Fabrication of Microfluidic Devices to Study the Fusion Kinetics of Influenza Virus to Biomimetic Membranes

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Abstract:

The aim of this work is to mimic influenza fusion to biomimetic membranes in microfluidic channels, using total internal reflection microscopy (TIRF) to track individual virus fusion events. Using this single particle approach, we can also distinguish between hemifusion and pore formation. Fusion of the influenza to the biomimetic membrane is initiated at low pH. We have integrated a proton uncaging technique to initiate a rapid pH drop in the microfluidic channel. This assay allows us to obtain accurate kinetic information, such as the hemifusion and pore formation rate constants, and the number of fusion proteins required to initiate fusion.

Summary of Research:

Influenza is a membrane-enveloped virus, which infects cells using membrane fusion [1] to release its genome, as illustrated in Figure 1. There are two steps in the influenza fusion process, the merging of the outer lipid monolayers (hemifusion), followed quickly by opening of the fusion pore through which the viral RNA is released. Inhibition of these steps is currently the target of antiviral and antibody therapy and it is important to understand the kinetics of this process in a controlled, in-vitro environment. We use microfluidic devices to mimic the endosomal environment. Using vesicle-vesicle fusion, solid supported lipid bilayers (SLBs) are formed in microchannels. The SLBs contain sialic acid residues to which the virus binds. The virus is dual labeled [2] with a self-quenching membrane dye, R18, an internal dye, sulforhodamine B (SRB), to monitor hemifusion and pore formation respectively. An illustration of the microfluidic device used is shown in Figure 2. Fusion is typically initiated by flowing acidic buffer a high flow rate through the microchannels. This high shearing flow can damage the membrane as well as removing virus particles from the SLB. The pH change is also slow and uncoordinated, which has a significant effect on fusion statistics at a single virion level. To overcome this limitation we use a proton-uncaging technique to reduce the pH in the channel on the order of microseconds, in a quiescent environment. The channel is filled with a water-soluble, photoisomerizable compound, o-nitrobenzaldehyde (o-NBA) which upon UV irradiation releases a proton into solution [3].

Upon analysis of the kinetic data obtained using the uncaging method, we see that the hemifusion rate constant ($k_H$) and decay rate ($k_{HD}$) are in agreement with published data as seen in Figure 4. However, the number of proteins (N) required to initiate hemifusion was shown to be between 1 and 2, which is lower than previous reported values of 3. Control experiments showed that N is sensitive to the rate of acidification, and slower acidification times that occur during acidic buffer exchange smear the time distribution of fusion events.

We can now use this method to determine the effects of laboratory adaptation on influenza fusion kinetics and how this affects virulence of different strains. Influenza is typically passaged in eggs to increase the yield of virus for vaccine and antiviral development. These passages result in lab adaptations, which change the virus morphology and the receptor specificity from human to avian type receptors. Preliminary results using a clinical strain indicate that clinical strains exhibit slower fusion kinetics and are independent of pH below pH 5.5, in comparison to lab-adapted strains.

References:

Figure 1: A) TEM of influenza particle. Influenza is membrane enveloped virus which contains three transmembrane proteins on the viral surface. The most abundant protein, hemagglutinin (HA) mediates entry into the cell. B) Influenza particle binds to sialic acid receptors on the cell surface via the HA protein in the viral membrane. The virus is then engulfed into the cell via endocytosis. The endosome becomes more acidic and at a critical pH (5.5) the HA undergoes a conformational change and the viral and endosomal membrane fuse. A pore opens and the RNA is released into the cytosol and transported to the nucleus for replication.

Figure 2: A multichannel microfluidic device containing influenza virus bound to a solid supported lipid bilayer. The channel is filled with a solution of o-NBA (hexagons) and flow is stopped. The channel is then irradiated with a short UV pulse and a proton is cleaved from each o-NBA molecule, reducing the pH in the region of the UV laser and initiating fusion.

Figure 3: Images of single virus particle undergoing hemifusion and pore-formation. At t = 0 acidification occurs and 32 seconds later the virus hemifuses (as denoted by fluorescence dequenching) and 20 seconds a pore forms in the same virion and the internal dye is released.

Figure 4: Fusion kinetics of influenza as a function of pH. Hemifusion rate constants obtained using acidic buffer exchange (black circles) are in good agreement with literature values. When fusion is initiated using o-NBA (diamonds) at three different calibrated pH values, the hemifusion rate constant are comparable to those attained using buffer exchange. However, N, is seen to be significantly reduced when o-NBA method of initiation is used.