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VACCINE DEVELOPMENT & MODERN APPROACHES FOR COVID 19

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Abstract
Most of the infectious diseases due to pathogens are caused by the mucosal tract penetration. Hence, vaccines delivered directly to the mucosal tissues can defend pathogenic infections and provide protection at the first site of infection. Thus, mucosal, specifically, oral delivery is becoming the most ideal mode of vaccination. However, oral vaccines have to overcome numerous barriers such as the extremely low pH of the stomach, the presence of proteolytic enzymes and bile salts as well as low permeability in the intestine. Several formulations based on nanoparticles like liposomes, solid solutions, emulsions, VLPs are currently being used to prepare stable oral vaccine formulations. In current days different companies are trying to develop oral vaccine for COVID 19 also. This review briefly discusses several vaccine development criteria their mechanisms and various aspects of oral nanoparticles-based vaccine design that should be considered for improved mucosal and systemic immune responses.
Keywords: Vaccine development, nanoparticles, liposome, VLP, COVID 19, mucosal immunity.

Introduction

After the penetration of pathogenic microorganisms in our body, they (germs) instantly start to invade and multiply to extend their soldiers against body's immunity system resulting infection. Even though the immunity system has its own mechanisms to battle against such harmful germs by using its macrophages, T-lymphocytes also as B-lymphocytes, maximum times this fail in case of very harmful pathogens. According to the statistics of WHO within the year 2016, infectious diseases are still now causing severe mortality globally, especially in developing countries whereas infections are liable for more than 30% of total death among top ten causes of human mortality [1]. Nowadays, researchers are more likely tend to develop vaccines to combat and eradicate deadly infectious diseases due to their extreme capability to fight against pathogens alongside immunity. Moreover, vaccines are used against various lethal infectious diseases from 1796 when Jenner for the primary time introduced smallpox vaccine till now successfully [2]. Vaccines are usually a kind of biological accumulation of antigens that take a step to activate adaptive immunity by mimicking an infection, thus, vaccines obviate harmful microorganisms also as impede microbial evolution. The infection which is caused by vaccines usually doesn't cause illness of a private, but his/her body remember of this infection by treating this vaccine a threat and creates antibody and memory cells for further facing that infections causing microbes [3]. Immunization through vaccination is useful not just for vaccinated personnel but also

his/her surrounding society by producing herb immunity [4]. Surprisingly, about 90% of total pathogens cross thin and simply vulnerable mucosa to invade cellular mechanisms via handling digestive, pulmonary and genitourinary systems credit go to the large surface area of mucous. [5]

Hence, primary targeting site sort of a mucosal barrier for vaccine delivery would be the acceptable decision against infection-causing pathogens. Once first defensive position is going to be strong only a couple of pathogens can cross the barrier. Researchers already proved that vaccine delivery targeting mucus membrane can easily produce mucus antibody IgA against pathogens [6] also as increase cellular immune by secreting systemic antibody IgG [7,8]. Thus, when one site of the mucosal barrier is going to be protected, others site is going to be automatically protected through mucosal inner safeguard network [9]. Through oral vaccine delivery, it's possible to focus on the mucosal barrier instead of other routes of administration due to oral delivery of vaccines facilitate both IgA and IgG secretion in the body. Moreover, oral delivery isn't the sole potential for the simplest protective mechanisms but also its other merits like enhanced patient compliance, price-effectiveness, large-scale production, no harm and infection from the needle and so one. In contrast, IV/IM/SC administrated vaccines aren't ready to stop pathogens within the mucosal barrier. Development of oral vaccines can be a novel approach by considering their physiological barriers & pharmacokinetics (ADME). However, most of the potential vaccines are administered through injection whereas a very few numbers of vaccines are available for oral delivery [10].

Conventional Vaccine Types

Scientists are trying from the years to develop various types of vaccines against harmful infectious pathogens with an identical function to produce robust immune responses against pathogens. For vaccine development against a specific virus infection, it's important to know about both how germs attack the cells and the way our immune system response. Vaccines can be categorized as the following. [11]

Live Attenuated Vaccine

Among all types of vaccines, Live attenuated vaccines are considered as the most effective & affordable. This type of vaccines is generally developed from weakened viruses. The mechanism of live attenuated vaccine is asymptomatic infection without having illness & patient complication resulting into increased antibody formation against a particular antigen of pathogens. Live attenuated vaccines can provide lifetime safe guards after only single or highly two doses of inoculation. These vaccines have been found to modulate all types of immune response by secreting both IgA and IgG. [12]

Killed Whole cell vaccines

To overcome different complication of live attenuated vaccines, killed whole cell vaccines are used which are also known as inactivated vaccines. Inactivated vaccines are more protective & static in comparison to live attenuated vaccines. The mechanism of actions behind inactivated vaccine is that due to their inactivated state they cannot replicate further within the body, but our immune system can recognize them & produce respective antibody against those antigens. [13,14,15]

Next-generation vaccines enabled through advances in nanotechnology

Viruses are objects of nanoscale, therefore can be regarded as naturally occurring nanomaterials; according to that definition, LAVs, IVs and subunit & others are parts nanotechnologies. Identical sizes of Nanoparticles and viruses make nanotechnology approachable in vaccine development and immune engineering. Nanoparticles, mimic the structural features of viruses and chemical biology, biotechnology and nano-chemistry enables the development of next-generation vaccine technologies.

Advancement in nanotechnology has been resulted into invention of following kinds of vaccines-

Nucleic acid-based vaccines

Development of genetic codes for in vitro production of identical viral protein is a promising alternative to conventional vaccine development approach. Both DNA & mRNA based vaccines are developed by this technology and also being used for current COVID19 pandemic. Though these technologies have high scalability, safety speed stability but they carry significant risk of failures in clinical trials like other approaches [15]. Till date there is no licensed DNA or RNA vaccine used clinically. All though there is a particular advantage of these kind of vaccines is that in addition to antibody and $CD4^+$ T cell responses, they also have $CD8^+$ cytotoxic T cell eliciting responses, which plays a key role for pathogen eradication [16,17,18].

As example of DNA vaccines in current days, Inovio Pharmaceuticals have started working with their COVID 19 vaccine which is on Phase I clinical trial has commenced 6 April 2020.

Another rising company on the way for a Phase I clinical trial is Entos Pharmaceuticals, Inc. a company based in Alberta, Canada. mRNA vaccines are generally produced through in vitro transcription, which eliminates the need for pathogenic cells [19]. More interestingly DNA vaccines offer higher stability & efficacy over mRNA vaccines; the mRNA is non-integrating and therefore poses no risk of insertional mutagenesis. Additionally, the half-life, efficacy and immunogenicity of mRNA can be tuned through established modifications [20]. For example, researchers at the Imperial College of London and Arcturus Therapeutics have invented self-amplifying RNA technology to prolong the short half-life of the RNA and thereby boost S protein expression levels [21]. Nanotechnology-based approaches offer trafficking of the vaccine to appropriate cellular populations and subcellular locations. Some synthetic nano-carriers like cationic liposomes and polymeric nanoparticles are being used for the delivery of DNA vaccines across cell membranes; targeted formulations of such can further enhance nuclear translocation of the plasmid DNA [22-25].

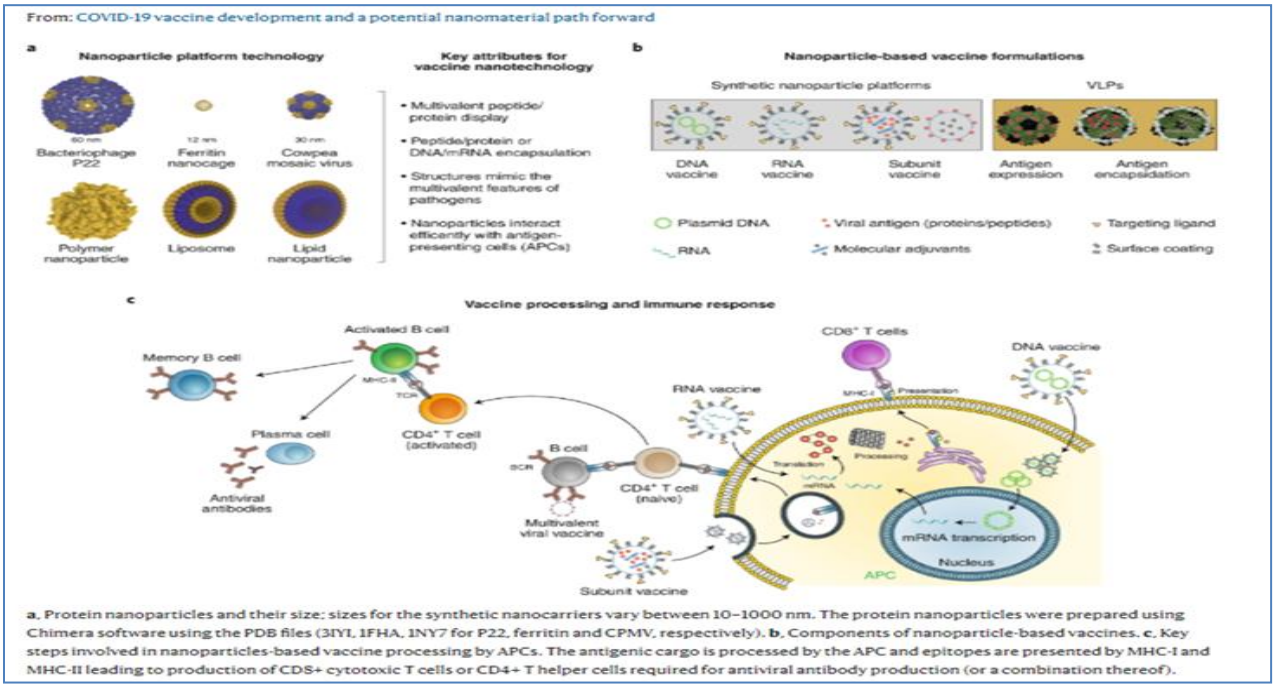

Fig.1: Nnao-particle based vaccine technologies [25]

Moderna's mRNA vaccine is developed based on a lipid nanoparticle platform, but there are many other evolving nanotechnologies for the delivery of nucleic acid vaccines (several structures are shown in Fig. 1). Further some others cationic nano-emulsions, dendrimers, liposomes or polysaccharide particles has been utilized for improving the stability and delivery of mRNA based vaccines [23,24].

Sub Unit vaccines

Unlike traditional live attenuated or killed vaccines in which either live or killed pathogens are used, subunit vaccine contains the only antigen of specific pathogens. Where there is no chance of mutant formation. Sometimes only epitope of antigen is used as vaccine in which paratope (antigen binding protein) of antibody is bind.

For example based on current senario contemporary SARS-CoV-2 subunit vaccine candidates are formulated using full-length S protein or S1/S2 subunits with adjuvants. The flag holder amongst inventors, Novavax had started developing on 25 May 2020. Also, one more competitor Sanofi Pasteur/GSK, Johnson & Johnson, Vaxine, and the University of Pittsburgh also there in this race from December, 2020. Others, includes University of Queensland and Clover Biopharmaceuticals were also independently developing subunit vaccines engineered to present the perfusion based on S protein using the molecular clamp technology [26] and the Trimer-tag technology [27]. Further, other groups are exploring subunit vaccines using only the RBD of the S protein.

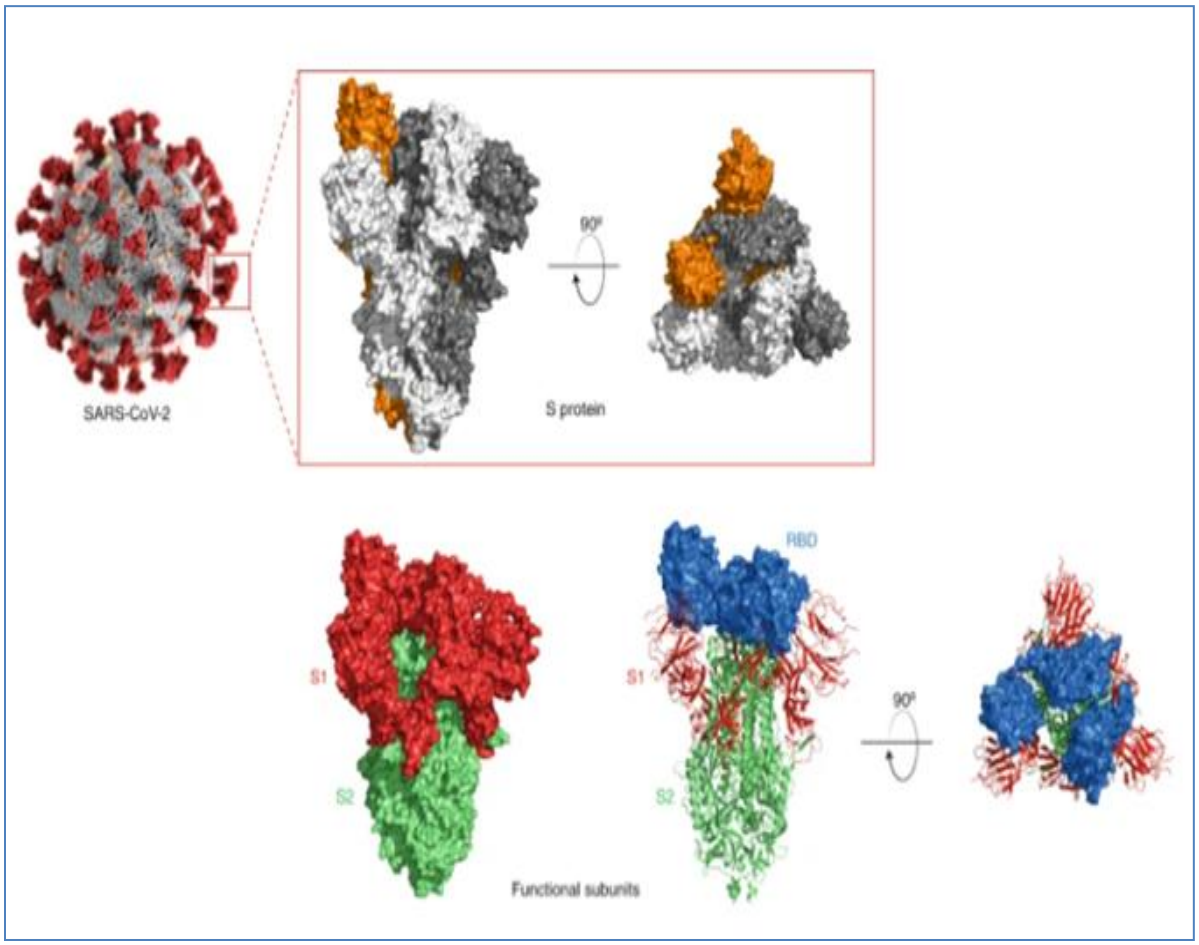

Fig. 2: The spike protein S, protruding from the coronavirus SARS-COV-2 is the primary target for various ongoing vaccine development efforts. [25]

Polysaccharide vaccines

In polysaccharides vaccines, particular parts of pathogenic polysaccharide capsule or pure cell membrane of sugar coat, such as surface protein of polysaccharide is used. Sometimes polysaccharide coated bacterial or viral antigens are also used to elicit immune response through vaccination [28]. Though it is very easy to prepare anti capsular antibody polysaccharide vaccine [29], it is not that much useful due to slow antibody generation, and produced immunogenicity is very poor with poor immune memory.

Conjugated vaccines

To counteract with underdeveloped immunity of children that cannot work against polysaccharide antigens and immature immune system conjugated vaccines has been developed. The modern technology chemically linked a protein carrier (diphtheria or tetanus toxoids) from a different agent with microbial polysaccharide cell wall to enhance immunogenicity for a long time even in children. The chemical linkage is the main reason why polysaccharide-protein conjugation vaccines are better rather than polysaccharide vaccines. To be specific, T cells of our immune system (both adult and child) first detect the protein carriers of polysaccharide-protein conjugation vaccines as well as alert B cells about the entrance of pathogenic antigens as foreign materials in our body [30]. Then, plasma B cells produce a huge amount of antibody to destroy such threats. Then remaining memory B cells act as a defensive soldier against the pathogens for future. [31]

Peptide-based vaccines

An important consideration for vaccine designing is safety. Many vaccines rely on immunological introduction of whole structural moieties, for example, full-length S protein, which will act as large component of potent epitopes leading to a broad spectrum of antibody formation and immune responses. Though, further studies on SARS and MERS vaccines have pointed to the risk of antibody-dependent enhancement (ADE) of infection. [32,33,34] Further research shows, presence of non-neutralizing antibodies contributing to increased infections which latter can lead to life-threatening allergic inflammations [35,36]. However, there is no clear evidence for that, immunological history from patients may point toward possible ADE for SARS-CoV-2, suggesting that high IgG level correlate with worse outcomes [37,38]. Therefore, developing peptide epitope vaccines targeting the SARS-CoV-2, S protein may yield a safer vaccine. Various B- and T-cell based epitopes of the SARS-CoV-2 S protein have already been recognized and projected in silico [39,40,41]. Significantly, when serum from convalescent COVID-19 patients was screened for neutralizing antibodies, experimentally-derived peptide epitopes will confirm useful epitope regions and will help to invent more optimal antigens in second-generation SARS-CoV-2 peptide-vaccines; Currently National Institutes of Health (NIH) has funded La Jolla Institute for Immunology (LJI) for this perspective. [42,43]

Peptide-based vaccines are the simplest form of vaccines that can be easily designed, with rapid validation & quick scalability [44]. Peptide-based vaccines can be formulated from peptides and adjuvant mixtures of peptides, which can be delivered by an appropriate nanocarrier or can be conjugated with nucleic acid vaccine formulations. Several peptide-based vaccines and peptide–nanoparticle conjugated vaccines targeting chronic diseases and cancer are under clinical trial [45,46]. In addition, for the development of peptide-based COVID-19 vaccines, industries and academics has predicted B- and T-cell epitopes in their development of subunit vaccines against SARS-CoV-2; for example- OncoGen. University of Cambridge/DIOSynVax is also using immune informatics-derived peptide sequences of S protein in their vaccine development [47,48]. Peptide based vaccines are dependent on adjuvant compatibility and required proper carrier for delivery for efficacy, and nanoparticles can serve both these roles. By

introducing emerging invention of targeting lymph nodes (LNs) and subcellular locations, efficacy of nanoparticle vaccine can be improved and their immune profiles can be modified to address specific diseases. For example, the new innovative solution of 'albumin hitch hiking' elicited the natural trafficking ability of albumin to LNs [49]. Recently, the intrinsic ability of nanoparticles to target specific subsets of LN-resident dendritic cells (DCs) and macrophages has used to design a dual targeting Hepatitis B virus (HBV) vaccine. The supplementing immune responses generated by these cellular subsets resulted in an enhanced efficacy of viral vaccine in a chronic HBV mouse model [50]. Subcellular localization of the antigen also acts as critical determinant of the ensuing immune response. Vaccine designing parameters such as encapsulated antigen, surface displayed antigens govern the others presentation of the antigen. Where the first requires degradation or disassembly of the nanocarrier and therefore mimic viral infection leading to cellular immune response, the latter leads primarily to humoral immune response generated by the externally displayed viral proteins [51]. See Table 1 for examples of modern approaches for the development of COVID 19 vaccines-

Table 1: Modern aproaches For COVID 19 [52]

Developere	***Vaccine***	***Vaccine type***	***Clinical Trial Registry***
Sinovac	Formalin inactivating whole virus particles combined with an alum adjuvant	Inactivated vaccine	NCT04383574
Beijing Institute of Biological Products, Sinopharm	Inactivated vaccine of SARS-CoV-2	Inactivated vaccine	ChiCTR2000032459
Wuhan Institute of Biological Products, Sinopharm	Inactivated vaccine of SARS-CoV-2	Inactivated vaccine	ChiCTR2000031809
Institute of Medical Biology, Chinese Academy of Medical Sciences	Inactivated vaccine of SARS-CoV-2	Inactivated vaccine	https://www.who.int/who-documents-detail/draft-landscape-of-covid-19-candidate-vaccines
Novavax	Stable, pre-fusion S protein given with adjuvant, Matrix-M	Subunit vaccine	NCT04368988
CanSino Biological Incorporation, Beijing Institute of Biotechnology, Canadian Center for Vaccinology	Recombinant SARS-CoV-2 intramuscular vaccine that incorporates the adenovirus type 5 vector (Ad5-nCoV)	Non-replicating viral vector vaccine	NCT04313127 NCT04341389 NCT04398147
University of Oxford, AstraZeneca	Chimpanzee adenovirus vaccine vector (ChAdOx1)	Non-replicating viral vector vaccine	NCT04324606 NCT04400838
Shenzhen Geno-Immune Medical Institute	Approach 1: modified dendritic cells expressing SARS-CoV-2 minigenesApproach 2: artificial antigen-presenting cells expressing SARS-CoV-2 minigenes	Non-replicating viral vector vaccine	NCT04276896 NCT04299724

Inovio Pharmaceuticals	Optimized DNA vaccine given via electroporation	DNA vaccine	NCT04336410
Symvivo	bacTRL-Spike oral DNA vaccine encoding S of SARS-CoV-2	DNA vaccine	NCT04334980
Moderna	Prefusion stabilized S protein mRNA vaccine	RNA vaccine	NCT04405076
BioNTech, Pfizer, Fosun Pharma	Lipid nanoparticle mRNA vaccines	RNA vaccine	NCT04368728

Correlation between Oral vaccine & Nano-particles

Oral vaccine delivery systems are generally particulate containing emulsions, micro particles or nano-particles or liposomes and have identical dimensions to the pathogens, against which immune system evolved to fight. Increasingly more sophisticated drug delivery systems are being developed; using which immunity stimulatory adjuvant may be utilized with antigens. The rational for this purpose is to ensure that both the adjuvant & antigen of pathogens are deliverable to the antigen-presenting cells. Enhancement of adjuvant activity through using nano particulate delivery system is particularly exciting, as it has synergistic effects & these effects can be correlated with their increased potency to develop robust immune response within effectors organ. Nano-particle also offers the enhancement of their uptake through cells by penetration due to their identical surface area, thus nano-technology becomes a wide field of exploration & evolution for pharmaceutical science. [53]

Barriers to Oral delivery of vaccines

Numbers of barriers are associated with successful induction of immunity in the mucosal surfaces. One of them is that vaccine antigens delivered through the mucosa have tendency to get diluted in mucosal secretions, which may limit effective deposition onto the mucosal epithelia. Additionally, antigens delivered through the mucosal surfaces have a tendency to be captured within the mucus layer and subsequently will get degraded by proteases or nucleases [53]. The acidic pH of the gastrointestinal tract is also act as barrier for successful oral immunization. Mucosal tissues are highly colonized by commensal microbes, which also can significantly influence mucosal immune regulation and serves as a barrier to optimal mucosal immunity [54]. Subunit vaccine formulations composing proteins, DNA/RNA, or polysaccharides are prone to degradation and may lose their potency during passage through the mucosa. Therefore, they need sufficient protection for optimal efficacy. Now day's mucosal vaccines have found to be more efficacious, if the particulates of such vaccines can mimic the physicochemical properties of opportunistic pathogens, with respect to shape, size & surface charges. So an effective vaccine should have some capabilities & designs to elicit mucosal immunization like (i) overcoming mucosal barriers, (ii) targeting mucosal APCs or M cells for adequate antigen processing and T- or B-cell activation, and (iii) capability of modulating the kinetics of antigen and adjuvant presentation for induction of proper immunological memory responses. Nanotechnology-based drug delivery systems are capable of overcoming physiological barriers of the mucosa, can efficiently target immune cells, and control antigen kinetics. [55,56]

Nanotechnology-based solutions for oral vaccination

Nano-technology based drug delivery is attractive solution for targeted delivery of antigens on the effectors organs. Using nanotechnology, different properties of vaccine antigens like solubility, stability & surface properties can be easily modified to get required efficacy of vaccines. This capability of nanotechnology has been created a great interest in the field of vaccinology. Vaccine

antigens can either be encapsulated in or surface absorbed on nanoparticles. Through encapsulation, nanoparticles can be evolved to a method of delivering antigens, which may either gets degraded fast upon administration or elicit a brief, local immune response. Encapsulation of antigens into nanoparticles allows introduction of antigens to APCs in a parallel way as during natural infections and may elicit similar immune responses. Size range between 10–1000 nm in the nanoscale provides a high surface area-to-volume ratio and a high diffusion rate, which is appropriate for the delivery of vaccine antigens to mucosal sites like GIT, respiratory tract, Urogenital tract.

Additional advantages of nanoparticle-based antigens delivery, over conventional systems, include (i) targeted delivery of antigens; (ii) improved antigen penetration; (iii) adequate and sustained antigen concentration at the effectors sites of mucosa; (iv) enhanced bioavailability; and (v) improved immunization, which may be either immunity elicitation via pro-inflammatory cytokines or immune suppression via anti-inflammatory cytokines.

Different types of nanoparticle-based delivery systems have been invented for vaccine delivery to mucosal surfaces. These inventions include liposomes, polymeric nanoparticles, lipid-polymer hybrid nanoparticles, emulsions, virus-like particles (VLPs), dendrimers, and immune stimulatory complexes (ISCOMs) (Table 2). Design and development of an efficient and safe delivery system does not only require an understanding of biomaterial but also the carrier (antigen + adjuvant), the target, and the desired immunomodulation [57].

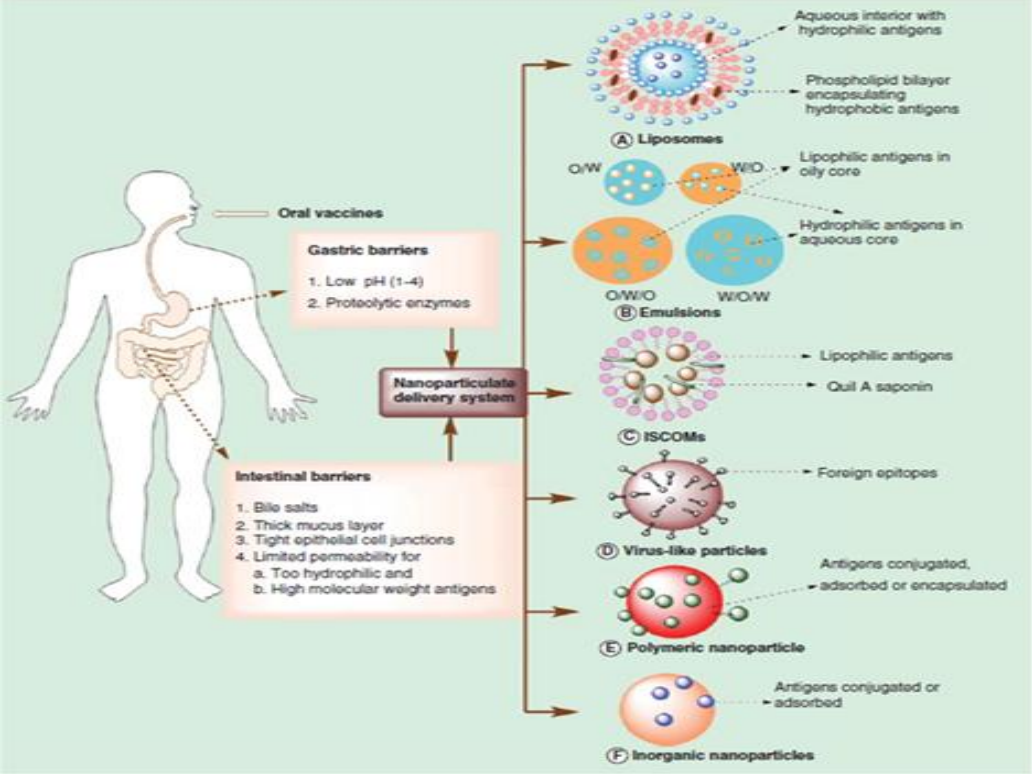

Fig 3: Nanoparticles used for oral vaccines [58]

Nanoparticles must have to be safe materials that are sterile, nonreactive, and biocompatible, and they should have optimal encapsulation or conjugation, capability, and penetration ability to the local mucosal layers to deliver antigen [59]. In addition, nanoparticles should have ability to protect the antigen from the harsh pH or enzymatic activity of the mucosa,

which can otherwise result in antigen degradation [60], and deliver the antigen to the right APCs for effective activation of the immune system [61]. Therefore an effective generation of an immune response against a vaccine antigen requires knowledge about antigen uptake and binding, release kinetics, and the mechanisms of generation of mucosal immunity, which should be integrated in the design of the nanoparticle carrier [62].

Table 2: Examples of nanoparticulate drug delivery systems. [62]

Drug delivery system	Composition	Antigen/pathogen	Mucosal route	Animal model	Immunity type
Lipid-based nanoparticles					
Liposomes	DOTAP and dimethyl aminoethyl carbamate; DDA and TDB (CAF01); CAF01 and CpG; phosphatidylcholine, cholesterol, and chitosan; DPPC, DPPS, and cholesterol; DOPC and cholesterol	*Streptococcus pneumonia*; *Leishmania amazonensis* antigens; streptococcal C5a peptidase, Ag85B; influenza A virus; *Salmonella enteritidis*; H56	Nasal, oral, sublingual, pulmonary	Mice	IgA, IgG, Th17; IFN-γ; IgA and Th7; Th1; IgA and IgG
ICMVs	DOPC, MPB, and MPLA	Ovalbumin	Pulmonary	Mice	T_{em}
Solid lipid nanoparticles	DPPC and MPLA	Hepatitis B	Rectal, oral	Rats	IgA, IgG, IFN-γ, IL-2
Cubosomes	Monoolein and Quil-A	Ovalbumin	Oral	Mice	IgG, CTL
Emulsions	Nanoemulsion $W_{80}5EC$; isopropyl myristate, Cremophor EL-35 and PEG	H1N1 influenza, respiratory syncytial virus, methicillin-resistant *Staphylococcus aureus*, HIV gp120	Nasal	Mice, rats	IgG and IgA, Th1
ISCOMs	Phosphatidylcholine, cholesterol, antigen, Quil A	*Mycobacterium tuberculosis*, human T-cell lymphotropic virus type 1, diphtheria toxoid	Pulmonary, nasal, oral	Mice	IgG and IgA; Th1
Natural polymer-based nanoparticles					
Chitosan	Mannosylated chitosan, chitosan, trimethyl chitosan	*Mycobacterium tuberculosis* Hsp65, swine influenza A virus, hepatitis B, Group A *Streptococcus*	Nasal	Mice, pigs	IgA, Th1; IgA, IgG, and Tem
Gamma polyglutamic acid	Polyglutamic acid and trimethyl chitosan; polyglutamic acid, chitosan, and cholera toxin subunit A1	Group A *Streptococcus*, influenza A virus	Nasal	Mice	IgA and IgG; Th1 and Th2
Hyaluronic acid	Hyaluronic acid microspheres (HYAFF) and enterotoxin from *Escherichia coli* (LT), DOTAP and hyaluronic acid	Influenza hemagglutinin, ovalbumin	Nasal	Mice, rabbits, micropigs	IgA and IgG; IgG and CD8 + T cells

Pullulan	Cholesteryl group-bearing pullulan; TNF-α and cholesteryl group-bearing pullulan	*Clostridium botulinum* type-A neurotoxin, *Streptococcus pneumonia*, influenza A virus	Nasal	Mice, macaques	IgA and IgG; Th2 and Th17; IgG1 and IgA
Synthetic polymer-based nanoparticles					
PLGA	PLGA and MPLA; PLGA, DDA, and MPLA; PLGA and MPL; PLGA and hydroxypropyl methylcellulose phthalate; PLGA and Eudragit; PEG and PLGA	Ovalbumin; HspX/EsxS fusion protein of *Mycobacterium tuberculosis*, H5N1 influenza; *Helicobacter pylori*, HIV envelop protein, Hepatitis B	Oral, nasal, colorectal	Mice	IgG and IgA; IgA, Th1 and Th17; IgA and Th1
PEI	Polyethyleneimine, polyethyleneimine-triethyleneglycol, deacylated PEI	H7N9 Influenza, HIV gag, HIV envelop protein	Nasal	Mice, chickens	IgG, IgA, Th1 and Th2: IgG, Th1, CTL;
PCL	poly-ε-caprolactone, PCL and chitosan, PCL and PEI, or PCL and PEG	Hepatitis B, ovalbumin, *Streptococcus equi*	Nasal	Mice	IgG and IgA
PPS	Polypropylene sulfide; Pluronic-stabilized PPS	Ovalbumin, H1N1 influenza	Nasal	Mice	IgG and IgA; CTL
Dendrimers	G4-PAMAM-NH_2	HIV-1 gp120	Nasal	Mice	IgG and IgA
Lipid-polymer hybrid nanoparticles	DOTAP and hyaluronic acid; PLGA, CAF01, and chitosan; PLGA, DOPC, DOTAP, and DSPE-PEG	*Yersinia pestis*, *Chlamydia trachomatis*, HIV-1 gag	Nasal	Mice	IgG and CD8 + T cells; IgG and IgA
VLPs	H1N1 influenza, phosphatidylcholine, and phosphatidylethanolamine; DCPC, respiratory syncytial virus; H5N1 Influenza and Matrix M	HIV-1 gp41, respiratory syncytial virus, H5N1 Influenza	Nasal	Nonhuman primates, mice	IgA, IgG, antibody-dependent cell cytotoxicity; Th1
Gas-filled microbubbles	DSPC and PEG	*Salmonella enterica typhimurium*, ovalbumin	Nasal	Mice	IgA, Th1, and Th17

Oral delivery of vaccines

Infections are causing by pathogens, viruses a major cause of human mortality. In last few decades, vaccines have been the greatest achievement in the medical field and have saved more lives than any other available drugs. Vaccination always serves as the best route of drug administration for combating infectious disease owing to their efficacy & cost effectiveness. Vaccines are generally composed of either live attenuated, inactivated or killed organisms or minimal fractions of infection causing pathogens. Most of the pathogens [~90%] invade inside the body tissue via mucosal route, through respiratory tract, GI tract, Respiratory tract or urogenital tract. So, oral vaccination can also be very effective. But any vaccination through needle is invasive & painful, sometime different patient complication arises. And needled-vaccine generally produce robust & effective immune response at cellular & systemic level to prevent the disease, local effect, whereas oral vaccine delivery produces cellular, systemic & mucosal effect against pathogens. Body Mucosal systems are more susceptible to pathogens due to larger exposed area & easily invadable than the skin due to thinner composition. Now as a first line defense against pathogens, first priority should be protection of mucosal surfaces from pathogens. A major immunoglobulin Secretary IgA [S IgA] plays important role against pathogen invasion at the mucosal sites. More interestingly mucosal

immunity gained at one side has been found to develop immune response throughout other remote mucosal system of the body. However, Immunity produces at distal sites will be lesser than the main effectors sites or the site of administration. Now days to overcome many patient complication researchers & academics have been found to shown interest to develop an alternative oral vaccine without utilization of needle. [62]

Advantages of Oral vaccines:

- Affordable cost
- Easy administration without help of medical professionals and special devices.
- Non-invasive & safe.
- No needle associated risk or injury.
- High Scalability.
- Highly stable under lyophilization and no need of cold-chain Storage.
- Sufficient Protection of antigens against different physiological barriers (proteolytic enzymes, low pH and bile salts)
- High antigen encapsulating capacity due to nano sized particles
- Compatible with Strong mucosal adjuvant due to nano technology.
- Prolonged exposure of antigens to antigen-presenting cells
- Optimum particle sizes can easily cross intestinal lumen
- Sufficient targeting ability to microfold-cells of Intestine.
- In addition, oral vaccines are superior to injectable vaccines due to their potency to produce both antigenic-specific systemic antibodies (IgG) in blood and mucosal antigen specific (IgA) antibodies.
- Adequate safety profile & very less patient compliance till now.

Oral vaccines For COVID 19

Vaccination through Needle has lots of patient complication from the perspective of patient's psychology & also from the physiological point of view. But you know what pharmaceutical science & it's inventions are unstoppable. Starting from an inactivated polio vaccine, developed few years ago by Jonas Salk, in 1955 & another attenuated live oral polio vaccine, developed by Albert Sabin Its continuously evolving [63]. Now nanotechnology is another charm, researchers are working on. People whose are scared of needles and injections would be delighted to hear that soon they may be able to take the COVID-19 vaccine in the form of a capsule. [64]

Interesting fact is that multiple pharmaceutical companies are working now to develop an oral vaccine for COVID 19. One of these new forms of the COVID-19 vaccine is a capsulated form that can be administered orally. This COVID-19 vaccine capsule is being developed by Indian pharma company "Premas Biotech". Premas Biotech in collaboration with American firm Oramed Pharmaceuticals Inc had announced on March 19, 2021 that they are developing an oral COVID-19 vaccine candidate that has shown efficacy upon administration of a single dose [64]. A single dose of the Oravax COVID-19 capsule and been found effective and its efficacy has been proven by tests on animals as part of the vaccine's pilot study. The oral vaccine produces both systemic & cellular immunity by producing Neutralizing Antibodies (IgG) as well as (IgA) immune response. These protect the gastrointestinal and respiratory tracts against infection.

Premas' protein-based Virus Like Particle (VLP) COVID-19 vaccine has founds to provide triple protection against three parts of the SARS CoV-2 virus. These are - Spike S, Membrane M, and Envelope E targets. But! It unable to provide protection against Nucleocapsid N antigen [64]. As addressed by Dr Prabuddha Kundu, serves as the Co-founder and Managing Director of Premas Biotech. The VLPs in the vaccine are produced using Premas's proprietary Crypt platform while its collaborator Oramed Pharmaceuticals Inc has the world's leading oral Protein Delivery Platform (POD). Thus Oravax COVID-19 vaccine candidate thus combines and harnesses the true potential of the two unique platforms. The current observations regarding the oral vaccine are based on the preliminary results of

animal studies. Clinical trials are expected to be launched in the second quarter of 2021. Indian firm Bharat Biotech is also currently developing a COVID-19 vaccine in the nasal form in collaboration with the University of Wisconsin. Clinical trials for this have already begun.

Vaxart developed an oral vaccine which induces higher $CD8^+$ T-Cell responses than others vaccine as seen in case of Moderna or Pfizer vaccines in comparative experiment conducted by the Company IgA antibodies triggered in the mucosa, show broad cross-reactivity. Vaxart is developing oral recombinant vaccines that is administered able by tablet rather than by injection. Latest data obtained from its Phase I trial showed the evidence suggesting that VXA-CoV2-1 is able to trigger mucosal immunity and includes both the S and N SARS-COV-2 proteins and also has broad cross corona virus activity. The evidence found from clinical trial phase I for Vaxart's vaccine that its is capable of elicit CD8+ T-cell response, as measured by IFN-g and TNF-a induction which is also more efficacious than Moderna or Pfizer mRNA vaccine. [65]

Conclusion:

Vaccine designing & development techniques have improved a lot through different advancement of nanotechnologies with pace of time. Even though very few approaches of vaccine designing have been found to be "must have" requirements, but many have failed also. That's why there are very a few vaccines available clinically in the market so far. Most interestingly, Oral vaccine is most convenient so far due to their ability to impact immune system with higher efficacy. Now a day's oral vaccine is also getting different exposure due to their less patient complication. Different multinational companies like Prema's Biotech, Vaxart are involving themselves in development of oral vaccines for COVID 19 also.

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