vaccine which induces the humoral immunogenic response in mice with aluminium hydroxide gel used as adjuvants [38] (Table 1, entry 13). Further they proposed generation of lymph node T-cell proliferation and cytokine production such as IL-2, IL-4, IL-5, IFN-γ and TNF-α [38].

He and co-workers [39] designed inactivated vaccine by β-propiolactone which stimulated neutralizing antibodies to obstruct SARS-CoV entry [39] (Table 1, entry 14). Moreover, they observed that, S protein of receptor binding domain is a major component to induce potent neutralizing antibodies with need of appropriate caution to avoid the harmful immune or inflammatory responses [39]. Zhong et al. [40], evaluated inactivated SARS-CoV vaccine potency in rhesus monkey which indicated humoral and mucosal immunity [40] (Table 1, entry 15). Similarly, Zhou and co-workers [41] studied inactivated SARS-CoV vaccine potency in rhesus monkey which stimulated humoral and mucosal immunity [41] (Table 1, entry 16). Zhang et al. [42], observed production of immune response (specific antibodies) after 15 days of immunization by inactivated vaccine combined with various three kinds of adjuvants (namely Freund's, $Al(OH)_3$ and CpG) [42] (Table 1, entry 17). Spruth and group [43] proposed two step inactivation by formaldehyde and UV ray to design inactivated vaccine which produces high levels of neutralizing antibodies and stimulates interferon-γ as well as interleukin-4 production in mice [43] (Table 1, entry 18). Qin et al. [44], inactivated SARS-CoV by use of β-propiolactone and tested in monkeys which displayed prevention of replication of virus with sufficient induction of antibodies [44] (Table 1, entry 19).

Tsunetsugu-Yokota group [45] proposed inactivated SARS-CoV vaccine designing by UV-ray and formalin treatment which showed strong immune response (IgG and interleukin-4 generation) in mice [45] (Table 1, entry 20). Further, Tsunetsugu-Yokota group [46] developed inactivated vaccine by UV-inactivation of SARS coronavirus which can be used against corona-virus disease [46] (Table 1, entry 21). Gai et al. [47], investigated the influence of various immunization protocol for inactivated SARS virus which indicating significant production of IgG and IgA antibodies by an intraperitoneal immunization than intranasal immunization [47] (Table 1, entry 22). Roberts and co-workers [48] tested the efficiency of β-propiolactone inactivated SARS-CoV vaccine in mice and golden Syrian hamsters which displayed boosting of antibodies after multiple dosages [48] (Table 1, entry 23). Agarwal et al. [49], injected an inactivated MERS-CoV vaccine to mice, which indicated the production of neutralizing antibodies in mice [49]. However, inactivated MERS-CoV vaccine displayed hypersensitive-type lung pathology risk which involves the lung mononuclear infiltration and increased eosinophil promoting [49] (Table 1, entry 24).

In conclusion, the inactivated vaccine is considered as safe compared to live-attenuated form due to absence of living pathogens and their inability of possible re-infection [22]. The chances of reverting back into virulent phases are much less in case of inactivated vaccines than live attenuated vaccine [37–39]. However, mode of presentation of unexpected immune response (than that of actual pathogenecity) is the major limitation of inactivated vaccine [22,44]. In case of inactivated coronaviruses vaccine, some reports showed inflammation and lung lesion with eosinophil infiltration [39,49]; whereas, few articles reported that, inactivated vaccines lead to create weaker immune response or delayed immune response [42] with requirement of multiple dosages [48], since actual infection is not established. Thus, multiple/high/frequent dosage, weaker and unexpected immune response is the major limitation associated with use of inactivated vaccines.

3.2. Second generation vaccine

3.2.1. Protein subunit vaccine

A protein subunit vaccine involves the use of synthetic or isolated or recombinant or derived highly antigenic protein base subunits with the short antigen segment which offers safer vaccine designing approach [23]. Various protein subunit vaccines are successfully formulated against various pathogens such as influenza virus, hepatitis B, pneumonia and meningitis etc [23,50–55]. In case of coronavirus vaccine, various kind of proteins in full or segmented form are reported in literature which involves the receptor binding domain or membrane protein or nucleo-capaside protein or spike protein or envelop protein [50–59] (Table 2,

Table 2
Second generation vaccine for coronavirus disease.

Entry	Virus, Vaccine, year	Animal model	Antigen	Study/finding	Author [Ref.]
1	SARS-CoV, Subunit, 2004	Rabbit	Recombinant fusion protein consist of 193-amino acid (318–510) residues and IgG1-Fc fragment	Demonstrated recombinant fusion of protein residues (318–510) from receptor-binding domain which produced immune response (neutralizing antibody) in immunized rabbits.	He et al. [50]
2	SARS-CoV, Subunit, 2005	BALB/c mice	Recombinant S2 fragment with amino acid residues with Freund's adjuvant.	Observed high level of antibodies, Th1-and Th-2 type of imunogenic responses for immunized S2 subunit residues (681–1120) in mice with Freund's adjuvant.	Guo et al. [51]
3	SARS-CoV, Subunit, 2006	NM	S fragments consist of amino acid residues S74-253, 294–739, 1129–1255.	Investigated effect of intron and exon splicing enhancers for upgrading of protein expression in the mammalians which can be useful in designing of SARS-CoV subunit vaccine.	Chang et al. [52]
4	SARS-CoV, Subunit, 2007	NM	B cell epitope peptide of SARS-CoV S2 spike protein	Designed epitope peptide of SARS-CoV S2 (expressed in E.coli) which induced antigenicity of S2 protein.	Feng et al. [53]
5	SARS-CoV, Subunit, 2007	129S6/SvEv mice	Spike protein amino acid residues 318-510	Proposed remarkable production of immunogenic response (IgG2 antibodies and cellular immune response) for the SARS subunit vaccine (given subcutaneously to mice) which consists of spike protein amino acids S318-510 in saline, with alum + CpG oligodeoxynucleotides as adjuvants.	Zakhartchouk et al. [54]
6	SARS-CoV, Subunit, 2013	Mice	Trimeric recombinant spike protein	Compared immunogenic response and vaccine efficiency of various monomeric and trimeric recombinant S proteins of SARS-CoV which stimulated neutralizing antibody.	Li et al. [55]
7	MERS-CoV, Subunit, 2015	Human	Protein containing amino-acid residues from 377 to 588 of receptor binding domain	Studied receptor binding-domain subunit vaccine and optimized antigen-doses to acquire strong immune responses (humoral and cellular) with minimal antigen dose.	Tang et al. [56]
8	MERS-CoV, Subunit, 2016	Mice	Different epitopes of receptor binding domain with a glycan.	Engineered vaccine offered increased efficiency by producing immune response in protecting transgenic mice by MERS-CoV virus.	Du et al. [57]
9	MERS-CoV, Subunit, 2020	NM	MERS-CoV- S1 subunit	Developed S1 sub-unit vaccine which displayed the potent antibody responses after almost 15 days of immunization.	Kim et al. [58]
10	SARS-CoV-2 Subunit, 2020	NM	Recombinant antigen consist of adjuvant, B-cell epitope, cytotoxicand helper T-lymprocyte joined by linker	Studied multi-peptide based epitope subunit-vaccine (consist of 33 efficient antigenic epitope from major three types of proteins) which has major role in host-receptor recognition in SARS-CoV-2 infection.	Kalita et al. [59]
11	SARS-CoV, Vector base, 2005	Wistar rats	Adenovirus carrying N-terminal segment of S1gene of SARS-CoV	Analyzed a vector base recombinant vaccine (adenovirus with SARS-CoV S1 spike protein expression) which induced specific humoral immunogenic response in rats after subcutaneous or intranasal immunization.	Liu et al. [60]
12	MERS-CoV, Vector base, 2019	Mice	Recombinant adenovirus encoding the spike S1 subunit	Proposed use of recombinant adenovirus encoding MERS-CoV S1 subunit which showed immunogenic (humoral and cellular) responses in mice.	Ababneh et al. [61]
13	MERS-CoV, Vector base, 2019	Mice	Adenovirus-vectored consist of full length spike glycoprotein MERS-CoV	Tested immunogenicity by adenovirus-vectored vaccine consist of complete spike protein of MERS-CoV which generated humoral and cellular response against MERS-CoV.	Folegatti et al. [62]

entries 1–10). He et al. [50], demonstrated recombinant fusion of protein residues (318–510) from receptor-binding domain which efficiently produced immune response (neutralizing antibody) in immunized rabbits [50] (Table 2, entry 1). Guo and co-workers [51] observed high level of antibodies, Th1-and Th-2 type of imunogenic responses for immunized S2 subunit residues (681–1120) in mice with Freund's adjuvant [51] (Table 2, entry 2). Chang and group [52] investigated effect of intron and exon splicing enhancers for upgrading of protein expression in the mammalian cells which can be useful in designing of SARS-CoV subunit vaccine [52] (Table 2, entry 3). Feng et al. [53], investigated epitope peptide of SARS-CoV S2 (expressed in E.coli) which induced antigenicity of S2 protein [53] (Table 2, entry 4). Zakhartchouk et al. [54], noted remarkable production of immunogenic response (IgG2 antibodies and cellular immune response) for the SARS subunit vaccine (given subcutaneously to mice) which consists of spike protein amino acids S318-510 in saline, with alum + CpG oligodeoxynucleotides as adjuvants [54] (Table 2, entry 5). Li and group [55] compared immunogenic response and vaccine efficiency of various monomeric and trimeric recombinant S proteins of SARS-CoV which stimulated neutralizing antibody [55] (Table 2, entry 6). Tang et al. [56], developed receptor binding-domain subunit MERS vaccine and optimized antigen-doses to acquire strong immune responses (humoral and cellular) with minimal antigen dose [56] (Table 2, entry 7). Du and co-

workers [57] showed that, different epitopes of receptor binding domain with a glycan engineered vaccine offered increased efficiency by producing immune response in protecting transgenic mice by MERS-CoV virus [57] (Table 2, entry 8). Kim et al. [58], developed S1 sub-unit MERS vaccine which displayed the potent antibody responses after almost 15 days of immunization [58] (Table 2, entry 9). More recently, Kalita and group [59] developed multi-peptide epitope subunit-vaccine (consist of 33 efficient antigenic epitope from major three types of proteins) which displayed major role in host-receptor recognition in SARS-CoV-2 infection [59] (Table 2, entry 10).

In conclusion, the receptor binding domain is the most widely used protein segment in coronavirus vaccine design due to its efficient immunogenic response as a vaccine candidate [50–59]. These vaccines do not contain the viral genetic materials, while they include only essential antigenic protein component to stimulate the immunogenic response [50–52]. Various reports mentioned induction of neutralizing antibodies [50], IgA, IgG [54,56,57], Th-1 and Th-2 [51] type of immunogenic response by subunit coronavirus vaccine. The major advantage of these vaccines are the lesser chance of adverse impact, since actual naturally occurring viral components are not available in it and hence considered as more safer than first generation vaccines [23]. Besides this, subunit vaccine designing can offer an opportunity to vaccinate against multiple epitopes (of genes subunit) from the similar or different kinds of pathogens/strains [59]. However, some reports concluded poor or delayed immunogenic response (due to absence of several other viral components) which may be sometimes overcome by use of appropriate adjuvant [51,54,58]. Due to definite immunogenic components of protein subunits, production can be readily possible in outbreak situation and can be enhanced by use of adjuvant which is the major outstanding features of subunit vaccines [54].

3.2.2. Vector based vaccine

Production of the vector-based vaccines is proficient in creating immunogenic responses [24]. Various viral vectors are used as a delivery tool for the vaccination such as modified vaccinia Ankara virus, adenovirus, adeno associated virus, retro virus vector, lenti virus vector, sendai virus etc. which can be able to elicit the immune responses [24,60]. Very few reports are available for the vector base vaccine since it is a perfectly recombinant vaccine which involves pathogenic harmful antigenic component into non-pathogenic vector virus [60–62] (Table 2, entry 11–13). Liu et al. [60], designed a vector base recombinant vaccine (adenovirus with SARS-CoV S1 spike protein expression) which induced specific humoral immunogenic response in rats after subcutaneous or intranasal immunization [60] (Table 2, entry 11). Ababneh et al. [61], suggested use of recombinant adenovirus encoding MERS-CoV S1 subunit which was showed immunogenic (humoral and cellular) responses in mice [61] (Table 2, entry 12) Folegatti and workers [62] tested immunogenicity by adenovirus-vectored vaccine consist of complete spike protein of MERS-CoV which generated the humoral and cellular responses against MERS-CoV [62] (Table 2, entry 13).

In conclusion, S gene/spike proteins are specifically reported to code in adenovirus vector which induces the immune response [60–62]. The viral vector base vaccine is more advantageous than first generation vaccine since it vaccinate the live virus by recombination of antigenic protein component of pathogenic virus into non-virulent vector [24,61]. Thus it mimics the possible natural pathogenic infection with subsequent cellular and humoral immunogenicity [60–62]. The major challenge in designing of this kind of vaccine is to know the exact epidemiology, genotoxicity and virology of both viruses (pathogenic and vector virus) [24,62]. Hence it is difficult to design rapid vector base vaccine for the newly emerging viruses like SARS-CoV-2. Further, major limitation is the hampering and delaying of actual expected immune response against pathogenic virus, since, primary and pre-existing immune response is mainly acquired due to vector virus which is known as the pre-existing immune response [24]. Besides this, there is a risk of mutation and unexpected virulence ability of engineered vectored virus.

3.3. Third generation vaccine

3.3.1. Nucleic acid vaccine

Nucleic acid vaccines make available stable antigenic expression (into delivery plasmid by genetic engineering) which is known to stimulate relatively lesser but constant immune responses [25]. Further, nucleic acid vaccines are cloned antigenic protein materials that mimic the natural infection and can be manufacture relatively in a short period of time [63–70]. The nucleic acid vaccine is considered as safer alternative than that of inactivated and live-attenuated vaccines which are used currently to acquire immunity against dengue, malaria, typhoid and anthrax etc [25,71]. These vaccines possess the potential advantages and can be designed against newly emerging viruses by encoding gene sequence (Table 3 entries 1–14) [63–76]. Zhao and group [63] showed induction of conserved N protein of SARS virus by designed prophylaxis DNA vaccine which produces IL-2, γ-interferon, cytotoxic T lymphocytes and $CD8^+$ response [63] (Table 3, entry 1). Li et al. [64], used spike gene fragments to develop the DNA vaccine against SARS-CoV which able to develop (delayed) immune response (IgG, cytotoxic T lymphocytes and $CD8^+$) in rats in between 3 and 7 weeks [64] (Table 3, entry 2). He and co-workers [65] constructed eukaryotic expression of plasmid encoding SARS-CoV partial S gene of virus which demonstrated production of immune response (serum IgG and γ-interferon) after 2 weeks in mice [65] (Table 3, entry 3). Zakhartchouk et al. [66], investigated the synergetic influence of the recombinant DNA and killed virus vaccines together to know efficient immune response against SARS-CoV [66] (Table 3, entry 4). They observed induction of T-helper type-1 and type-2 immune response. Huang and researchers [67] designed DNA vaccine which able to generate long-term protection and immune response (induction of $CD4^+$ and $CD8^+$ T cell responses in both lymphoid and nonlymphoid system) in mice [67] (Table 3, entry 5).

Wang and co-workers [68] designed DNA vaccine by encoding S1 and S2 subunit which able to induce the immune response (specific antibody) in mice [68] (Table 3, entry 6). Callendret et al. [69], proposed improved immunogenic response (neutralizing antibodies) by S-protein DNA vaccines in mice [69] (Table 3, entry 7). Zakhartchouk et al. [70], tested various four formulations (pLL-70, pcDNA-SS, pcDNA-St, pcDNA-St-VP22C) which uses different gene fractions for designing of DNA vaccines for SARS-CoV. Among all these formulations, pcDNA-SS (codon-S-gene) and pcDNA-St-VP22C (codon-N-gene) based DNA vaccine produced the strong immune response against SARS-CoV in mice [70] (Table 3, entry 8). Dutta and group [71] utilized three fragments of N proteins (N1, N2 and N3) to express in E.coli for designing of DNA vaccine which produced strong immune response (IgG and IgG-1 antibodies) in mice after immunization [71] (Table 3, entry 9). Wang et al. [72], designed multi-epitope (from S and M protein) DNA vaccine which induces the polyvalent immune response against SARS-CoV virus in mice [72] (Table 3, entry 10). Martin and researchers [73] mentioned production of neutralizing antibodies, $CD4^+$ and $CD8^+$ T cell response through multiple dose DNA vaccine against SARS-CoV virus [73] (Table 3, entry 11). Lu et al. [74], reported stimulation of high level antibodies, Th-1 response, γ-interferon (through $CD8^+$) and interleukin-2 (through $CD4^+$) by 3a gene DNA vaccines through electroporation against SARS-CoV virus in mice. Further they

Table 3
Third generation vaccines against coronavirus disease.

Entry	Virus, Vaccine, year	Animal model	Antigen	Study/finding	Author [Ref.]
1	SARS-CoV, Nucleic acid, 2005	BALB/c mice	Plasmid pCI-N, encodes full-length N gene.	Studied induction of conserved N protein of SARS virus by designed prophylaxis DNA vaccine which produces IL-2, γ-interferon, cytotoxic T lymphocytes and $CD8^+$ response.	Zhao et al. [63]
2	SARS-CoV, Nucleic acid, 2005	Wistar rats	Plasmid containing the S gene encodes N- and C-terminal of the Spike protein.	Used spike gene fragments to develop the DNA vaccine against SARS-CoV which able to develop (delayed) immune response (IgG, cytotoxic T lymphocytes and $CD8^+$) in rats in between 3 and 7 weeks.	Li et al. [64]
3	SARS-CoV, Nucleic acid, 2005	BALB/c mice	Plasmid, pVAX-S1, encoded partial S gene	Constructed eukaryotic expression of plasmid encoded SARS-CoV partial S gene of virus which demonstrated immune response (serum IgG and γ-interferon production) after 2 weeks in mice	He et al. [65]
4	SARS-CoV, Nucleic acid, 2005	Mice	Plasmid encoding SARS-CoV S protein and propilactone inactivation	Investigated the synergetic influence of the recombinant DNA and killed virus vaccines together to know efficient immune response against SARS-CoV. They observed induction of T-helper type-1 and type-2 immune response.	Zakhartchouk et al. [66]
5	SARS-CoV, Nucleic acid, 2005	Mice	A pool of peptides overlapping entire SARS-CoV S protein	Designed DNA vaccine which able to generate long-term protection and immune response (induction of the $CD8^+$ and $CD4^+$ T cell responses in both non-lymphoid and lymphoid system) in mice.	Huang et al. [67]
6	SARS-CoV, Nucleic acid, 2007	Mice	Plasmid encoding S1 and S2 (pIRCTL-S1 and pIRCTL-S2)	Designed DNA vaccine by encoding S1 and S2 subunit which able to induce the immune response (specific antibody) in mice.	Wang et al. [68]
7	SARS-CoV, Nucleic acid, 2007	Mice	Pasmid vectors for S gene expression	Proposed improved immunogenic response (neutralizing antibodies) by S-protein DNA vaccines in mice.	Callendret et al. [69]
8	SARS-CoV, Nucleic acid, 2007	C57BL/6 mice	pLL-70 with S gene; pcDNA-SS with S gene (12–1255); pcDNA-St with S gene (12–532); pcDNA-St-VP22C with N codon portion	Tested various four formulations (pLL-70, pcDNA-SS, pcDNA-St, pcDNA-St-VP22C) which uses different gene fractions for designing of DNA vaccines for SARS-CoV. Among all these formulations, pcDNA-SS (codon-S-gene) and pcDNA-St-VP22C (codon-N-gene) based DNA vaccine produces the strong immune response against the SARS-CoV in mice.	Zakhartchouk et al. [70]
9	SARS-CoV, Nucleic acid, 2008	BALB/c mice	Three gene fragments of SARS-CoV N protein cloned into pVAX-1: N1 (1–422); N2 (1–109); N3 (110–422)	Utilized three fragments of N proteins (N1, N2 and N3) to express in E.coli for designing of DNA vaccine which are producing strong immune response (IgG and IgG-1 antibodies) in mice after immunization.	Dutta et al. [71]
10	SARS-CoV, Nucleic acid, 2008	Mice	Multi-epitope S 437–459 and M 1–20 in DNA vaccine	Designed multi-epitope (from S and M protein) DNA vaccine which induces the polyvalent immune response against SARS-CoV virus in mice.	Wang et al. [72]
11	SARS-CoV, Nucleic acid, 2008	NM	Plasmid encoding the SARS-CoV spike glycoprotein	Mentioned production of neutralizing antibodies, $CD4^+$ and $CD8^+$ T cell response through multiple dose DNA vaccine against SARS-CoV virus.	Martin et al. [73]
12	SARS-CoV, Nucleic acid, 2009	Mice	Open reading frame SARS-3a gene and bat like SARS-CoV open reading frame 3a gene	Reported stimulation of high level antibodies, Th-1 response, γ-interferon (through $CD8^+$) and interleukin-2 (through $CD4^+$) by 3a gene DNA vaccines through electroporation against SARS-CoV virus in mice. Further they proposed that, spike genes play an important role in vaccine designing, while slight modification in spike protein affects effectiveness of vaccine.	Lu et al. [74]
13	MERS-CoV, Nucleic acid, 2015	Mice	MERS-CoV spike protein synthetic DNA vaccine	Designing of synthetic DNA vaccine against MERS virus which induces the potent cellular immunogenic response in mice.	Muthumani et al. [75]
14	MERS-CoV, Nucleic acid, 2017	Mice	DNA vaccine encodes the 725 S amino-acid residues of MERS-CoV	Developed S1 encoded (725 amino acids) DNA vaccine against MERS-CoV which induces secretion of γ-interferon and other cytokines by $CD4^+$ and $CD8^+$ T cells in mice.	Chi et al. [76]
15		Mice			

Table 3 (*continued*)

Entry	Virus, Vaccine, year	Animal model	Antigen	Study/finding	Author [Ref.]
	SARS-CoV, Nano-base, 2010		S protein of SARS-CoV on polyethylenimine nanocarrier	Proposed nano-based vaccine for the intranasal immunization which induces SARS-coronavirus spike proteins to produce humoral and immune response (IgG, IgA, γ-interferon, interleukin-2) in mice. The nano-polymer polyethylenimine was used as a vaccine carrier.	Shim et al. [77]
16	SARS-CoV, Nano-base, 2012	Mice	Plasmid DNA loaded biotinylated chitosan nanoparticles as a carrier for N protein of (SARS-CoV)	Studied efficacy of plasmid DNA encoded N protein antigen loaded on chitosan nano-polymeric carrier for non-invasive intranasal immunization against SARS-CoV which induces mucosal IgG and IgA antibodies (at the point of entry of virus).	Raghu-wanshi et al. [78]
17	MERS-CoV, Nano-base, 2019	NM	Virus-like particle mimetic nanovesicles	Investigated the designing of virus-like nano-particles mimetic nano-vesicles which displaying the potency of vaccine designing. They designed three recombinant proteins (S, E and M) of MERS-CoV which can acted as a major platform for vaccine designing.	Kato et al. [79]

proposed that, spike genes play an important role in vaccine designing, while slight modification in spike protein affects effectiveness of vaccine [74] (Table 3, entry 12). Muthumani et al. [75], reported designing of synthetic DNA vaccine against MERS virus which induces the potent cellular immunogenic response in mice [75] (Table 3, entry 13). Chi et al. [76], designed S1 encoded (725 amino acids) DNA vaccine against MERS-CoV which induces secretion of γ-interferon and other cytokines by $CD4^+$ and $CD8^+$ T cells in mice [76] (Table 3, entry 14).

In conclusion, N gene, S gene, S1 gene, S2 gene or multiple epitope genes are reported in literature to design engineered nucleic acid vaccine for coronaviruses [63–76]. Highly efficient immunogenic response is reported by various researchers about the use of nucleic acid vaccine against coronavirus which includes production of IL-2, γ-interferon, cytotoxic T lymphocytes, $CD4^+$, $CD8^+$ [63–65,67,73,76], neutralizing antibodies [68–71], Th-1 and Th-2 [66] type response. The major advantage of nucleic acid vaccine is the use in combination with other vaccine platforms (such attenuated or inactivated), no risk of infection, improved heat and shelf-life stability [66]. The major limitation of the nucleic acid vaccine is the limited immune response attributed to specified or engineered genetic material, tedious genetic engineering task, local pain at site of injection, pyrexia and essential need of adjuvant for long time immunity [64,65].

3.3.2. Nano-material based vaccine

The newly advanced methodology and technology in vaccine designing is to use of the nano-materials as a carrier of antigenic component [77–79]. The adsorption, entrapment and conjugation are the basic three interactions that are associated in between antigen and nano-particles [77–79]. Various kinds of nano-material are widely used such as nano-polymer, liposomes, inorganic nano-particles, carbon base nano-materials and quantum dots etc [77]. These nano-materials are broadly used in designing of various vaccine candidates against pathogenic disease such as taxoplasmosis, malaria, HIV, ebola, and influenza etc [78,79]. Very few reports are available for the use of nano-based vaccine against coronavirus [77–79] (Table 3, entries 15–17). Shim et al. [77], designed nano-based vaccine for the intranasal immunization which induces SARS-coronavirus spike proteins to produce humoral and immune response (IgG, IgA, γ-interferon, interleukin-2) in mice. The nano-polymer polyethylenimine was used as a vaccine carrier [77] (Table 3, entry 15). Raghuwanshi et al. [78], investigated efficacy of plasmid DNA encoded N protein antigen loaded on chitosan nano-polymeric carrier for non-invasive intranasal immunization against SARS-CoV which induces mucosal IgG and IgA antibodies (at the point of entry of virus) [78] (Table 3, entry 16). Kato et al. [79], investigated the designing of virus-like nano-particles mimetic nano-vesicles which displayed the potency of vaccine designing. They designed three recombinant proteins (S, E and M) of MERS-CoV which acted as a major platform for vaccine designing [79] (Table 3, entry 17). The major limitation of the nano-based vaccine is cellular toxicity of nano-material and need of the adjuvant for enhanced performance of vaccine [79]. Further various physico-chemical properties (size, shape, charge and surface area) of nano-materials are greatly affect the nano-vaccine development [78,79].

In conclusion as of now, no licensed vaccine is available against coronavirus disease. Various vaccine development strategies (live-attenuated, inactivated, protein subunit, vector base, nucleic acid, nano-based) of SARS and MERS may be helpful to direct vaccine designing against SARS-CoV-2 virus (COVID-19). The most commonly used antigen were receptor binding domain protein segment to acquire the immunity. Some studies reported that, S protein are highly antigenic while some studies showed that, N proteins are highly immunogenic in nature. Thus the combination of multi-protein segments can be more effective in order to acquire assured immunogenic response. Related literature review suggested that, neutralizing antibodies may play a key role to get protection against coronavirus disease which can be efficiently acquired by antigenic spike protein material. Most of the reports showing, induction of the humoral and cellular immune response against vaccinated spike protein antigen in mice. Further, some, researchers have reported induction of antigen-specific $CD4^+$/$CD8^+$ T cells response in mice due to receptor binding domain spike protein antigen. Thus, vaccination may be the promising approach in order to create an immunogenic response against coronavirus infection and to control the spread of infection. Nucleic acid vaccines are seen to be superior than live attenuated vaccines, however, there is chances of development of extreme lower immune response due to unavailability of actual natural antigenic material. Further, vector base vaccination provides a platform of creation of novel mutant pathogenic strain due to use of recombinant vector strain and pathogenic virus. Nano-material based vaccine development is at the preliminary stage of research which involves the synthesis of virus like nano-particles, use of nano-carriers for the vaccine delivery. However, modern synthetic vaccines are not enough sufficient to produce immunogenic response due to unavailability of the actual antigenic component. Further some papers reported major drawbacks such as requirement of frequent-multiple-high dosages, delayed response and adverse side effects which are accounted as a possible reason for not getting approved

any coronavirus vaccine by FDA. The available overview and recent advances of coronavirus vaccine development proposed that, (i) receptor binding domain based protein subunit vaccine designing is a possible potential, ideal and safer option to design rapid vaccine against SARS-CoV-2 virus (COVID-19), (ii) E protein can be deleted during the vaccine designing due to its virulence ability, (iii) preservation of highly antigenic receptor binding epitope and removal of immune damaging epitope from spike protein may offers better approach to fabricate an efficient vaccine against SARS-CoV-2 (iv) more conserved M protein or N protein epitope can be synergetically used with S protein epitope to get enhanced immunogenicity (v) inactivated, nucleic acid and subunit vaccine may be better and quicker option to design vaccine within short period of time.

4. Possible clues/ target sites for vaccine designing against coronavirus

Various vaccine designing efforts are undertaken from 2003 to till date to design a successful vaccine candidate against coronavirus [5,12,13]. Despite of several available literature reports, NO vaccine is approved to use commercially against coronavirus disease (SARS and MERS) [5,12,13]. In reality, Universal spirit and prevailing need of vaccine development slowly faded after upshot of the SARS epidemic, possibly due to NO new cases are reported for the SARS after year 2005 (Fig. 1) [13,18]. However in current situation of pandemic COVID-19, various prior coronavirus vaccine designing efforts are getting new momentum for rapid vaccine development [26–79]. Looking to thrust of vaccine designing, the available past/ historical efforts or experience of vaccine development against SARS and MERS will be of great value in present worldwide pandemic COVID-19 scenario considering (i) the gene sequence homology of SARS-CoV and SARS-CoV-2 (ii) urgent need of vaccine to control existing pandemic (iii) short time span and huge capital investment of basic preliminary research & development.

The M protein, E protein, S protein and N protein showed almost 90%, 94%, 76% and 90% similarity in between the gene sequencing of SARS-CoV and SARS-CoV-2 [80,81]. Similarity of S protein is found to be lesser which might be attributed to mutation in SARS-CoV virus throughout the period which is a possible clue as well as the challenge in designing of rapid vaccine [81,82]. Hence, availability of prior art literature for epitope study may be the biggest clue and anticipation for the rapid vaccine designing [50–79]. Various previous attempts regarding to vaccine designing have been reported to use S protein or N proteins or M protein or combination of S/N/M protein or gene segment (with or without E gene) in order to induce the potential immune response against coronavirus [50–79].

The S glycoprotein (has two subunits S1 and S2) binds to ACE-2 through the receptor-binding domain and showed induction of sufficiently high level of antibodies in host [9,39,50,52,56,59]. The S1 subunit is responsible for the binding to host cell receptor (ACE-2), whereas S2 subunit is responsible for the fusion process [9,81–83]. The S glycoprotein or S1 segment or S2 segment induces different kinds of immunogenic responses which can individually also work as a potential vaccine candidate [39,50,52,54]. The S1 subunit is more immunogenic and hence producing more kind of antibodies than that of S2 subunit [9,60,68,81]. Thus, the receptor binding domain epitopes (S protein segment) is a major component which is extensively reported in literature with the animal model response and can be useful to consider as a possible antigen for COVID-19 vaccine as they not only induces the humoral immunity but also elicit T-cell immune responses [82,83]. Various researchers He et al. [50], Guo et al. [51], Chang et al. [52], Feng et al. [53], Zakhartchouk et al. [54], Li et al. [55], Liu et al. [60], Li et al. [64], He et al. [65], Zakhartchouk et al. [66], Huang et al. [67], Wang et al. [68], Callendret et al. [69], Zakhartchouk et al. [70], Martin et al. [73], Shim et al. [77], Kato et al. [79], are reported to use S protein in full form or in segmented form as an antigen against SARS-CoV virus. Similarly, Tang et al. [56], Du et al. [57], Kim et al. [58], Kalita et al. [59], Ababneh et al. [61], Folegatti et al. [62], Muthumani et al. [75], Chi et al. [76], also mentioned importance of the spike protein as an antigen for the development of vaccine against MERS-CoV.

The use of N protein segment of SARS-CoV may be another option for the vaccine designing against coronavirus, which is reported to generate lesser immunogenicity than S protein [61,71,78]. This N protein is reported to use as an antigen by Zhao et al. [63], Dutta et al. [71], and Raghuwanshi et al. [78], as it is able to generate specific antibody and cellular immune response [63,71,78,84]. The M protein can be considered as a potential protein for vaccine designing as it induces long-term memory humoral immune response as well as high-titer antibody responses [72]. However, M protein is associated with the virulence ability and regeneration of viral particles [82,85] hence very few reports are available to use M protein as an antigen in vaccine designing. Similarly the E protein is also responsible for the morphogenesis of virus, virulence capacity as well as viral assembly and hence most commonly not used as an antigen for vaccine designing [28,31,33,34,82,85]. Netland et al. et al. [28], Jimenez-Guardeño et al. [31], Regla-Nava et al. [33], and DeDiego et al. [34], reported to use first generation (live attenuated) vaccine with deletion or lack of E gene in order to avoid the virulence ability. Thus, deletion of the E gene block viral production and reduces the viral number almost 200 times inside the cell [34,86,87].

5. Current vaccine development scenario against SARS-CoV-2 virus (COVID-19)

At present various international pharmaceutical biotech companies and research organizations are actively involved in development of vaccine against SARS-CoV-2 virus [88–105]. According to the WHO (as of now June 8, 2020), about 114 vaccine candidates are under pre-clinical stage, Table 4 showed various clinical trials regarding to COVID-19 vaccine development which are in trial phase I or II or III [88]. It involves various generations/kinds of vaccine development and clinical trials such as use of live-attenuated BCG vaccine for investigation of influence of BCG vaccination on healthcare workers in order to get protection from severity of COVID-19 (Table 4, entries 1,2) [89–91]. It is proposed that, BCG vaccine may offers protection against SARS-CoV-2 infection which may be due to induction of innate heterologous immune response [89]. Furthermore, it is observed that, impact of COVID-19 is higher in some countries (such as US and other European countries like Italy) wherein BCG vaccine is not compulsory or not involved in the common public vaccination programmes [89–91]. Hence, anticipation about the BCG vaccine to work (at some extent) against SARS-CoV-2 is increased which may possibly offers some kind of mysterious heterologous immunity against SARS-CoV-2.

Some vaccine developers are investigating the safety and immunogenicity of inactivated SARS-CoV-2 antigen in group of healthy people of different ages which are in development phase I or II (Table 4, entries 3–6) [92–95]. Developments of viral (adenovirus or lentivirus) vector based vaccine (Table 4, entries 7–13) [96–102] and nucleic acid vaccine (Table 4, entries 14–16) are on high priority of the research in designing of effective vaccine against COVID-19 [103–105]. Developments of any kind of vaccine involves various steps/stages such as identification of effective antigen, lab-scale antigen engineering/synthesis, safety concern, animal model study, human trails, efficacy trials, large scale synthesis, regulatory clearance and huge capital investment [13–15,17]. Thus vaccine

Table 4
Current scenario about COVID-19 vaccine development.

	Trial No.	Organization/Developer	Phase	Registration date	Objective/Information	Author [Ref.]
1	NCT04328441 Live attenuated	UMC Utrecht	Phase III	31-Mar-20	To investigate the influence of BCG vaccination on healthcare workers in order to get protection from COVID-19.	[90]
2	NCT04327206 Live attenuated	Murdoch Childrens Research Institute	Phase III	31-Mar-20	Determination of impact of BCG vaccination for reduction of COVID-19 severity in pandemic.	[91]
3	ChiCTR2000031809 Inactivated	Wuhan Institute of Biological Products co., Ltd.	Phase II	11-Apr-20	Investigation of safety and immunogenicity of inactivated COVID-19 vaccine in group of healthy people of different ages.	[92]
4	NCT04352608 Inactivated	Sinovac Research and Development Co., Ltd.	Phase I/II	20-Apr-20	To determine the safety and immunogenicity of trail inactivated COVID-19 vaccine in group of healthy peoples having age range of 18–59 years.	[93]
5	ChiCTR2000032459 Inactivated	Beijing Institute of Biological Products Ltd.	Phase I/II	01-May-20	To assess safety as well as immunogenicity of inactivated COVID-19 vaccine	[94]
6	NCT04383574 Inactivated	Sinovac Research and Development Co., Ltd.	Phase I/II	12-May-20	To assess safety as well as immunogenicity of inactivated COVID-19 vaccine	[95]
7	NCT04276896 Non-replicating Vector	Shenzhen Geno-Immune Medical Institute	Phase I/II	17-Feb-20	To study immunogenic response and safety concern of non-replicating vector COVID-19 vaccine.	[96]
8	NCT04299724 Vector base	Shenzhen Geno-Immune Medical Institute	Phase I	05-Mar-20	To develop universal lentiviral vector base vaccine for investigation of safety and immune reactivity of COVID-19 vaccine.	[97]
9	NCT04313127 Vector base	CanSino Biologics Inc.	Phase I	15-Mar-20	To develop Adenovirus Type 5 Vector base vaccine for study of safety, reacto-genesis and immune reactivity of COVID-19 vaccine.	[98]
10	ChiCTR2000030906 Vector base	Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Phase 1	18-Mar-20	To investigate the influence of Adenovirus Type 5 Vector base novel coronavirus vaccine in a group of healthy adults having age range 18–60 years	[99]
11	NCT04324606 Non-replicating vector	University of Oxford	Phase I/II	27-Mar-20	To investigate proficiency, safety and immunogenicity of COVID-19 vaccine in age group of 18–55 years.	[100]
12	NCT04341389 Vector base	Insitute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Phase II	10-Apr-20	To investigate the influence of Adenovirus Type 5 Vector base novel coronavirus vaccine in a group of healthy adults	[101]
13	ChiCTR2000031781 Vector base	Insitute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Phase I/II	10-Apr-20	To investigate the influence of Adenovirus Type 5 Vector base novel coronavirus vaccine in a group of healthy adults having age range 18–60 years.	[102]
14	NCT04336410 Nucleic acid	Inovio Pharmaceuticals	phase I	07-Apr-20	To investigate the nucleic acid vaccine against COVID-19.	[103]
15	2020-001038-36 Nucleic acid	BioNTech RNA Pharmaceuticals GmbH	Phase I/II, 2-Part, Dose-Escalation Trial	14-Apr-20	To study the safety and immunogenicity of various four vaccines against COVID-19.	[104]
16	NCT04368728 Nucleic acid	Biontech SE	phase I/II	30-Apr-20	To study safety and immunogenicity and efficiency of RNA vaccine against COVID-19.	[105]

designing is the lengthy process (take at least 18–36 months) which involves the multiple pauses in order to observe, to analyze and to conclude the clinical trials data [13–15]. However, some testing and trials can be done in parallel mode (such as animal model and human trails) with high financial risk in urgency to save the time [14,17]. In case of the coronavirus vaccine, existing knowledge about SARS-CoV and MERS-CoV vaccine designing may be crucial to consider which may offer clues about target sites for rapid vaccine development against SARS-CoV-2 virus (COVID-19) [13,15,16]. Thus the next few months are very crucial for development and anticipation of successful vaccine to combat COVID-19.

6. Other potential factors responsible for the development of COVID-19 vaccine

Several other factors are also responsible for the rapid designing of vaccine. In vaccine development phases, animal model testing, determination of route of administration and use of the adjuvant are theree crucial factors for timely release of the vaccine candidate.

6.1. Selection of appropriate animal model

The selection of animal model has some basic objectives such as (i) to characterize the disease/viral pathogenesis (ii) to characterize immunogenicity, (iii) to assess development of anti-viral/anti-disease vaccines responses and (iv) to observe clinical symptoms after challenge [106–110]. Further, animal model is used to assess pre-clinical efficiency of vaccine which involves the vaccine-dose, vaccine-safety, vaccine-formulation and route of administration [106–110]. Advanced computational bio-analytical-methods are used to determine the appropriate animal model and to avoid time as well as costing of unnecessary animal model experiments [106,107]. In case of coronavirus disease vaccine development, various animal models are reported to determine the pre-clinical efficacy of vaccine which involve mice [27,30,37,55,61,75,76] (BALB/c mice [28,35,48], hACE2 Tg mice [28], 129S6/SvEv mice [54], C57BL/6 mice [70]), wistar rat [60], rabbit [64], golden Syrian hamster [48], African green monkey [26] and rhesus monkey [40,41,44].

Thus in context of the rapid vaccine designing, animal model plays a crucial role in giving details of the cellular and humoral immune response [107]. However sometimes, different kinds of immune response can also be demonstrated by the animal model which is not expected in humans [108]. Hence it is challenging task to select the appropriate animal model and to develop the safer vaccine rapidly based on animal models in order to control the pandemic [107,109]. Furthermore, coronaviruses are the zoonotic origin virus and may have different kind of animal response than that of humans which may involves the restricted virus multiplication and less severity of the symptoms [26,44,55,109]. Thus, selection of the animal model is having great importance which needs to be screened in such a way that immunogenic response of animal model should be closely associated or related with human [106–109]. The success of the pre-clinical assessment of vaccine

depends on the animal model and hence various non-human primates can also use as animal model to test vaccine efficacy for rapid development [106–108,110].

6.2. Route of administration

Efficacy of the vaccine also depends on the selective route of the vaccination; various routes of administration of vaccine are available which can assist designing of effective vaccine [111–115]. Coronavirus disease is associated with the respiratory tract; hence it would be advantageous to induce memory response against respiratory tract infection [78]. Raghuwanshi et al. [78], investigated efficacy of plasmid DNA encoded N protein antigen loaded on chitosan nano-polymeric carrier for non-invasive intranasal immunization against SARS-CoV which induces an efficient mucosal and systemic immune response at the point of entry of virus [78]. Liu et al. [60], designed a recombinant vaccine involving adenovirus with expression of SARS-CoV S1 spike protein which can be able to induce an effective immune response against SARS-CoV in rats after subcutaneous or intranasal immunization. Hu et al. [113], was studied comparative analysis of immunogenicity induction via different routes of administration. They mentioned that, oral route of administration offered better immune response whereas; combination strategy of administration (oral + intramuscular) could be more impactful to generate the cellular and humoral immune response. However, Gai et al. [47], investigated the influence of the various immunization protocol for inactivated SARS-CoV virus which indicated significant production of IgG antibodies by an intraperitoneal immunization than intranasal immunization [47]. Recently Zhao et al. [114], observed that, intranasal route of administration offered effective cellular immune response in respiratory tract and better protection level in mice. Leyva-Grado et al. [115], proposed direct local administration into the respiratory tract which displayed better immunization efficiency. The route of vaccine administration is selected based on criteria of (i) lesser adverse impact and (ii) generation of effective and quick immunogenicity [112–115]. Various routes of administration are listed in Table 5 which are used during coronavirus vaccine development in last 17 years. For live attenuated coronavirus vaccination intranasal route was proposed by Bukreyev et al. [26], and Escriou et al. [30], (Table 5, entries 1,2). For inactivated coronavirus vaccine intranasal [43,47], subcutaneous [38,45], intramuscular [40,41], and intraperitoneal [47] route of vaccination was reported (Table 5, entries 3–9). Zakhartchouk et al. [54], mentioned intradermal route of administration for protein subunit vaccine of coronavirus (Table 5, entry 10). Intramuscular [61], intranasal [60,61] and subcutaneous [60] mode of vaccination was reported for vector base vaccine of coronavirus (Table 5, entries 11–14). Intramuscular/subcutaneous mode of vaccination is reported by various researcher for the nucleic acid vaccine [63,64,67,70,72] (Table 5, entries 15–19). More recently, for nano-based vaccine intranasal [77,78] and intramuscular [77] mode of vaccination is reported by Shim and Raghuvanshi et al. [77,78], (Table 5, entries 20–22). Thus different responses are attributed by different types of routes of vaccine administration which need appropriate screening; and hence it is the challenging task in this time of race to design successful rapid vaccine.

6.3. Selection of efficient adjuvants

Adjuvant is an essential component used in vaccine designing to boost the immune response with minimum amount of antigen, to regulate the immunogenicity and to offers better protection by mean of long-period impact of vaccine [116–118]. More specifically, the use of adjuvant manages (i) the vaccine dosages (ii) promotes slow release of antigen, (iii) retains antigenecity of antigen for longer time and (iv) activate selective pathways of immunity against vaccine antigen [116–118]. The actual mechanism of the adjuvant functioning is not well known, but its use offers more benefits for effective functioning of vaccine [116–118]. Various kinds of the adjuvants are reported in the literatures (Table 6, entries 1–10) that are used in coronavirus vaccine development. It involves aluminum hydroxide [35,36,38,43], Freund's adjuvant [42,51], oligodeoxynucleotides [47], 8AS01B [48], AS03A [48], and MF59 [56] as adjuvants. Tang et al. [35,36], Xiong et al. [37], Gai et al. [47], and Zakhartchouk et al. [54], reported that, antibody production was augmented by the use of adjuvant [35–37,47,54]. However, Spruth et al. [43], did not observe any significant impact of adjuvant on antibody induction in coronavirus vaccine designing [43].

The uses of the adjuvant make it possible to immunize to more number of people as it facilitates the requirement of small dosages

Table 5
Mode of administration used in croronavirus vaccine development.

Entry	Route of administration	Vaccine types	Author [Ref.]
1	Intranasal	Live attenuated vaccine	Bukreyev et al. [26]
2	Intranasal	Live attenuated vaccine	Escriou et al. [30]
3	Intranasal	Inactivated vaccine	Spruth et al. [43]
4	Intranasal	Inactivated vaccine	Gai et al. [47]
5	Intramuscular	Inactivated vaccine	Zhong et al. [40]
6	Intramuscular	Inactivated vaccine	Zhou et al. [41]
7	Subcutaneous	Inactivated vaccine	Takasuka et al. [38]
8	Subcutaneous	Inactivated vaccine	Tsunetsugu-Yokota et al. [45]
9	Intraperitoneal	Inactivated vaccine	Gai et al. [47]
10	Subcutaneous	Protein subunit vaccine	Zakhartchouk et al. [54]
11	Intranasal	Vector based vaccine	Liu et al. [60]
12	Intranasal	Vector based vaccine	Ababneh et al. [61]
13	Intramuscular	Vector based vaccine	Ababneh et al. [61]
14	Subcutaneous	Vector based vaccine	Liu et al. [60]
15	Intramuscular	Nucleic acid vaccine	Zhao et al. [63]
16	Intramuscular	Nucleic acid vaccine	Li et al. [64]
17	Intramuscular	Nucleic acid vaccine	Huang et al. [67]
18	Intradermal	Nucleic acid vaccine	Zakhartchouk et al. [70]
19	Intramuscular	Nucleic acid vaccine	Wang et al. [72]
20	Intranasal	Nano-based vaccine	Shim et al. [77]
21	Intranasal	Nano-based vaccine	Raghu-wanshi et al. [78]
22	Intramuscular	Nano-based vaccine	Kato et al. [78]

Table 6
Use of adjuvants in croronavirus vaccine development.

Entry	Adjuvant	Vaccine type	Finding about use of adjuvant	Author [Ref.]
1	Aluminum hydroxide	Inactivated	The antibody levels induced by the vaccine with aluminum hydroxide were higher than those without aluminum hydroxide.	Tang et al. [35]
2	Aluminum hydroxide	Inactivated	The antibody levels induced by the vaccine with aluminum hydroxide were higher than those without aluminum hydroxide.	Tang et al. [36]
3	Aluminum hydroxide gel (alum)	Inactivated	Use of alum augmented the serum IgG production was	Takasuka et al. [38]
4	$Al(OH)_3$, oligodeoxy-nucleotides and Freund's adjuvant.	Inactivated	Tested various adjuvant, use of Freund's adjuvant in vaccine formulation is effective	Zhang et al. [42]
5	Aluminum hydroxide	Inactivated	No significant effect of adjuvant aluminum hydroxide on the immunogenicity of vaccine.	Spruth et al. [43]
6	Oligodeoxynucleotides	Inactivated	Use of oligodeoxynucleotides in inactivated SARS-CoV vaccine formulation induces the IgG antibodies.	Gai et al. [47]
7	8AS01 B and AS03 A	Inactivated	Use of 8 AS01 B adjuvant is more effective than AS03 A adjuvant in vaccine formulation	Roberts et al. [48]
9	Freund's adjuvant	Subunit vaccine	Use of Freund's adjuvant in vaccine formulation is effective	Guo et al. [51]
10	Alum plus CpG oligodeoxynucleotides (ODN)	Subunit vaccine	Alum plus CpG oligodeoxy-nucleotides displayed increase of IgG2a antibody and INF	Zakhartchouk et al. [54]
11	MF59 adjuvant	Protein subunit	MF59 as adjuvant increases the performance of vaccine	Tang et al. [56]
12	Aluminum hydroxide	Nucleic acid vaccine	Aluminum hydroxide as an adjuvant increases the efficacy of vaccine	Zakhartchouk et al. [66]

[116,117]. However in case of respiratory disease related vaccine, choice of adjuvant plays an important role, since some nano-particles, lipid and inulin based adjuvants causes toxicity to the lung tissue which are rich in immune cells and macrophages [119–121]. Honda-Okubo et al. [119], reported significant eosinophilic immunopathology associated with the use of delta inulin based adjuvant in animal model mice. Wang et al. [120] reported the lung toxicity attributed due to nano-particle based adjuvant compounds. Further Raetz et al. [121], reported cytotoxicity of the lipid based adjuvants. Other common side effects of use of adjuvants include myalegia, pyrexia, allergic action, rashes, and rarely neurotoxicity [122]. Hence, the use of adjuvant should be well optimized in vaccine designing; the ideal adjuvant is biocompatible in nature, biodegradable, should not harm cells in any way and do not induce any kind of allergic/side effects [116–118,123]. Thus it is always challenging task to select the appropriate adjuvant for the respiratory disease related vaccine designing. Various FDA approved adjuvants are available commercially with high purity which can be used selectively and safely (with proper screening) in vaccine designing to improve the performance of vaccine.

7. Opportunities and challenges

An ideal designed vaccine should able (i) to generate a potential neutralizing-antibody response against different viral strains of same pathogen (ii) to offer well protection against infection as well as transmission (iii) to produce quick immunogenic response with lower amount of antigen dosage (iv) to be used/injected in various groups of ages safely without any allergic or adverse effects or inflammatory effects. In the prevailing COVID-19 scenario, rapid development of a safer and efficient vaccine against coronavirus disease-2019 is an urgent need in order to control this ongoing pandemic. However, there are several challenges that are associated with the vaccine development against SARS-CoV-2 (COVID-19).

Virus characteristic and virulence ability of newly emerging viruses: It is always difficult to find out genomic sequence, mode of actual virus entry, mechanism of virus action, mutation, target organ, development of immunity, variation in adaptive immunity, asymptomatic nature, delaying of onset of symptoms and re-infection mechanism are the basic challenges for newly emerging viruses which needs careful study and analysis to design future possible vaccine.

Vaccine efficiency and optimization: The development of coronavirus vaccine after SARS and MERS outbreak is delayed due to unavailability of the suitable animal model which displayed restricted clinical manifestation and severity of disease. Due to high virulence ability of SARAS-CoV-2 coronavirus, the challenge-study of vaccine development generally not performed in human directly. Several challenges are associated with the selection of animal model such as presence of the natural immunity about testing pathogen, or absence of actual natural receptor for testing pathogen or unexpected pathogencity / immunogenicity against tested pathogen etc. Selection of various modes of administration involve oral, intramuscular, intradermal, subcutaneous, intranasal, and intraperitoneal mode. Most of viruses enter into the human via respiratory/digestive/genital tract in which mucosal vaccine may play an important role which can able to generate the immunogenic response at the site of occurrence of infection. However, it is always a challenging task to determine effective and appropriate route of administration for high efficiency of vaccine. Use of the adjuvant is recommended to get enhanced immunogenicity. Biological activity of adjuvant, antigen-adjuvant interaction, selection of adjuvant, mechanism of the adjuvant, adjuvant formulation, adjuvant dosage, physicochemical parameters of adjuvant are the major challenging issues related to use of the suitable adjuvant during the rapid development of SARS-CoV-2 vaccine.

Vaccine safety: The safety of the vaccine against various pathogenic strains can be investigated by the repetitive and different animal model experiments as well as clinical trials which are considered as the biggest challenge in the race of development of SARS-CoV-2 vaccine in small time duration. Further, several previous literature reported antibody dependant adverse events such as lung injury, pro-inflamatory cytokine lung mononuclear infiltration, increased eosinophil and neutrophil influx in the animal model test of SARS and MERS vaccine [33,34,49]. Moreover, antigen-dependent enhancement factor (which promote virus infectivity) is also a big concern. Live attenuated and inactivated vaccine may show reversion of the virulence. Hence, vaccine target profile must be provided with details of safety consideration to avoid adverse immunogenic effects. Thus safety is a major concern for designing of vaccine against newly emerging pathogens. Beside this, vaccine must offer long term protection and immune response against pathogenic virus with small amount of antigen dose, since

large and frequent dosage are difficultly approved by the FDA.

Vaccine designing time-span: Vaccine designing (within short period of time) itself is the biggest challenge to control pandemic situation which involves series of operations such as determination of antigen, antigen potency, route of immunization, animal model study, immune-response study, clinical trials, safety concern, regulatory approval, licensing, patenting, bulk-production, and target product profile etc. All these operations are must to design vaccine which at least required on an average 18–20 months (or more) to develop a successful vaccine. Previous documented literature about SARS/MERS vaccine development may be helpful to guess possible antigen, animal model, adjuvant, and safety concern in quick designing of SARS-CoV-2 vaccine.

Possible COVID-19 vaccine and other vaccination schedule: Vaccine design must be suitable for all kinds of the age group while target population for the vaccination should be prioritized. This vaccine should not be interfere with other vaccination protocol in paediatrics which required further extensive efforts to determine the interference of possible SARS-CoV-2 (COVID-19) vaccine with other vaccination protocol or vice-a-versa. In this short time span of vaccine developing phase, it is hardly possible to identify the probable interference of COVID-19 vaccine on other vaccination schedules due to time consuming study and protocol.

Vaccine scale-up and commercialization: The bio-processing scale-up of vaccine with the high purity of antigen is also a bigger challenge, since, all trial and error experiments are performed by lab made (small scale) antigen synthesis/production. Whereas, large scale production sometimes hamper (in race of rapid/quick production) by the purity of the antigen product which may largely affect the safety and induction of cellular immune response of vaccine with substantial adverse effects. Further, vaccine should have sufficient antigen and shelf-life stability. Vaccine production should be in substantial stock, and considering the global health concern vaccine must be made available with minimal charges (less profit). For this, various multinational companies and National Medical or Virology research organization need to take initiative. Thus, quality control, technology transfer, trouble-free scale-up, unpredictable side effects of newly developed vaccine and high cost investment are the major concerns to develop the rapid vaccine against newly emerging viruses in present pandemic scenario. Any kind of influence or pressure regarding to hurriedly and quack development of vaccine may result in adverse events or even complete failure of vaccine designing project. There should not be any kind of timeline for vaccine designing against SARS-CoV-2 virus, however this task should be treated on high priority.

8. Conclusion

A safe, efficient, preventive or prophylaxis vaccine is urgently needed to control recent COVID-19 pandemic or possible future coronavirus outbreak. Several efforts have been attempted in the last 17 years to design a successful vaccine against coronavirus. However, NO vaccine is approved till date against coronavirus. Looking to the present COVID-19 pandemic, vaccination approach may be of high interest to avoid the further infection/transmission and future outbreak of coronavirus. The full length genome phylogenetic analysis suggested that genomic sequence of SARS-CoV-2 is almost 78–80% similar to that of SARS-CoV; further both these viruses bind to same host cell receptors ACE-2. Hence it is expected that, previously taken efforts and literature/data/experience about SARS-CoV vaccine designing may play a crucial role in rapid vaccine development against SARS-CoV-2 virus. In view of this, the present review article summarizes existing related literature information about the type of vaccine, antigen, immunogenic response, animal model, route of administration, and adjuvants for designing of coronavirus vaccine which may be of great importance. The most commonly used antigen for vaccine development were receptor binding domain spike protein segment which is recognized as highly antigenic in nature to produce humoral and cellular immune response. Further, more conserved M protein or N protein epitope can also be used synergetically with S protein epitope to get enhanced immunogenicity along with lymph node T-cell proliferation and cytokine production such as IL-2, IL-4, IL-5, IFN-γ and TNF-α. In present scenario of COVID-19, various research and pharmaceutical organizations have undertaken the challenging task of rapid vaccine designing against coronavirus which are in pre-clinical or initial phase of development. However, a successful vaccine development may take at least some months or years. In context of this, the sorted information of previous literature reports (which is condensed in this review) may be crucial to guess the possible antigen, animal model, route of vaccination, selection of adjuvant and safety concern. Various opportunities and challenges are associated with the rapid designing of vaccine which are also addressed in order to develop the successful vaccine.

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Declaration of competing interest

On the behalf of all authors, I corresponding author confirms that there is no conflicts of interest.

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