Microscopic Visualization of Insect Cell–Bubble Interactions. II: The Bubble Film and Bubble Rupture

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In this paper, the second in the series, the use of a microscopic, high-speed video system to study the interactions of two suspended insect cells strains, Trichoplusia ni (TN-368) and Spodoptera frugiperda (SF-9), with rupturing bubbles is reported. Events such as the adsorption of cells onto the bubble film and the mechanism of bubble rupture were observed. On the basis of these observations and the experimental and theoretical work of other researchers on bubble rupture and cell death as a result of sparging, it is proposed that cells are killed by the rapid acceleration of the bubble film after rupture and the high levels of shear stress in the boundary layer flow associated with bubble jet formation.

Introduction

In the previous paper in this series (Bavarian et al., 1991), results were presented which indicated that insect cells absorb to rising air and oxygen bubbles and to the air–medium interface. In this paper, microscopic, high-speed video images of cells interacting with the bubble film (liquid layer between the air above the bubble resting on the top air–liquid interface and gas within the bubble) and bubbles rupturing will be presented. In addition, the theoretical and experimental work of bubble and film rupture mechanisms by other researchers will be discussed in light of the effect these processes have on suspended animal and insect cells.

The first reported work on bubble rupture mechanism was by Lord Rayleigh (Rayleigh, 1891). In one of the first reported uses of high-speed photography, he photographed the rupture of soap films. From these observations he related the potential energy in the film before rupture, resulting from surface tension, to the velocities at which the film recedes after rupture.

Stuhlman (1932) suggested that the ejected liquid drops that arise when a bubble ruptures originate from liquid jets that form almost instantly after bubble rupture. Kientzler et al. (1954), also using high-speed photography, verified that once a bubble ruptures a liquid jet forms as a result of liquid flowing into the bubble cavity. The breakup of this jet results in the ejection of five liquid drops to heights significantly higher than the bubble diameter.

MacIntyre (1968, 1972) demonstrated that the liquid in this jet originates in the bubble film and a thin layer of liquid surrounding the remainder of the bubble, the bubble cavity. Both the bubble film and the thin liquid layer are subjected to extremely high accelerations and velocities as the bubble breaks.

In 1970, Blanchard and Syzdek (1970, 1972) made a significant observation: the concentration of bacteria in these drops that originate from the liquid jet was 10–1000 times higher than in the bulk solution. Bezedek and Carlucci (1972) also observed concentrations factors of bacteria in the jet drops from natural seawater of up to 100. Quinn et al. (1975) reported that in addition to bacteria significant increases in concentrations (up to 300X) of micrometer-size latex particles are observed in jet drops. Each group of authors conclude that this increase in concentration in the jet drop was the result of a higher concentration of bacteria or particles adsorbed to or within a thin liquid layer surrounding the gas–liquid interface of the bubble. In regard to animal or insect cells, cells either adsorbed to or in this thin liquid layer surrounding a rupturing bubble will be subjected to extremely high hydrodynamic forces, which, as will be discussed, are probably sufficient to kill cells.

Materials and Methods

The cells, medium, and method of cultivation that were used in this work have been reported previously (Bavarian et al., 1991).

Figure 1 is a schematic diagram of the experimental apparatus. As in the previous work (Bavarian et al., 1991), a high shutter speed video camera connected to a high-band U-Matic video recorder was used to record the images. Again, both an Olympus BMHJ microscope and a Nikon Micro-Nikkor lens (105 mm, f/2.8) were used to magnify the images.

To view bubbles interacting with the air–medium interface, a plastic spectrophotometer cuvette (Fisher Scientific, Pittsburgh, PA) was used. To conserve cell suspension, the cuvette was cut in half so that the liquid volume was approximately 1.5 mL. The dimension of the modified cuvette was 10 × 10 × 20 ×10⁻³ m. Through a plastic cap a 22 gauge hypodermic needle was positioned such that the needle end would inject bubbles into the center of the cuvette. The whole system was thoroughly washed with distilled water. Air was delivered to the needle through silicone tubing (Masterflex N-06411-13) connected to a pump (Masterflex, Cole-Parmer, Chicago, IL.).

The capability to visualize cells on the bubble film is based on the microscope optics, the intensity and lighting positions, and the position of the liquid meniscus. The lighting system consisted of a fiber-optic light source (Fiber-Lit, Series 180, Dolan-Jenner Industries, Inc.) connected to a specially designed telescopic lens system mounted on a moving table. This lighting system could
focus the light emitted from the tip of the fiber cable into a 1 mm diameter area. Since a fiber-optic light source was used, cold illumination was obtained. The cuvette was slightly overfilled with cell suspension so that a convex down surface resulted. This allowed bubbles to always rise to the same spot on the interface. It also allowed the adjustment of the light source so that the light beam would either illuminate the cells on the bubble film or both the cells and the bubble film. The light beam approached the interface horizontally while the microscope was mounted either vertically, viewing the interface from the top, or horizontally, 90° from the light source. This illumination in conjunction with the dark phase optics of the BMHJ microscope allowed cells to appear bright with a dark background, thereby enhancing the image of the cells on the bubble film. This arrangement of light, interface, and microscope is crucial to view the cells on the film.

To visualize the bubble rupture mechanism, the lower power Micro-Nikkor lens system was used. It was mounted either horizontally or at an angle to the interface. The telescopically focused light source and/or a combination of high-power light bulbs was used for illumination.

Results

Two types of observations were performed: (1) visualization of the attachment of cells on the bubble film and (2) the bubble rupture phenomena.

Entrapment of Cells on the Bubble Film. Figure 2 shows a typical set of events that occurs as a bubble approaches the interface. The cell suspension consisted of SF-9 cells in exponential growth. Figure 2a shows the bubble approaching the interface. Since the light beam was positioned above the medium-air interface, the bright spot in the upper right corner is the interface that has been elevated to the level of the light beam. Note that the small bright spots in Figure 2a corresponds to SF-9 cells, while in Figure 2b the SF-9 cells appear as streaks on the bubble, indicating that a majority of the cells in the fluid above the bubble are rapidly flowing over the top of the bubble. In addition, in Figure 2b a small bubble can be observed being ejected outward, which corresponds to the small bubble in approximately the center of Figure 2a. The shutter speed in these images was 1/500 s, indicating that the rate of the small bubble ejection was approximately 0.15 m/s. In Figure 2c, cells that had not flowed off the bubble are observed to be attached to the bubble film. These cells appear as bright spots against the darker bubble and background. The curved, bright ellipses on the left side correspond to secondary and tertiary focusing rings from the telescopic light source. The ellipses on the right are reflections of the rings on the left. By adjusting the focus of the microscope it was possible to conclude that the bubble film is in focus in this figure.

The approach of the bubble, and thus the high pressure field generated in front of the rising bubble, drains the liquid in between the bubble and the interface. Consequently, most of the cells are carried by the draining liquid; however, some finite volume of liquid remains. This finite volume, and cells contain therein, form the bubble film. By adjusting the illumination beam we have observed that the cells on this film will become illuminated before the bubble film, thereby indicating that the cells are on the top of the film and the film thickness is significantly less than the thickness of the cell. From the potential flow model, this volume of cells and medium originated in or near the central line of motion of the bubble.

Bubble Film Rupture. Once a disturbance ruptures the film, the hole created by the disturbance rapidly expands. The liquid from this expanding hole rolls up to form a toroidal ring (Ranz, 1959). The advancing toroidal ring will either expand until it reaches the bubble edge or partially break up into two groups of drops: a group that moves horizontally and a second group that moves vertically.

Figure 3 shows two sets of photographs, panels a and
Figure 3. Photographs of two sets of video images, panels a and b (tap water) and panels c and d (TNM-FH medium and cells) of bubble films before and during rupture. (Shutter speed 1/10 000 s.)

Mechanism of Bubble Rupture and of Jet Formation. Figure 4 is a composite of eight different $3 \times 10^{-3}$ m bubble ruptures showing the sequence of events that takes place after the bubble film ruptures. A composite was necessary, since although a high shutter speed camera was used, images are only recorded every 1/60 s, while the rupture event takes place in approximately $1.7 \times 10^{-3}$ s. Once the film ruptures, liquid flows down the side of the cavity and forms an upward and a downward jet. The upward jet can be observed in Figure 4c–f. Once the jet reaches a height of approximately one bubble, the jet breaks up into individual drops, which are ejected upward. One of these drops is seen coming back downward in Figure 4g. Very similar observations of jet formation were made by Kientzler (1954). Figure 5 shows photographs of three video images of a $1.5 \times 10^{-3}$ m bubble rupturing in TNM-FH medium containing SF-9 cells. The jet formed is clearly observed in Figure 5b.

Discussion

The rupture of a bubble at an air-liquid interface releases a tremendous amount of energy when one considers the volume and time span in which this event takes place. MacIntyre (1972) has estimated that up to 6 erg of potential energy is converted to kinetic energy and viscous dissipation loss when a $1.7 \times 10^{-3}$ m bubble ruptures. This corresponds to a constant power of 0.5 mW. He also reported that the inner bubble surface (the bubble cavity wall) would experience an acceleration approaching $10^6$g after the bubble burst.

It is highly probable that this intense release of energy at bubble rupture is capable of destroying cells sufficiently close to the rupturing bubble. The location of a cell relative to a rupturing bubble can be classified into two regions: (1) cells adsorbed to the bubble film when the film breaks and (2) cells adsorbed to or very near to the bubble cavity wall. The rupture process in each of these regions is discussed below in the context of how a cell could be destroyed.

Rupture of the Bubble Film with Cells Attached. The adsorption of cells on the bubble film is quite common; every bubble on the air-medium interface, which was microscopically observed, had cells attached to the film. This is in contrast to the microscopic studies of Handa et al. (1989), in which no cell-interface attachment was observed. In 1986 Tramper et al. suggested that cells might adsorb onto bubble films; however, no actual observations were recorded.

On the basis of video images of bubbles rising to the interface, a majority of the cells that are attached to the bubble film become attached as the bubble rises through the interface and the film cap thins. Once the cells are adsorbed onto this film, three possible mechanisms can damage the cells on this interface: (1) forces (elongational, shear) in the film, (2) extended cell contact with gas above the medium, or (3) the rupture of the bubble film with the associated forces involved.

The possible effect of elongational forces on cells attached to the bubble film has been observed with TN-368 cells, which are significantly larger than SF-9 cells. Attached TN-368 cells have been observed to become deformed with time on the bubble film, and in some cases the leakage of what appears to be intracellular components has also been observed (data not shown).

It is not known what effect extended contact of cells with air will have on the viability and metabolism of cells; however, in most cases bubble rupture takes place rapidly enough to prevent prolonged exposure.
The third mechanism is probably the most plausible for the destruction of cells attached to the bubble film. The breakage of bubble films has been studied both theoretically and experimentally (Blanchard, 1963; Culick, 1960; Ranz, 1959; Rayleigh, 1891), and the results of this work, applicable to attached cells, are summarized below.

As a bubble either approaches or rests at the interface, the film cap thins until the bubble ruptures. The film thickness at rupture has been reported to vary from 500 Å to 1.5 × 10^{-6} m. As described previously, once a hole develops in the film, it rapidly expands, with the material from this expanding hole rolling up to form a toroidal ring (Ranz, 1959). The velocity of this expanding toroidal ring can be determined by conducting a force and energy balance. Culick (1960) related the potential energy associated with the surface tension to two energies: the kinetic energy of the expanding torus and the energy lost as a result of the inelastic acceleration of the stationary film to the velocity of the torus. This velocity is constant and is equal to

\[ v = (2\sigma/\rho h)^{1/2} \]  

As eq 1 indicates, the velocity of the ring is a function of the surface tension and thickness of the film. Both of these values have been reported to vary over a film (MacIntyre, 1972) and make this equation approximate; however, reasonable agreement with experimental results has been obtained (Culick, 1960; Ranz, 1959). The film velocity for a bubble in seawater with a film thickness of 2 μm has been reported to be 8 m/s (MacIntyre, 1972).

This velocity is sufficiently rapid that the film outside of this toroidal ring is unaware of the film rupture (Ranz, 1959). The region of the outer boundary of the expanding toroidal ring that experiences this acceleration from a stationary state to the velocity of the ring has been shown...
Figure 5. Photographs of video images of a single bubble rupture in TNM-FH medium containing TN-368 cells.

Table I. Toroidal Ring Velocity, Distance of Acceleration, and Acceleration the Liquid Would Experience

<table>
<thead>
<tr>
<th>film thickness, μm</th>
<th>distance from point of rupture, μm</th>
<th>ring diameter, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>4.2</td>
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<tr>
<td>1</td>
<td>50</td>
<td>5.8</td>
</tr>
<tr>
<td>1</td>
<td>250</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
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<td>10</td>
<td>250</td>
<td>42</td>
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</tbody>
</table>

Table II. Diameter of Toroidal Ring as a Function of Film Thickness and Distance from Point of Film Rupture

\[
\delta \approx h (\mu v / \sigma)
\]

where \(\delta\) is the distance of acceleration (Culick, 1960).

Figure 6. Scale drawing comparing the relative sizes of SF-9 cells and the toroidal ring for different film thicknesses after the ruptured film has receded 50 μm.

Mechanism of Jet and Jet Drop Formation. The mechanism of bubble breakup has been reviewed by Mac-
Once this symmetric, boundary layer flow reaches the bottom of the cavity, a stagnation point is created. The high pressure at this stagnation point generates two opposing vertical jets (as required by a momentum balance): one that flows upward into the air and a second that flows downward into the liquid beneath the cavity. The downward flow has been observed experimentally by MacIntyre (1972), using dyes. For a 1.7-mm bubble, this downward flow has an initial velocity of approximately 0.70 m/s. The downward jet is similar to the jet formed as a result of the collapse of bubbles formed during cavitation. It is a well-known fact that these jets are strong enough to erode metal and other hard surfaces.

As the second, upward jet accelerates upward, it forms capillary ripples (Figure 4c,d), which leads to its breakup into vertically ejected droplets by Rayleigh–Taylor instabilities. The diameter, ejection velocity, and drop diameter of the first drop have been shown (Blanchard, 1963) to be a function of the original bubble diameter. While the drop diameter is approximately equal to the 1.3 power of the bubble diameter, the ejection velocity for the first drop is a decreasing function of bubble diameter, and neither is a function of liquid salinity. For seawater, the initial ejection velocity ranges from 8000 cm/s for a 7.0 $\times 10^{-5}$ m bubble to 900 cm/s for a 1 $\times 10^{-3}$ m bubble. The liquid material in this jet has been shown experimentally to be composed of materials originating in the bubble film and a thin shell surrounding the bubble cavity (MacIntyre, 1972, 1968; Blanchard 1963; Quinn et al., 1975). In addition, these jet drops have been shown to be charged.

Blanchard (1963) determined that the only significant source of energy that could account for the kinetic energy of the drop ejection is the surface free energy, $E_s$, which is defined as

$$E_s = 4\pi R^2$$

(4)

While the amount of energy increases with the square of the bubble radius, the ejection velocity of the drop decreases with increasing radius. This can best be understood when one considers that, as described above, the driving force for the boundary layer flow in the bubble cavity is the pressure difference created as a result of the toroidal ring and its associated high curvature geometry at the edge of the cavity. From Laplace's equation, the pressure difference decreases as the two principal radii increase. Therefore, the rupture of a smaller bubble creates a higher pressure difference, and consequently a faster boundary layer flow and jet drop ejection velocity, than a larger bubble.

It has been reported (Handa et al., 1985) that smaller bubbles are much more damaging to animal cells in a bubble column than larger bubbles. It is possible that this higher level of damage is the result of the higher velocity of the boundary layer flow and the correspondingly higher fluid dynamic forces.

Unlike the radius, $\Delta P$ is directly proportional to the surface tension, $\sigma$, of the liquid, as is the surface free energy of the bubble. Handa et al. (1986) reported that the surface tension of animal cell growth medium, RPMI, decreases with the addition of 5% fetal calf serum (FCS) and with the addition of 5% FBS plus 0.1% Pluronic PE 6800 by 29% and 43%, respectively. They also reported that cell damage decreased with the addition of FCS and Pluronic to the growth medium. Other researchers (Kunas and Papatiosakas, 1989; Murhammer and Goочек, 1988; Kilburn and Webb, 1968; etc.) have also observed that the addition of serum and/or other compounds that lower the surface tension of the medium protects cells. It is possible that the protection these additives provide to the cells is

Figure 7. Diagram of the bubble cavity interface at 6.67 $\times 10^{-4}$ s time sequences after bubble rupture. The figure inset is a 10-fold scale enlargement of the boundary layer flow next to the bubble cavity interface [Partially adapted from MacIntyre (1972).]
the result of the lowering of the hydrodynamic forces associated with the bubble rupture.

**Implications of Jet Formation to Cells.** As discussed above, the liquid flow down the walls of the cavity can be characterized by boundary layer flow. On the assumption that the velocity profile can be approximated by the first term in a power series and that the liquid experiences a constant acceleration, MacIntyre (1972) approximated the flow profile as

\[ u(t, \eta) = a t e^{-2.37 \eta} \]

where

\[ \eta = z / (2ut)^{1/2} \]

and \( z \) is a positive radial direction perpendicular to the cavity surface and \( t \) is time after the film ruptured.

As described previously, Figure 7 contains a 10X enlargement of the air–liquid interface 1 X 10^{-3} s after the bubble film ruptured. Within the inset the size and magnitude of the fluid boundary layer flow are represented, based on calculations from eq 5. Both the figure and the inset are drawn to scale so that the size of a 2.0 X 10^{-5} m cell, relative to the bubble and the fluid boundary layer, can be compared. Significant fluid motion penetrates the fluid for at least 3 cell diameters.

The shear stress in this boundary layer flow can be determined by taking the derivative of eq 5 and multiplying by the liquid viscosity. Based on data of MacIntyre (1972) for a 1.7 X 10^{-3} m bubble rupturing in seawater, the velocity and shear stress as a function of the penetration depth, \( z \), at three time increments are presented in Figure 8.

As can be observed, very high levels of both velocity and shear stress are experienced by the fluid in this boundary layer flow. It should be noted that the calculations used in both Figure 7 and 8 are based on seawater, which has a surface tension similar to that of water, while the surface tension in growth medium is significantly less. As previously shown, this lower surface tension would result in a lower rate of energy release upon bubble loss, and consequently, the fluid velocity would probably be less. It is also important to note that, in addition to high levels of shear stress, a cell would experience a gradient of shear stress sufficient for cell lysis.

It is highly probable that cells close to or adsorbed to the bubble cavity interface will be destroyed by these shear stresses. Goldblum et al. (1990) reported that significant cell lysis is observed over a range of shear stress of 0.12–13.1 N/m^2 at time scales of seconds to minutes. While it is not known if a time scale on the order of milliseconds (as produced during a bubble rupture) is sufficient to lyse cells, the level of shear stress in these boundary layer flows can be in the range of 200–300 N/m^2, which is over an order of magnitude greater than those reported by Goldblum et al. (1990). It is also not known what effects a shear stress gradient would have on the cell.

Finally, it is interesting to note that Handa et al. (1985, 1989) observed that cells near rupturing bubbles oscillate and in some cases they are ejected tangentially from a bubble when it ruptures. It is highly probable that this oscillation and tangential ejection is the result of the cell being in this boundary layer flow yet sufficiently far from the interface that they did not experience a level of shear stress sufficient for cell lysis.

**Conclusion**

On the basis of the microscopic observations reported above and the theoretical and experimental work of other researchers on bubble rupture, it is suggested that the forces associated with bubble rupture result in (1) the destruction of cells adsorbed to the bubble film as a result of the large release of potential energy as the film breaks and (2) the destruction of cells in the boundary layer flow into the bubble cavity after bubble film rupture.

Trumper et al. (1986) suggested that a hypothetical “killing volume” is associated with each bubble in a sparged bubble column. It is proposed, on the basis of preliminary calculations, that this killing volume consists of the medium and cells that make up the bubble film and the thin shell surrounding the bubble cavity in which the previously described boundary layer flows.

Present and future work in our laboratory is investigating whether these two mechanisms are the primary mechanisms of cell death, as well as the protective effect of medium additives such as FCS and Pluronic.

**Notation**

- \( E_s \): surface free energy, J
- \( g \): gravitational acceleration
- \( h \): film thickness, m
- \( R \): bubble diameter, m
- \( r_1 \): principal radius of curvature, m
- \( r_2 \): principal radius of curvature, m
- \( t \): time after bubble rupture, s
- \( u \): velocity of expanding toroidal ring, m/s
- \( v \): fluid velocity tangential to cavity surface, m/s
- \( \delta \): distance of film acceleration, m
- \( \Delta P \): pressure difference as a result of surface curvature, Pa
- \( \rho \): density of liquid in film, kg/m^3
- \( \sigma \): liquid surface tension, N/m
- \( \nu \): kinematic viscosity, m^2/s

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**Literature Cited**


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