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Short Communication


# COVID-19 mRNA vaccines and pathological cell-cell fusion: an unintended consequence

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The Pfizer and Moderna COVID-19 vaccines are composed of lipid nanoparticles (LNP) containing a modified messenger RNA (mRNA) that encodes for the Spike S1 protein [1-3]. The LNP transfection likely involves particle engulfment by the host immune cells due to their resemblance to apoptotic bodies, vesicles with externalized phosphatidylserine (ePS). As the LNPs are decorated with PS-like ionizable phospholipids, including 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), they encourage human phagocytes into internalizing them [4-6].

The LNP technology, to put it simply, mimics viral envelopes with ePS, a universal "eat me" signal, that directs immune cells to engulf the particle [7,8]. However, as ePS is also a potential "fuse me" signal, LNP may inadvertently facilitate the formation of pathological syncytia [9,10]. Moreover, ePS may activate a disintegrin and metalloprotease 10 and 17 (ADAM 10)(ADAM 17), master regulators of syncytia formation, contributing further to the unintended consequence of cell-cell fusion [11,12].

LNP-incorporated mRNA comprises an enormous technological success that goes beyond vaccines, opening new avenues for developing "smart" therapeutics that can be delivered with pinpoint precision to specific subcellular structures [13]. The development of such therapeutics is anticipated to redefine clinical pathways, including for non-communicable diseases. However, are these therapies ready for worldwide application in their present molecular form?

The question has been asked before, often in relation to the potential toxicity of lipid formulations used in the past, especially as part of the delivering cancer therapeutics [14,15]. However, in the following sections, we take a closer look at a novel perspective, namely the mRNA vaccines' structure and composition and at their unintended biological consequences derived from pathological cell-cell fusion.

## Messenger RNA vaccines, an overview

To elicit the formation of neutralizing antibodies, exogenously administered mRNA must avoid two key obstacles: hydrolysis by extracellular RNAases and recognition by cytosolic innate immune sensors, including toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I) protein [16,17]. The former is accomplished by hiding the nucleic acid backbone into LNPs, while the latter by attaching nucleobases, such as N1-methylpseudouridine (m1Ψ) to the mRNA [18,19](Figure 1). The coding region of the mRNA is flanked by two untranslated regions (UTRs) followed by a polyadenylated (polyA) tail at 3' and a cap at 5' for further structural stabilization and protection (Figure 1) [17,19,20].

As mRNA vaccines are based on pre-fusion epitopes, the fusion pathology may be undeterred, allowing viral infection by syncytia formation to continue unabated [3,16]. This is significant, as it could account for the reoccurrence of COVID-19 symptoms in fully vaccinated individuals [3,21,22]. In addition, this may explain the rare post-vaccination events associated with cell-cell fusion, including giant cell myocarditis, giant cell arteritis, and Creutzfeldt-Jakob Disease, recorded in Vaccine Adverse Event Reporting System (VAERS) database (please see section "Vaccine core: the synthetic mRNA") [23-27].

### What is cell-cell fusion?

Cell-cell fusion is a physiological or pathological process in which one or more adjacent cells merge their plasma membranes,

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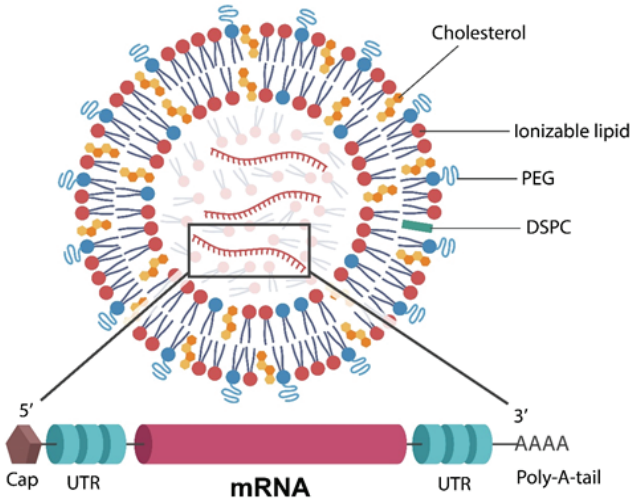


***Figure 1.*** *N1-methylpseudouridine (m1Ψ)-modified mRNA (in the rectangle) is surrounded by a lipid nanoparticle (LNP) comprised of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and an ionizable lipid. Polyethylene glycol (PEG) is conjugated with the lipid molecules to increase the mRNA duration of action. The mRNA encodes for the full-length S antigen and is flanked by two untranslated regions (UTRs) and a polyadenylated (polyA) tail at the 3' end for stabilization. A cap at the 5' end offers further protection from exonuclease recognition.*

cytoplasm, nuclei, and intracellular organelles, generating multinucleated syncytia often with novel, emerging properties [28]. Under normal circumstances, cell-cell fusion occurs during fertilization and placentation as well as during the formation of myoblasts and osteoclasts [29]. Likewise, in the central nervous system (CNS), astrocytes engender physiological syncytia via connexin-mediated cytosolic exchange [30,31].

Several viruses, including SARS-CoV-2, exploit host physiological fusogens as these molecules induce premature cellular senescence and immunosuppression, phenotypes hospitable to viral replication and, immune evasion [32,33]. Indeed, cell-cell fusion is triggered by ePS, a marker of low immunogenicity, exploited by the SARS-CoV-2 virus to enter into host cells undetected [9,34-36]. Virus-upregulated intracellular calcium (Ca2+) activates transmembrane protein 16F (TMEM16F), that in turn flips PS from the inner to the outer layer of cell membranes, promoting fusion [9].

Viruses, including SARS-CoV-2, can enter host cells via endocytosis or cell-cell fusion, processes driven by cell-penetrating peptides (CPPs), suggesting that endocytosis inhibition may not always prevent infection [37-39]. Endocytosis requires viral protein attachment to a cell surface receptor and internalization of the entire virus/receptor complex. For example, SARS-CoV-2 binds to host angiotensin-converting enzyme-2 (ACE-2) via its S1 protein followed by endocytosis. On the other hand, ePS activates ADAM17, inducing cell-cell fusion via furin cleaving site (FCS) located within the S2 protein, a pathway independent of S1/ACE-2 attachment [40-43].

## Vaccine core: the synthetic mRNA

In contrast to traditional vaccines that present a plethora of viral proteins to the host immune cells, COVID-19 mRNA-based therapeutics are limited to the antigen of interest (AOI) and elicit antibodies primarily against S1 receptor binding site (RBS) [1,12,44]. For the complete success of the above method, it must be assumed that the SARS-CoV-2 virus cannot ingress host cells by an alternative pathway. However, several studies have highlighted other potential routes of viral ingress, including metalloprotease, integrins, glucose-regulating protein 78 (GRP78), antibody-dependent enhancement, cell-penetrating peptides, and possibly HERV activation [45-47]. These entry points will be discussed in more detail after a brief examination of LNP components.

## LNPs

Although transfection data is mostly proprietary, interrogation of LNP components can provide clues about the mRNA ingress into human cells [6]. For example, LNPs contain ionizable lipids, phospholipid 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and cholesterol that can attract host phagocytes to internalize the particle [48-50]. Both ionizable lipids and DSPC resemble PS, communicating to phagocytes readiness for engulfment [51]. Aside from comprising an established "eat me" signal, ePS can also convey "fuse me" cues to host phagocytes that can contribute to the unintended consequence of pathological syncytia formation [10]. The LNP component, cholesterol, is also a promoter of pathological cell-cell fusion as it can alter the asymmetry of cell membranes [50]. Moreover, as cell-cell fusion leads to premature cellular senescence and iatrogenic immunosuppression, it may partly explain the immune dysfunction documented in some vaccinated individuals [52-54].

### Polyethylene glycol (PEG)

PEG was added to the LNP to stabilize and prolong the mRNA duration of action (Figure 1). The extensive utilization of PEG over the past few decades, suggests that preexisting antibodies could trigger hypersensitivity to vaccines containing this molecule [55,56]. Aside from allergy, PEG is also an established chemical fusogen that can generate pathology by promoting polynucleation, aneuploidy, and genomic instability [57-60]. In addition, PEG upregulates intracellular Ca2+, activating the transmembrane protein 16F (TMEM16F), a lipid scramblase that flips PS on the cell surface, triggering fusion, premature cellular senescence, and immunosuppression [61-63]. As these phenotypes are virus-friendly, PEG-induced cell-cell fusion may inadvertently facilitate not only SARS-CoV-2 but also other viral infections [61,65-67]. Furthermore, ePS-activated ADAM 10 and 17 promote syncytia formation via metalloprotease pathway [40,42,43].

PEG was never used in an approved vaccine therefore, its presence in Pfizer-BioNTech and Moderna -1273 therapeutics raised concerns, especially regarding anaphylactic and fusogenic adverse effects [53,68,69]. Moreover, PEG promotes temporary permeabilization of the blood-brain barrier (BBB), a property exploited by the pharmaceutical industry for CNS delivery systems [70-72]. This may account, at least in part, for the rare VAERS-reported neuropsychiatric symptoms, including neurodegenerative disorders [73-75]. Furthermore, earlier studies have demonstrated that PEG may interfere with the conformational stability of proteins, indicating that syncytia, cellular senescence and, dysfunctional proteostasis are highly intertwined [76-78]. While the attention to PEG and the need to further study its relation to the potential vaccine adverse reactions is logical and appropriate, it must be noted that excipients other than PEG might be also be involved in such reported adverse reaction events [79].

### DSPC and ionizable lipids

To deliver the liposome cargo to human immune cells, mRNA therapeutics must trick host phagocytes into internalizing LNPs by phagocytosis [80,81]. This is accomplished by decorating the liposomal particles with ionizable lipids and DSPC, an anionic phospholipid that mimics ePS and conveys readiness for phagocytosis [82,83]. This "eat me" signal is exploited by many viruses, including SARS-CoV-2, to enter host immune cells by engulfment [51].

Pfizer and Moderna vaccines were designed to "imitate" dying cells or apoptotic bodies by utilizing ionizable lipids and DSPC delivering mRNA directly to the immune cells' translation machinery [5]. However, DSPC's resemblance to PS may inadvertently activate ADAM 10 and 17, promoting pathological cell-cell fusion and subsequent pathology [40].

Taken together, LNPs mimicking ePS are engulfed by host immune cells, and generate anti-S1 antibodies by delivering the mRNA cargo to host ribosomes. However, in some cases the disruption of plasma cell asymmetry may inadvertently engender iatrogenic syncytia by activating the metalloprotease pathway.

## Potential non-RBS modalities of SARS-CoV-2 infection

In the sections below, we take a closer look at SARS-CoV-2 infection by RBS/ACE-2 independent pathways that may escape neutralization by mRNA vaccines.

### Infection by fusion, the metalloprotease pathway

SARS-CoV-2 infection can be disseminated from cell to cell by pathological syncytia, a FCS-dependent route also known as the metalloprotease pathway [84,85]. This infection modality may promote higher infectivity than the RBS/ACE-2 route as FCS deletion was demonstrated to attenuate infectivity [86]. Moreover, FCS activates ADAM10 and 17, master regulators of cell-cell fusion, enhancing both syncytia formation and contagiousness [11,12]. Thus, the metalloprotease pathway may be resistant to mRNA vaccines that were designed primarily to neutralize pre-fusion epitopes, likely explaining the emergence of COVID-19 symptoms in fully vaccinated individuals [2,3,40-43,87]. Furthermore, as pathological cell-cell fusion was associated with premature cellular senescence and immunosuppression, the metalloprotease pathway may account for the dysfunctional immune responses observed in some vaccinated individuals [35,36,52]. Syncytia-related pathology may also contribute to other VAERs-documented post-vaccination events, including giant cell myocarditis, arteritis, and neurodegeneration [23,24,27,88].

### Antibody-dependent enhancement

Antibody-dependent enhancement (ADE) is a mechanism of increased viral infection in the presence of partially neutralizing antibodies that can activate immunoglobulin G (IgG) Fc-gamma receptors (FcγRs) [89-92]. This "Trojan horse" infection modality occurs when the SARS-CoV-2 virus hijacks antibodies to infect immune cells and decrease host antiviral defenses [93,94]. This infection route is routinely employed by many viruses, including the human cytomegalovirus (HCMV) known for usurping host T lymphocytes and macrophages [95-98].

The ADE infection pathway presents with the following characteristics: 1. direct correlation with the disease severity, 2. independent of S1/ACE-2 attachment, and 3. probably unaffected by the mRNA vaccines [95,99].

### Cell-penetrating peptides

COVID-19 proteomic studies show that SARS-CoV-2 expresses many cell-penetrating peptides (CPP) that can promote viral entry and may be undeterred by the mRNA vaccines [100]. Indeed, many enveloped and unenveloped viruses contain CPPs and employ them for ingress host cells [101].

Aside from SARS-CoV-2, several other viruses, including the H5N1 avian influenza, dengue virus, and human papillomavirus can enter host cells via CPPs, indicating a common viral entry route [102,103]. In addition, CPPs are being utilized as pharmacological vehicles for intracellular delivery of therapeutics, highlighting the capability of these molecules to cross cell membranes [104]. CPPs can upregulate intracellular Ca2+, promoting both pathological syncytia and protein misfolding [105-108].

### Viroporins

Viroporins are hydrophobic, voltage-independent viral proteins known for piercing plasma membranes, triggering cell death. Many viruses, including SARS-CoV-2, express viroporins and promote infectivity, as these proteins are known for mediating viral entry and exit [109,110]. A recent in silico study found that the SARS-CoV-2 virus expresses three viroporins, the E antigen, open reading frame 3a (ORF3a), and ORF8a, highlighting potential, non-RBS, routes of viral ingress that may be refractory to mRNA vaccines [111]. Interestingly, vaccine-mediated neutralization of S1 protein may contribute to the accumulation of other viral proteins, including viroporins, in the extracellular space (ECS), probably opening alternative entry portals for viral ingress [110,112]. In addition, viroporin channels disrupt the ionic homeostasis of host cells, upregulating intracellular Ca2+ that in turn, promotes pathological cell-cell fusion [113].

### HERVs

FCS was reported to activate HERVs, primarily type W and K, triggering not only cell-cell fusion but also hyperinflammatory responses and dysfunctional proteostasis [114-117]. HERVs are viral fossils embedded in human DNA that can be "awakened" by the infection with exogenous viruses, cancer, or neuropsychiatric conditions [118-120].

Several studies demonstrated that SARS-CoV-2 can activate HERV-W, an ancestral gene that encodes for the physiological placental fusogen syncytin-1 responsible for the merger of trophoblasts during the early pregnancy [114,115]. This suggests that the reproductive post-vaccine events may be triggered by the FCS pathology. Virus-upregulated syncytin-1 may promote aberrant cell-cell fusion throughout the host tissues and organs, including the brain [121,122]. Interestingly, Omicron variant convalescent sera contain anti-FCS antibodies, suggesting that it may neutralize not only the pathological cell-cell fusion but also HERV activation [123,121].

## Conclusions

The mRNA vaccines were approved on an emergency basis to combat COVID-19 pandemic. These vaccines also represent the first administration of LNPs at large scale. Taken together, they constitute milestones in the development of a novel and much promising therapeutics delivery field. Having said that, at the time of the emergency approval, the S2 viral antigen was insufficiently studied, and the FCS-mediated fusion pathology was mostly unknown. These aspects are now starting to attract

attention, in an effort to best understand the underlying cellular mechanisms, pathways and potential unintended consequences.

The Pfizer and Moderna vaccines elicit powerful neutralizing antibodies against the RBS located on S1 protein and block viral entry by endocytosis. However, the S2-dependent metalloprotease pathway and other potential entry portals may not be adequately addressed by these therapeutics. Residual COVID-19 symptoms, often conceptualized as vaccine adverse effects, could be caused by FCS-mediated pathology. ADAM inhibitors and/or Omicron convalescent sera may effectively eradicate the SARS-CoV-2 virus by inhibiting metalloprotease pathway.

## *Disclaimer*

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