Controlled Release of Dexamethasone from PLGA Microspheres Embedded Within Polyacid-Containing PVA Hydrogels

Submitted: May 12, 2004; Accepted: October 4, 2004; Published: September 2, 2005

Izabela Galeska,¹ Tae-Kyoung Kim,² Siddhesh D. Patil,² Upkar Bhardwaj,² Debjit Chattopadhyay,¹ Fotios Papadimitrakopoulos,¹ and Diane J. Burgess²

¹Nanomaterials Optoelectronic Laboratory, Department of Chemistry, Polymer Program, Institute of Materials Science, University of Connecticut, 97 N Eagleville Road, Storrs, CT 06269

²Department of Pharmaceutical Sciences, University of Connecticut, 372 Fairfield Way, Storrs, CT 06269

ABSTRACT

The development of zero-order release systems capable of delivering drug(s) over extended periods of time is deemed necessary for a variety of biomedical applications. We hereby describe a simple, yet versatile, delivery platform based on physically cross-linked poly(vinyl alcohol) (PVA) microgels (cross-linked via repetitive freeze/thaw cycling) containing entrapped dexamethasone-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres for controlled delivery over a 1-month period. The incorporation of polyacids, such as humic acids, Nafion, and poly(acrylic acid), was found to be crucial for attaining approximately zero-order release kinetics, releasing 60% to 75% of dexamethasone within 1 month. Microspheres alone entrapped in the PVA hydrogel resulted in negligible drug release during the 1-month period of investigation. On the basis of a comprehensive evaluation of the structure-property relationships of these hydrogel/microsphere composites, in conjunction with their in vitro release performance, it was concluded that these polyacids segregate on the PLGA microsphere surfaces and thereby result in localized acidity. These surface-associated polyacids appear to cause acid-assisted hydrolysis to occur from the surface inwards. Such systems show potential for a variety of localized controlled drug delivery applications such as coatings for implantable devices.

KEYWORDS: hydrogels, microspheres, controlled drug delivery, dexamethasone

INTRODUCTION

There is a growing interest in drug delivery systems that can provide site-specific and continuous therapeutic drug levels for extended periods of time. These systems can be

envisioned as adhesive patches or implantable devices. The goal of the present research is to obtain an approximately zero-order release profile of dexamethasone over a 1-month period using a hydrogel system that has potential for future application as a coating for implantable devices such as glucose biosensors. Dexamethasone is intended to control the inflammatory response and tissue repair process at the implant site. Gel-forming polymers, eg, poly(vinyl alcohol) (PVA),¹ are of interest as such drug delivery systems, since they can provide soft, permeable, and hydrophilic interfaces with body tissues. In this capacity, PVA hydrogels have been used as drug delivery vehicles, alone,² as well as in combination with other delivery systems, to provide desirable release profiles.³ Typically, the degree of PVA hydrogel cross-linking, which is intimately linked with the mechanical properties and water content, greatly influences drug release properties.¹ Cross-linking has been achieved by annealing at elevated temperatures (120°C-130°C),^{4,5} γ -irradiation,⁶ and/or by chemical means.¹ For many applications involving both small molecular weight (MW) drugs^{1,3} and protein therapeutics,^{1,2} chemical cross-linking is considered undesirable because the complete removal of any unreacted, often toxic, cross-linking agents is very difficult.

Over the past 2 decades, research aimed at the fabrication of mechanically strong hydrogels has led to the development of a well-established physical cross-linking scheme involving repetitive freezing and thawing of aqueous PVA solutions.^{7,8} This technique promotes the formation of ordered microcrystalline domains as a result of enhanced intra- and interpolymer interactions in the unfrozen regions of the PVA-water system.⁸ This type of cross-linking circumvents the need for chemical cross-linking agents and has led to PVA gels that are bio-inert and exhibit long-term in vivo compatibility.^{9,10}

The close resemblance of PVA hydrogels to human soft tissue (high water content,¹¹ rubbery and elastic nature¹²) has made them a material of choice for many biomedical applications, such as intervertebrate disc nuclei¹³; artificial articular cartilage^{14,15}; contact lenses¹⁶; matrices for cell immobilization^{17,18}; and mucoadhesives^{19,20}; as well as for the controlled release of drugs,²⁰⁻²² growth factors,²³ and proteins. Drug release rates from PVA hydrogels tend to be

Corresponding Author: Diane J. Burgess, Department of Pharmaceutical Sciences, University of Connecticut, 372 Fairfield Way, Storrs, CT 06269; Tel: (860) 486-3760; Fax: (860) 486-4998; E-mail: d.burgess@uconn.edu

relatively rapid (minutes to weeks) depending on the pore size, extent of cross-linking, and the nature of the incorporated drug and typically follow first-order kinetics.²³ To overcome this limitation, other controlled-release delivery systems (eg, microspheres, nanoparticles, liposomes) can be entrapped within these PVA hydrogels without any detrimental effects that could occur in the presence of organic solvents and chemical cross-linking agents. The incorporation of microspheres and other particulate delivery systems can have advantages, such as isolation of the drug, slower drug release rates, and achievement of different drug release profiles, as well as incorporation of multiple drugs in different microsphere populations. Although microspheres alone can be used to achieve long-term drug release of weeks to months,^{24,25} such systems typically do not result in constant drug release profiles. Microspheres usually exhibit an initial rapid “burst” release as a result of surface-associated drug.²⁴ Furthermore, microsphere systems based on the commonly used poly(lactic-co-glycolic acid) (PLGA) polymer usually display a “triphasic” release profile, with an initial burst, followed by a minimal release phase, before entering into an approximate superlinear release profile when sufficient polymer degradation has occurred.^{26,27} For many drug delivery applications, such a profile is undesirable, and in some cases the initial burst release can raise safety and efficacy concerns.

We presently report the development of a composite (PVA-hydrogel/PLGA-microsphere) drug delivery system, which exhibits an approximately zero-order controlled-release profile over a period of 1 month. These composites are intended for use as biocompatible coatings for implantable devices such as glucose biosensors. Dexamethasone was selected as a model drug based on its potential for suppressing both the acute and chronic inflammatory response following device implantation. The incorporation of a small amount of surface active polyacids, poly(acrylic acid) (PAA), humic acids (HAs), and Nafion (perfluorosulphonic acid/polytetrafluoroethylene copolymer) into the composite hydrogel matrix was determined essential in achieving erosion of the PVA-embedded PLGA microspheres, as determined by scanning electron microscopy (SEM) and interfacial tension measurements. Furthermore, a detailed characterization of the effect of the number of freeze-thaw cycles on the mechanical and hydration properties as well as the drug release profile were conducted.

MATERIALS AND METHODS

Preparation of PVA Gel by the Freeze-thaw Method

PVA (99% hydrolyzed, MW 133 kd, Polysciences Inc, lot 440424 (Warrington, PA) was used in all experiments as a 5% wt/wt solution in Millipore (Billerica, MA) deionized water. The PVA films were prepared by casting 5 mL into

5-cm diameter covered Petri dishes and freezing at -20°C for 16 hours, followed by thawing for 8 hours at room temperature. The gels were subjected to as many freeze-thaw cycles (N_{ft}) as desired.

Preparation of Dexamethasone Microspheres

PLGA (50:50; MW 60 000; Resomer RG504) was a generous gift from Boehringer Ingelheim (Ridgefield, CT). PVA (MW, 30 000-70 000), dexamethasone and all other reagents were purchased from Sigma Chemical Co. (St Louis, MO).

One hundred milligrams of dexamethasone was dispersed in 4 mL methylene chloride containing 1 g of PLGA at 10 000 rpm using a homogenizer. This mixture was poured into 20 mL of 1% wt/wt PVA solution and further homogenized at 10 000 rpm. The microspheres were hardened in 300 mL of 0.1% wt/wt PVA solution with vigorous stirring under vacuum, washed with 0.1% wt/wt PVA solution, and collected by filtration. The microspheres were subsequently dried and stored under vacuum until further use. The encapsulation efficiency of all microsphere batches was determined by dissolving in methylene chloride. Dexamethasone was analyzed by high-pressure liquid chromatography (HPLC) at 246 nm using a C18 column (Nova-Pak, 4×150 mm, Waters, Bedford, MA) at 25°C , and a 1:1 mixture of 2 mM acetate buffer (pH 4.8) and acetonitrile as the mobile phase. An encapsulation efficiency of 52.1% (± 0.3) was obtained.

Fabrication of PVA Hydrogel Composites Containing Dexamethasone and Dexamethasone-loaded PLGA Microspheres

Twenty milliliters chilled 5% wt/wt PVA solution (prepared as described above) and 100 mg of dexamethasone were homogenized at 10 000 rpm and poured into a mold ($24.5 \times 57.5 \times 0.75$ mm). PVA hydrogels containing dexamethasone microspheres were prepared with 20 mL of chilled 5% wt/wt PVA solution and 1 g of dexamethasone-loaded microspheres, followed by homogenization at 5000 rpm. The molds containing the dexamethasone/PVA dispersions and dexamethasone microsphere/PVA dispersions were sealed with parafilm and subjected to freeze-thaw cycling, as described previously. The hydrogel films containing dexamethasone or dexamethasone encapsulated in microspheres were air dried after 1 to 5 freeze-thaw cycles. The hydrogel films used for subsequent characterization and in vitro release contained either 5 mg of dexamethasone in PVA or 50 mg of microspheres loaded with 5 mg of dexamethasone.

HAs (for elemental and functional group analysis see Galeska et al,²⁸ Wershaw,²⁹ and Stevenson³⁰), Nafion (5%

wt/wt solution of perfluorosulphonic acid/ polytetrafluoroethylene copolymer in lower aliphatic alcohols (1-propanol and ethanol) and water), and PAA (MW, 450 kd) purchased from Sigma-Aldrich (St. Louis, MO) were used as modifiers in the PVA gels. One hundred milligrams of the polymeric acid (HAs, Nafion, and PAA) was mixed with 100 mL of 5% wt/wt PVA solution, (approximately 2% wt/wt additive content with respect to PVA) and processed with the microspheres as described above.

In Vitro Release Study

The hydrogel films ($24.5 \times 57.5 \times 0.75$ mm), prepared as described above, containing either dexamethasone or dexamethasone microspheres were immersed in jars containing 100 mL of 0.01 M phosphate-buffered saline (PBS) (pH 7.4) and incubated at 37°C ($\pm 0.5^\circ\text{C}$) under constant agitation (100 rpm). The release of dexamethasone was periodically monitored by extracting 500- μL aliquots for 2 to 4 weeks and replenishing with 500 μL of PBS. PVA hydrogels without dexamethasone were used as a control. The concentration of dexamethasone was monitored (using HPLC analysis as described below), and the buffer solution was removed and replaced with fresh buffer as necessary to maintain dexamethasone concentration below 10% solubility (sink conditions). Release from the dexamethasone microspheres alone was determined by placing 50 mg of microspheres in 3 vials containing 1 mL PBS (pH 7.4) for each of the following time intervals: 2, 4, 7, 8, 9, 10, 12, 14, 16, 18, 23, 28, and 30 days. The concentration of dexamethasone was monitored and replenished with fresh buffer to maintain sink conditions, as above. All the in vitro release studies were conducted in triplicate (independently prepared films and different batches of microspheres), and mean values and standard deviations were calculated.

Surface Activity Determination

The polymeric acids (HAs, Nafion, and PAA) were mixed with PLGA microspheres and dispersed in distilled water at the same concentration as used in the PVA-hydrogel/PLGA microsphere composites (1 g of microspheres in 20 mL of deionized water). These dispersions were subjected to 3 freeze-thaw cycles, following which the microspheres were filtered and rinsed with copious amounts of deionized water. The ability of the polymers to modify the surface of the microspheres was investigated by comparing contact angle and zeta potential before and after the addition of the polymeric acids.

The contact angle studies (20- μL drop size) were performed using a Ramé-Hart goniometer (Mountain Lake, NJ). Freshly prepared and modified PLGA microspheres were annealed in a vacuum at 200°C for 24 hours to form a film.

The contact angle measurements were repeated 10 times on several film positions and averaged to account for film inhomogeneities.

Zeta potential studies were conducted using a Zetaplus apparatus (Brookhaven Instruments, Hotsville, NY). The as-prepared and polyacid-modified PLGA microspheres (as above) were dispersed (1 mg/mL) in deionized water or PBS, pH 7.4. This dispersion was then added to the Zetaplus electrophoresis cell, and the electrophoretic mobility was measured and the data converted to zeta potential values. These experiments were repeated 10 times, and the mean values and standard deviations calculated.

The surface tension of the polymeric acids was determined using a Kruss K12 Tensiometer (Hamburg, Germany), in the Wilhelmy Plate mode. Time-dependent changes in the surface tension were monitored automatically. The polymeric acids (2% wt/wt) were dissolved in either deionized water or PBS buffer, and the surface tension was recorded continuously at $25^\circ\text{C} \pm 0.5^\circ\text{C}$ until the fluctuation in the data was less than 0.1 mN m^{-1} . These experiments were performed 5 times and mean values and standard deviations calculated.

Characterization of PVA Hydrogel

The swelling of the air-dried PVA gels was characterized using 2 methods: (1) monitoring the dimensional changes as a function of immersion time under a constant load of 20 mN using a Perkin-Elmer Thermomechanical Analyzer (TMA) (Wellesley, MA), and (2) monitoring weight change. The percentage strain (% strain), as obtained from TMA, was defined as the percentage change in length divided by the original length: $(L - L_0)/L_0 \times 100\%$. The swelling experiments were performed in PBS buffer at room temperature and also at 37°C ($\pm 1^\circ\text{C}$). The percentage weight change was defined as the percentage change in mass during swelling: $(M - M_0)/M_0 \times 100\%$. Air-dried PVA samples (M_0) (approximately $10 \times 10 \times 0.75$ mm) were weighed and immersed either in 20 mL deionized water or in PBS buffer and maintained at 37°C for 96 hours in a thermostated water bath; following which excess fluid from swollen samples was carefully removed and the weight change ($M - M_0$) with respect to the dry mass recorded.

The Young's moduli of the as-fabricated and microsphere-loaded PVA gels were obtained from uniaxial tensile testing using TMA. Rectangular samples (approximately 4-mm wide and 15-mm long and 0.75-mm thick) were used for all tensile tests. The force was linearly ramped from 30 to 100 mN at a constant rate of 25 mN min^{-1} and the Young's modulus was ascertained from the resulting stress-strain curves. The Young's modulus was measured for air-dried and fully hydrated samples (PBS buffer).

Figure 1. Degree of swelling (% weight change) of PVA gels immersed in water and PBS buffer (pH 7.4) at 37°C, as a function of the number of freeze-thaw cycles; $n = 3$, where n is the number of repeat experiments conducted. ($P < .05$ shows significant difference between water and PBS groups as analyzed using Student t test.)

A Perkin-Elmer differential scanning calorimeter (DSC 7, Wellesley, MA) was used to determine the degree of crystallinity of air-dried PVA gels in the presence and absence of acidic additives and PLGA microspheres. DSC scans were performed at 5°C/min from 25°C to 250°C. The degree of crystallinity (X), ($X = \Delta H / \Delta H_C \times 100\%$) was calculated by dividing the heat required to melt 1 g of dry sample (ΔH - obtained by integrating the area under the endothermic peak between 190°C and 240°C) by the standard enthalpy (ΔH_C) for 100% crystalline PVA (138.6 J/g).³¹ All crystallinity values were normalized with respect to the actual PVA content in the sample.

Morphological Characterization of Degraded Microspheres

Microsphere degradation was monitored using a Phillips environmental scanning electron microscope (ESEM) (FEI Company, Hillsboro, OR) operated at 20 keV. Both as-prepared microspheres and hydrogel-dispersed microspheres were treated as described in the in vitro release experiments, and their morphology was compared at various intervals over a 4-week study period. Microspheres were recovered from the hydrogel by dry-fracturing the polymer matrix and brushing them off the fractured surface. Microscopy specimens were prepared by placing the microspheres on carbon tape-covered microscopy stubs and coating them using a Polaron sputter-coating system (Quorum Technologies, Newhaven, East Sussex, UK) (at 0.04-0.06 Torr for 20 seconds) with a thin layer of Au/Pd to increase conductivity and image quality.

RESULTS AND DISCUSSION

Mechanical Properties of PVA Hydrogels

Prior to freeze-thaw assisted cross-linking, aqueous solutions of 99% hydrolyzed PVA were preheated to c.a. 80°C to facilitate complete polymer dissolution. PVA hydrogels with a lower degree of hydrolysis (below 89%) were soluble in water below 37°C and therefore were deemed inappropriate for use in this study.

Figure 1 depicts the degree of swelling of PVA gels, in water and PBS, as a function of freeze-thaw ($N_{f/t}$) cycling. In both environments the weight change was inversely related to the $N_{f/t}$ cycles, corroborating other reports.³² Typically, hydrogels subjected to 1 $N_{f/t}$ cycle had water uptake of up to 500% of their dry weight, whereas hydrogels with 5 $N_{f/t}$ cycles exhibited c.a. 350% swelling. This differential water uptake can be related to the extent of physical cross-linking, which decreases porosity⁷ and alters fluid uptake.⁷ Although the fluid uptake was lower in high ionic strength media (ie, PBS buffer) for samples subjected to 1 and 2 freeze-thaw cycles, the overall trend was similar to that observed in water. This decreased fluid uptake in high ionic strength media may be a result of antichaotropic effects (ie, reinforcement of hydrogen bonding within the gel), previously reported in the presence of inorganic (eg, NaCl, NaF, Na_3PO_4 , Na_2SO_4) and a few amino acid salts.³³

The dimensional changes of the PVA gels upon hydration in PBS buffer, as a function of immersion time, are depicted in Figure 2. An initial induction time (varying from 0.5 to 20 minutes), characterized by the plateau before the onset of the dimensional change, was observed for all samples tested at 25°C and is thought to arise from the slower fluid diffusion into the dry film and depends on film thickness and microstructure. Typically, the specimens attained

Figure 2. Typical percentage strain of the PVA gel (subjected to $N_{f/t} = 3$ freeze-thaw cycles) upon immersion in PBS buffer (pH 7.4) at 25°C and 37°C as a function of time. ($n = 3$, where n is the number of repeat experiments conducted.)

Figure 3. Young's modulus for PVA hydrogels determined at 25°C as a function of the number of freeze-thaw cycles for: (○) dry gels as-fabricated; (■) dry gels MS-loaded; (▲) fully hydrated gels as-fabricated, and (▽) fully hydrated gels MS-loaded. ($n = 3$, where n is the number of repeat experiments conducted. $P < .05$ shows significant difference between PVA and MS/PVA at different freeze-thaw cycles under dry and wet conditions as analyzed using Student t test.)

fully hydrated dimensions after approximately 200 minutes of immersion (a 35% increase in length for $N_{f/t} = 3$). However, for the same experiments performed at physiological temperature (37°C), not only was no induction time observed but the PVA gels reached the same fully hydrated state substantially faster (in less than 30 minutes) pointing to almost instantaneous swelling at body temperature with equilibrium swelling being attained within 30 minutes.

Figure 3 depicts the Young's modulus for dry and fully hydrated PVA films in the presence and absence of microspheres as a function of $N_{f/t}$ cycles. In general, these gels have an order of magnitude larger modulus after the first freeze-thaw cycle, and reach an asymptotic value after $N_{f/t} = 3$ cycles, corroborating observations of Takamura et al²¹ and Takamura and Ishii.³⁴ The modulus for the dry samples changed from 2 ($N_{f/t} = 1$) to 20 to 40 MPa ($N_{f/t} = 5$), which can be attributed to the gradual ordering and crystallization of the macromolecular chains upon repetitive freezing and thawing.^{7,32,35} The crystalline regions within the gels act as stress transfer points, redistributing external stress similarly to chemically or irradiatively induced cross-links. Additionally, the PLGA microspheres dispersed within the dry PVA matrix act as a filler,^{36,37} which is apparent from the slight reinforcement of gels prepared with 1, 2, and 3 freeze-thaw cycles. In contrast to dry samples, the fully hydrated gels exhibit an order of magnitude smaller modulus, which is comparable with that of soft human tissue.³⁸ Surprisingly, the reinforcing effect of the microspheres was not observed in the hydrated state, where the Young's modulus was lower than that of the fully hydrated PVA gels prepared with 3, 4, and 5 freeze-

thaw cycles. This discrepancy may be related to the poor adhesion between the microspheres and the solvated polymer matrix, and possibly dewetting (slippage at the interface), which may occur owing to limited hydration of the relatively hydrophobic PLGA microspheres.³⁶

In Vitro Release From Hydrogels and Hydrogel/Microsphere Composites

Based on the above results, PVA gels subjected to either 1 or 2 freeze-thaw cycles were determined to be inadequate in terms of their mechanical properties and therefore were not considered suitable for further investigation. Consequently, the in vitro release studies were conducted on samples that had undergone 3 to 5 freeze-thaw cycles. The in vitro release of dexamethasone from the PVA hydrogels alone (no microspheres) was determined to be inversely proportional to the number of freeze-thaw cycles (shown in Figure 4) for $N_{f/t} = 3$ and 5, respectively. The effect of the number of freeze-thaw cycles on drug release rates is in agreement with literature data on release from such PVA hydrogels.²⁰ The release of dexamethasone from these gels into pH 7.4 PBS reached 100% release within 10 days for $N_{f/t} = 3$ and followed approximately first-order release kinetics.

PLGA microsphere-encapsulated dexamethasone embedded within the PVA matrix (for $N_{f/t} = 3$) released only approximately 6% in pH 7.4 PBS after 30 days (Figure 5). For the purpose of comparison, the release profile of dexamethasone-loaded microspheres into pH 7.4 PBS in the absence of the PVA gel is also shown in Figure 5. As expected, release of dexamethasone from the microspheres alone into PBS revealed an initial "burst" followed by a slower release phase. Apparently, the presence of the surrounding PVA hydrogel has a profound effect on the

Figure 4. Cumulative release of dexamethasone from PVA hydrogels as a function of the number of freeze-thaw cycles. (PBS buffer, pH 7.4, 37°C; $n = 3$, where n is the number of repeat experiments conducted.)

Figure 5. Cumulative release of dexamethasone (Dex) from (●) PLGA microspheres (MS); and MS incorporated into the PVA hydrogel matrix: with (○) no additives; and in presence of (Δ) PAA; (□) HAs; and (*) Nafion ($N_{f/t} = 3$; PBS buffer, pH 7.4, 37°C; $n = 3$, where n is the number of repeat experiments conducted.)

release profile of PLGA microspheres and, as shown in Figure 4, this extremely slow release profile cannot be attributed to drug diffusion through the PVA hydrogel matrix, since release of drug alone (no microspheres) from the PVA was complete in 10 days. Such behavior may result from a dramatic decrease in the acid-catalyzed, self-accelerated ester bond cleavage that has been established as a prevalent mechanism for PLGA microsphere degradation and consequent drug release.³⁹ Typically, as the PLGA degrades there is a build up of acidic oligomeric by-products of PLGA, and these prefer to remain within the nonpolar environment of the PLGA microspheres rather than partition into aqueous release media. These by-products then self-catalyze bulk degradation of the PLGA.³⁹⁻⁴² However, in the presence of the PVA hydrogel, the oligomeric by-products are speculated to partition into the hydrogel (since this presents an environment that is more polar than PLGA, yet less polar than water) and, therefore, are unavailable for self-catalysis of the PLGA. A second possible explanation for the decreased release of dexamethasone from the microspheres embedded in the hydrogels is that microcrystalline PVA domains form at the PLGA microsphere surfaces, thereby significantly decreasing the ability of water to partition into the microspheres and, therefore, decreasing polymer degradation and drug release.

To enhance PLGA degradation and consequent erosion-controlled drug release within the PVA hydrogels, surface active polyacids (such as PAA, Has, and Nafion) were added to the hydrogel matrix to slightly increase acidity in the localized environment of the PLGA microspheres. As illustrated in Figure 5, the addition of these polyacids significantly accelerated the release of dexamethasone

from PLGA microspheres embedded within PVA hydrogels. This resulted in an order of magnitude increase in the release rate of dexamethasone as compared with that for PLGA microspheres entrapped in the PVA hydrogel alone. Among these polyacids, HAs were shown to have the most pronounced effect on the release kinetics, resulting in ~75% drug release within 1 month, with an approximately zero-order release profile. The other 2 polyacids exhibited a slightly lower release rate than HAs (~60% release within 30 days), which, as discussed below, is believed to originate from differences in the associated surface acidity of the PLGA microspheres within a buffered (ie, PBS/PVA) media. There was no significant difference between the release rates of dexamethasone from microspheres embedded in Nafion- or PAA-modified gels.

As shown in Figure 6, the 3 polyacids exhibit a similar extent of surface activity. Furthermore, zeta potential and contact angle measurements (Table 1) suggest that these polyacids preferentially adsorb onto the PLGA microsphere surfaces. The as-prepared PLGA microspheres contain surface-associated low MW PVA surfactant rendering a relatively hydrophilic interface. Nafion,⁴³ which possesses a fluorinated backbone, and thus the highest hydrophobicity among the polyacids studied, exhibits the highest contact angle. The differences in the zeta potentials of these polyacids can be explained on the basis of their relative acidity. Nafion, a strong polyacid, is completely ionized when in contact with PBS buffer, thereby exhibiting the highest zeta potential. This is followed by HAs, which is a stronger acid than PAA.²⁸

The strong UV-visible absorption of HAs (with a profound brown coloration) provided further experimental evidence of the pronounced surface association of HAs on the PLGA microspheres embedded in PVA hydrogels. Unlike HA-loaded PVA gels, where HA gradually diffused out within a few days, those gels with incorporated PLGA microspheres retained the HAs for periods of more than a few

Figure 6. Effect of acidic additives (ie, HAs, PAA, and Nafion) on surface tension at the air-aqueous (water or PBS) interface. ($n = 5$, where n is the number of repeat experiments conducted. $P < .05$ shows significant difference between PVA/water and different additives as analyzed using Tukey's test.)

Table 1. Contact Angle and Zeta Potential Measurements for PLGA Microspheres As-prepared and with Acidic Additives Adsorbed on their Surfaces*

	PLGA MS	PLGA MS + Poly(acrylic acid)	PLGA MS + Humic Acids	PLGA MS + Nafion
Contact Angle (°)	60.9	67.1	71.7	82.9
± SD	9.14	7.63	2.63	1.43
Zeta Potential (mV)	-5.8	-27.8	-58.0	-69.3
± SD	2.13	2.74	2.32	3.46

*PLGA indicates poly(lactic-co-glycolic acid); MS, microspheres; and n = 10, where n is the number of repeat experiments conducted. $P < .05$ shows significant difference between PLGA MS and other groups as analyzed using Tukey's Multiple Comparison test.

months, as observed by their strong brownish coloration and the absence of dissolved HAs in the release media.

Variations in PVA-hydrogel crystallinity as a result of the presence of the various additives must be taken into consideration as this could affect their release profiles. Figure 7 illustrates the degree of crystallinity of dried PVA gels (after 3 freeze-thaw cycles) as a function of additives with concentrations proportional to those used in the in vitro release studies. With the exception of PAA, the remaining polyacids (HAs and Nafion) decrease PVA's crystallinity by about one third. This finding is currently attributed to either (1) the stronger acidity of Nafion and HAs with respect to PAA, and/or (2) the dramatically dissimilar polymer repeating units of Nafion and HAs as compared with PVA (whereas the backbone architectures for PAA and PVA are structurally similar). Upon incorporation of PLGA microspheres, there is no significant difference in PVA crystallinity within error. However, upon incorporation of all 3 polyacids into the PVA-hydrogel/PLGA-microsphere composites, there is a 10% to 20% increase in the degree of crystallinity. This increased crystallinity can only be explained by the PLGA surface activity of the polyacids, which prevents them from mixing with the PVA and lowering its crystallinity as observed in the absence of microspheres for HAs and Nafion. Furthermore, the increase in PVA crystallinity is in accordance with recent reports indicating that exposure of PVA to hydrophobic surfaces promotes microcrystal formation.⁴⁴

Among these polyacids, HAs-modified PVA hydrogels exhibit the highest degree of crystallinity (about 60%) in the presence of PLGA microspheres (see Figure 7). Although higher PVA crystallinity is expected to decrease dexamethasone diffusion away from the hydrogel, the order of magnitude higher release rate of dexamethasone from PVA hydrogels alone when compared with microsphere/hydrogel composites renders diffusion considerations irrelevant.

Two possible explanations for the faster drug release in the presence of HAs might be at play. First, HAs is a stronger acid than PAA, with pK_a values at (1) 3.2, and (2) 5.8

and 6.8 based on the presence of (a) aromatic carboxylic groups ortho to phenolic substitutions, and (b) weak aromatic and aliphatic carboxylic acids, respectively.²⁸ Since these polyacids induce PVA crystallinity (see Figure 7), the local vicinity at the microsphere/polyacid surface might experience lower pH than the surrounding hydrogel matrix, which is expected to be at pH 7.4 (PBS). Thus the multiple pK_a values of HAs, as compared with PAA, are expected to have an important role in maintaining a slightly stronger localized acidic environment in the immediate vicinity of the microspheres, thereby promoting faster PLGA surface erosion and hence increasing dexamethasone release rate. Second, HAs, but not PAA and Nafion, contain significant quantities of trace heavy metal ions (ie, Mn, 175 ppm; Ti, 164 ppm; Sr, 139 ppm; Ba, 90 ppm; V, 10 ppm; Zr, 9 ppm; Cu 6 ppm; and Cr, 2 ppm) that might catalyze the acid-assisted degradation of the PLGA microspheres.³⁹

Figure 7. Comparison of degree of crystallinity (as obtained from DSC) for dried samples of (1) as-fabricated PVA hydrogels (PVA); (2-4) PVA hydrogels containing PAA, HAs, and Nafion (Naf), respectively; (5) PVA hydrogels containing PLGA MS; and (6-8) PVA hydrogels containing PLGA MS along with PAA, Has, and Naf, respectively. ($N_{ft} = 3$; $n = 3$, where n is the number of repeat experiments conducted. $P < .05$ shows significant difference between as-prepared PVA hydrogels and PVA hydrogels modified with polyacids with/without MS as analyzed using Tukey's test.)

In order to further investigate the effects of polyacids on PLGA microsphere degradation, when embedded in PVA microgels, a morphological study was conducted using HAs as a model polyacid. Figures 8-A1 and 8-A2 illustrate low and high magnification ESEM images of nondegraded PLGA microspheres. As can be seen from these photomicrographs, there is a considerable particle size variation with a volume-weighted mean size of ~ 40 micrometers. The as-prepared microspheres appear round with a fairly smooth surface and no evidence of significant porosity. Following 4 weeks submersion in PBS buffer, (Figures 8-B1 and 8-B2), these microspheres show a significant degree of degradation. As expected, degradation proceeds faster internally than externally for the as-prepared microspheres in PBS buffer due to internal build up of acidic by-products that self-catalyze the PLGA degradation process, resulting in the formation of hollow cores.^{39,42} On the other hand, the PLGA microspheres embedded in PVA hydrogels, which have been immersed in PBS for 4 weeks, show similar or less external porosity with little internal degradation as evident from the fractured microspheres shown in Figures 8-C1 and 8-C2. This finding is consistent with the minimal drug release rate observed from these systems when embedded in neat PVA hydrogels (shown in Figure 7). However, in the case of PLGA microspheres that had been embedded in HAs-modified PVA hydrogels and immersed in PBS for 4 weeks, an entirely new surface morphology is witnessed. As can be seen in Figure 8-D1, these microspheres exhibit significant surface pitting, which appears as crater-like dimples a few micrometers in diameter. This finding is in agreement with HAs surface association (presented above), followed by pronounced surface catalyzed degradation. Degradation also occurs in the interior of these microspheres (see Figure 8-D2), and although it is not as extensive as in the case of the non-PVA embedded systems, it is clearly greater than in the absence of polyacids.

CONCLUSION

A study was performed on PVA hydrogels to delineate their structure-property relationships in conjunction with their in vitro release performance using dexamethasone as a model drug. The physico-chemical and mechanical properties of these gels (ie, modulus, swelling, crystallinity, and drug release) was dependent on the number of freeze-thaw cycles (N_{ft}) used to physically cross-link the PVA matrix and was determined to plateau at $\sim 3 N_{ft}$. These gels, in their dried state exhibit sufficient hardness to withstand the forces associated with handling and implantation and rapidly hydrate and swell to c.a. 350% in aqueous environments, resulting in pliable gels with Young's modulus comparable with that of soft human tissue (0.1 to 4 MPa).

Figure 8. ESEM photomicrographs of PLGA microspheres: (A1, A2) as-prepared, nondegraded, no-gel; (B1, B2) after 4 weeks submersion in PBS; (C1, C2) embedded in PVA gel, no additives after 4 weeks submersion in PBS; and (D1, D2) embedded in PVA gel with humic acids after 4 weeks submersion in PBS.

When dexamethasone was incorporated into plain PVA hydrogels, 80%-100% release was attained within 2 weeks depending on the number of N_{ft} cycles. On the other hand, PLGA microsphere-encapsulated dexamethasone, embedded within PVA hydrogels, resulted in minimal release

(approximately 6%) over a period of 1 month. Incorporating polyacids within gels containing PLGA microspheres resulted in an order of magnitude increase in the dexamethasone release rate (to 60% to 75% release over a 1-month period). Polyacid-induced acceleration of drug release was linked to the surface activity of these polyacids towards PLGA microspheres. This appeared to cause a localized increase in acidity in the vicinity of the microsphere surfaces, which in turn appeared to alter their degradation characteristics from an inside-out (in the case of PLGA microspheres alone) to an outside-inwards model when embedded in polyacid modified PVA hydrogels. These systems show potential for a variety of localized controlled drug delivery applications, such as biocompatible coatings for implantable devices.

ACKNOWLEDGMENTS

The authors thank the NIH (grant no. 1R01RR14171), the Juvenile Diabetes Research Foundation International (grant no. JRFI-5-2001-687), and the Patterson Trust Foundation for financial support of this research.

REFERENCES

- Hassan CM, Peppas NA. Structure and applications of poly(vinyl alcohol) hydrogels produced by conventional cross-linking or by freezing/thawing methods. *Adv Polym Sci*. 2000;153:37-65.
- Hassan CM, Stewart JE, Peppas NA. Diffusional characteristics of freeze/thawed poly(vinyl alcohol) hydrogels: applications to protein controlled release from multilaminate devices. *Eur J Pharm Biopharm*. 2000;49:161-165.
- Cascone MG, Zhu Z, Borselli F, Lazzeri L. Poly(vinyl alcohol) hydrogels as hydrophilic matrices for the release of lipophilic drugs loaded in PLGA nanoparticles. *J Mater Sci Mater Med*. 2002;13:29-32.
- Peppas NA, Merrill EW. Differential scanning calorimetry of crystallized PVA hydrogels. *J Appl Polym Sci*. 1976;20:1457-1465.
- Peppas NA, Merrill EW. Determination of interaction parameter χ_1 for PVA and water in gels cross-linked from solutions. *J Polym Sci Polym Chem Ed*. 1976;14:441-464.
- Danno A. Gel formation of aqueous solutions of poly(vinyl alcohol) irradiated with a cobalt-60 source. *J Phys Soc Jpn*. 1958;13:722-727.
- Yokoyama F, Masada I, Shimamura K, Ikawa T, Monobe K. Morphology and structure of highly elastic poly(vinyl alcohol) hydrogel prepared by repeated freezing and melting. *Coll Polym Sci*. 1986;264:595-601.
- Lozinsky VI. Cryotropic gelation of poly(vinyl alcohol) solutions. *Russ Chem Rev*. 1998;67:573-586.
- Tamura K, Nakamura T, Okada K. New hydrogel from poly(vinyl alcohol) and its fundamental study for medical applications. *Jap J Artif Organs*. 1984;13:1197-1201.
- Tamura K, Ike O, Hitomi S, Isobe J, Shmizu Y, Nambu M. A new hydrogel and its medical application. *Trans Am Soc Artif Intern Organs*. 1986;32:605-608.
- Stauffer SR, Peppas NA. Poly(vinyl alcohol) hydrogels prepared by freezing-thawing cycling processing. *Polym*. 1992;33:3932-3936.
- Lozinsky VI, Vainerman ES, Domotenko LV, et al. Study of cryostructurization of polymer systems. VII. Structure formation under freezing of poly(vinyl alcohol) aqueous solutions. *Coll Polym Sci*. 1986;264:19-24.
- Bao QB, McCullen GM, Higham PA, Dumbleton JH, Yuan HA. The artificial disc: theory, design and materials. *Biomaterials*. 1996;17:1157-67.
- Ushijima M, Oka M, Hyon S-H, et al. Study on the improvement of wear-resistant properties of artificial articular cartilage. *Seitai Zairyo*. 1996;14:201-204.
- Oka M, Ushio K, Kumar P, et al. Development of artificial articular cartilage. *Proc Inst Mech Eng [H]*. 2000;214:59-68.
- Kuriaki M, Nakamura K, Mizutani J. Application of transparent poly(vinyl alcohol) gel to contact lens. *Kobunshi Ronbunshu*. 1989;46:739-743.
- Lozinsky VI, Zubov AL, Titova EF. Poly(vinyl alcohol) cryogels employed as matrices for cell immobilization. 2. Entrapped cells resemble porous fillers in their effects on the properties of PVA-cryogel carrier. *Enzyme Microb Technol*. 1997;20:182-190.
- Lozinsky VI, Plieva FM. Poly(vinyl alcohol) cryogels employed as matrices for cell immobilization. 3. Overview of recent research and developments. *Enzyme Microb Technol*. 1998;23:227-242.
- Mongia NK, Anseth KS, Peppas NA. Mucoadhesive poly(vinyl alcohol) hydrogels produced by freezing/thawing processes: applications in the development of wound healing systems. *J Biomater Sci Polym Ed*. 1996;7:1055-1064.
- Peppas NA, Mongia NK. Ultrapure poly(vinyl alcohol) hydrogels with mucoadhesive drug delivery characteristics. *Eur J Pharm Biopharm*. 1997;43:51-58.
- Takamura A, Ishii F, Hidaka H. Drug release from poly(vinyl alcohol) gel prepared by freeze-thaw procedure. *J Control Release*. 1992;20:21-27.
- Patil SD, Papadimitrakopoulos F, Burgess DJ. Dexamethasone-loaded PLGA Microspheres/PVA Hydrogel Composites for Inflammation Control. *Diabetes Technology and Therapeutics*. 2004;6:887-897.
- Bourke SL, Al-Khalili M, Briggs T, Michniak BB, Kohn J, Poole-Warren LA. A photo-crosslinked poly(vinyl alcohol) hydrogel growth factor release vehicle for wound healing applications. *AAPS PharmSci*. 2003;5(4):E33.
- Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. *Int J Pharm*. 2004;282:1-18.
- Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric Systems for Controlled Drug Release. *Chem Rev*. 1999;99:3181-3198.
- Kim T-K, Burgess DJ. Formulation and release characteristics of poly(lactic-co-glycolic acid) microspheres containing chemically modified protein. *J Pharm Pharmacol*. 2001;53:23-31.
- Hickey T, Kreutzer D, Burgess DJ, Moussy F. Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices. *Biomaterials*. 2001;23:1649-1656.
- Galeska I, Hickey T, Moussy F, Kreutzer D, Papadimitrakopoulos F. Characterization and biocompatibility studies of novel humic acid based films as membrane material for an implantable glucose sensor. *Biomacromolecules*. 2001;2:1249-1255.
- Wershaw RL. *Humic Substances II. In Search of Structure*. New York, NY: John Wiley & Sons; 1989.

30. Stevenson FJ. *Humus Chemistry. Genesis, Composition, Reactions*. New York, NY: John Wiley & Sons; 1994.
31. Peppas NA, Hansen PJ. Crystallization kinetics of poly(vinyl alcohol). *J Appl Polym Sci*. 1982;27:4787-4797.
32. Urushizaki F, Yamaguchi H, Nakamura K, Namajiri S, Sugibayashi K, Morimoto Y. Swelling and mechanical properties of poly(vinyl alcohol) hydrogels. *Int J Pharm*. 1990;58:135-142.
33. Lozinsky VI, Zubov AL, Titova EF. Swelling behavior of poly(vinyl alcohol) cryogels employed as matrixes for cell immobilization. *Enzyme Microb Technol*. 1996;18:561-569.
34. Takamura A, Ishii F. Preparation of freeze-thaw poly(vinyl alcohol) emulsion gel suppository. *Yakugaku Zasshi*. 1987;111:45-50.
35. Watase M, Nishinari K. Rheological and DSC changes in poly(vinyl alcohol) gels induced by immersion in water. *J Polym Sci: Polym Phys Ed*. 1985;23:1803-1811.
36. Nielsen LE, Landel RF. Particulate-filled polymers. In: Landel RF, ed. *Mechanical Properties of Polymers and Composites*. 2nd ed. New York, NY Marcel Dekker; 1994;377-460.
37. Bucknall CB. Applications of microscopy to the deformation and fracture of rubber-toughened polymers. *J Microsc*. 2001;201:221-229.
38. Yamada H. *Strength of Biological Tissues*. Baltimore, MD: Williams & Wilkins, 1970.
39. Vert M, Li S, Garreau H, et al. Complexity of the hydrolytic degradation of aliphatic polyesters. *Angew Makromol Chem*. 1997;247:239-253.
40. Li SM, Garreau M, Vert H. Structure-property relationships in the case of the degradation of massive aliphatic poly-(α -hydroxy acids) in aqueous media, part 1: poly(DL-lactic acid). *J Mater Sci Mater Med*. 1990;1:123-130.
41. Li SM, Garreau H, Vert M. Structure-property relationships in the case of the degradation of massive poly(α -hydroxy acids) in aqueous media. Part 2. Degradation of lactide-glycolide copolymers: PLA37.5GA25 and PLA75GA25. *J Mater Sci Mater Med*. 1990;1:131-139.
42. Fu K, Pack DW, Klibanov AM, Langer R. Visual evidence of acidic environment within degrading poly(lactic-co-glycolic acid) (PLGA) microspheres. *Pharm Res*. 2000;17:100-106.
43. Gierke TD, Hsu WY. Perfluorinated ion exchange membranes. In: Yeager HL. *Perfluorinated Ionomer Membranes*. Washington, DC: American Chemical Society; 1982;283-307.
44. Kozlov M, Quarmyne M, Chen W, McCarthy TJ. Adsorption of Poly(vinyl alcohol) to Hydrophobic Substrates: A General Approach for Hydrophilizing and Chemically Activating Surfaces. *Macromolecules*. 2003;36:6054-6059.