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Membrane tension may define the deadliest virus infection

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**Rapid Communication**

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**ABSTRACT**

This manuscript describes the potentially significant role of interfacial tension in viral infection. Our hypothesis is based on evidence from drop coalescence hydrodynamics. A change in membrane tension can trigger fusion between the virus-carrying vesicles and the host cell. Viral infections can be reduced if such fusion was inhibited. Membrane fusion itself is ubiquitous in cell biology including virus fusion, membrane trafficking, and neurotransmitter release [1]. The ability to mediate biological membrane fusion processes is nontrivial and, thus, the fundamental mechanisms governing this process need to be understood, including the role of relevant hydrodynamic mechanisms.

Fusion is initiated through a certain arrangement of protein structures with or without the presence of chemical potentials. The spontaneous curvature of the contacting membranes may play a significant role in pore formation. Once a stable, which is not momentarily closed, aqueous pore is established, its subsequent dilation is governed by the fluid dynamics of liquid drop coalescence [2]. Membrane fusion is a complex, multiscale process guided by a complex balance of hydrodynamic and biochemical forces. Therefore, this complex phenomena demands multidisciplinary approach such as protein structures, thermodynamics, curvature mechanics [3], and fluid dynamics. Findings can be used to develop therapeutic strategies to inhibit viral membrane fusion.

1. **Introduction**

One of the most significant processes of viral infection is the fusion between the virus-carrying vesicles and the host cell. Viral infections can be reduced if such fusion was inhibited. Membrane fusion itself is ubiquitous in cell biology including virus fusion, membrane trafficking, and neurotransmitter release [1]. The ability to mediate biological membrane fusion processes is nontrivial and, thus, the fundamental mechanisms governing this process need to be understood, including the role of relevant hydrodynamic mechanisms.

Fusion is initiated through a certain arrangement of protein structures with or without the presence of chemical potentials. The spontaneous curvature of the contacting membranes may play a significant role in pore formation. Once a stable, which is not momentarily closed, aqueous pore is established, its subsequent dilation is governed by the fluid dynamics of liquid drop coalescence [2]. Membrane fusion is a complex, multiscale process guided by a complex balance of hydrodynamic and biochemical forces. Therefore, this complex phenomena demands multidisciplinary approach such as protein structures, thermodynamics, curvature mechanics [3], and fluid dynamics. Findings can be used to develop therapeutic strategies to inhibit viral membrane fusion.

2. **Membrane mechanics: hemifusion or fusion?**

Membrane proteins can locally alter surface tension [4] and, therefore, can work as a fusion-favorable site [5] by lowering its energy barrier [6]. Fusion proteins alone [7], or upon receiving activation potential [8,9], can provide enough energy to trigger fusion by forming hourglass-like shapes at the merger site. Depending on the spontaneous curvature [10] of the membrane, a stable hemifusion or an intermediate hemifusion can occur, which leads to membrane fusion through rupturing of the hemifusion diaphragm. Hemifusion could also be caused by spontaneous negative curvature [11]. When a proximal leaflet with negative spontaneous curvature wraps from the outside, it becomes more unstable because it is bent in the opposite way. On the other hand, the distal leaflet wraps from inside, it becomes less unstable as it is bent in the same way. Lipids in the distal leaflet are packed tightly and make the membrane more stiff which can withstand stress in the diaphragm during the retraction of proximal leaflet [12]. Spontaneous neutral or positive curvature may lead to fusion by expanding a pore.

3. **Fusion pore expansion vs hydrodynamics of drop coalescence**

3.1. **Hydrodynamics of drop coalescence**

We recently investigated the hydrodynamics of two dimensional drop coalescence [2]. High pressure gradients from bulk water drop to the bridge (i.e. pore) region \((AP, \text{Fig. 1})\) drives the coalescence (i.e. fusion) process. We determined that initial dynamics of coalescence (i.e. pore dilation) was dominated by surface tension and local viscous effects; subsequent pore-formation dynamics are more significantly...
impacted by other competing forces and interactions. We predicted pore growth rate through a theoretical scaling argument which was validated for different viscous fluids.

A brief summary of our model, including its relationship with pore formation, is described next. Early dynamics of coalescence, at the beginning of pore propagation, depends on surface tension ($\sigma$) and viscosity ($\mu$) of the drop. The pore dilation ($r$) with respect to time ($t$) relates as $r = \frac{\pi}{2} \sqrt{\frac{\mu}{\sigma}} \overline{t}$, refer to Appendix for this derivation. While early dynamics is universal, later dynamics is significantly influenced by the size of the drop ($R$), density of the drop ($\rho$) and the surrounding medium, and longitudinal curvature of the pore. For coalescence of two similarly-sized drops in air, pore growth rate can be shown as the following,

$$\frac{dr}{dt} = -\frac{Oh}{2} \left( \frac{1}{r} + \frac{2}{A^2} \right) + \sqrt{2 + \frac{2}{A^2} - \left( \frac{2}{A} + 1 \right) \frac{1}{r} - \frac{2\rho}{4} \left( \frac{1}{r} + \frac{2}{A^2} \right)^2}$$

(1)

where $Oh$ represents Ohnesorge number and is expressed as $Oh = \frac{\rho}{\sqrt{\rho \sigma v}}$. Parameter $A$ relates to longitudinal curvature, $r$ and $t$ represents scaled (dimensionless) pore radius and propagation time. While this mathematical representation may successfully describe multi-scale fusion propagation, it may not be physically intuitive to observe the dynamics at later time based on this expression itself. Graphical representation of this transient phenomena, with prior knowledge of all the governing hydrodynamic parameters, is needed as demonstrated previously for different viscous fluids [2].

3.2. Early dynamics of pore expansion

From early dynamics ($r = \frac{\pi}{2} \overline{t}$) of pore dilation, we can see that it is a function of membrane tension and fluid viscosity. Fluid viscosity represents the rigidity of the vesicle’s membrane. Stable pore formation, therefore, may depend on membrane spontaneous curvature [12], membrane tension, and membrane rigidity. Greater membrane rigidity causes greater flow resistance. Therefore, once a pore is formed, it may subsequently close due to instability from collapsing catenoid. This phenomenon can be seen in a Nanodisc-Black lipid membrane experiment when an opening pore subsequently closed causing flickering events [13]. Membrane tension and rigidity can be altered by membrane proteins and cholesterol [14], and can cause variability in fusion pore expansion dynamics at the early stage. It is also possible that membrane tension of fusing vesicles is approximately zero initially; however, upon pore formation, transformational changes may locally increase membrane tension.

3.3. Later dynamics of pore expansion- effect of membrane tension differences

Surface tension guides later hydrodynamics after the preceding curvature-based forces have subsided. For example, membrane tension variation among virus and host cells can alter the dynamics significantly. If membrane tension of the virus vesicle is higher than that of the cell membrane ($\sigma_v > \sigma_c$, Fig. 1), vesicle lumen inside the pore will form a liquid column due to Marangoni effect on the membrane (see Fig. 12 in [2]) and genetic material will be penetrated further away from the cellular membrane; here, fusion will happen irreversibly. However, if the membrane tension of the virus vesicle is less than that of the cell membrane ($\sigma_v < \sigma_c$), genetic material will reside next to the cell membrane and reversible virus budding [15] may occur. This can be viewed from the rebound dynamics of a drop on a bath having different surface tension [16,17], see Fig. 2).

4. Coronavirus membrane fusion mechanism

During membrane fusion process, the coronavirus is attached to the host cell mediated by the spike (S) protein held in the viral membrane. The S1 subunit of S protein binds to angiotensin-converting enzyme 2 (ACE2) which is abundantly present on lung cells [18]. The model coronavirus is proposed to have two entry pathways when the virus binds to its cell receptor-plasma membrane (early entry) and endosome (late pathway) [19] such as clathrin- and nonclathrin-mediated endocytosis [20,21]. Once the virus is internalized via endocytosis, they are enveloped inside a vesicle and wait to deliver RNA to the cell interior until a favorable condition, such as low pH, proteases, ions, intracellular receptors, and/or lipid composition, is met [22]. Within the endosome, the fusion energy barrier between the virus membrane and virus-carrying-vesicle membrane should be higher as vesicles are likely inflated which may inhibit fusion because of greater membrane tension [23]. It has been previously shown that the uncleaved MERS-CoV S proteins slowly change their conformations after receptor binding and, thus, virus traffic to the late endosome [24]. Therefore, endosome fusion can be slow, arbitrary, and involves multiple steps while cell membrane fusion can be fast and highly regulated. Depending on the membrane

Fig. 1. Resistance due to rigidity of virus membrane and role of membrane tension difference. A) At the early stage of pore propagation, highly-bent membranes remain close to each other. The majority of the flow is resisted due to viscous shearing of the membrane at the merging interface region. Therefore, an increase in rigidity slows down the pore dilation rate. Velocity gradient, perpendicular to the pore propagation direction, is presented by different sizes of arrows. Early dominance (when $r \ll 1$) of the pore expansion dynamics is due to pressure force caused by highly-bent longitudinal curvature ($\frac{2}{A}$) and depends on membrane tension and local viscous effect (membrane rigidity). B) Later, other forces become significant and pore expansion dynamics can be significantly changed due to membrane tension difference between vesicle and the cell membrane. For example, if membrane tension of the vesicle ($\sigma_v$) is greater than that of the cell membrane ($\sigma_c$), dilation can be halted and vesicle lumen inside the pore will form a liquid column – this way vesicle content would be released towards the cell’s interior, away from cell membrane.
tension difference between virus envelop and the host cell (as explained in Section 3, Fig. 3), the membrane fusion can generate liquid column and thus irreversible RNA transmission can happen inside the cell by releasing them further away from the cell membrane. Microtubule can easily pick delivered RNA from inside the cell, though it may not do the same for viral RNA near the plasma membrane due to the hydrodynamic interactions of RNA molecules with the membrane.

In Section 3, we have shown that propagation for early dynamics of pore expansion was \( r = \sigma \mu t \). Therefore, early dynamics on pore propagation is dominated by membrane tension and rigidity. While greater...
membrane tension can increase the energy barrier and may work as fusion inhibitor, it enlarges more rapidly [23] once a fusion favorable site has been initiated. Membrane tension can be influenced by temperature [25], pH [26], membrane proteins [27], and possibly the size of the vesicle. Therefore, it is imperative to investigate the role of membrane tension for this fusion process.

5. Membrane tension increases with decreasing temperature

As we discussed in Section 4, there may have different ways of virus entry into the cell, however, virus replication may be the most probable case when viral RNA is released towards the cell’s interior, away from cell membrane. This can happen when the membrane tension of the virus vesicle is slightly greater than that of the cell membrane – lower temperatures may induce this favorable scenario for the virus. Below a critical temperature, tension builds up in the membrane of an otherwise tension-free vesicle [28], possibly by changes in the membrane chemical structure [29]. It has been shown previously that in mouse airway cells the rhinovirus (i.e. the common cold virus) replicates preferentially at cooler temperatures found in the nasal cavity (33 °C) compared to warmer core body temperatures (37 °C) [30]. The architecture of each virus is different, thus, the critical temperature below which membrane tension builds up can be different for each virus. Fusion can still happen, though, without building tension on the virus surface. However, at elevated temperatures the viral RNA may reside, as discussed earlier, near to the cell membrane which may not be adequately transported to cell replication machinery. With fewer viral replication, patients may exhibit milder symptoms or could be asymptomatic due to body’s better immune response [31].

6. Factors affecting nasal airway temperature

Exposure of dry and cold air can significantly lower nasal cavity temperatures relative to body temperature. Dry air helps to lower the temperature through evaporation of the mucosa layer. For a review of heat transfer and water exchange within the respiratory tract, readers are referred here [32]. The following will describe how air conditioning, atmospheric conditions, and uses of masks may impact temperature-dependent viral replication.

6.1. Air conditioning system

There are studies that suggest that air conditioning plays a role in viral transmission. However, the relationship between air conditioning and nasal airway temperature is not clear. There was a study where four pairs of infected and uninfected guinea pigs were placed in climate-control chambers (5–20 °C, 20–80% RH) and airflow was directed from the infected towards the uninfected guinea pigs [33,34]. Influenza virus transmission was favored cold and dry conditions where infection was prominent at low temperature (5 °C) and low humidity (~20–35% RH).

There is recent evidence that SARS-CoV-2 (COVID-19) may be transmitted through aerosols [35]. We can take a closer look at recent studies where the inhalation of cold, dry air may have played a significant role in each infection event. We admit that the following conjectures are challenging, if not impossible, to precisely align with our previous discussion. Though, in the absence of a clear study showing the temperature dependence on coronavirus infection, we discuss how temperature within each scenario could have impacted the resultant viral transmission.

In a restaurant at Guangzhou, China, ten customers (out of 83) were infected, sitting at the same table or in close proximity to the original index-patient [36]. Coincidentally, the three tables that had infected cases were aligned with airflow from an adjacent air-conditioning system. Another study of that same incidence [37] showed that viral transmission likely occurred throughout the restaurant due to the location of the exhaust fan on the other side of the restaurant. Therefore, each patron in the restaurant likely got exposed to a lower dose of the virus. However, it is important to note here that researchers have found no correlation between viral load and severity of the SARS-CoV-2 [38]. While throat swab samples from the contacts were negative for the viral infection by reverse transcription PCR, there was no serologic studies on them. Perhaps the temperature of the three-table infection zone was sufficiently low for efficient viral replication.

6.2. Atmospheric condition

Smuls were thought to be the most vulnerable for coronavirus because of high density of the population, open sewers, non-existent waste disposal systems, poor economic conditions, and other unsanitary conditions [39]. One serological surveillance suggests that more than half of the slum dwellers in Mumbai, India were infected by COVID-19 during the first-half of July 2020 [40]. However, the death rate among those who were infected was extremely low (0.05%), while death rate in India was 2% (as of 9/24/2020 [41]). During that time, average highest daily temperature was 88 °F and lowest was 77 °F (and an average relative humidity of 81–98%) [42]. Another news reported on slums located in Dhaka, Bangladesh [43] reported few, if any, COVID-19 cases and deaths; the weather during the time of this study was humid and hot (May to July, average temperature was 85 °F and average relative humidity was 82%) [44]. Surprisingly, some of the slums were located in regions with the highest number of reported COVID-19 cases for that city (Mirpur). With lower testing facilities [45], people were likely tested who showed symptoms. This report suggests that temperature and humidity may play a role in viral replication, as slums are typically without air conditioning and have greater outdoor air circulation.

Recent studies suggest that incidence of disease declines with increasing temperature (data from January 22, 2020 to April 3, 2020 in USA) [46]. However, another study found positive association of diurnal temperature and negative association of humidity with COVID-19 deaths (January 20, 2020 to February 29, 2020 in Wuhan, China) [47]. Another study found a link between decreasing temperatures and high precipitation with the spread of the Spanish flu pandemic in 1918 and 1919 [48]. Perhaps, heavy rainfall, particularly in mid- to low-latitude regions, significantly lowered local temperatures [49] which may have caused right condition for virus replication.

Could our hypothesis be applied to the different waves of death during Spanish flu? Deaths were highest at early November and significantly reduced from middle of December to February in London, United Kingdom; a second wave was seen to emerge in the middle of February. The trends on death were similar across the cities of New York, Paris, and Berlin [50,51]. This signifies that approaching winter months were more deadly than during freezing winter itself. We hypothesize that elevated membrane tension of the virus caused by cold temperatures either inhibit fusion by creating a greater energy barrier, or, after fusion, the virus RNA resides near the membrane due to the lack of liquid column formation during fusion (Fig. 2, for $\sigma_v = 0.3$ drop rebounds after partially coalescence, unlike complete coalescence for $\sigma_v = 0.91$ [17]).

6.3. Use of masks

When we inhale, we intake surrounding air. However, as we exhale, we release 100% humid air. Therefore, a mask can provide added protection by creating an elevated temperature and humid environment inside nasal cavity. For example, it was shown previously that the use of mask (N95 FFR and N95 FFR/EV) results in deadspace air temperature and relative humidity levels that are markedly elevated above ambient levels [52]. From recent studies, we have seen that masks may not fully protect the user from viral transmission but it has been associated with an increased rate of asymptomatic cases [53,54], though the reason is
not fully understood. Therefore, it is possible that mask helps in lowering viral replication by keeping nasal cavity temperature close to the body temperature. As temperature of the mucosa layer does not go down, interfacial tension of viral membrane remains zero or close to zero.

7. Future directions- characterizing membrane tension at different conditions

Measuring tension and rigidity of the merging membranes can be a key step in learning fusion dynamics. However, measuring membrane properties for a very small structure (such as 100 nm vesicle) can be very challenging. Therefore, reconstituting the virus vesicle into a giant unilamellar vesicle (GUV) [55] can be an important step to conduct microscale observations and experiments. At this larger scale, several techniques for membrane tension measurements can be employed such as optical measurements [56], aspiration techniques [57], or nanoindentation using AFM [58]. Another important aspect of GUV systems is that pore propagation can be visualized microscopically and different transient fusion processes can be monitored. Virus attachment, docking, and fusion triggering can be determined by using nanodisc-based planar bilayer electrophysiology [13], which facilitates the study of pore assembly and disassembly, where the effect of protein assembly and role of viscosity of the medium can be evaluated.

8. Conclusion

Viral entry inside the cell may have many different routes. However, for infectious membrane fusion, membrane tension likely plays the driving role in fusion dynamics [59]. Therefore, we need to have a better understanding of the role of membrane properties during fusion dynamics. Membrane properties are a function of the medium pH, membrane proteins, cholesterol [60], and temperature. As aerosol droplets of different sizes can land on different parts of the respiratory tract [61], the aforementioned properties, regulated by age effect and/or underlying health condition, at different parts of the track can be crucial in regulating membrane tension. Here, we mostly discussed the temperature effect in the upper respiratory tract and possible sources of the temperature effect.

Knowing the membrane properties and using theoretical arguments we could predict the dynamics of membrane fusion and potential viral transfer; for example, for a given scenario if RNA will be located near the cell membrane or further into the cell’s interior. When the upper respiratory tract is relatively cold compared to body temperature, inhaling virus-laden droplets may lead to increased virus replication [30]. This can possibly be caused by the higher membrane tension difference between the virus envelop and the host cell. For the case of zero membrane tension, membrane fusion can be caused by membrane proteins in a concerted effort. Morphological changes of those proteins during this type of fusion process should be conducted in an organized fashion. In such case, molecular diffusion, due to concentration gradient, will happen after the completion of fusion. This may restrict viral genetic material to reside near the cell membrane which may form virus outside of the cell through virus budding process while not replicating a new one. Therefore, understanding the role of membrane tension for both the virus and host cells may provide insight as to why some patients may experience different viral loads, influencing the severity of their subsequent infection.

CRediT authorship contribution statement

Md Mahmudur Rahman: Conceptualization, Investigation, Formal analysis, Writing - original draft. Stuart J. Williams: Visualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no conflict of interest.

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Appendix A. Early stage coalescence

For two equal sized similar drops coalescing in air, propagation dynamics can be expressed by, from eq. 2 in [2],

$$\left(2 + \frac{2}{A^2} - \left(2 + \frac{1}{A^2} - \frac{1}{T} \right) \right) \frac{dr^2}{dt} + \frac{\frac{Oh}{T} dr^2}{dt} + \frac{2 \frac{Oh}{T} dT}{dt}$$

(A)

where, $F = \frac{\frac{Oh}{T}}{T}$, $I = \frac{1}{T}$ and $T = \sqrt{\frac{A^2}{\frac{Oh}{T}} e}$. At the early stage ($T < 1$) of coalescence, $\frac{dT}{dt}$ term is far greater than $\frac{dr}{dt}$ term which signifies that viscous resistance is dominant in the mixing interface region. Therefore, at the very early regime, the scaling law converges to $T = \frac{dT}{dt} x$, which further can be simplified as $r = \frac{1}{2} t$. While this relationship is shown here for coalescence in air, it will hold for coalescence in any viscous medium because any added $1/T$ term will not contribute at the early stage ($T < 1$) dynamics.

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