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Poly(anhydride-ester) and Poly(N-vinyl-2-pyrrolidone) Blends: Salicylic acid-releasing blends with hydrogel-like properties that reduce inflammation

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Abstract

Polymers such as poly(N-vinyl-2-pyrrolidone) (PVP) have been used to prepare hydrogels for wound dressing applications but are not inherently bioactive. For enhanced healing, the release of physically admixed therapeutics from hydrogels has been evaluated, but with limited control over drug release profiles. To overcome these limitations, PVP was blended with salicylic acid-based poly(anhydride-esters) (SAPAE) and shown to exhibit hydrogel properties upon swelling. In vitro release studies demonstrated that the chemically incorporated drug (SA) was released from the polymer blends over 3–4 days in contrast to 3 hours, as observed with diffusion-controlled hydrogels. Generally, blends of higher PVP content displayed greater swelling values and faster

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SA release. The polymer blends significantly reduce the inflammatory cytokine, TNF-α, in vitro without cytotoxic or anti-proliferative effects, further demonstrating their potential as a wound dressing with enhanced healing and decreased scar tissue formation.

Keywords

Biodegradable; poly(anhydride-esters); hydrogel; drug delivery system; blending

1. Introduction

Hydrogels are crosslinked networks comprised of polymers, such as poly(N-vinyl-2-pyrrolidone) (PVP) (Figure 1A), that imbibe large amounts of water without dissolving due to the presence of physical (e.g., intermolecular interactions and entanglements) or chemical crosslinks (e.g., covalent bonding) [1–5]. These systems are becoming increasingly important for a variety of biomedical applications [1, 6–8] because they are biocompatible and have properties similar to those of natural living tissue given their high water content and pliable consistency [2, 9]. Hydrogels are particularly suitable as wound dressing materials due to their intrinsic softness, flexibility, adhesion to healthy skin without affecting the wound bed, and ability to prevent nerve-ending exposure, thereby decreasing pain [7, 10].

PVP is commonly used to prepare hydrogels due to its mechanical and water-absorption properties when chemically crosslinked [5, 11]. One major drawback, however, is that PVP alone does not exhibit inherent bioactivity and thus cannot reduce inflammation associated with wounds. The ability for wound dressings to address such inflammation and prevent scarring would be advantageous, as prolonged inflammation and proliferative phases of the wound healing process greatly increase the occurrence of hypertrophic and keloid scars [12].

To combat issues associated with inflammation, researchers have investigated combined hydrogel therapies that utilize bioactives. One such example includes a topical therapy in which salicylic acid (SA), a non-steroidal anti-inflammatory drug, was admixed with a hydrogel dressing and shown to be efficient in reducing hypertrophic scars [13]. Additionally, researchers have developed PVP/poly(vinyl alcohol) (PVA) membranes with admixed SA to control drug release and mitigate systemic side effects [14]. Utilizing these PVP blends as controlled drug delivery systems is limited by the relatively rapid SA release, where >50% SA was released within the first 4 hours, presumably due to the high water content and large pore size [1, 14]. Ideally, the drug release profile would be tunable to deliver a nearly instantaneous dosage followed by sustained drug release to maintain drug concentrations at therapeutic levels without causing toxicity issues[15].

SA-based poly(anhydride-esters) (SAPAEs) are innovative degradable materials that have been evaluated over the past decade for SA delivery [16–18]. Compared to other biodegradable delivery systems, they are unique in that the bioactive molecules are chemically incorporated into the polymeric backbone via linker molecules (Figure 1B), rather than physically admixed [19–21], leading to higher drug loading (up to 85%) [17]. Moreover, upon hydrolytic degradation, SA and biocompatible linker molecules are released in a controlled, sustained manner [17] contributing to their utility as drug delivery systems.

Despite SAPAEs' inherent bioactivity upon degradation and controlled SA release profiles, they can be brittle and lack the swelling properties necessary to form hydrogels. An approach that combines the bioactivity of SAPAE with the hydrogel characteristics of PVP was investigated.

Herein, various ratios of PVP and SAPAE were blended to generate materials with hydrogel behavior as well as sustained SA delivery when hydrated. SAPAE enables high SA loading and sustained release of SA, whereas PVP provides the mechanical properties by forming a soft material with increased plasticity and hydrophilicity. After the two polymers are blended, characterization and degradation studies on the blended films were performed. Among many potential applications of these polymer blends, a bioactive hydrogel-like material that reduces inflammation and prevents scar formation is a highly desirable "smart" wound dressing.

2. Experimental Section

2.1. Materials

Acetic anhydride and hydrochloric acid were purchased from Fisher (Fair Lawn, NJ). The Poly(N-vinyl-2-pyrrolidone) K90 (Mw 360,000), salicylic acid (ACS reagent ≥99,0%) tetrahydrofuran (anhydrous, ≥99,9%, inhibitor-free), pyridine (anhydrous, 99,8%), adipoyl chloride (98%), triethylamine (≥99,9%), triphosgene (98%), dichloromethane (anhydrous, ≥99,8%), N,N-dimethylformamide (anhydrous, 99,8%) and chloroform (anhydrous, 99,8%) used to produce the polymeric blends were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received. Poly[1,6-bis(o-carboxyphenoxy)-hexanoate] (SAPAE) was prepared according to method previously described [22].

2.2. Formulation of Blended Polymer Films

Solutions of PVP were stirred for 48 hours in anhydrous dimethylformamide/anhydrous chloroform (1:1 v/v) at room temperature. SAPAE of varying amounts (Table 1) were each added separately to the PVP solutions and stirred for 24 hours. These blends were prepared at 3 different ratios of 7:3, 6:4, and 5:5 PVP:SAPAE. The resulting solutions (15 % w/v total polymer, 1 mL) were cast onto Teflon plates (28 mm diameter) and dried in a vacuum oven to form films (n=3). Miscibility analyses were performed via scanning electron microscopy (SEM) and differential scanning calorimetry (DSC). SEM images were obtained using an AMRAY-1830I microscope (AMRAY Inc.) after films were dried in a vacuum, stored under N_2, and coated with Au/Pd using a sputter coater (SCD 004, Blazers Union Limited). DSC measurements were carried out on TA Instrument Q200 to determine glass transition (T_g) temperatures of the blended films. Measurements on samples (4–6 mg) heated under nitrogen atmosphere from −10 °C to 200 °C at a heating rate of 10 °C/min and cooled to −10 °C at a rate of 10 °C/min with a two-cycle minimum were performed. TA Instruments Universal Analysis 2000 software, version 4.5A was used to analyze the data. The Fox equation, Equation (1), was used for this binary system to calculate the predicted T_g values for the blends where $T_{g,blend}$ pertains to the miscible blend, $T_{g,i}$ to the noted pure components, and w_i to the weight fraction of noted component *i*.

$$\frac{1}{T_{g,blend}} = \frac{W_{PVP}}{T_{g,PVP}} + \frac{W_{SAPAE}}{T_{g,SAPAE}} \quad \text{Equation (1)}$$

2.3. Swelling Values of Blended Polymer Films

The films were immersed in deionized water for 2, 6, and 24 hours (separately) at room temperature. The first time point (2 hours) was chosen as this was the time at which SA release reached detectable levels. Swelling values (Q) were calculated according to Equation (2), where w_s and w_d represent the weight of swollen and dried films, respectively.

$$Q = \frac{W_s - W_d}{W_d} \quad \text{Equation (2)}$$

2.4. Rheological Testing and Mechanical Properties

Differences in mechanical properties of the films were monitored using oscillatory rheology. Oscillatory time sweep experiments were carried out on PVP:SAPAE films of three different blend ratios of 7:3, 6:4, and 5:5 PVP:SAPAE. Each time sweep step was carried out for 9 hours. For each ratio, a film was soaked in deionized water for 2 hours prior to the measurements to swell the films. Then, the film was removed from the water and placed between the upper plate and the lower Peltier plate of a TA Instruments ARG2 stress-controlled rheometer. A stainless steel serrated parallel plate geometry (20 mm) was chosen as the upper plate. Samples (20 mm diameter) were used. Water evaporation from the swollen blended polymer films was prevented by covering the sides of the plate with low viscosity mineral oil. After completion of the oscillatory time sweep step, dynamic frequency sweep and dynamic strain sweep tests were also carried out in order to observe blend properties after a period of water degradation and to confirm that the time sweep measurements were performed in the linear viscoelastic regime, respectively. The gap height used for the experiments was 400 μm. The time sweep measurements were carried out at a constant angular frequency of 6 rad/s and strain of 1 %. The frequency sweep measurements were carried out at a constant oscillatory strain of 1 % and the strain sweep measurements were carried out a constant oscillatory frequency of 6 rad/s.

2.5. In Vitro Salicylic Acid Release

To determine whether the chemically incorporated SA is advantageous over physically incorporated SA, PVP:SAPAE films were used to represent the chemically incorporated SA systems. An inactive control polymer (ICP), a SA-based poly(anhydride-ether) (Figure 2), was used to prepare PVP:ICP blended films at 7:3, 6:4, and 5:5 ratios. These control polymers were similarly prepared to PVP:SAPAE films in that ICP replaces SAPAE in the formulation. Additionally, the theoretical SA amount for each ratio (see Table 1) was added with the appropriate amount of ICP to the solution 24 hours before casting. Specifically, for the PVP:ICP ratios, 0.163, 0.217, 0.271 g of SA were added to the 7:3, 6:4, and 5:5 ratios, respectively.

Films (80 ± 5 mg) were immersed in 20 mL glass scintillation vials containing 10 mL phosphate buffered saline (PBS) at pH 7.40. The vials were stored at 37 °C with agitation at 60 rpm in a controlled environment incubator shaker (New Brunswick Scientific Excella E25) to mimic physiological conditions. A 5 mL aliquot of the spent media was removed and replaced with 5 mL of fresh PBS every 24 hours to ensure sink conditions. The spent media was analyzed by UV spectrophotometry using a Perkin Elmer Lambda XLS spectrophotometer to monitor SA release. Measurements were obtained at $\lambda = 303$ nm, the maximum SA absorbance that did not overlap with PVP or other polymer degradation products. Data were calculated against a calibration curve from standard SA solutions in PBS. All sets of experiments were performed in triplicate, carried out until drug release was no longer detected, and normalized to 100 %.

2.6. TNF-α Secretion Assay

Human blood-derived monocytes (Blood Center of New Jersey) were used to determine the efficacy to decrease inflammatory cytokine secretion. The cell isolation and purification protocol used was previously described by Kim et al. (2009) [23]. Briefly, peripheral blood mononuclear cells were collected from blood of healthy donors by density gradient separation using ficol at a density of 1.077 (GE Healthcare, Piscataway, NJ). Red blood cells were lysed by incubation in Ammonium-Chloride-Potassium (ACK) lysing buffer for 5 min, washed with PBS and counted. Monocytes were cultured on 175 cm^2 flasks (BD, Franklin Lakes, NJ) at a concentration of 8×10^6 cells/mL in RPMI-1640 media (GIBCO BRL, Rockville, MD). RPMI media was supplemented with 10 % fetal bovine serum (FBS) (GIBCO BRL), 100 units/mL penicillin (GIBCO BRL), 100 ug/mL streptomycin (GIBCO BRL) and 400 mM L-glutamine (GIBCO BRL). Monocytes were allowed to adhere for 2 hours and then washed 3 times with PBS to remove non-adherent cells. Monocytes were cultured for 7 days at 37 °C and 5 % CO_2 in RPMI supplemented with 5 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) (R&D Systems, Minneapolis, MN) to generate macrophages. After 7 days of culture, macrophages were washed once with PBS and then detached with trypsin-EDTA (GIBCO) for 30 minutes at room temperature. Cells were re-suspended in culture medium (RPMI), counted, re-plated at 8×10^3 cells/well in a 96 well plate, and allowed to attach overnight. The following day, the media was replaced with the various sample groups: polymer-containing media (0.1 mg/mL dissolved PVP or PVP:SAPAE blend, 10 ng/mL lipopolysaccharide (LPS), 1% DMSO), a positive control (10 ng/mL LPS, 1% DMSO), and a negative control (no LPS, 1% DMSO). All cell studies were performed in triplicate. TNF-α secretion was achieved by activation of the macrophages with LPS (10 ng/mL). After 48 hours, media was collected and TNF-α secretion was determined with an ELISA kit against human TNF-α (BioLegend, San Diego, CA). A CellTiter 96®AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) was used to ensure that differences in cell viability did not account for differences in TNF-α secretion.

2.7. In Vitro Cytotoxicity and Proliferation Assay

Cytocompatibility of the polymer blends was performed by culturing NCTC clone 929 (strain L) mouse areolar fibroblast cells (ATCC, Manassas, Virginia) in media containing the dissolved polymer blends. These L929 fibroblast cells are a standard cell type for

cytocompatibility testing as recommended by ASTM [24]. The polymer blends were dissolved in dimethyl sulfoxide (10 mg/mL; DMSO, Sigma, St. Louis, MO) as a stock solution and serially diluted with cell culture media to a 0.1 mg/mL concentration). Cell culture media consisted of Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, St. Louis, MO), 10 % v/v fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 1 % L-glutamate (Sigma) and 1 % penicillin/streptomycin. The polymer-containing media was distributed into a 96-well plate and seeded at an initial concentration of 2,000 cells per well. The media with dissolved polymer was compared to DMSO-containing media (1 % v/v) and media alone. For the L929 fibroblasts, cell viability was determined by using a CellTiter 96®AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) at 24, 48, and 72 hours. After 2 hours incubation, the absorbance was recorded with a microplate reader at λ = 490 nm. Cell numbers were calculated based upon a standard curve created 24 hours after original seeding.

2.8 Statistical Analysis

Cell studies were conducted in experimental triplicate. The results were then evaluated using a one-way ANOVA followed by Bonferroni's all-pairs comparison, with significance criteria assuming a 95% confidence level ($P < 0.05$). Standard deviation is reported in the form of error bars on the graphs of the final data.

3. Results and Discussion

These polymer blends exhibit hydrogel properties over several days: the materials first swell in water, the SAPAE component begins to hydrolytically degrade, the PVP begins to solubilize, and the hydrogel films eventually fall apart and/or solubilize. This time window is advantageous (e.g., hours to days) for topical wound care applications. As polymeric materials that swell until dissolution, similar behavior is observed in physical hydrogels that are not covalently crosslinked.[25] While most recently published hydrogel work involves molecular design of specific covalent or physical crosslinks [26], the PVP-SAPAE hydrogel-like films are simply prepared by blending in organic solvents.

3.1. Formulation of PVP:SAPAE Blended Films

SAPAEs were blended with PVP and cast into films at ratios of 7:3, 6:4, and 5:5 PVP:SAPAE. Additional crosslinking methods (i.e., ultraviolet or gamma irradiation) were not necessary to generate hydrogel-like materials because the films became soft, stable three-dimensional structures upon swelling in water. This structural stability decreased after immersion in water; hydrolytic degradation of the SAPAEs yields completely dissociated films over 3 weeks at 25 °C and 6 days at elevated temperatures (37 °C). The lack of solid residues and complete PVP solubilization also indicate the physical interaction between SAPAE and PVP.

To elucidate the blend structures, polymer miscibility was determined on the copolymer blends. The surface morphology was homogenous; as observed in the SEM images (Figure 3A–C) of the non-hydrated films, all three blend ratios exhibit uniform morphologies without phase separation or aggregation, clearly indicating polymer miscibility.

Correspondingly, the DSC results for pure polymers and blends are summarized in Figure 4 as “experimental”. DSC analyses showed single, intermediary T_g values for the blends relative to the pure polymer values. Figure 4 demonstrates the behavior of the measured T_g against PVP weight fraction and compares values predicted by Fox analytical equation for a binary system [27]. In short, the T_g increases with increasing PVP content. Fox’s equation proves a relationship between the individual homopolymers and respective blends’ T_g values. Accordingly, the measured T_g values are very close to the values predicted for a miscible polymer blend. These findings support the miscibility nature of the two polymers.

3.2. Swelling Values of PVP:SAPAE Blended Films

When comparing swelling values, the PVP concentration affected swelling; higher PVP content led to higher swelling values as demonstrated by monitoring film thickness before and after swelling in water (Table 2). This effect is likely due to the PVP hydrophilicity and its ability to attract water. Notably, PVP alone dissolves in an aqueous medium; yet when blended with SAPAE, PVP dissolution occurs at a much slower rate, as SAPAE interacts with the PVP via physical interactions.

To formulate a physically crosslinked hydrogel-like material, entanglements and intermolecular interactions between PVP and SAPAE must take place. Fourier transform infrared spectroscopy analyses of the blends (not shown) indicated that dipole-dipole interactions were not responsible for the miscibility, as no clear signs of absorption band shifting were observed. Therefore, it is proposed that hydrophobic interactions are likely responsible for the polymer miscibility. As such, the observed swelling values (compared to immediate dissolution) may result from the intermolecular interactions between the hydrophobic portion of PVP and the hydrocarbon chains of SAPAE.

3.3. Rheological Testing and Mechanical Properties

Results from the oscillatory rheological experiments show distinct differences in the mechanical properties of polymer films at the three different blending ratios PVP:SAPAE (7:3, 6:4 and 5:5). Stiffer hydrogel-like films displayed enhanced structural integrity, which is useful for applications such as drug delivery implants or patches, as they are less susceptible to displacement or site removal by biological fluids.

The time sweep measurements show a steady, uniform decline in the storage modulus, or G′ (Pa), values (Figure 5). The G′ (Pa) is an indicator of the elastic or solid nature of the hydrogel-like blended films. The water imbibed by the films during soaking for two hours brings about this effect. The initial values of G′ (Pa) for 7:3, 6:4, and 5:5 PVP:SAPAE films are around 600 Pa, 6000 Pa, and 50,000 Pa. Thus, an order-of-magnitude increase in stiffness is observed for steadily increasing SAPAE concentrations. Comparing the two components, SAPAE is more hydrophobic relative to PVP. This aspect likely leads to more intramolecular and intermolecular interactions among the SAPAE molecules, in turn, leading to more tightly bound networks for samples with higher SAPAE concentrations and stiffer polymer blends with high values of G′ (Pa). The exponential decrease in the G′ (Pa) values can be attributed to the degradation of the SAPAE molecules. As the SAPAE is susceptible to degradation by the entrapped water in the film, the steady uniform decline in

the stiff or elastic nature of the blended films is attributed to SAPAE degradation. At the end of the time sweep measurements, the frequency sweep measurements carried out on all three films indicate some dependence of G′ on applied angular frequency. This data indicates that the gel-like nature of the films reduces over time, as the gels begin to lose their integrity over a period of days at room temperature. The initial and final values of G′ are directly related to the PVP content in the films. This observation of mechanical properties correlates with the swelling ratio values, i.e., higher PVP content provides a higher swelling ratio and more flexible blended polymer films. The correlation between composition and mechanical integrity is appropriate for the PVP/SAPAE. In brief, we hypothesize that the decreases in storage modulus is due to the degradation of SAPAE in the blends. For all samples the observed values of G″(Pa) ≪ G′(Pa) showing that after the water absorption the materials behave as gels. Hence, these materials behave as gel-like solids lacking flow characteristics, and the evaluation of their shear rate and/or strain dependence on viscosity does not provide relevant information.

3.4. In Vitro Salicylic Acid Release

The polymer blends presented herein have properties suitable for a wound dressing, but would also be beneficial as a drug delivery system if SA were released in a controlled manner. Therefore, the PVP:SAPAE blends' in vitro SA release rates were analyzed. The hydrogel-like polymer blends exhibited 100 % SA release over 3–4 days (Figure 6). The correlation between the PVP content and SA released per day was observed; as PVP content increased, SA release rate increased. The release kinetics can be compared by the slope of release curve, where 7:3 > 6:4 > 5:5. The 7:3 polymer blends reached 100 % SA release by day 3, whereas ratios 6:4 and 5:5 released 100 % SA by day 4. After 3–4 days, no residual polymer films were visible and no SA was detected in the degradation media. The SA release curves correlate with the swelling values in Table 1: higher swelling values correspond to faster SA release rates. Swelling values are a measure of water uptake by each film; therefore, higher swelling values correlate with higher hydrophilicity and higher water diffusion through the blend. Moreover, SA release rates are expected to be dependent on water contact with hydrolytically labile anhydride and ester bonds in the SAPAEs.

The PVP:ICP blends with admixed SA were used as a control to ascertain whether the chemical incorporation of SA via SAPAEs is truly advantageous over physical incorporation for sustained SA release. Note that the ICP has an ether bond (Figure 2) relative to the hydrolysable ester bond of SAPAE; otherwise, the polymers are similar. Moreover, the UV spectrum of the ICP's degradation product (diacid, Figure 2) does not overlap with the spectra of free SA. As expected, the physically mixed SA was released within 3 hours (Figure 7) from the PVP:ICP films compared to 3–4 days for PVP:SAPAE samples, demonstrating the advantage to using the SAPAEs over a solely diffusion-controlled delivery system.

3.5. TNF-α Secretion Assay

Human blood-derived macrophages were activated by LPS to elicit the secretion of TNF-α. Dissolved blended polymer films (0.1 mg/mL) were monitored for their effect on the amount of secreted TNF-α (Figure 8). The PVP sample increased the amount of TNF-α

secreted when compared to the LPS control. This increase in inflammatory cytokines in the presence of high molecular weight PVP is consistent with previous literature [28]. The SAPAE hydrogel-like polymer blends showed a reduction in TNF-α, compared to the PVP alone with the 5:5 and 6:4 ratios exhibiting a significant decrease in TNF-α secretion when compared to the LPS control. When correlated with drug loading, the SAPAE blends inhibited TNF-α secretion in a dose-dependent manner, with greater amounts of SA resulting in lowered TNF-α. Cell viability was determined by MTS assay to ensure that differences in TNF-α secretion were not due to differences in cell number; no statistical differences were found in cell viability between the groups (data not shown).

3.6. In Vitro Cytotoxicity and Proliferation Assay

While the TNF-α studies indicated that the dissolved films were not toxic to macrophages at 48 hours, the effect on other relevant cell types needed to be studied. L929 fibroblasts were used to determine the PVP:SAPAE blends' effect on cytotoxicity and cell proliferation. Fibroblasts were chosen for several reasons including: the broad use of fibroblasts by many research teams; the broad applicability of fibroblasts, particularly in wound healing applications; and our long-track record using these cells to assess new materials. While we recognize that this cell line is not ideal for the final application, it is appropriate as a cytotoxicity screening tool for material libraries. The cells were cultured in the presence of dissolved polymers for three days. At 0.1 mg/mL, a concentration at which anti-inflammatory effects were observed, the polymer blends were not statistically different from the DMSO controls (1%) in terms of both cell viability and proliferation (Figure 9). This study indicates that these hydrogel-like materials would be effective as wound dressings without adversely affecting healing. It is also important to consider that all polymeric decomposition products are bioactive and/or biocompatible, eg. PVP, SA and adipic acid, and relevant/appropriate for wound dressings. In addition, the obtained results from TNF-α combined with in vitro cytotoxicity and proliferation assay demonstrate that, even in the presence of trace amounts of possible residual solvents from the preparation of the blended polymer films, the system does not present toxicity.

4. Conclusion

A molecularly blended polymer material that exhibits hydrogel properties after swelling and that can be simply prepared by blending can be advantageous. Formulation of SAPAEs with PVP resulted in miscible film blends that, upon hydration, present properties similar to those observed for physically crosslinked hydrogels at room temperature. These experiments demonstrate that the hydrogel-like polymer blends release SA, an anti-inflammatory and analgesic agent, in a controlled manner over a sustained time period. This blending and subsequent hydration of PVP and SAPAE provides a biocompatible drug delivery system with good handling and swelling capabilities, porosity, and sustained drug release without a burst release, thus providing a foundation for these blends as promising wound dressings. However, to infer that these polymer blends can effectively be used as an ideal wound dressing it becomes necessary to perform in vivo studies since the composition of wound fluid may interfere differently in the swelling process determined in vitro and therefore in the releasing profile of salicylic acid.

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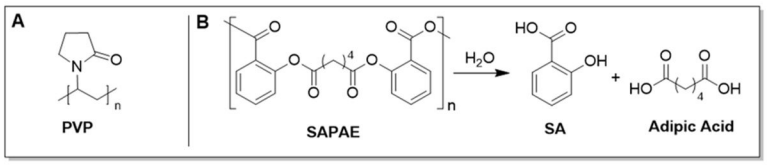

Figure 1.
Chemical structures of PVP (**A**) as well as SAPAE and its degradation products (**B**).

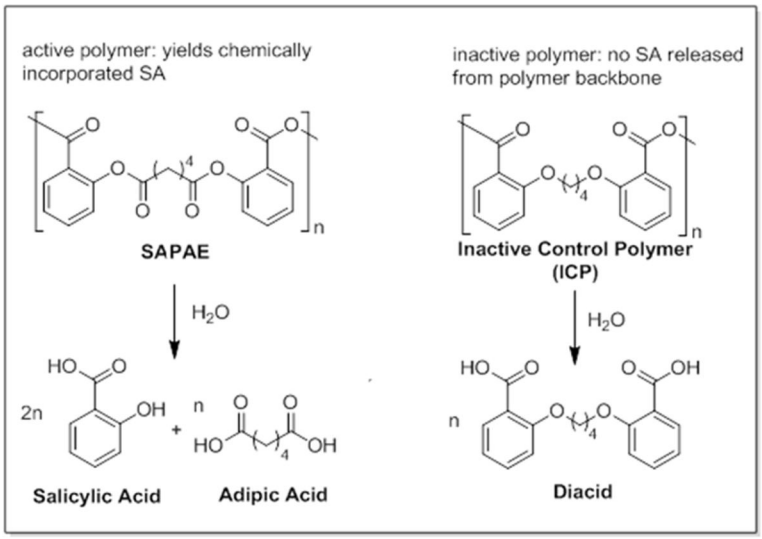

Figure 2.

Hydrolytic degradation of SAPAE, an active poly(anhydride-ester) that releases SA and adipic acid compared to the degradation of inactive poly(anhydride-ether), referred to as inactive control polymer (ICP).

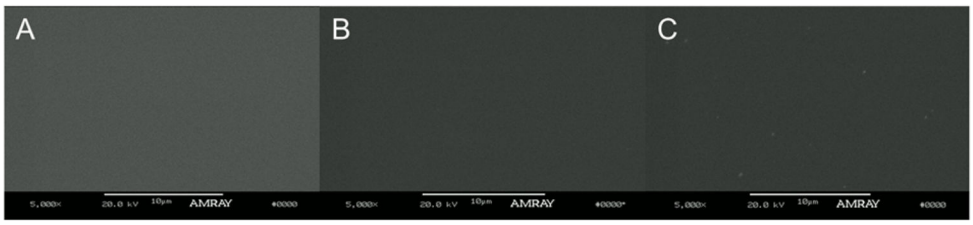

Figure 3.

SEM image of blended PVP:SAPAE 7:3 (A), 6:4 (B) and 5:5 (C) films prior to hydration at 5000x magnification with a homogenous morphology (no phase separation).

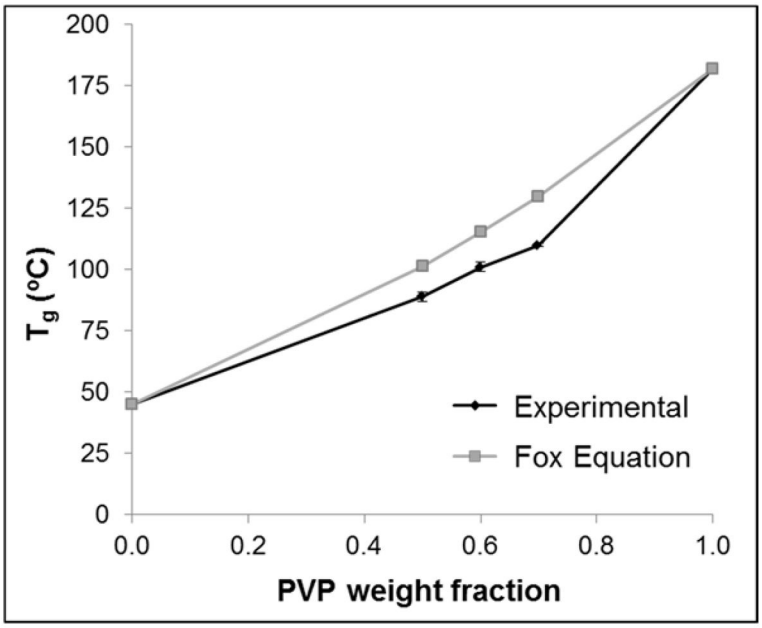

Figure 4.

Experimental glass transition temperatures for pure PVP, SAPAE and the copolymer blends as a function of PVP weight fraction compared to values predicted by the Fox equation.

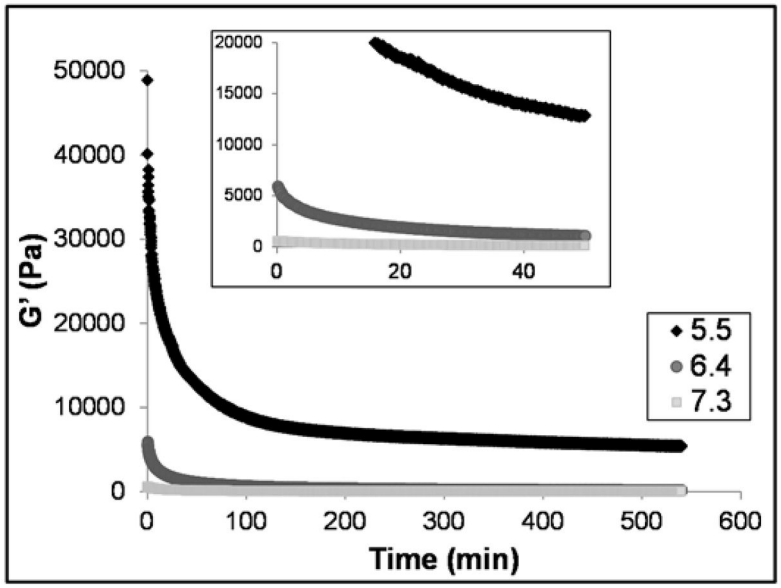

Figure 5.

For three different PVP:SAPAE ratios, storage modulus as monitored by time sweep measurements at 1 % strain and 6 rad/s angular frequency. The y-axis label on the inset graph is "G′ (Pa)" and x-axis label is "Time (min)".

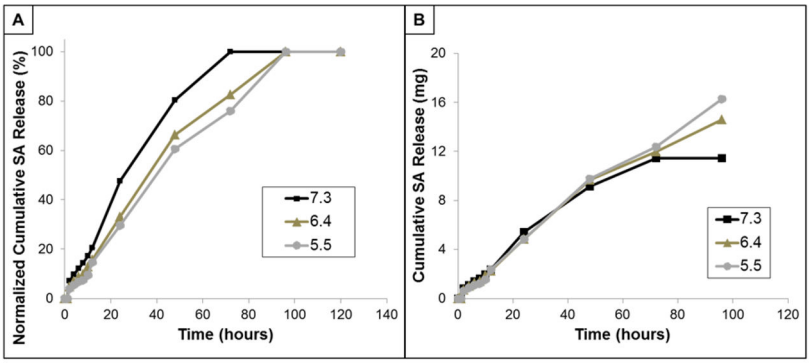

Figure 6.

At three different PVP:SAPAE ratios, in vitro SA release from blended polymer films represented as normalized cumulative SA release (**A**) and cumulative mass of SA (**B**). For each data point, three samples were measured and the averages graphed. The standard deviations were smaller than or equivalent to the data point symbols.

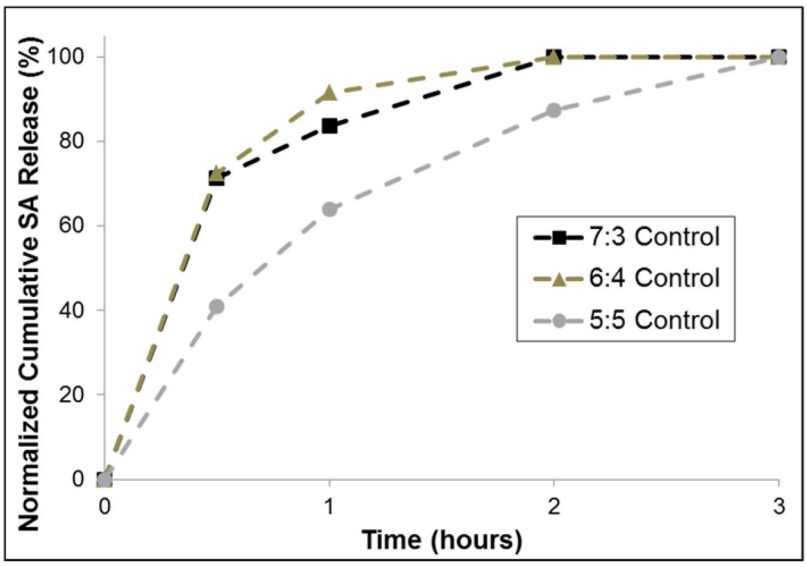

Figure 7.

SA was physically admixed into three ratios of PVP:ICP blends. For each data point, three samples were measured and the averages graphed. The standard deviations were smaller than or equivalent to the data point symbols.

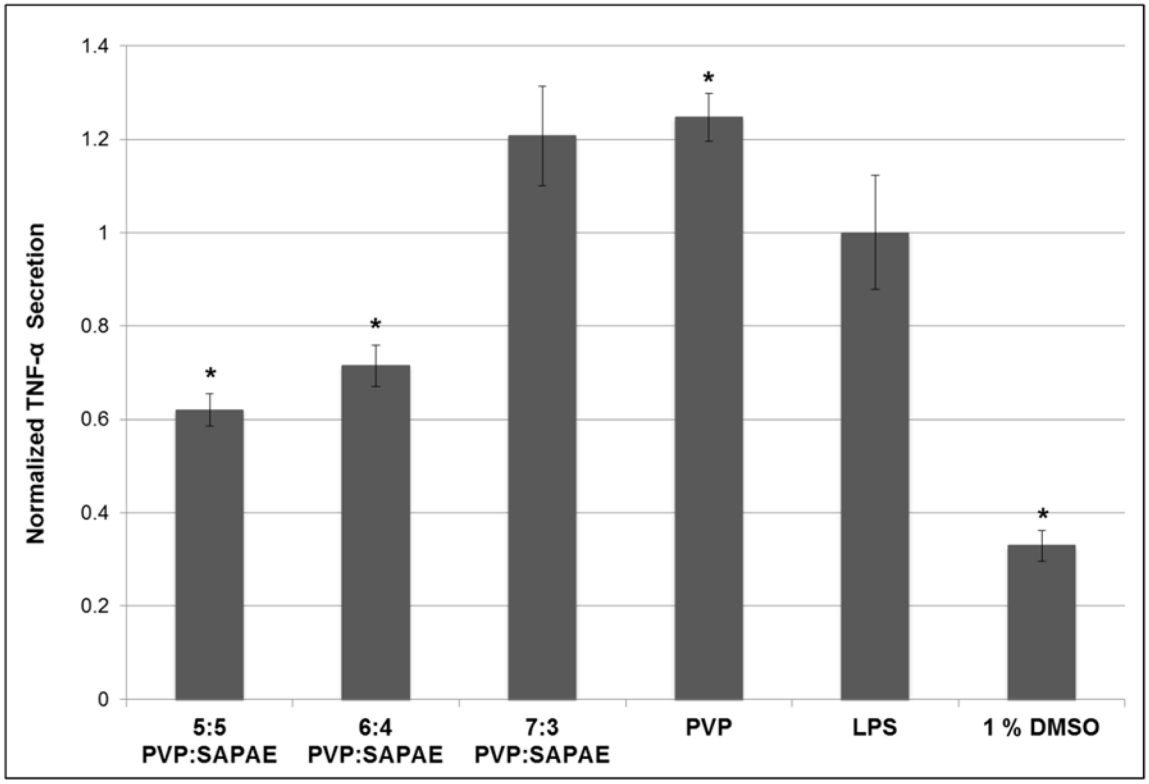

Figure 8.

TNF-α secretion (normalized to the positive LPS control) from macrophages. The PVP group elicited significantly higher TNF-α than the LPS control, while the 5:5 and 6:4 PVP:SAPAE samples significantly decreased TNF-α secretion compared to the positive LPS.

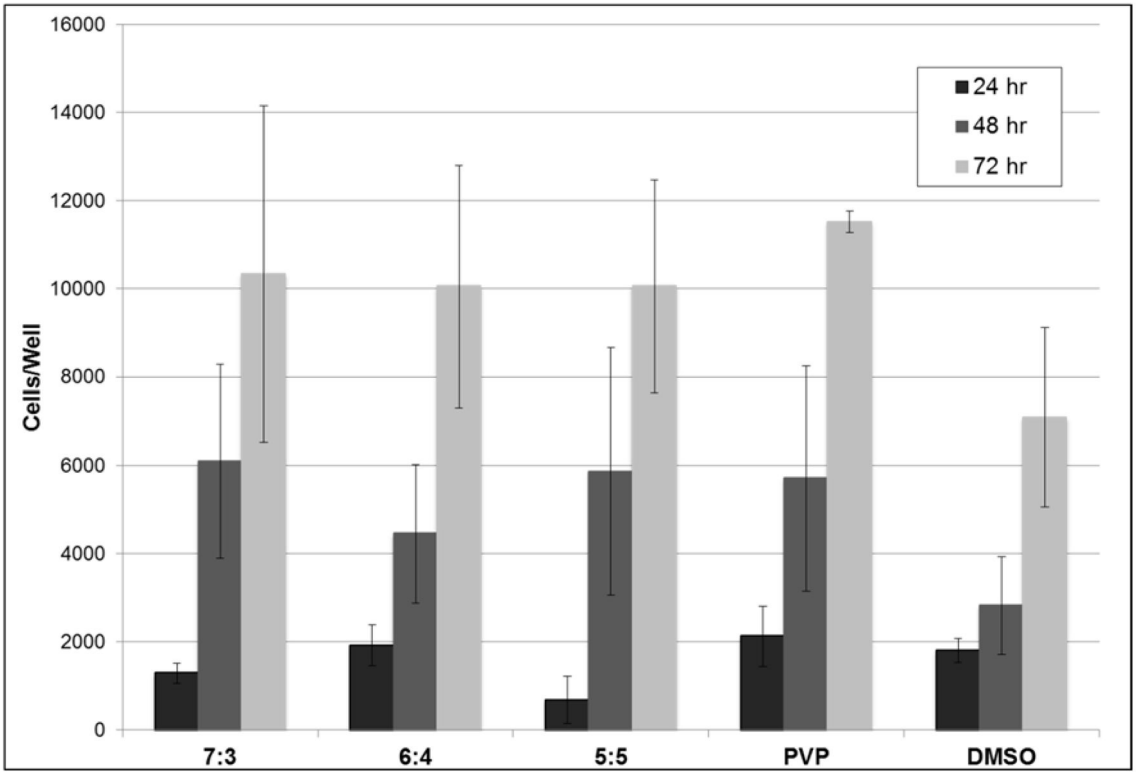

Figure 9.

L929 cell viability at 24, 48, and 72 hours in the presence of dissolved polymer blends. The hydrogel-like materials did not exhibit cytotoxicity compared to the DMSO control.

Table 1

For each copolymer blend, relative amounts of PVP and SAPAE used and maximum, theoretical amount of SA.

Blend ratio PVP:SAPAE	PVP Amount (g)	SAPAE Amount (g)	Calculated SA Amount (g)
7:3	0.525	0.225	0.163
6:4	0.450	0.300	0.217
5:5	0.375	0.375	0.271

Table 2

Swelling values (*Q*) for the PVP:SAPAE blends at various ratios according to Equation (1) (n=3).

PVP:SAPAE Ratio	PVP:SAPAE (*Q* after 2 hours)	PVP:SAPAE (*Q* after 6 hours)	PVP:SAPAE (*Q* after 24 hours)
7:3	7.7 ± 1.0	8.5 ± 0.5	11.1 ± 0.9
6:4	4.7 ± 0.3	5.3 ± 0.3	7.2 ± 0.8
5:5	3.6 ± 0.4	4.0 ± 0.6	4.9 ± 0.1