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Cinephotographic observations of particle removal from a surface by acoustic cavitation

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It is commonly believed that ultrasonic cleaners remove particles from a surface through acoustic cavitation, presumably in which a pulsating bubble interacts directly with the particle. In this study, we have used a high-speed movie camera to observe the removal of biofilm attached to a solid surface during exposure to a cavitation field. The biofilm consisted of *Streptococcus mutans*, a common oral bacterium, grown on a glass slide and observed under magnification to be both thinly coating the glass surface and clustered in larger colonies. The cavitation field was created by an Ultreo™ toothbrush which combines both vibrating bristles and an ultrasound transducer with a waveguide, operating at a frequency of 324 kHz. When the waveguide was immersed in water containing bubbles from an ultrasound contrast agent (Optison™), visual observations could be made with the naked eye of biofilm removal. With high-speed microcinephotography, it was possible to observe bacteria removal by the direct interaction of a cavitation cloud (cluster of cavitation bubbles) and the colony. These observations will be presented along with our interpretations of the data. [Work supported in part by Ultreo™, Inc.]

Introduction

Ultrasonic cleaners are well-known to the general public; yet, the precise physical mechanism through which they accomplish cleaning is not fully understood. Of course, most physical scientists believe that it is acoustic cavitation that results in the removal of particles from the surface of an object to be cleaned; however, the literature is replete with papers [see for example, Menon, (1990), Olson (1988) and Geers and Hasheminejad (1991)] that argue that particles are removed by the direct result of acoustic radiation force. Indeed, it is not uncommon for cavitation to be discounted as an effective particle removal mechanism in the semiconductor cleaning industry in which “megasonics” (ultrasonic cleaners operating in the megahertz frequency range) is used to remove microscopic particles from the silicon wafer surface.

In order to remove particles from silicon wafers, as well as other surfaces, the adhesive forces must be overcome by the removal forces. Forces between solids are predominantly attractive and cause adhesion of particles to each other and to surfaces. The principal interactions that are encountered in particle-surface adhesion include molecular interactions (van der Waals forces), electrostatic effects, capillary condensation, liquid bridges, double-layer repulsion, and chemical bonds such as polar or metallic bonds (Ranade, 1987). Of these, electrostatic forces are comparable in magnitude to Van der Waals forces for submicron particles. The electrostatic adhesive forces on the particle depend primarily on the charge states of the particle and the surface. In wet cleaning processes such as megasonic cleaning, the wet surfaces decrease the electrostatic forces, thereby reducing significantly their contribution to particle adhesion. Particles are either attracted or repelled from the wafer surface depending upon the electrokinetic properties of the particle in the cleaning solution. That is, the zeta potential of the cleaning solution determines if particles are deposited or removed due to the formation of an electrical double layer on the wafer surface.

There are two principal mechanisms by which ultrasonic (including megasonic) cleaners may accomplish cleaning. The first is a direct interaction of the sound field with the attached particle, i.e., the oscillating acoustic field exerts periodic forces directly on a particle attached to a boundary or surface. Under this hypothesis, these oscillating forces eventually overcome the attractive attachment forces and free the particle. There are two types of direct-effect forces. The first is due to a drag imposed on the particles by the displacements of the particle velocity associated with the acoustic field. In an acoustic field, a small portion of the fluid will oscillate back and forth, exerting a drag force on obstacles in the flow. However, it is likely that acoustic particle velocities (at megasonic cleaning intensities) are of insufficient displacement to result in significant particle motion [Kinsler and Frey, 1962].

The second type of direct force imposed upon local inhomogeneities located within the liquid is that of acoustic radiation pressure. These radiation pressure forces, sometimes called Primary Bjerknes Forces [Crum, l975] could potentially result in particle removal. According to Menon, [1990], these forces are principally responsible for megasonic cleaning. However, recent papers by Olson [1988] and by Geers and Hasheminejad [1991] define the conditions necessary for optimal particle removal and their theories "...suggest that very high frequencies (~1 GHz) will be required to remove submicron contaminants from wafers" [Olson, 1988].

A second mechanism for consideration for a submicron particle removal mechanism is that of acoustic cavitation. This modality has three different specific ways in which particles can be removed. The first mechanism could be described as acoustic microstreaming. In this case, a microscopic air bubble pre-existing in the liquid (or nucleated on a solid surface) undergoes stable, large-amplitude pulsations which in turn cause rapid movement of the liquid as it tries to follow the oscillating bubble boundary. If any asymmetry exists in the flow pattern (brought about by, say, a nearby boundary), intense microstreaming patterns develop which can lead to significant shear stresses imposed along the boundary. This microstreaming phenomenon is normally associated with the presence of *stable* cavitation activity, which generally occurs when the cavitating liquid is saturated with gas. However, microstreaming must also occur for more vigorous cavitation as it is related to the volume oscillations of cavitation bubble. Acoustic microstreaming is a well-known phenomenon and quantitative approaches exist for evaluating the forces available for particle removal [Elder, 1959; Nyborg, 1965; Kashkoush and Busnaina, 1993].

A second cavitation-related source of debridement is through a phenomenon that shall be called microjet impact. If an oscillating bubble is undergoing relatively

large displacement excursions, then the bubble-wall collapse velocities can also be quite large and the motion is inherently unstable. In this case, the bubble is said to be inertially-controlled and any asymmetry in the flow field around the bubble normally results in an asymmetric (non-spherical) bubble collapse. Given this scenario, one side of the bubble tends to collapse faster than the other, resulting in the development of a microscopic liquid jet that propels itself through the bubble, penetrates the opposite side and violently impacts the very boundary that caused the asymmetry to develop in the first place. This liquid jet, which can attain supersonic velocities, is thought to be the principal mechanism for cavitation erosion. Jet formation is associated with the presence of *inertial* cavitation, which generally occurs when the cavitating liquid is somewhat degassed and the acoustic pressure amplitudes are large. For more information on this phenomenon, see for example, papers by Naude and Ellis, [1961]; Lauterborn, [1988]; Crum, [1979], and Crum, [1988].

A third cavitation mechanism is a result of the violent implosion of the collapsing gas bubble. When the bubble implodes, it can create a shock wave in the liquid surrounding the bubble. The local pressures in this shock wave are known to be as high as a few kilobars--pressures sufficient to damage even the surfaces of metals. If the impinging shock wave can damage the surface, it can clearly loosen lightly attached contamination. Although most cavitation researchers believe jet impacts are the principal mechanism for cavitation damage, it is not possible to eliminate shock wave effects as a possible damage source. Shock waves are typically produced by inertial, rather than stable cavitation and are therefore more likely to be present when the cavitating liquid is moderately degassed or when the acoustic pressure amplitude is very large. Studies of this type of acoustic cavitation damage have been performed by Ellis, [1966] and Tomita, et al., [1986].

Finally, it is often (incorrectly) assumed that all forms of cavitation are associated with a single, distinct gas or vapor-filled bubble. In most forms of cavitation, the oscillations of the bubble are so violent that the bubble breaks into smaller bubbles during collapse and a cavitation cloud or bubble cluster is formed. With a cloud the potential exists to amplify any or all of the effects described above.

An opportunity to explore ultrasound-induced particle removal from a surface arose when we were engaged by Ultreo™, the manufacturers of a truly ultrasonic toothbrush, to examine the ability of their toothbrush to clean "beyond the bristles". The toothbrush, including the transducer waveguide that generates an acoustic field with a maximum pressure amplitude of 0.62 MPa (in water) at 323 kHz is shown in Fig. 1. One of the deficiencies of brushing with a standard manual toothbrush is that it is difficult to clean the interdental spaces between the teeth—that's why you should floss! The Ultreo™ toothbrush contains an ultrasound transducer embedded within the bristles. It is claimed that interactions between the acoustic field and the ever-present bubbles in the mouth will help remove biofilms (the undesirable plaque) from areas missed by traditional bristle contact, e.g., plaque that grows between the teeth or in tooth irregularities too small for the bristles to penetrate. Herein we report on an investigation, mostly with microcinephotography, in which we examine the removal of bacteria colonies from a glass surface by the Ultreo™ toothbrush.

Fig. 1. Photograph of the Ultreo™ toothbrush with an enlargement of the head, showing the transducer waveguide.

Materials and Methods

Biofilms were formed within a box on a microscope slide that made up the base of the box. The box was then filled with filtered water to a height of about 8 mm, and placed on the stage of an inverted microscope. The Ultreo™ toothbrush was placed facing down in the water at a sufficient depth to cover both the bristles and the waveguide. The end of bristles were about 2 mm from the microscope slide. The transducer on the brush was centered over the 10x objective and was 5 mm from the slide surface. Four fiber optic lights were placed around the brush facing down on the slide and objective. Just prior to experiment 3 drops of Optison™ contrast agent were added to the water, and the toothbrush was activated. Because the bristles were completely covered with water, they did not necessarily generate bubbles on their own. The image seen through the microscope was recoded by a Photron ultima APX-RS digital high speed camera. Frame rates and shutter speeds are reported in the figure captions, but a short shutter was used to resolve fast moving bubbles within a fairly long capture time at slower frame rates. Figure 2 is a photograph of the experimental arrangement.

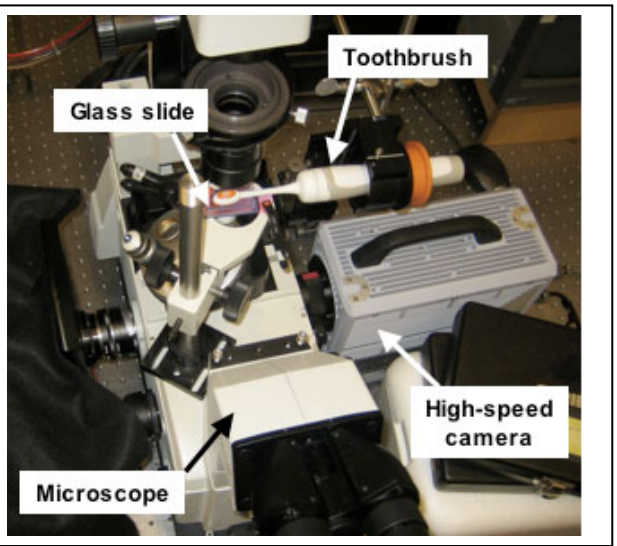

Fig. 2. A biofilm, grown on a slide in a small box of water with contrast agent, is centered on the inverted microscope. The toothbrush and lighting face down. The camera is attached to a port on the microscope.

As a model biofilm for experimentation, *Streptococcus mutans* was grown on a glass slide surface. Slides were coated with sterile porcine gastric mucin for 60 minutes prior to biofilm growth. Trypticase Soy Broth supplemented with 10% sucrose was added to the chamber slide. Slides were incubated at 37ºC for 48 hours and subsequently rinsed in distilled water to prepare them for experimentation. To enhance visualization of the biofilm on the slide surface, a dental disclosing solution (dye) was added to the biofilm for 1 minute. After dyeing, the slides were rinsed to remove non-adherent dye and prepared for microcinephotography.

Results And Discussion

As the toothbrush was activated above the glass slide containing the biofilm, high speed photography was performed, while viewing though the microscope. Movies were taken at different framing speeds and at different levels of magnification. These movies were shown during the oral presentation and demonstrate the removal of the biofilm by the Ultreo™ toothbrush. In this document, still frames from these movies are provided below that show the ability of the Ultreo™ toothbrush to remove surface biofilms.

Shown in Fig. 3 is a magnified view of the surface of a glass slide on which a biofilm of the bacterium (*Streptococcus mutans)* has been grown. When the Ultreo™ toothbrush was activated, the biofilm was rapidly eroded from the surface. The total time between Frames A and D was approximately 15 seconds. Note that in Fig. 3, the bristles were vibrating, generating significant hydrodynamic fluid motion that could potentially remove biofilm as has been claimed by some power toothbrushes. We observed significant biofilm removal only under the waveguide and not underneath the bristles near the tips of the brush head. Since the bristles of the toothbrush did not touch the surface of the slide, but were approximately 2 mm away, this slides shows strong evidence of "beyond the bristles" cleaning.

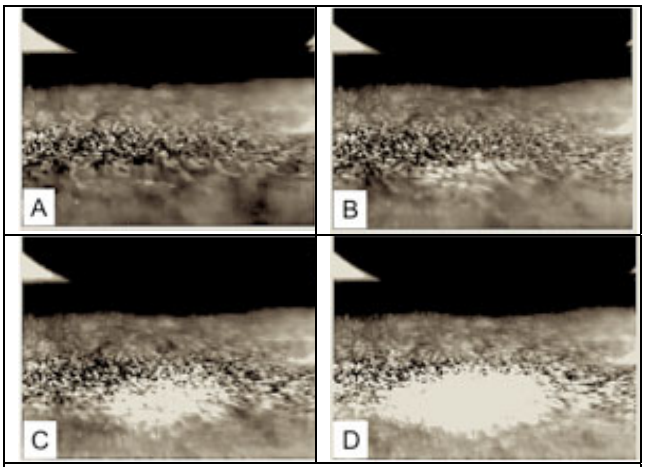

Fig. 3. Show in this figure are frames from a movie in which the Ultreo™ toothbrush is directed at the surface of a glass slide covered with a biofilm of *Streptococcus mutans*. The frames are separated by approximately 5 seconds in time and the exposure time of each frame was 10 μs. This is a magnified view of the surface; the width of a full frame is about 8 mm.

We sought next to focus the microscope directly on the surface of the eroded area to determine if the physical mechanism that resulted in biofilm erosion could be elucidated. Shown in Fig. 4 is a higher magnification view of the surface of the biofilm. In this case, various frames from a high speed movie are shown that captured direct evidence of the involvement of cavitation in the removal of a surface particle. In this figure, the small individual white spots are colonies of the *Streptococcus mutans* bacterium. These colonies are approximately 50 μm in size.

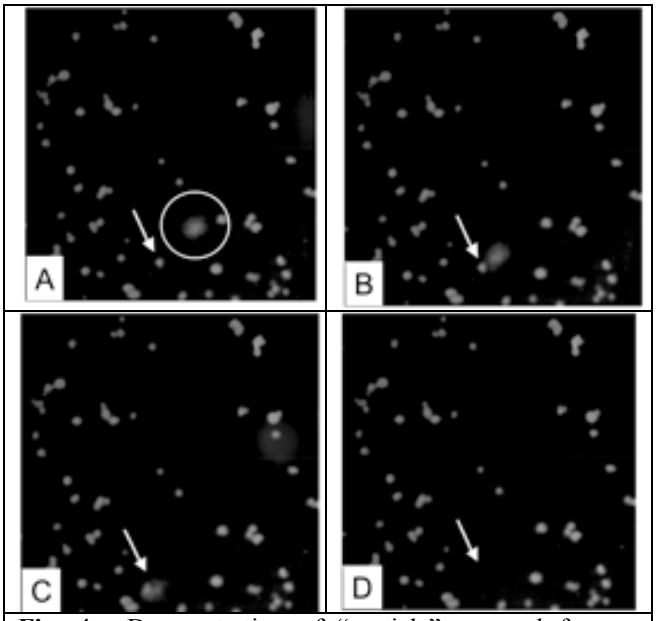

Fig. 4. Demonstration of "particle" removal from a surface by direct interaction of a cavitation cloud and a bacteria colony. The various frames in this figure show bacteria colonies of *Streptococcus mutans* growing on a glass surface (shown by the white spots—these colonies are about 50 μm in diameter). In frame A-C, a cavitation cloud (indicated by the white circle) moves toward one of the colonies (indicated by the white arrow), and interacts with the colony in frame C. It is seen in frame D that the colony has been completely removed from the surface. No other colonies were removed during this observation period. The exposure time for an individual frame is 166 μs, the time between frames is 333 ms, and the width of an individual frame is 1.7 mm.

In frame A, a diffuse image, almost certainly a cavitation cloud, or bubble cluster (enclosed in the white circle), is seen. In Frame B, the cloud has moved in the 7 o'clock direction and approaches a bacterium colony, indicated by the white arrow. In Frame C, there appears to be an interaction between the cavitation cloud and the colony, and in Frame D, it is seen that the colony has now been removed from the glass surface. It is likely that due to radiation pressure forces, the cloud is moving across the surface of the glass slide and when it encounters a colony, the vigorous action of cavitation within the bubble cloud completely removes the colony from the surface—there is no apparent residue of the colony remaining. Cavitation cloud dynamics is a very complex phenomenon; within the cloud cavitation jets, intense shock waves, as well as violent fluid microstreaming can occur; thus, from these data, it is not possible to isolate the specific aspects of cavitation that give rise to particle (bacteria colony)

removal. The event shown in Fig. 4 was not a unique event; at least 6 separate movies were recorded and each contained at least 2 dozen cavitation-removal events; over 30 movies that were taken showed interactions between a cavitation cloud and a bacteria colony, and each interaction resulting in the removal of the colony. A particularly interesting one is shown in Fig. 5. In this case, the cavitation cloud appeared for only one frame, and showed a plume of material "erupting" from the site of colony-cloud interaction.

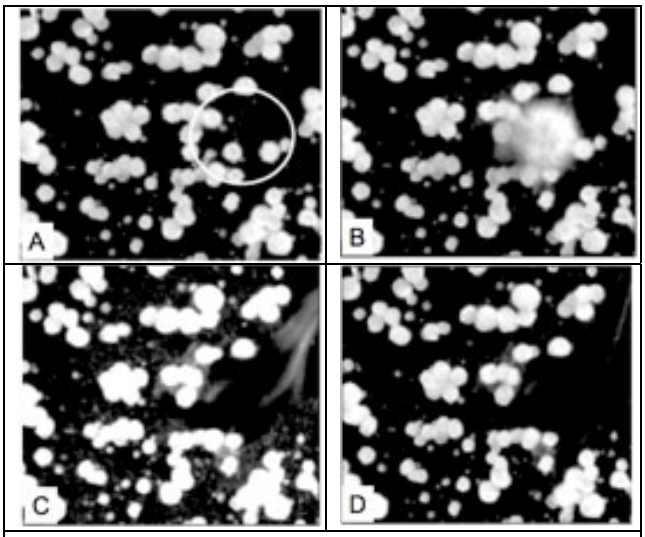

Fig. 5. An event similar to that shown in Fig. 4 in which is observed a direct interaction between a cavitation cloud and a bacteria colony. Note the complete removal of the colony as a result of this interaction. For this case, the exposure time was 333 ms, the time between frames was 4 ms, and the width of a full frame was 1.7 mm.

In all of our many observations of the effect of the Ultreo™ toothbrush on bacteria colony removal, we did not make a single observation of colony removal without the direct interaction of a visible cavitation cloud and a bacteria colony. We conclude from these observations that the hydrodynamic flow generated by the movement of the bristles themselves do not remove surface biofilms as effectively as cavitation clouds excited by the ultrasound.

Conclusions

Using microcinephotography, we have demonstrated that an Ultreo™ toothbrush can remove bacteria from the surface of a glass slide. The mechanism that enables this removal appears to be acoustic cavitation in which a cloud of bubbles interacts directly with the colony. In over 6 replicate experiments that were made, this cavitation cloud—bacteria colony interaction was observed hundreds of times and recorded to disk over 30 times; however, there was not a single observation in which a colony was seen to be removed without this direct cavitation event.

The results in this study complement previous studies by Krefting, et al., (2004) in which, similar to this effort, high-speed cinephotography was used to observe the direct physical removal of particles from a surface by an ultrasonic cleaner, operating at a frequency of 40 kHz. In those observations, bubble clusters, called "smokers", were seen to have strong erosive action in removing particles from a glass interface. Related work by Ohl, et al., (2006), in which laser-induced bubbles were used to examine particle removal. suggested that acoustic micro-streaming, associated with a cavitation jet impacting the surface, generates a strong shear flow that is effective in removing particles from the surface.

We generalize our observations from this study to conclude that it is very likely that ultrasonic cleaners (including megasonic cleaners) remove particles though the cavitation-particle interactions and that other non-cavitation effects such as induced hydrodynamic flow or direct radiation force play a minor if any role in particle removal.

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