The role of artificial cells in the fight against COVID-19: deliver vaccine, hemoperfusion removes toxic cytokines, nanobiotherapeutics lower free radicals and pCO₂ and replenish blood supply

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ABSTRACT
This review concentrates on how artificial cells can contribute to helping patients with COVID-19. Artificial cells have led to mRNA vaccines with more improvements to come. Excessive cytokines in severe COVID-19 can damage organs leading to death. Artificial cell-based collodion macroporous activated charcoal adsorbent can effectively remove middle molecular weight range molecules in patients. A novel hemoperfusion device based on collodion membrane macroporous synthetic resin effectively removes cytokines and recovery in COVID-19 patients. This has been approved as an emergency treatment for COVID-19 in China, Europe, and Canada. A recent nanobiotherapeutic containing haemoglobin and up to six times the concentration of red blood cell enzymes: catalase, superoxide dismutase and carbonic anhydrase. In an animal study, this can effectively lower the damaging increase in free radicals and the removal of increased tissue pCO₂. This can also help as blood substitute for the severe and critical problem of COVID-19 pandemic donor blood supply crisis.

KEY MESSAGES
- COVID-19 and its variants have resulted in major pandemics, severe sicknesses, and deaths around the world. COVID-19 and its variants has only started less than 3 years ago, and it is even more recently that we know more about its mechanisms, requirements, prevention, and treatment. This being the case, this is the first review on the present status and future perspectives of the use of the principle of artificial cells for COVID-19 related to vaccines, treatment, and critical donor blood supply crisis.

Why do we need artificial cells for COVID-19?

General: COVID-19
In December 2019, there was an outbreak of coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Epidemiology, clinical characteristics, risk factors for mortality and clinical course have been described [1]. This has become a pandemic with major outbreaks around the world. What is worse is that new variants develop and within 2 years, many countries have already experienced 6 waves. Many patients with very severe COVID-19 have died despite ventilator and oxygenator support. Furthermore, the pandemic has resulted for the first time a severe donor blood supply crisis. Thus, it is important to (1) develop suitable vaccines (2) Find a possible way of treatment (3) way to solve the critical donor blood supply crisis. This paper describes examples of the use of the idea of artificial cells towards these aims. First, what are artificial cells?

What are artificial cells?
Artificial cells are attempts to mimic some of the properties of biological cells for use in medicine. This author prepared the first artificial cells by enclosing the content of red blood cells inside ultrathin polymer membranes of cellular dimensions (Figure 1) [3,4]. He then extended this research by going outside the box with variations in contents, membrane composition, and configurations (Figure 1). Contents of artificial cells include haemoglobin, enzymes, cells, vaccines, compartments, cytosol, organelles, magnetics, adsorbent, insulin and later, stem cells, gene, DNA, mRNA, silver, gold, miromagnets, biotechnological products and others (Figure 1). Membrane composition include polymeric membrane, lipid membrane, biodegradable membrane, crosslinked protein membrane, conjugation, lipid-polymeric membrane, PEGalated membrane and others (Figure 1). It has since been developed around the world into many configurations and dimensions under different names for different specific applications (Figure 1) [2,5–19]. One can now tailor-made Artificial...
Cells to suit specific applications. It has now evolved into many different areas including blood substitutes, hemoperfusion, nanomedicine, nanobiotherapeutics, drug delivery, regenerative medicine, cell/stem cell encapsulation, nanoparticles, liposomes, bioencapsulation, and other areas. Artificial cell is now a very large area and reviews on artificial cells are available elsewhere [2,5,7]. Figure 1 is a summary of the area.

The role of artificial cells in the fight against COVID-19

The present paper is not a review of the very large area of artificial cells that has already been reviewed in 2019 [2]. It is only the present status and future perspectives of the use of artificial cells for COVID-19 since the 2019 pandemic: (1) as carriers for vaccine (2) Novel artificial cell-based hemoperfusion for the removal of damaging cytokines and for the partial support of kidney and liver failure (3) Nanobiotherapeutics for the removal of oxygen radicals and CO2 (4) for blood supply in pandemic donor blood supply crisis (Table 1).

Artificial cell as carrier for COVID-19 vaccine

Introduction

Many excellent and detailed reviews have been written on COVID-19 vaccines [14,20–22]. The successful development of the COVID-19 vaccine is the result of research by many groups around the world over many years. It takes time for an idea or basic research to be developed for eventual clinical use and we cannot wait for the need to come before doing serious research and development. This author first proposed the use of artificial cells for vaccine in the mid-1970s [12,13]. It was too early for its time and there was not much follow-up research on this. Similarly, there was not too much interest in synthetic mRNA, but researchers also persisted and came out with a suitable mRNA [14]. However, there are problems with mRNA that include (1) the destruction of mRNA by ribonucleases when injected (2) the negatively charged mRNA has problem entering the cell because of the negative potential across the cell membrane. Fortunately, centres around the world have been developing artificial cells as carriers for other biologics and therapeutics and the combined effort of many centres around the world over many years have resulted in readily available solution (Figures 2 and 3) [2–13,15–22].

Development towards a suitable carrier for mRNA

Components of the membrane of artificial cells. In 1968 a group reported that they were able to use of the original 1964 artificial cell method [3] to prepare single bilayer...
membrane vesicles [16]. In 1972, artificial cells with protein-lipid membrane or polymer-lipid membrane were prepared [4]. Microspheres of onion-like concentric multilamellar lipid bilayers were originally used as membrane models in basic research [17]. This was later developed into drug carrier [18]. However, these onion-like multi-lamellar structures limit the loading of drugs. In 1976 researchers used ‘ether evaporation’ method to form single bilayer lipid membrane vesicles [19]. This ‘ether evaporation method’ is an extension of one of the original methods using ether for the preparation of artificial cells [3, 4]. These lipid membrane artificial cells, also called liposomes, have since been extensively studied and used as drug delivery systems around the world [2, 6, 9–11, 20–22]. All the above research has resulted in the availability of suitable vaccine carriers and the rapid progress and success of the mRNA and other vaccines (Figure 2).

**Final version of the mRNA vaccine**

Thus, mRNA is enclosed within PEG-lipid positively charged membrane nanodimension artificial cells with the following functions (Figure 2) The enclosing membrane excludes ribonucleases from destroying the enclosed mRNA. The PEG component prevents the nano artificial cells from being removed quickly by macrophage. The positive charge surface facilitates the entrance of the nano artificial cells across the negatively charged membrane potential by endocytosis into the cell in the form of endosome and is later released to carry out its function (Figure 3).

**Future**

The present widely used mRNA vaccines are successful. However, much improvement for longer term function is on the horizon with other approaches in addition to mRNA and the use of other configurations and membrane material for artificial cell including biodegradable polymeric artificial cells and other configurations [2, 9–11, 20–22].

**Removal of damaging cytokine in COVID-19 patients**

**Cytokines STORM in COVID-19**

Patients with very severe COVID-19 may die despite ventilator and oxygenator support due to ‘cytokine storm’, a sudden and massive increase in cytokines including interleukin (IL)-6, IL-2, IL-7, IL-10, and tumour necrosis factor [1, 23]. Pro-inflammatory IL-6 is the most important causative cytokine in CRS (Cytokine release syndrome) also called ‘cytokine storm’ can damage organs resulting in multi-organ failure and death of the patients [1, 23]. Thus, it is important to remove these inflammatory cytokines. However, these are rather large molecules. Furthermore, these patients are severely sick, and the treatment must involve methods that
do not aggravate the condition. We have earlier developed artificial cell containing adsorbents like activated charcoal or resin [24]. This has been used to prepare artificial cell based hemoperfusion device [4,24–27], that is (1) effective in removing from blood molecules in the larger middle molecular weight range and (2) Unlike haemodialysis it did not cause hypotension in severely ill patients.

**Artificial cell-based hemoperfusion for toxin removal and partial kidney and liver support**

**Basic principle**

My analysis of the effect of diameter and membrane thickness of artificial cells on mass transfer shows unexpected results [24]. Figure 4 shows the theoretical mass transfer of a fixed volume of 10 nm membrane thickness artificial cells with different diameters. This is compared to an artificial kidney (haemodialysis) machine available at that time with a mass transfer of 1. At the micro diameter, the theoretical mass transfer of artificial cells can be 100 times that of an artificial kidney. It is even higher at the nanodimension. This forms the basis of using artificial cells as highly efficient micro/nano dialyser/bioreactor (Figure 4).

**Collodion membrane-activated charcoal**

For example, 70 g of 90-micron diameter artificial cells containing activated charcoal are retained inside a small container by screens at either end. The artificial cell membrane separates the adsorbent from damaging the blood cells and prevents adsorbent particles from being released to cause adverse effects. The sorbent in the artificial cells removes toxins or drugs from the blood of patients perfusing through
the column (Figure 4). The result is a miniaturised cup size hemoperfusion device with much higher efficiency than a washing machine size haemodialysis machine (Figure 4). In addition to adsorbent artificial cells can also contain other bioreactors (Figure 4).

Clinical use of artificial cell hemoperfusion (collodion membrane activated charcoal) in patients

The author personally carried out a study on the use of artificial cells containing adsorbents for hemoperfusion. This included personally carried out scaled up, animal testing and clinical trial in patients. He showed the safety and effectiveness for using this first in animals then in patients. Figure 5 shows the result of one of the many patients the author has treated [25,26,28]. This is a suicidal patient who ingests 3 times the lethal dose of a sleep pill, methyprylon [25]. Five hours of standard haemodialysis treatment cannot lower the drug level and the patient remained comatose, hypotensive with cardiac arrests. When the author started hemoperfusion treatment the plasma methyprylon level decreased rapidly in 2 h and the patient was no longer comatose nor hypotensive and shortly recovers completely. This was also carried out successfully in 12 other drug poisoning patients [25,26]. He has also used this for the partial support of kidney failure and liver failure patients [26,28]. Thus, FDA has approved this artificial cell based hemoperfusion for routine clinical uses.

It is important to note that the activated charcoal adsorbent is macroporous and 10 nm thin collodion membrane coating does not obstruct the openings (Figure 5) [4]. On the other hand, thick membrane coating would interfere with the ease of entrance of molecules to be removed (Figure 5).

Commercial devices and a recent novel synthetic microporous resin with collodion membrane for cytokine removal

General

Hemoperfusion is now an accepted routine clinical use for the treatment of patients around the world. A 2017 book [27] by specialists around the world shows that this approach is being used extensively around the world. There are now at least 10 commercial devices around the world [28]. Each company has their own proprietary methods. There are variations in the type of adsorbent, membrane material and membrane thickness and as a result, they are different in effectiveness [28]. There is a new commercial hemoperfusion device (HA330) not included in the 2017 review that has been approved for emergency use for COVID-19 in China, Europe and Canada. This is based on the use of collodion membrane with novel adsorbent in the form of synthetic styrene divinylbenzene copolymers macroporous resin [29,30].

Synthetic macroporous resin with collodion membrane (HA330), for patients with elevated systemic cytokines

Before the onset of COVID-19 this commercial device has been studied in other conditions with the elevation of
systemic cytokines [31–34]. For example, a detailed study in a porcine acute respiratory distress syndrome model shows that hemoperfusion cartridge (HA330), with a blood flow of 200–250 ml/min reduces the circulating and alveolar levels of proinflammatory cytokines, improves oxygenation and helps the recovery of lung injuries in the exudative phase [35]. Another study in patients with multiple organ dysfunction syndrome (MODS) shows the effectiveness of hemoperfusion [36]. Hemoperfusion significantly improves the biochemical indicators, inhibits the activation of NK-xB and significantly reduces the levels of inflammatory cytokines IL-6 and TNF-α in peripheral blood of the patients. A similar effect on the reduction of systemic cytokines are observed in patients with extrapulmonary sepsis-induced acute lung injury [37], Leptospirosis with acute respiratory failure and acute kidney injury [31] and septic shock patients [32].

Use in COVID-19 patients

Very severe cases of COVID-19 can progress rapidly to death. The emergency nature is such that no large-scale control clinical trial has yet been published. However, based on the above large scale clinical result showing its effectiveness for the removal of cytokots in other conditions with cytokine storms, this has been approved for emergency use for severe cases of COVID-19 in China, Europe and Canada.

Case reports of emergency treatments are available [33,34,38]. A Case report of a COVID-19 patient with cytokine storm on day 18 after admission with an elevation of particularly IL-6, IL-8 and TNF-α. Hemoperfusion starts on day 23 and lowers systemic cytokines, especially IL-6, IL-8, and TNF-α and on day 33 lab result reaches normal level and the patient fully recovers [34]. Another case report is a COVID-19 patient with severe acute respiratory distress syndrome (ARDS) with high plasma level of interleukin-1 (IL-1), IL-6, IL-8, and tumour necrosis factor alpha [38]. The chest X-ray shows a recovery of both lungs following the completion of 3 sessions of hemoperfusion. The inflammatory cytokines 48 h following the last session of hemoperfusion shows the decrease of IL-1, from 523.3 pg/mL to 38.25 pg/mL, IL-6 from 226.35 pg/mL to 210.18 pg/mL, IL-8 from 886.5 pg/mL to 482.4 pg/mL. The patient is finally transferred to the ward with an acceptable clinical condition. Another case report of the successful recovery of severe COVID-19 with cytokine storm treated with hemoperfusion [34]. On day 12 after admission, the IL-6 (198.66 pg/mL) is almost 10 times the normal range (5.9 pg/mL). Treatment includes a double plasma molecular adsorption system and plasma exchange.

levels of interleukin 6 (IL-6) and interferon γ (INF) reach peak on day 14 after admission and falling to near normal on day 16. On day 36 the patient is asymptomatic at rest and had no shortness of breath [34]. One looks forward to published results from control clinical trials in COVID-19 patients [39].

Oxygen radicals, elevated tissue pCO2 and donor blood supply crisis in COVID-19

General

Body response to coronavirus includes the release of high levels of oxygen radicals [40–42]. These high levels of oxygen radicals damage body tissue including the lung and the heart resulting in organ damage and failure. The hypothesis of free radicals playing a crucial pathogenic role in damage in COVID-19-induced pneumonia suggests an antioxidative therapeutic strategy for COVID-19 [40]. Others suggest the potential role of free-radical processes in biomolecules damage during COVID-19 and ways of their regulation [41]. There is also a group that proposes that COVID-19: captures iron and generates reactive oxygen species to damage the human immune system [42]. Damage to the lung also interfere with the normal removal of CO2 and the Extracorporal Oxygenator is more efficient in exchange of oxygen rather than CO2. Inability to remove CO2 can result in the increase of pCO2 in the skeletal muscle and heart muscle resulting in worsening of the patients’ condition [43,44]. There is also severe shortage of donor blood supply, for example, the US Red Cross declares ‘first-ever blood crisis amid Omicron surge’ with resulting severe shortage of blood supply and the delay of many lifesaving procedures [45]. Artificial cells in the form of nanobiotherapeutics can be used to solve these problems (Table 2).

Nanobiotherapeutic in COVID-19 for controlling free radicals and pCO2 and also for blood supply in COVID-19 donor blood supply crisis

Nanobiotherapeutics have been developed originally as red blood cell substitutes. Red blood cells have three major functions: (1) transport oxygen from the lung to the tissue, (2) remove damaging oxygen radicals and (3) carry carbon dioxide CO2 from the tissue to the lung to be removed. This has been developed in 3 progressing steps: Step 1. Oxygen carrier; Step 2. oxygen carrier with antioxidant properties and Step 3. oxygen carrier with antioxidant function and CO2 removal functions. Originally these are only to replace red
blood cells and not to function as nanobiotherapeutics. We have recently prepared a nanobiotherapeutic with up to 6 times enhancement of red blood cell functions by using enzyme concentration of superoxide dismutase catalase and carbonic anhydrase that are six times that of rbc. This can now function also as a nanobiotherapeutic to enhance the removal of oxygen radicals and CO.

**Oxygen carriers**

We prepared Polyhemoglobin-Catalase-superoxide dismutase (PolyHb-SOD-CAT) as an oxygen carrier and antioxidant to remove oxygen radicals (Figure 6) [52]. This is effective in removing oxygen radical. Thus, in a 90 min haemorrhagic shock- cerebral ischaemia rat model, there was no brain edema with the infusion of PolyHb-SOD-CAT (Figure 6) [53]. There was brain edema with infusion of PolyHb or with infusion of Hb, SOD and CAT that are not crosslinked. Ischaemic small intestine releases damaging oxygen radicals when reperfused with PolyHb. However, PolyHb-SOD-CAT reperfusion does not increase oxygen radical release (Figure 6) [54]. These results show the possible use of this nanobiotherapeutic for removing damaging oxygen radicals.

The work of Hsia’s group using conjugated haemoglobin containing synthetic antioxidants (PNPH) is another way to solve the problem [55]. Simoni et al. uses pharmacological agents [56]. Zal uses HEMO2life [57]. Alayash’s group uses haptoglobin [58]. Bulow’s group uses recombinant foetal hemoglobin [59]. Ascorbic acid is another approach [60].

**Oxygen carriers + removal of oxygen radicals + removal of CO**

Increase of tissue pCO₂ results in a decrease in the recovery of haemorrhagic shock animals [43]. Furthermore, increase in tissue pCO₂ is correlated with the degree of myocardial ischaemia [44]. In COVID-19 patients, damage to the lung...
interfere with the normal removal of CO$_2$ and the Extracorporeal Oxygenator is more efficient in exchange of oxygen rather than CO$_2$. Our recent 3rd step is to prepare a Polyhemoglobin-Catalase-superoxide dismutase-carbonic anhydrase (PolyHb-SOD-CAT-CA) complex (Figure 7) [46]. This soluble nanobiotechnological complex contains all three major rbc enzymes at up to 6 times the enzyme concentration of rbc. This results in up to 6 times the rbc functions [46]. In a 90-min haemorrhagic shock animal model (Figure 7) [46]. It is better than whole blood in removing elevated intracellular pCO$_2$, avoid ischaemia in the heart, and the histology of the heart and intestine (Figure 7).

This result shows the effectiveness in preventing cardiac side effect that is even better than blood. This is because the enzyme concentration can be up to 6 times that of red blood cells (Figure 7).

**Avoid the critical and severe COVID-19 donor blood supply crisis**

So far, we have discussed the therapeutic uses of the above nanobiotherapeutics to remove free radicals and elevated tissue pCO$_2$. However, COVID-19 Pandemic has also caused a severe crisis for donor blood supply. Careful checking of donor blood and donors can prevent the transmission of COVID-19 from donor blood. However, this also decreases the number of qualified donors since those tested positive or those with exposures cannot donate blood. Shut down and avoidance of possible contacts also resulted in the unavailability of blood donors. For example, a recent publication [61] shows that during the pandemic, whole blood donation in a Chinese blood centre dropped by 67% and 81% of potential donors are worried about contact with COVID-19 during donation. The result is a crisis for blood supply during pandemic. The US Red Cross also declares a first-ever blood crisis amid Omicron surge with resulting severe shortage of blood supply and the delay of many lifesaving surgical procedures [45]. The third-generation blood substitutes, PolyHb-SOD-CAT-CA, as described above no longer has problem related to cardiac side effects and is better than donor blood in the following ways (Figure 8). Unlike donor blood, it has no blood group and can be given immediately on the spot. Donor blood can only be stored for 1 day at room temperature and 42 days at 4°C centigrade. Lyophilised PolyHb-SOD-CAT-CA can be stored for 320 days in room temperature and longer at 4°C centigrade. Donor blood cannot withstand pasteurised, but lyophilised PolyHb-SOD-CAT-CA can be heat pasteurised at 68°F for 2 h to inactivate inactive organisms.
The rbc enzymes can be extracted from bovine rbc inexpen-
sively [62]. We studied in rats 4 weekly 5% blood volume infu-
sion of bovine PolyHb- SOD-CAT-CA followed by 30% volume exchange transfusion [47]. The result shows long term safety with no immunological problems. Measurement of histamine and tryptase also show no anaphylactic reaction (Figure 8). When used in patients bovine PolyHb itself shows no immunological problems [50]. In PolyHb-SOD-CAT-CA, the large excess of haemoglobin molecules bioencapsulated the small fraction of enzyme molecules (Figure 8) [47,48].

Thus, PolyHb-SOD-CAT-CA can help to solve the critical and severe donor blood supply crisis, especially for the following situations [63,64].

- Cardic. Cancer and other urgent Surgery that requires the use of donor blood.
- Accidents, disasters, or conflicts resulting in severe blood loss require an immediate urgent blood transfusion.
- Arterial obstruction can lead to heart attack and stroke. Blood substitute being a solution can perfuse through partially obstructed vessels to reach the heart and brain.
- There are many medical conditions that may need donor blood. However, blood substitutes are only good for shorter term use in more acute conditions.
- If condition does not allow for rbc to be stored in the frig, lyophilised nanobiotherapeutic can be stored at room temperature for 1 year [65].

Thus, in addition to its therapeutic value, these nanobio-
therapeutics can also help to avoid the lack of donor blood supply during COVID-19 pandemics [45,61].

Highlights and discussions: COVID-19 and artificial cells

Artificial cells have resulted in successful carriers for mRNA vaccines but with needs for improvements for longer-term effect. In severe COVID-19, increase in cytokines, can damage organs and tissues leading to death. Our collodion membrane-activated charcoal adsorbent hemoperfusion can effectively remove middle molecular weight range molecules in patients. Recent commercial hemoperfusion of novel mac-
roroporous resin adsorbent with collodion membrane can
effectively remove cytokines in patients and in COVID-19 patients. It has been approved for emergency use for severe COVID-19 patients. A recent nanobiotherapeutic containing haemoglobin plus up to six times the concentration of red blood cell enzymes: catalase, superoxide dismutase and carbonic anhydrase. In an animal study, this can effectively lower the damaging increase in free radicals and increase the removal of increase tissue CO2. This can also help in the first ever critical donor blood supply crisis caused by the pandemic.

Ethical approval
This is a review article, thus for all references related to the source of research funds and ethical permissions in animal or human research, please refer to the original articles cited.

Author contributions
The author TMS Chang is the sole person responsible for the conception and design, analysis, and interpretation of the data; the drafting of the paper, revising it critically for intellectual content; and the final approval of the version to be published; he agrees to be accountable for all aspects of the work.

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