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HOW CHEMICAL ENGINEERS COULD APPLY TECHNOLOGY TO BATTLE COVID-19

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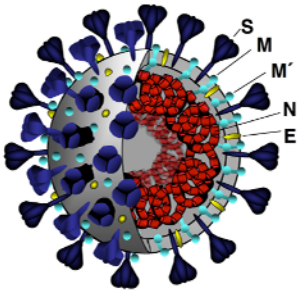
seek LIGHT

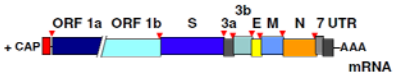
Contents

- What is coronavirus (CoV)
- What harmful caused by CoV?
- How coronavirus infect human
- Potential engineering solutions for new drugs and safe vaccines development

What is Coronavirus

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to pneumonia or more severe diseases

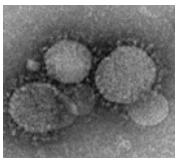




- Genome: linear single-stranded RNA +
- Size: 80 to 220 nm
- Shape: Spherical or helical
- S – spike (receptor binding cell fusion)
- E – envelope (small: envelope protein, not as abundant as S)
- M – membrane protein (transmembrane budding and envelope formation)

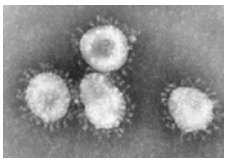
Table 1 Organisation of CoV species

Group	Species
α-CoV	HCoV-OC43 and HCoV-HKU1
β-CoV	COVID-19 (Dec 2019)
	Severe acute respiratory syndrome coronavirus (SARS-CoV)
	Middle Eastern respiratory syndrome coronavirus (MERS-CoV)
γ- CoV	Tylonycteris bat coronavirus HKU4
δ - CoV	Rousettus bat coronavirus HKU9



MERS-CoV

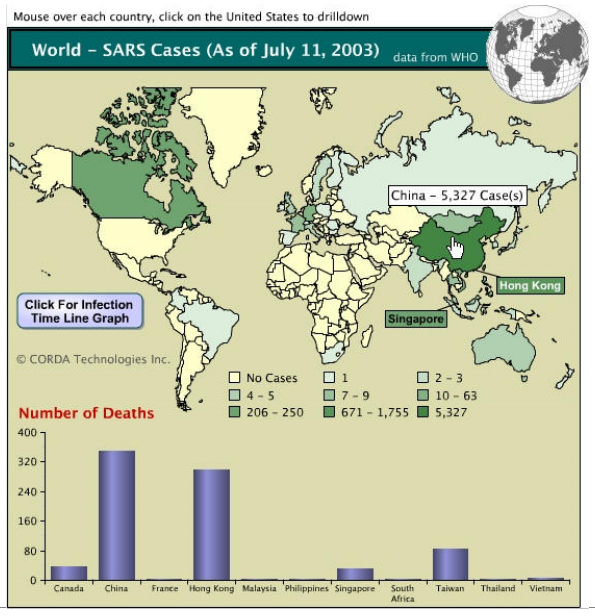
SARS-CoV

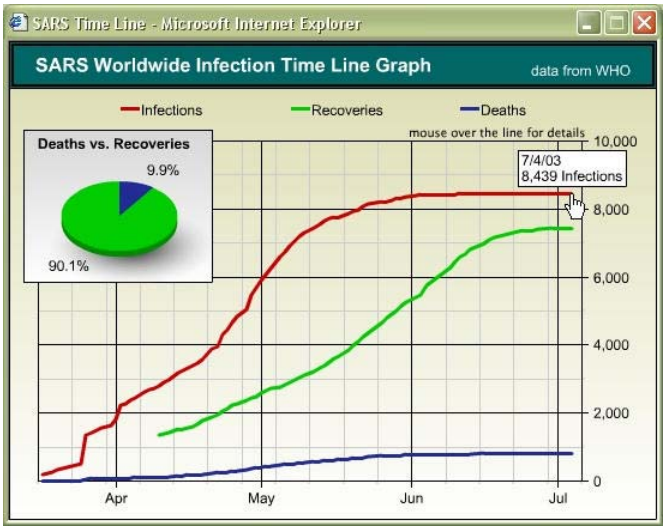


COVID-19

What harmful caused by CoV?

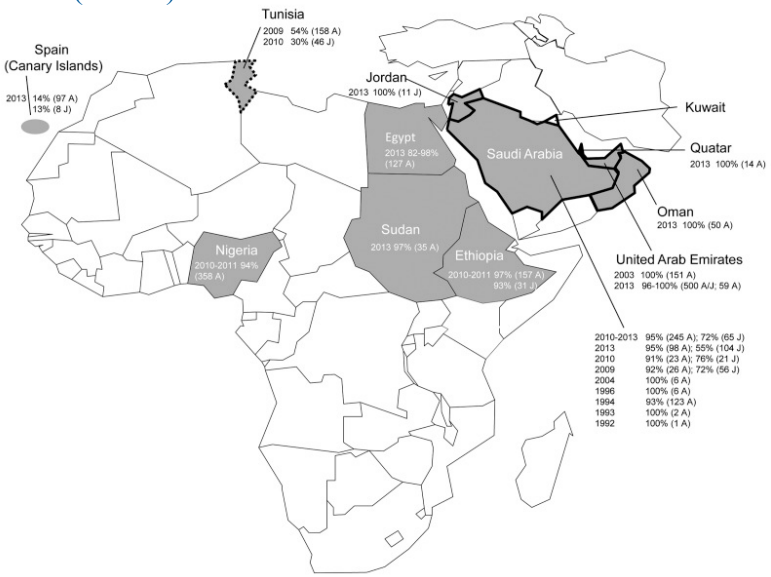
SARS-CoV:8,438 reported cases of **SARS** and 774 **deaths** in 2002 (9.5%)



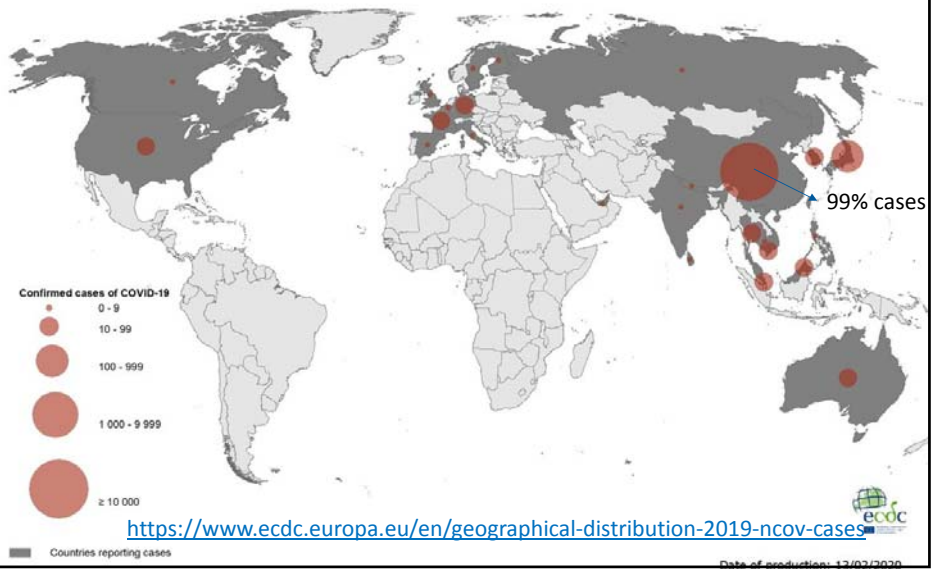


Corda's SARS worldwide infections/recoveries/deaths time line graph

MERS-CoV: 2,494 reported cases of **MERS-CoV** and 858 deaths since 2012 (34.4%)

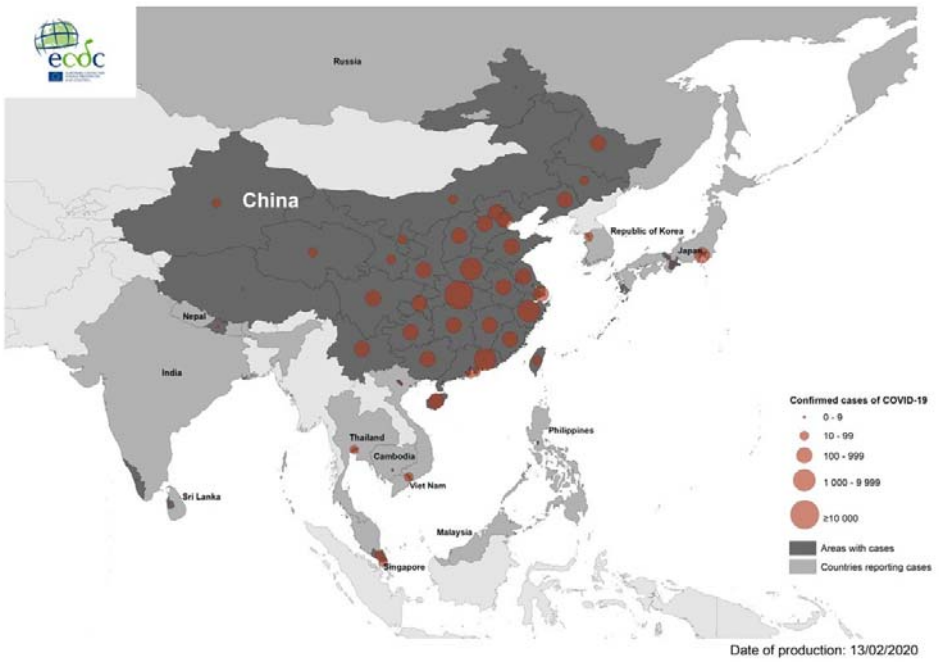


Geographic distribution of 2019-nCoV cases worldwide, as of 16 February 2020
69,267 reported cases, 1,669 death (2.4%)



https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases

Geographic distribution of 2019-nCoV cases in Asia, as of 16th February 2020

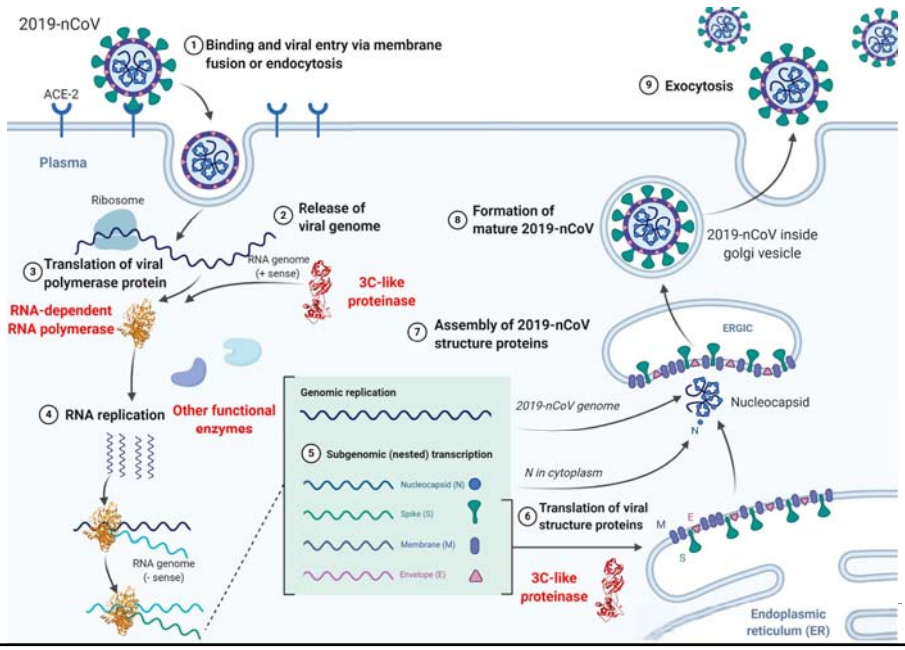


COVID-19

- Current knowledge: covid-19 has higher similarity with SARS-Co. Both binds with Angiotensin-converting enzyme 2 (ACE2).
- Current issue: No specific vaccine and targeted drugs to treat COVID-19
- Some claims: antibodies from plasma of patients who have recovered from the covid-19 contains highly potent antibodies that can kill and remove the virus
- Global negative economic impact

Roads are empty in Wuhan, where public transit has been shut down

How COVID-19 infect human

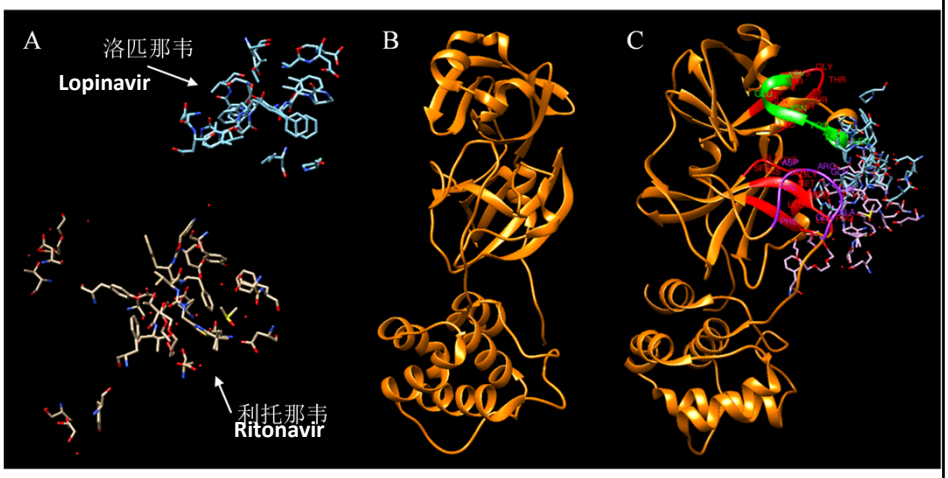


Potential Solutions

- Drug treatment—Inhibit the virus RNA replication
  - 3C Like Proteinase
  - RNA Dependent, RNA polymerase
  - Other functional enzymes
- Therapeutic monoclonal antibodies to neutralize CoV S-protein
- Prevent vaccines—Immuno response
  - Inactivated virus-based vaccines
  - Recombinant virus like particles (VLPs) based vaccines (eg chimeric VLPs vaccines containing epitopes to bind S protein receptor)

Small molecular drugs for SARS and MERS treatment

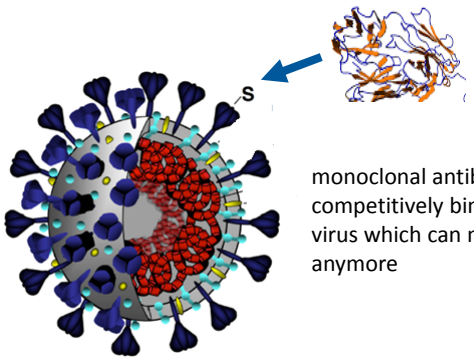
Diseases	Drugs	Target	Note
Severe Acute Respiratory Syndrome (SARS)	Lopinavir-ritonavir	3C-like proteinase	Its clinical efficacy has not been established
Severe Acute Respiratory Syndrome (SARS)	high-dose glucocorticoids and ribavirin	RNA dependent RNA polymerase	Neither treatment had a clear beneficial effect, and immediate and late toxicities were common
Severe Acute Respiratory Syndrome (SARS)	Remdesivir	RNA-Dependent RNA Polymerase	Experimental
Middle East Respiratory Syndrome (MERS)	(IFN)-alpha-2b and ribavirin	RNA dependent RNA polymerase	Treated animals had lower concentrations of serum and lung proinflammatory markers, fewer viral genome copies, and fewer severe histopathologic changes in the lungs.
Middle East Respiratory Syndrome (MERS)	oral lopinavir-ritonavir	3C-like proteinase	A placebo-controlled trial



A: Potential Drugs B: 3C Proteinase C: Binging of drug and 3C Proteinase

Monoclonal antibodies (mAbs) Treatment

mAbs



monoclonal antibodies (mAbs) competitively binding to the S protein of virus which can not fuse to the host cell anymore

Monoclonal antibodies (mAbs) with neutralizing epitopes of the SARS-CoV S-protein

- Neutralizing mAbs bind ACE2 receptor-binding domain (RBD) of the SARS S protein
- Chimeric mAbs

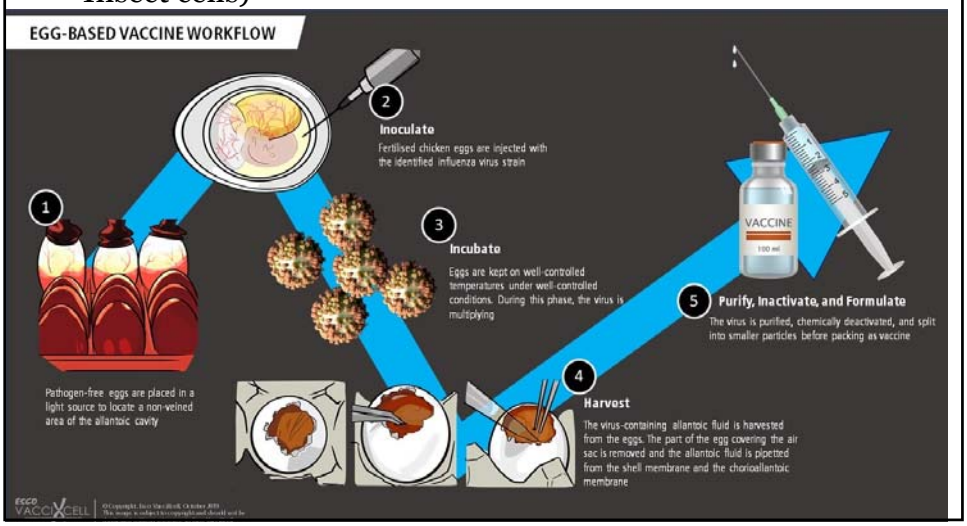
mAb	Isotype	Specificity	Affinity for S protein KD (nM)	Epitope[c]/mapping procedure	Neutralizing titre (nM)[a]	
					Tor3[b]	Tor2[j]
F26G3	G2a/k[a]	S protein[a]	0.83 (±0.26)	C/C-ELISA[d]	26	nd
F26G4	G2a/k[a]	unknown	nd	C/C-ELISA[d]	non	non
F26G6	G2b/k[a]	S protein[a]	nd	Phage	non	non
F26G8	G2a/k[a]	S protein[a]	0.83 (±0.26)	L/peptides[d]	non	non
F26G9	G2a/k[a]	S protein/RBD	10.2 (±3.5)	C/C-ELISA	1	nd
F26G10	G2a/k[a]	S protein/RBD	7.5 (±2.7)	C/C-ELISA	1	nd
F26G18	**G2b/k[a]**	**S protein[a]/RBD**	**1.79 (±0.50)**	**L/peptides[e]**	**0.075**	**2.07**
F26G19	G2a/k[a]	S protein[a]/RBD[b]	4.05 (±1.01)	C/Co-crystal[b]	1	nd
F20G7-5	G1/k	PA-toxin	nd	n/a	n/a	n/a
Chimeric F26G9	G1/k	S protein/RBD	2.69 (±0.50)	C/Nd	nd	nd
Chimeric F26G10	G1/k	S protein/RBD	3.62 (±1.46)	C/Nd	nd	nd
Chimeric F26G18	**G1/k**	**S protein/RBD**	**1.28 (±0.78)**	**L/peptides[f]**	**nd**	**2.47**
Chimeric F26G19	G1/k	Binding lost[k]	n/a	n/a	n/a	n/a

Potential Prevented Vaccines -Virus Vaccines

- **Inactivated (killed) virus vaccine**
  consisting of virus particles, bacteria, or other pathogens that have been grown in culture and then be activated, eg Flu-Shot
- **Live attenuated (weakened) virus vaccine**
  use pathogens that are still alive (but are almost always attenuated, or weakened), eg Flu Nasal Spray

Virus Production Processes

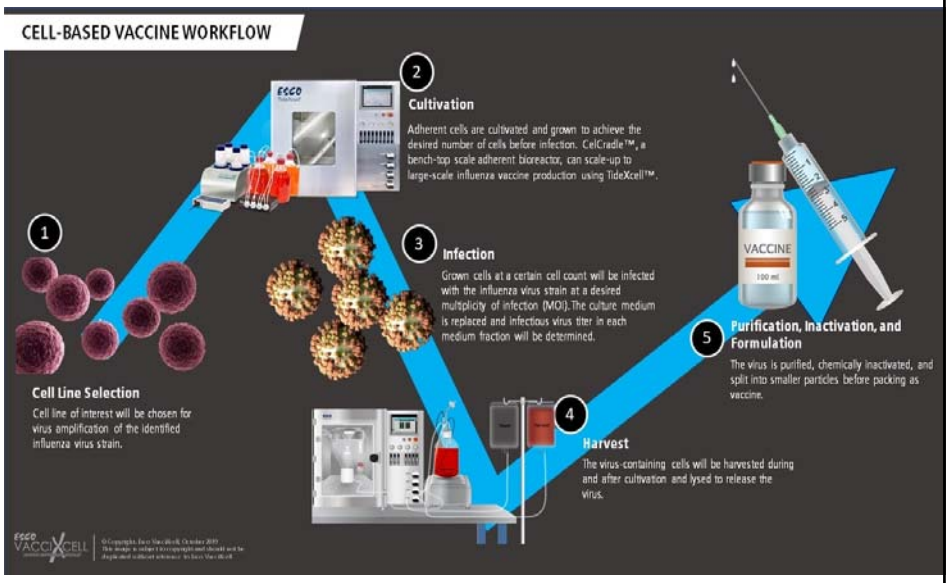
- Egg-based virus vaccine (Flu-vaccine)
- Cell-based virus vaccine (continuous cell lines Vero cells, Insect cells)



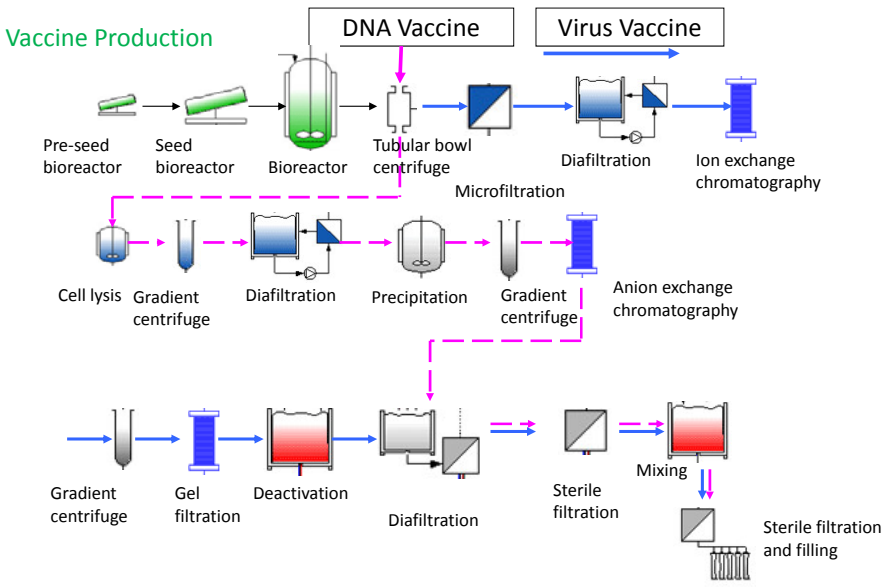
Time frame of Egg-based virus vaccine

Activity	Month 1	Month 2	Month 3	Month 4	Month 5
At WHO Collaborating Centres					
Identification of new virus	●				
Preparation of the vaccine strain	●———				
Verification of vaccine strain	●———	———			
Preparation of reagents to test vaccine	●———	———	———	———	———
At Manufacturer					
Optimization of virus growth conditions		●———	———		
Manufacture of bulk vaccine			●- - - - -	- - - - -	- - - - -
Quality control			●- - - - -	- - - - -	- - - - -
Vaccine filling and release				●- - - - -	- - - - -
Clinical trial (in certain countries)				●———	———
At Regulatory agency					
Review and release					●

Cell-based virus vaccine

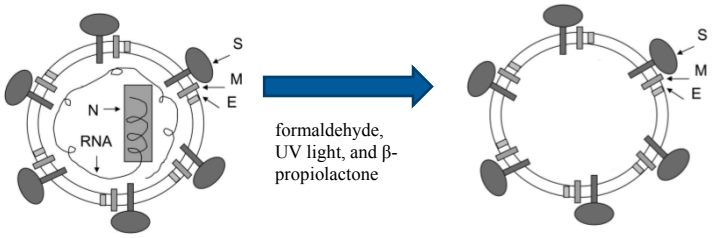


Unit Operation of cell-based virus/DNA vaccine



Barriers to Virus-based Vaccine

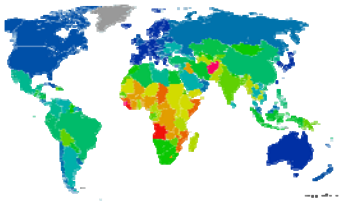
- Up to 6 month time frame for egg-based vaccine (Pandemic infection)
- Special operation facilities required but still not safe for the operation workers
- Potential risk to the patient (virus RNA, host cell protein)



Barriers to Virus-based Vaccine

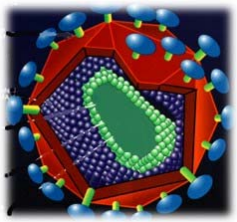
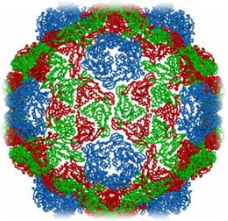
- Current production is slow & expensive. For example, flu vaccine is grown in eggs:
  - six-months required;
  - 100s of millions of specialized eggs;
  - not economic for 'surge' needs; and
  - flu may be deadly to chickens!

- Side-effects from current production methods:
  - Contamination – *e.g.* egg proteins in flu vaccine;
  - Preservatives – *e.g.* Thiomersal.
- Robustness of vaccines – issue for developing countries.



Better, Faster and Cheaper Vaccines *via* VLPs

- **Virus-like particles (VLPs)**
  - Virus shells – mimic Nature's way of interacting with cells.
  - No genetic material – cannot infect at all.
  - Can be engineered – vaccines with improved efficacy.
  - Can be grown in cell cultures (yeast or E Coli) – much faster and cheaper.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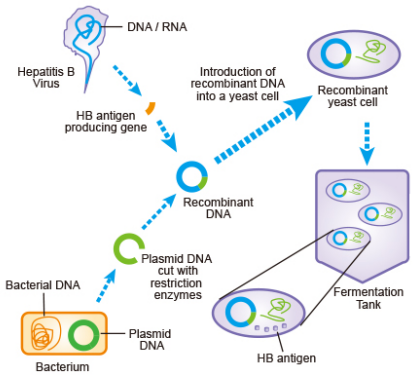
virus like particles (VLPs) based vaccines- successful cases

- Recombinant HBsAg (Hepatitis B Surface Antigen) Vaccine against Hepatitis B Virus



- Hepatitis B virus gene encoding major envelope protein (S protein)
- S protein has highest density of epitopes -> most immunogenic
- Under control of *P. pastoris AOX1* promotor
- *AOX1* promotor -> methanol-induced production

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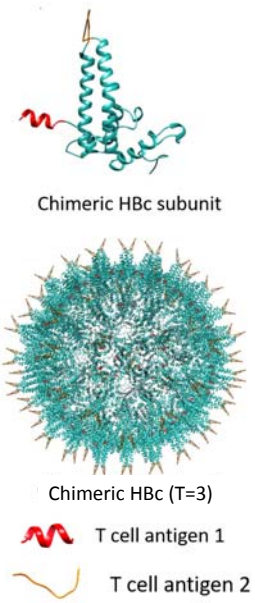
virus like particles (VLPs) based vaccines-successful cases

- Human Papillomavirus (HPV)-Gardasil® / Cervarix™
  1. Gardasil™ : The quadrivalent HPV4 vaccine (Merck and Co., Inc)- contains VLPs that are similar to those found in HPV types 6, 11, 16 and 18.
  2. To produce this vaccine, the L1 gene of these genotypes is expressed in *Saccharomyces cerevisiae* (yeast) and is used with an aluminum adjuvant

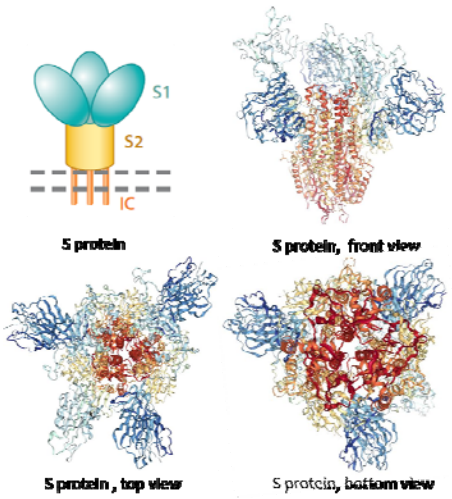
virus like particles (VLPs) based vaccines-Our Research Group

- Hepatitis B Core Protein VLP as Vaccine Carrier
  1. Hepatitis C Virus Infection Disease: Chimeric HBc-HCV vaccine.
  2. Epstein–Barr Virus Infection (cancer related) disease: Chimeric HBc-EBV Vaccine
- Current stage
  - ✓ Recombinant cell line development
  - ✓ Lab scale process to produce chimeric VLPs
  - ✓ In-vitro
  - ✓ In-vivo



virus like particles (VLPs) based vaccines-COVID-19?

- Current VLPs protein platform as coronavirus vaccine carrier??
- Current stage
  - ✓ ID the potential epitopes and suitable VLPs as carrier for COVID-19 vaccine;
  - ✓ ID suitable cell lines to express Chimeric VLPs



Clinical Trials for β-coronavirus vaccines

Row	Status	Study Title	Conditions	Interventions
1	Recruiting	A Clinical Trial to Determine the Safety and Immunogenicity of Healthy Candidate MERS-CoV Vaccine (MERS002)	• MERS	• Biological: ChAdOx1 MERS (adenoviral vectored vaccine expressing MERS-CoV spike protein)
2	Recruiting	Safety and Immunogenicity of a Candidate MERS-CoV Vaccine (MERS001)	• MERS	• Biological: ChAdOx1 MERS
3	Completed	Safety, Tolerability and Immunogenicity of Vaccine Candidate MVA-MERS-S	• MERS	• Biological: vaccine candidate MVA-MERS-S
4	Not yet recruiting	Randomized, Double-blind, Placebo-controlled, Phase Ib Study to Assess the Safety and Immunogenicity of MVA-MERS-S_DF-1	• MERS	• Biological: MVA-MERS-S_DF1 - Low Dose • Biological: MVA-MERS-S_DF1 - High Dose • Other: Placebo
5	Completed	Phase I Study of a Vaccine for Severe Acute Respiratory Syndrome (SARS)	• SARS	• Procedure: Blood Test • Procedure: Urine Test • Procedure: Physical Exam • (and 2 more...)
..	....	HBc VLPs as COVID-19 Vaccine carrier?????		.....

Challenges of Vaccine Production

- Low yield after multiple unit operation (cost)
- Molecular structure damaged in bioprocess (safety)
- Low through-put by traditional bioseparation process (pandemic infection diseases)
- Stringent quality requirements
  - Percentage purity
  - Absence of specific impurities (DNA and HCP (Host cell proteins)

Vaccine/recombinant proteins process from Lab-Scale to Pilot Scale

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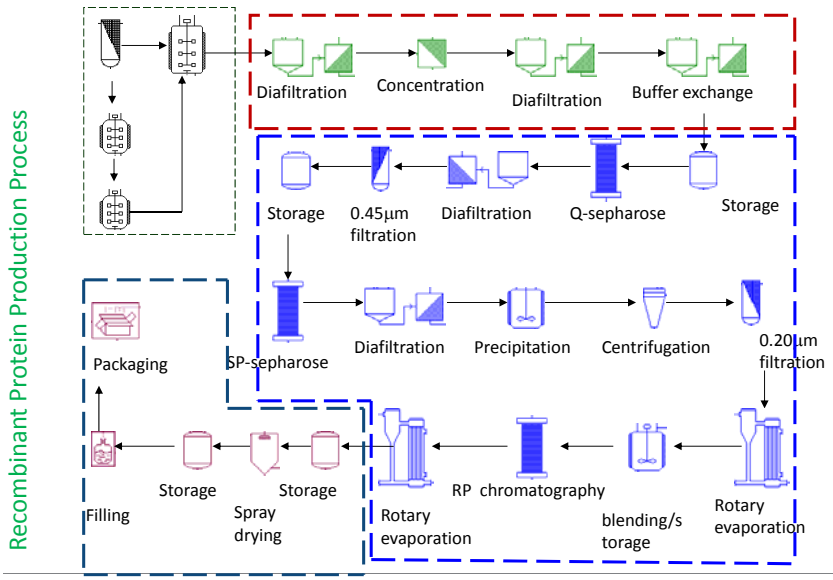
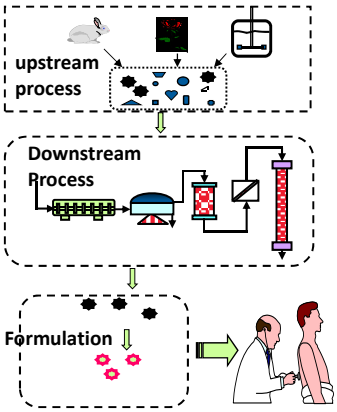


What Chemical Enginner could contribute?



- High regulatory requirements—quality and safety (FDA,TGA);
- Bioprocesses are not adequately developed for special need;
- The influence of fundamental process parameters is not well understood;
- No protocols for scale-up, technology transfer and raw material, formulation and process changes

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