An Investigation of the Effects of Ultrasound on Degradable Polyanhydride Matrices

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ABSTRACT: In vitro methodology has been developed to investigate the effects of therapeutic ultrasound on polymer erosion. Enhancement in the rate of polymer erosion was demonstrated using therapeutically acceptable levels of ultrasound on a model class of degradable polymers—polyanhydrides. It was found that ultrasound enhances polymer degradation as demonstrated by the enhanced decrease in polymer molecular weight during the induction period of erosion. Additionally, morphological changes on the surface of ultrasound exposed devices were assessed by environmental scanning electron microscopy and suggested that cavitation may cause the mechanical disintegration of the polymer surface.

Introduction

It has been demonstrated that ultrasound may be used to enhance the degradation rate of solid polymers.[2,3] This phenomena may have a number of applications including the degradation of polymeric waste and the release of incorporated agents from degradable polymers (e.g. drugs, fragrances).

Increasing awareness of the need for modulated and responsive drug delivery has stimulated a rapidly growing area of research.[4] There are a number of therapeutic situations which have been identified as requiring drug delivery profiles which are not currently provided by available delivery devices. Among the types of agents that have been cited as requiring temporal release include peptide/protein-based drugs[5] and vaccines.[6] Additionally, increasing evidence from the relatively new discipline of chronopharmacology has shown that many drugs previously thought to require constant delivery may benefit from more subtle delivery patterns under temporal control to obtain optimal therapeutic effects.[7]

The use of ultrasound to modulate the release of therapeutic agents from biodegradable polymeric carriers offers a particularly attractive means of achieving temporal release patterns at defined times and intervals. The envisaged implantable delivery device would be triggered externally and would not require the incorporation of additional substances in the polymer matrix such as enzymes, electrodes, or magnetic beads. Additionally, the use of an erodible carrier obviates surgical removal of the exhausted device. The use of polyanhydrides as degradable polymeric carriers has been described previously; they have been shown to be nontoxic and biocompatible and are currently being used in humans for the treatment of glioblastoma multiforme.[8]

It has been demonstrated both in vivo and in vitro that ultrasound may be used to enhance the release of agents from polymeric devices.[3] The effects of ultrasound have been shown with both erodible polymers, such as the polyanhydrides and poly(lactic acid)-based polymers, and nonerodible polymeric carriers such as ethylene/vinyl acetate copolymers. In all cases release rates due to ultrasound were higher than erosion rates. A major cause of these ultrasound-induced effects is acoustic cavitation (the formation, growth, and implosive collapse of bubbles in solution by a sound field).[3] A limitation of previous experiments was that the effects demonstrated used ultrasound intensities and frequencies (20–75 kHz) which would not be acceptable clinically. Utilizing previously adopted in vitro methodology where samples are typically irradiated through a glass container such as a beaker, we found no effects using therapeutic levels of ultrasound. In this paper we address this problem and describe an in vitro model system which we have developed using a therapeutic ultrasound generator. Using this methodology, the actual mechanisms by which cavitation enhances polymer erosion (the appearance of monomers in solution) are investigated. In particular the effects of cavitation on polymer degradation and the mechanical erosion of the polymer surface caused by ultrasound are studied (the term disintegration will be used rather than mechanical erosion in the rest of this paper to avoid confusion in terminology).

Experimental Section

Materials. Copolymer of 1,3-bis(*p*-carboxyphenoxy)propane (CPP) and sebacic acid (SA) in the ratio of 20:80 CPP:SA was received as a gift from Nova Pharmaceuticals (Baltimore, MD). The synthesis and purification of p(CPP:SA) 20:80 copolymer has been described previously.[9] Chloroform (HPLC grade) was obtained from EM Science (Gibbstown, NJ). Water used in these studies was freshly obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA). All other chemicals used were of analytical reagent purity.

Gel Permeation Chromatography. The molecular weight of polymer was determined relative to polystyrene standards (Polysciences, Warrington, PA) by gel permeation chromatography (GPC). A Perkin-Elmer GPC system (Perkin-Elmer, Norwalk, CT) consisting of the Series 10 pump, an LKB 2140 rapid spectral detector (Pharmacia LKB, Gaithersburg, MD) at 254 nm, and a PE 3600 data station was used. The samples were eluted with chloroform through a 30 cm × 0.75 cm PL Gel column with a particle size of 5 μm (Polymer Laboratories Inc., Amherst, MA) at a flow rate of 0.9 mL/min.

Device Fabrication. A hot-melt method of device fabrication which exposes the polymer to elevated temperatures for a short period of time was developed for these studies. Previous studies have shown that the melt method produces a highly compact structure which minimizes problems of diffusional release of

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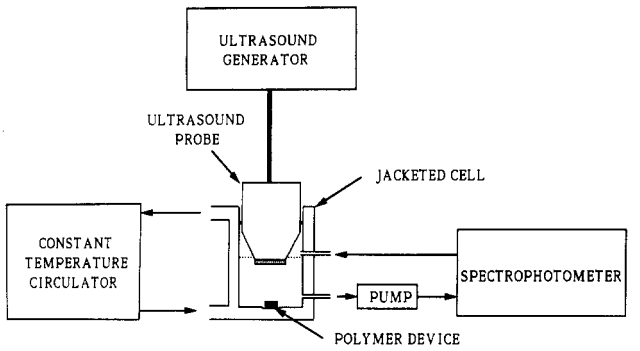

Figure 1. Schematic diagram of the apparatus used in ultrasonic studies.

incorporated substances during drug release which can occur with other device fabrication methods such as compression molding and solvent casting.[10] Polytetrafluoroethylene (PTFE) (AIN Plastics of New England, Norwood, MA) molds were used to fabricate devices. The low coefficient of friction of PTFE[11] provides a tight seal between the mold parts while allowing free movement. Polymer used was either spray-dried or milled. For the spray-dried polymer, a 3% solution of the copolymer in methylene chloride was spray-dried with a Büchi Model 190 spray dryer (Brinkmann Instruments Inc., Westbury, New York). The flow control was set between 600 and 700, with the inlet temperature at room temperature. For the milled polymer, polymer was ground in a MicroMill Grinder (Bel-Art Products, Pequannock, NJ) and sieved (Newark Wire Cloth Co., Newark, NJ) to a particle size of <250 μm. A weighed amount of polymer was placed into the mold and compressed tightly in a vise for 1 min (Panavise, Long Beach, CA) at room temperature. The mold was placed in an oven at 80 °C for 15 min. The mold was then compressed finger tight and allowed to cool in a desiccator at room temperature for approximately 15 min after which time the mold was dismantled and the device removed. The devices were stored under nitrogen at −20 °C until required. The disks used in this study were 0.8 cm in diameter and approximately 0.1 cm in thickness. The molecular weight loss produced by this method was estimated using GPC. The molecular weight of polymer samples before and after device fabrication method were determined; the loss in weight average molecular weight (M_w) by this method of device fabrication was found to be approximately 9%.

In Vitro Ultrasound Experiments. Using previous methodolgy where a sample was placed in a vial with the ultrasound source applied externally to the vial via a water bath we could detect effects using an ultrasonic bath at 75 kHz[3], but no effects could be demonstrated using a therapeutic frequency (1 MHz) and intensity (<2 W/cm²). A new methodology was designed in an attempt to examine the effect of methodology, particularly the significance of a barrier (glass vial) to the ultrasound source. In order to examine the effects of ultrasound continuously and in real time, a flow-through system comprising of a custom-built jacketed glass cell and a UV spectrophotometer (DU-65, Beckman Instruments, Inc., Fullerton, CA) was implemented (Figure 1). The cell was designed with a slight indentation in the base to retain the polymer device in a fixed position relative to the ultrasound probe. The ultrasound generator utilized in these studies was a Sonopuls 434 therapeutic unit (Enraf Nonius, Al Delft, The Netherlands). A 1-MHz collimating probe was used with an effective radiating area of 5 cm². Phosphate buffer (50 mL, pH 7.4) was pipetted into the cell and circulated through the spectrophotometer at a flow rate of 5.4 mL/min using a peristaltic pump (Rabbit-Plus, Rainin Instrument Co., Inc., Woburn, MA). The dead volume of the system was determined to be 4.3 mL. The effect of flow rate was examined by comparing the erosion profile of a control device with that of a device where the flow rate was varied in three increments at 2-h intervals from 4.3 to 6.8 mL/min. No differences were found in the profiles, indicating that the flow rate used provided adequate mixing. The temperature of the buffer was maintained at 37 °C using a constant temperature water circulator (RTE-210, NESLAB Instruments, Inc., Dublin, CA). A seal was maintained between the ultrasound probe and the rim of the jacketed cell using a viton O-ring (Aldrich Chemical Co., Inc., Milwaukee, WI). The appearance of CPP was used as a marker to follow polymer erosion by measuring buffer absorbance at 246 nm at 20-min intervals (SA does not absorb significantly at 246 nm).[9] The extinction coefficient of CPP was previously determined using a series of stock solutions of the diacid monomer prepared in buffer. Prior to experiments, the buffer was pumped through the system for at least 30 min to establish a baseline. To examine the baseline stability of the spectrophotometer, buffer alone and buffer containing approximately 0.01 mg/mL CPP were pumped through the system. In both cases no significant baseline drift was found over a period of 16 h. Experiments consisted of incubating devices in beakers containing buffer at 37 °C for 18 h and then transferring the devices to the jacketed cell at which point our studies were commenced. This was done to bypass the induction period of erosion.[12] The erosion rate without ultrasound was examined for a period of 2 h followed by exposure of the device to ultrasound at an intensity of 1.7 W/cm² for a period of 2 h and then another period of no ultrasound. Six replicates were performed.

Effect of Ultrasound on Induction Period. The induction period of polyanhydride erosion, though a relatively short period, has been shown to be very important; the induction period has been correlated to molecular weight changes and is a period during which polymer molecular weight (M_w) decreases rapidly to a value below approximately 5000.[12] In the present study the effects of ultrasound on the degradation of the polymer was evaluated by examining molecular weight changes produced by ultrasound in the induction period, since at later times the molecular weight is relatively low (M_w < 5000) and ultrasound induced effects may be difficult to detect. The jacketed cell was prepared as described above and the erosion of polymer devices (initial M_w = 65 400) both exposed (1.7 W/cm²) and nonexposed to ultrasound were monitored in the early stages of erosion. The experiments were performed in triplicate. Devices were removed at specific time intervals, rinsed with distilled water to remove buffer salts, and dried under vacuum overnight. The surface layer of devices was scraped off with a scalpel and the device core cut into small pieces and dissolved in chloroform (<10 mg/mL). The molecular weight of the polymer was determined by GPC. Experiments were performed in triplicate.

Studies of Polyanhydride Morphology. The morphology of polymer devices both exposed (1.7 W/cm² for 2 h) and nonexposed to ultrasound were examined by means of environmental scanning electron microscopy (ESEM)[13] (ESEM Model E-3, ElectroScan Corp., Wilmington, MA) to investigate the extent of disintegration of the polymer surface. To our knowledge, this is the first reported study of the use of this technique in the area of drug delivery systems. The ESEM allows samples (including insulators) to be imaged directly in their natural state and in a relatively low vacuum environment (~10 Torr water vapor pressure). The possibility of artifacts is minimized by obviating the need for sample modification or preparation (freezing, drying, and coating). Samples were removed from the jacketed cell and placed in distilled water for ca. 1 h to remove buffer salts. The samples were then transferred with a drop of water to the Peltier temperature control stage in the microscope chamber for imaging. The sample chamber was saturated with water vapor by controlling the chamber temperature and pressure. In order that the sample be imaged, water on the sample surface must be removed; this is achieved by either reducing the chamber vapor pressure or increasing the sample temperature in a controlled manner.

Results and Discussion

Effect of Ultrasound on Polyanhydride Erosion. Figure 2 is representative of the effect of ultrasound on the erosion of polyanhydride devices and compares the erosion profile of a device exposed to ultrasound for 2 h to a nonexposed device. As shown in Figure 2, the erosion rate increased significantly upon exposure to ultrasound and decreased on the removal of ultrasound. Figure 3 summarizes the results found in this study, comparing the erosion rates of the devices before, during, and after

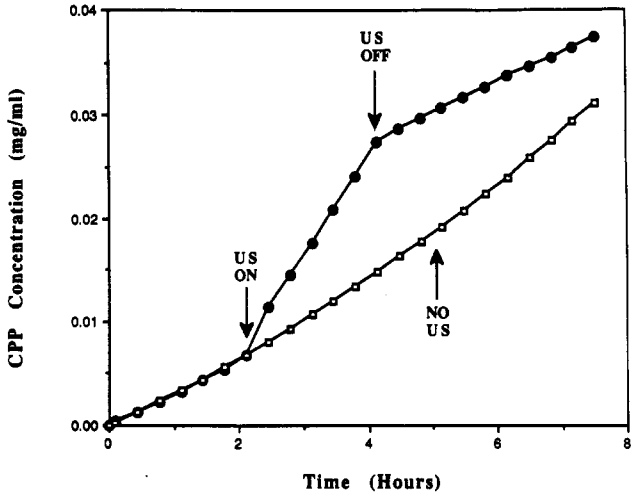

Figure 2. Effect of ultrasound on the erosion of a polyanhydride device compared to a nonexposed control.

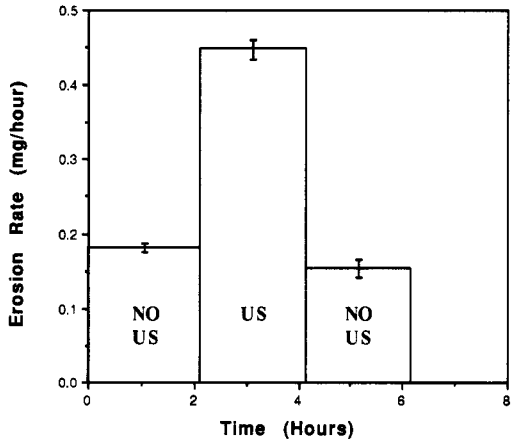

Figure 3. Effect of ultrasound (1 MHz, 1.7 W/cm^2) on the erosion rate of polyanhydride devices (±SD).

exposure to ultrasound. Whereas we were unable to obtain any effects utilizing our previously adopted methodology[3] where a glass barrier separates the ultrasound source from the sample at clinically acceptable frequencies, a 2.5-fold increase in the erosion rate was measured in the present study using a frequency of 1 MHz and an intensity of 1.7 W/cm^2 (both in a clinically relevant range). There are a number of reasons why we believe we were not successful in demonstrating an effect with the previous methodology using a therapeutic ultrasound generator. Firstly, there is an approximately 8-fold difference in the acoustic impedance of glass and water[14] which results in a significant portion of applied ultrasound energy (~60%) being reflected by the vial containing the polymer device. Additionally, the depth of penetration of high-frequency ultrasound (1 MHz) is less than that produced in a typical ultrasonic bath (75 kHz),[14] thus even though effects were found using 75-kHz ultrasound, 1-MHz ultrasound penetrates to a lesser degree and may even not penetrate the glass container. Moreover, the intensity of ultrasound within an ultrasound bath varies considerably depending on factors such as tank geometry, height of water, and continuous operation time; thus the ultrasound characteristics are not well defined.[15] The current in vitro methodology was designed taking these factors into consideration to improve the efficiency of the ultrasonic energy supplied to the polymer samples. This methodology is being used in our ongoing studies aimed at optimizing the enhancement produced by ultrasound.

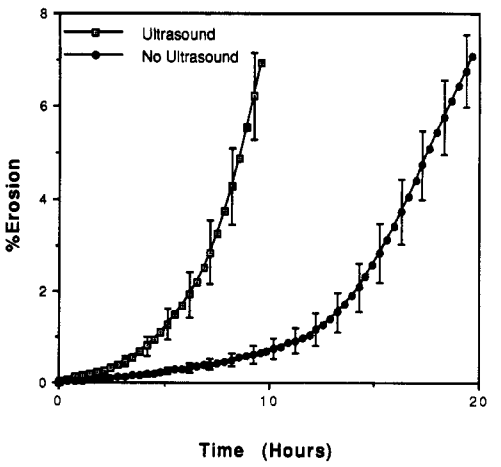

Figure 4. Effect of ultrasound on erosion during the induction period (±SEM, n = 3).

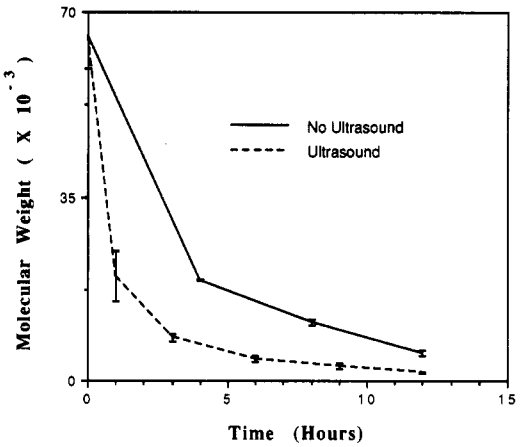

Figure 5. Effect of ultrasound on the decrease in molecular weight during the induction period (±SD).

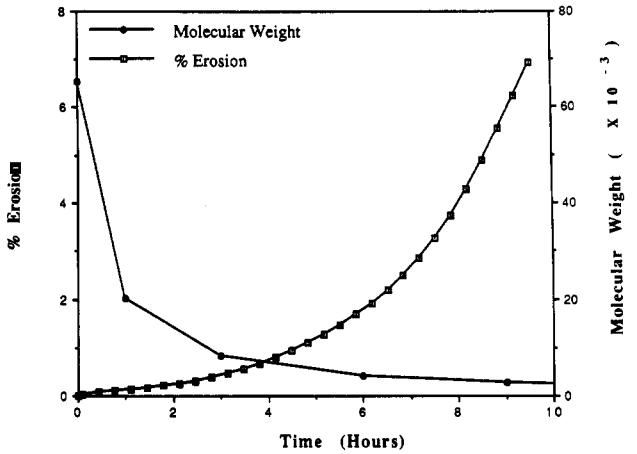

Figure 6. Relationship between % erosion and device molecular weight for an ultrasonically exposed device during the induction period.

It should be noted that our present studies are aimed at understanding the mechanisms by which ultrasound enhances polymer erosion in order to optimize the drug delivery modulation produced by ultrasound. It has previously been demonstrated that ultrasound produces a much greater effect on the release rate of incorporated molecules than on the erosion rate of devices,[3] thus the 2.5-fold increase in erosion rate produced by the therapeutic levels of ultrasound may translate to a much greater increase in drug release rates. This is currently under investigation.

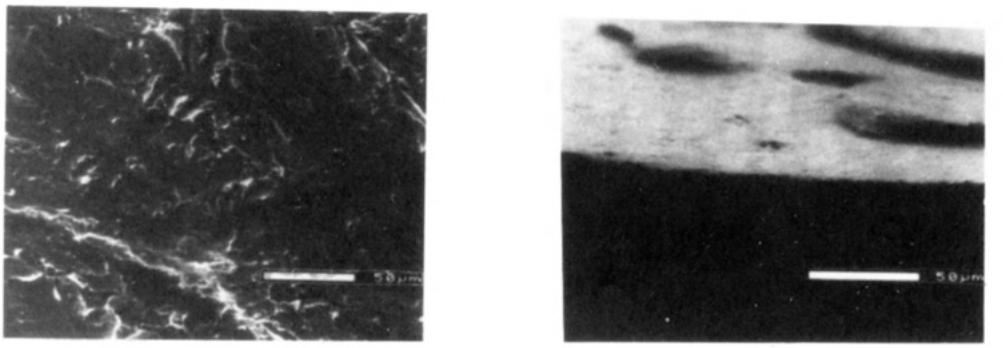

Figure 7. Environmental scanning electron micrographs of polymer devices prior to erosion: (a) surface and (b) tilted cross section.

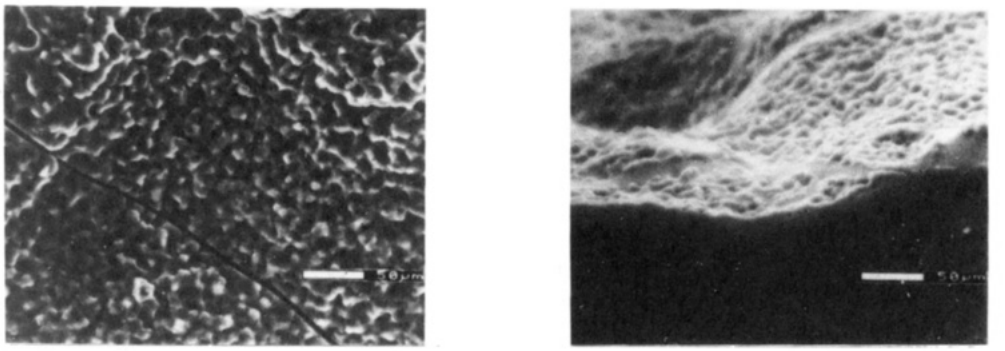

Figure 8. Environmental scanning electron micrographs of an ultrasonically exposed polymer device surface: (a) surface and (b) tilted cross section.

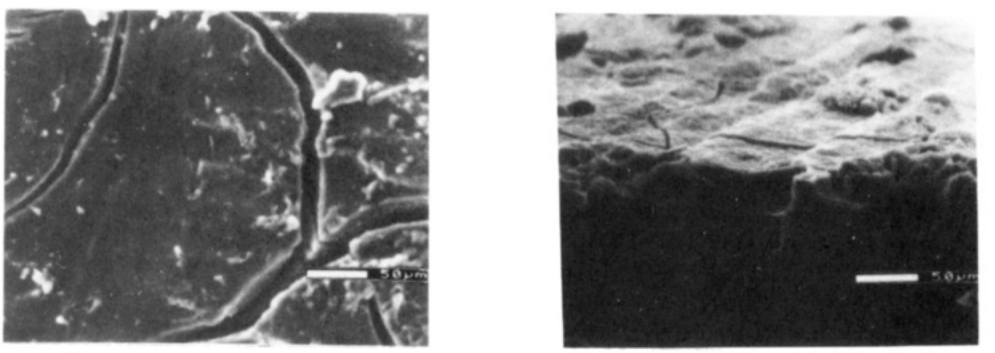

Figure 9. Environmental scanning electron micrographs of a nonexposed polymer device surface: (a) surface and (b) tilted cross section.

Effect of Ultrasound on Induction Period. The effects of ultrasound on the erosion profile during the early stages of device erosion are shown in Figure 4. Ultrasound significantly decreases the induction period of polymer erosion. The effect of ultrasound on the changes in polymer molecular weight were examined. Figure 5 compares the decrease in molecular weight (M_w) of devices both exposed and nonexposed to ultrasound. The effect of ultrasound is to increase the rate at which the molecular weight decreases. The decrease in molecular weight for ultrasonically exposed devices may be correlated to the induction period as shown in Figure 6. The results indicate that ultrasound enhances the rate of degradation within the polymer matrix. The actual mechanism is not known yet; however, potential mechanisms include facilitated penetration of water into the polymer matrix leading to enhanced anhydride bond hydrolysis, or possibly a direct rupture of either the anhydride bonds or other sensitive bond induced by ultrasound, or mechanochemical degradation.

ESEM Studies of Polyanhydride Morphology. Previous studies on the erosion of biodegradable polymer devices clearly demonstrated that acoustic cavitation is a major cause of the effects produced by ultrasound.[3] The actual mechanism by which cavitation mediates its effects however is uncertain, although temperature and mixing effects as a result of ultrasound were found to be relatively unimportant.[3] As we have shown in this study, ultrasound enhances degradation of the polymer during the induction period. However this may not be the sole mechanism by which ultrasound mediates its effects on polymer erosion. It has been shown that acoustic cavitation at solid–liquid interfaces results in markedly asymmetrical bubble collapse resulting in high-speed liquid jets directed at the solid surface with velocities estimated to be in excess of 100 m/s.[16] This effect causes the disintegration of solid

surfaces,[17-19] and it may be a mechanism by which ultrasound mediates its effects on polymer erosion. Another important phenomenon that plays a role in these effects is acoustic streaming, a microscopic turbulence which enhances liquid–surface mass transport.[17,18] It is these effects that are thought to cause the chemical activation of nickel and copper catalysts due to the removal of passivating oxide layers.[19] One aim of the present study was to investigate the surface morphology of ultrasonically exposed devices to see if any evidence for this effect could be found. Previous work in our laboratories revealed the existence of a well-defined erosion zone in degrading polyanhydride devices, the exact nature of which is uncertain but thought to be a layer of monomer and incompletely degraded polymer.[20,21] This zone is characterized as a porous and friable region which might therefore be susceptible to cavitation damage. Standard SEM was found to be unsatisfactory in the present study, since preparation techniques caused damage to the surface layer of the devices. Thus, ESEM was used to preserve the surface characteristics of the devices. Figure 7 shows a surface view and tilted cross section of a control device prior to erosion. Figure 8 shows a surface view and tilted cross section of an ultrasound-exposed device surface, and Figure 9 shows a surface view and tilted cross section of a nonultrasonically exposed device surface which had been immersed in buffer for the same period of time. There is a significant difference in morphology between ultrasound-exposed and -nonexposed surfaces. The morphology of the ultrasonically exposed surfaces is characteristic of other surfaces that have been subjected to cavitation effects where the surface damage produced was the result of high-velocity cavitation jets creating surface defects.[17,22] Additionally, damage to friable solids is expected due to the accompanying shock waves and microscopic turbulence (acoustic streaming).[17] Evidence from ESEM strongly indicates that these mechanisms may be the cause of the enhanced erosion found in the present study. Whether ultrasound propagates the erosion zone or merely removes it is uncertain at the moment. One consequence of this mechanism is that ultrasonically enhanced erosion is probably much greater than that measured in the present study; cavitation would be expected to produce small particles as a result of surface removal. These particles may be oligomeric or polymeric in nature and may not go into solution immediately. Thus the measured erosion rates may be an underestimate and explain why release rates of incorporated agents were found to be enhanced to a greater extent than the rate of polymer erosion.[6] An interesting finding in the present studies concerns the crack formation found when using ESEM. Previous SEM studies of the polyanhydrides have revealed cracks on the surface similar to those shown in the present study;[2,23] these cracks were thought to be an important avenue for water transport into the polymer. However, in the present study, it was found that the crack formation is probably an artifact of sample preparation. When samples are initially viewed under the ESEM, no cracks are visible, however as the surface starts to dry, cracks begin to form and start propagating throughout the sample surface. It is difficult to obtain micrographs before and during this process due to movement of the sample. The extent of this phenomenon varies from sample to sample and could be controlled to a certain extent by controlling the environment in the sample chamber.

The use of ESEM allows examination of samples in their natural state in a gaseous environment without sacrificing contrast or resolution due primarily to the differential pumping system employed and the development of a new type of detector.[13] ESEM may also be useful in the imaging of other biomaterials and biological samples due to the minimal amount of sample preparation required.

Conclusions

Methodology has been developed to investigate the effects of therapeutic ultrasound on the erosion of polyanhydride devices. It has been demonstrated that significant effects on polymer erosion can be achieved using therapeutically acceptable intensities and frequencies. This effect may be the result of more than one mechanism, including enhanced polymer degradation and mechanical disintegration of the polymer surface. Acoustic streaming, which accompanies cavitation, may also be involved. ESEM has been successfully applied as a nondestructive technique for the morphological analysis of polymer surfaces.

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