Mucociliary interactions and mucus dynamics in ciliated human bronchial epithelial cell cultures Movie captions

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The movie names correspond to the figure panels in the paper. The movies were made from confocal scans as cultures were scanned in many areas. To keep the keep the scan times short enough to survey the cultures regularly over the course of the experiment, only enough frames where taken to document the dynamics encountered. So many of the movies have few frames and are best viewed looped.

Movie 1A – Sparsely ciliated cultures. The movie shows the DIC image of a sparsely ciliated culture in top-down view. The superimposed fluorescence image shows mucus labeled with 20 nm beads (green) and 1 µm beads (red). From the DIC, it can be seen that ciliated cells were mostly separated from each other. Mucus labeled with beads could be seen only on cilia. Twenty nanometer beads were able to enter into the mucus, but 1 µm beads were restricted to its surface. This mucus moved in response to ciliary motion but remained attached while flow could be seen from non-attached 1 µm beads when they were first added.

Movie 1B – Sparsely ciliated cultures. The movie shows a sparsely ciliated culture in profile view. The top panel shows the fluorescence from 20 nm beads and the bottom panel shows the DIC image. Both the cilia and high concentrations of entrapped 20 nm beads could be seen in DIC. Comparing the DIC (cilia and beads) and the fluorescence (beads only) shows that the mucus was only associated with the cilia. Many of the mucus plumes extended far above the cilia.

Movie 2A – Mucus behavior on fully ciliated cultures. The movie shows the fluorescence on the culture surface just after the addition of red fluorescent 1 µm beads to the culture's apical surface. The fluorescence initially moved back and forth as the labeled mucus was attached to cilia. Soon after, some clumps grew and started to connect to each other and moved together. This can best be seen in the fluorescence in the center and right side of the movie. Scale bar, 30 µm.

Movie 2B – Mucus behavior on fully ciliated cultures. The movie shows the fluorescence on the culture surface after the addition of red fluorescent 1 µm beads and the formation of thick strands. Thick strands that extended for many hundreds of micrometers took several minutes to form. One particularly thick strand in the lower part of the movie can be seen moving along its axis. Small attached mucus clumps as seen in Movie 2A were no longer visible. Scale bar, 30 µm.

Movie 3AB – The effect of bead concentration on mucus dynamics. The movie shows the first of three stages during a slow increase in the concentration of 20 nm beads in the apical fluid. At this stage, no beads have yet been added. The left panel shows the fluorescence and the right panel shows the merged fluorescence/DIC. No mucus was seen and only slight autofluorescence could be seen. Scale bar, 40 µm.

Movie 3CD – The effect of bead concentration on mucus dynamics. The movie shows the second of three stages during a slow increase in the concentration of 20 nm beads in the apical fluid. At this stage, the beads just became visible. The left panel shows the fluorescence and the right panel shows the merged fluorescence/DIC. Very faint fluorescent clumps could be seen above the background. These moved back and forth with an amplitude consistent with attachment to cilia. It is not certain that the small labeled clumps actually become part of the long strands seen in Movie 2B since the extremely low level of fluorescence in Movie 3CD would not be observable with the settings used in experiments with higher bead concentrations. The beads in Movie 3CD may have become entrapped in tethered mucins. There were also large clumps of labeled mucus that occasionally flowed across the surface. This movie is longer than Movies 3AB and 3EF to capture these rare events. Scale bar, 40 µm.

Movie 3EF – The effect of bead concentration on mucus dynamics. The movie shows the culture after the final addition of beads during a slow increase in the concentration of 20 nm beads in the apical fluid. The left panel shows the fluorescence and the right panel shows the merged fluorescence/DIC. Fluorescent clumps were definitely present in highly ciliated areas. They were well defined and aligned in the direction of flow but many were still firmly attached to the surface. Mucus strands also flowed at a higher frequency and were thicker than previously seen but still did not extend across the entire field of view. These flowing strands were larger than the ones seen attached to the cell surface. So a progression from small labeled clumps, to small attached strands (clumps extending in the the direction of flow for several cell lengths), to larger flowing strands was associated with the increasing bead concentration. We expect that the clumps seen attaching and detaching in Movie 3EF contain gel-forming mucins. Scale bar, 40 µm.

Movie 4 – Two color sequential addition of beads. The movie shows a culture to which green 20 nm beads were added followed by red 20 nm beads. It shows a top-down view from images taken shortly before the profile view in Fig. 4 C. From left to right, panels show red fluorescence, green fluorescence, merged red and green, and merged fluorescence and DIC. The movie is arranged

in two parts created from two image sets taken a short time apart. In the first part, some highly ciliated areas were thinly labeled with green beads and can be seen in the lower left quadrant. After a pause, the second part shows the same area a bit later. Large amount of mucus passed leaving no fluorescence in the areas that had been thinly labeled. The red and green labeled mucus are mostly separate demonstrating that some of the mucus (green) was newly secreted during the experiment. Also a rolling motion that would tend to mix the older and newer mucus can clearly be seen on the left of the image while yellow can be best seen in the thickest mucus on the right side. Scale bar 23 µm.

Movie 5A – Exogenous MUC5B. The movie shows a culture with exogenous FITC-labeled MUC5B added to the apical solution. Before the experiment, the culture was treated with ATP to empty mucin stores and washed with DTT to solubilize the mucins and prevent new mucin production. A highly ciliated area is shown shortly after MUC5B addition. There was a continuous movement of mucus over the epithelium. Strands appeared less dense than those formed by beads. Importantly, focusing high above the cells showed that MUC5B was not arranged into strands until it made contact with the surface. Careful examination of the flow pattern of the strands shows that some portions suddenly stop and then start up again while other strands passing over the same area flow through continuously. This type of motion suggests that MUC5B is transiently attaching to the surface even in this highly dynamic part of the experiment. Scale bar, 20 µm.

Movie 5B – Exogenous MUC5B. The movie shows a culture with exogenous FITC-labeled MUC5B added to the apical solution. Areas with both high and low levels of ciliation are shown two hours after MUC5B addition. The area of lower ciliation can be discerned from the DIC and extends from the bottom center to the upper right corner of the image (see Fig. 5B, circled 3 and 4). Large clumps and strands appeared denser than earlier and occasionally attached to cilia and then detached after a pause. This behavior was strongest in areas of low ciliation and, over the duration of this movie, can be seen in the upper right quadrant. Thinly labeled areas similar to those found with low bead concentrations were also seen. So generally, all the patterns seen with beads also appeared with MUC5B addition although they were less distinct in both morphology and association with ciliation levels. Scale bar, 20 µm.