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Biodegradable Ferulic Acid-containing Poly(anhydride-ester): Degradation Products with Controlled Release and Sustained Antioxidant Activity

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Abstract

Ferulic acid (FA) is an antioxidant and photoprotective agent used in biomedical and cosmetic formulations to prevent skin cancer and senescence. Although FA exhibits numerous health benefits, physicochemical instability leading to decomposition hinders its efficacy. To minimize inherent decomposition, a FA-containing biodegradable polymer was prepared via solution polymerization to chemically incorporate FA into a poly(anhydride-ester). The polymer was characterized using nuclear magnetic resonance and infrared spectroscopies. The molecular weight and thermal properties were also determined. *In vitro* studies demonstrated that the polymer was hydrolytically degradable, thus providing controlled release of the chemically incorporated bioactive with no detectable decomposition. The polymer degradation products were found to exhibit antioxidant and antibacterial activity comparable to free FA and *in vitro* cell viability studies demonstrated that the polymer is non-cytotoxic towards fibroblasts. This renders the polymer a potential candidate for use as a controlled release system for skin care formulations.

Keywords

biodegradable; polymer; poly(anhydride-ester); ferulic acid; antioxidant; controlled release

INTRODUCTION

Exposure of skin to ultraviolet radiation (UV) causes oxidative stress, which can result in photosensitivity, senescence, and skin cancer.¹ The prevention of oxidative stress can be achieved using antioxidants as photoprotective agents that absorb UV radiation and scavenge free radicals responsible for causing damage.² Many phenolic compounds have

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Supporting Information Characterization measurements for polymer and precursors are included as well detailed antibacterial methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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gained considerable attention in recent years for their use in preventing oxidative stress.² Ferulic acid (FA), in particular, is a phenolic compound that exhibits anti-inflammatory, antimicrobial, and anticancer properties.³⁻⁷ As a photoprotective agent and antioxidant in biomedical and cosmetic formulations, FA also prevents harmful radiation effects both as a UV absorber and a free radical scavenger.^{2, 8} Although FA exhibits beneficial properties, it undergoes thermal, air, and light-induced decomposition through a proposed decarboxylation mechanism,⁹ which reduces its efficacy.^{10, 11}

To improve the physiochemical stability of FA in formulations, researchers have attempted to protect it from decomposition using controlled release technologies via physical incorporation into various types of matrices.¹² For example, FA has been physically incorporated into an organic-inorganic nanohybrid material for controlled release via diffusion, but the low FA concentrations exhibited minimal antioxidant activity.^{13, 14} To overcome this issue with physical incorporation, polymers containing cinnamoyl moieties chemically conjugated as pendant groups have been used to improve photostability, but exhibited low drug loading (ca. 50 %).^{15, 16} Polymers containing cinnamoyl groups in the main chain and polymerized with poly(ethylene glycol) derivatives, also exhibit lower drug loading and require enzymatic degradation.¹⁷ While other researchers have been successful with incorporating antioxidants into a biodegradable polymer backbone to increase drug loading (in some cases the polymer is comprised entirely of the antioxidant),¹⁸⁻²¹ this has yet to be achieved using ferulic acid with tunable drug delivery. In this work, we were successful at both protecting the unstable bioactive from decomposition and improving drug loading by chemically incorporating FA into a hydrolytically degradable polyanhydride backbone, enabling controlled FA release. This type of controlled release system can offer advantages compared to conventional formulations by reducing the frequency of applications and improving patient compliance.²²

This was achieved by synthesizing a biodegradable poly(anhydride-ester) containing FA in the backbone via solution polymerization methods and characterized using proton and carbon nuclear magnetic resonance (¹H and ¹³C NMR) and Fourier transform infrared (FTIR) spectroscopies. Thermal properties were evaluated using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Gel permeation chromatography (GPC) and mass spectrometry (MS) were used to determine weight averaged molecular weight (M_w) of the polymer and molecular weights of all polymer precursors, respectively. In addition, *in vitro* FA release studies were performed to study the polymer hydrolytic degradation. The antioxidant and antibacterial activities of the degradation products were evaluated and compared to free FA. The polymer cytotoxicity towards fibroblasts was assessed *in vitro*.

EXPERIMENTAL SECTION

Materials

Silica gel and fetal bovine serum were purchased from VWR (Radnor, PA) and Atlanta Biologicals (Lawrenceville, GA), respectively. 1 N hydrochloric acid (HCl), concentrated HCl, poly(vinylidene fluoride) and poly(tetrafluoroethylene) syringe filters, Wheaton glass scintillation vials, and 96-well plates were purchased from Fisher Scientific (Fair Lawn, NJ). Mouse areolar fibroblasts were purchased from ATCC (Manassas, VA). All other reagents, solvents, and fine chemicals were purchased from Aldrich (Milwaukee, WI) and used as received.

^1H and ^{13}C NMR and FTIR spectroscopies

^1H and ^{13}C NMR spectra were recorded on a Varian 400 MHz or 500 MHz spectrometer using deuterated chloroform (CDCl_3) with TMS as internal reference or deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) as solvent and internal reference. FTIR spectra were obtained using a Thermo Nicolet/Avatar 360 spectrometer, samples (1 wt %) ground and pressed with KBr into a disc. Each spectrum was an average of 32 scans.

Molecular Weight

Polymer precursors were analyzed via MS to determine molecular weights. A Finnigan LCQ-DUO equipped with Xcalibur software and an adjustable atmospheric pressure ionization electrospray ion source (API-ESI Ion Source) was used with a pressure of 0.8×10^5 and 150 °C API temperature. Samples dissolved in methanol ($< 10 \mu\text{g/mL}$) were injected with a glass syringe. GPC was used to determine polymer weight-averaged molecular weight and polydispersity using a Perkin-Elmer liquid chromatography system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 autosampler. Automation of the samples and processing of the data was performed using a Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software with a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Polymer samples were prepared for autoinjection by dissolving in dichloromethane (DCM, 10 mg/mL) and filtering through $0.45 \mu\text{m}$ poly(tetrafluoroethylene) syringe filters. Samples were resolved on a Jordi divinylbenzene mixed-bed GPC column ($7.8 \times 300 \text{ mm}$, Alltech Associates, Deerfield, IL) at 25 °C, with DCM as the mobile phase at a flow rate of 1.0 mL/min. Molecular weights were calibrated relative to broad polystyrene standards (Polymer Source Inc., Dorval, Canada).

Thermal Properties

DSC measurements were carried out on TA Instrument Q200 to determine melting (T_m) and glass transition (T_g) temperatures. Measurements on samples (4-6 mg) heated under nitrogen atmosphere from $-10 \text{ }^\circ\text{C}$ to $200 \text{ }^\circ\text{C}$ at a heating rate of $10 \text{ }^\circ\text{C/min}$ and cooled to $-10 \text{ }^\circ\text{C}$ at a rate of $10 \text{ }^\circ\text{C/min}$ with a two-cycle minimum were performed. TA Instruments Universal Analysis 2000 software, version 4.5A was used to analyze the data. TGA was utilized for determining decomposition temperatures (T_d) using a Perkin-Elmer Pyris 1 system with TAC 7/DX instrument controller and Perkin-Elmer Pyris software for data collection. Samples (5-10 mg) were heated under nitrogen atmosphere from $25 \text{ }^\circ\text{C}$ to $400 \text{ }^\circ\text{C}$ at a heating rate of $10 \text{ }^\circ\text{C/min}$. Decomposition temperatures were measured at the onset of thermal decomposition.

***t*-Butyl FA (2) Synthesis**

To prepare the *t*-butyl FA, (Scheme 1, **2**) a procedure adapted from Hu. et al.²³ was used where Meldrum's acid (2.5 eq) was dissolved in toluene (50 mL), tertiary butanol (2.5 eq) added, and the reaction heated to $100 \text{ }^\circ\text{C}$ with stirring for 5 hours. Without separation, the reaction was cooled to room temperature where vanillin was added followed by pyridine (2.5 mL) and piperidine (0.25 mL), and then heated to $75 \text{ }^\circ\text{C}$ with stirring for 24 hours. The reaction mixture was dried *in vacuo*, and the residue obtained was diluted in diethyl ether, washed with saturated aqueous sodium bicarbonate ($2 \times 200 \text{ mL}$), 1N HCl ($2 \times 200 \text{ mL}$), and distilled water ($1 \times 200 \text{ mL}$). The organic layer was then dried overnight over MgSO_4 , filtered, and the solvent removed *in vacuo* to yield crude product. This was purified on silica gel via flash chromatography using 4:1 hexane:ethyl acetate as eluent (see the supporting information for characterization data).

***t*-Butyl FA-containing Diacid Intermediate (3) Synthesis**

t-Butyl FA (**2**) (2 eq) was dissolved in anhydrous dimethylformamide (DMF) to which sodium hydride (NaH, 2.2 eq) was added slowly. After 30 minutes, adipoyl chloride (1 eq) dissolved in 10 mL DMF was added drop-wise at 20 mL/hr. Reaction progress was monitored by thin layer chromatography (4:1 hexane:ethyl acetate as eluent). Once completed, the reaction mixture was diluted with ethyl acetate (250 mL) and washed with deionized water (2 × 100 mL). The organic layer was collected, dried over MgSO₄, and the solvents removed *in vacuo*. This was purified on silica gel via flash chromatography using 4:1 hexane:ethyl acetate as eluent (see the supporting information for characterization data).

FA-containing Diacid (4) Synthesis

Compound **3** (1 eq) was dissolved in anhydrous DCM to which trifluoroacetic acid (TFA) (40 eq) was added and left to stir over-night. Solvent was removed *in vacuo* and the residue was triturated with DI water (300 mL), isolated via vacuum filtration, and dried *in vacuo* for 24 hours (see the supporting information for characterization data).

FA-containing Poly(anhydride-ester) (5) Synthesis

The polymer (**5**) was prepared using a modified version of a previously described procedure²⁴ (Scheme 1). Diacid **4** (1 eq) was dissolved in 20 mL anhydrous DCM under argon. After adding triethylamine (NEt₃, 4.4 eq), the reaction mixture was cooled to 0 °C. Triphosgene (0.33 eq) dissolved in 10 mL anhydrous DCM was added drop-wise (20 mL/h). The reaction was allowed to stir at 0 °C until CO₂ evolution ceased (ca. 6 h). The reaction mixture was poured over chilled diethyl ether (400 mL) and the precipitate was isolated via vacuum filtration. The residue was dissolved in anhydrous DCM, washed with acidic water (1 × 250 mL), dried over MgSO₄, concentrated, and precipitated with an excess of chilled diethyl ether (500 mL). The ether was decanted or filtered off via vacuum filtration, and the polymer was dried *in vacuo* at room temperature (see the supporting information for characterization data).

***In Vitro* FA Release from Polymer**

The release of **1** from polymer (**5**) was evaluated by *in vitro* degradation in phosphate buffered saline (PBS). Polymer discs (n = 3) were prepared by pressing ground polymer (45 ± 5 mg) into 8 mm diameter × 1 mm thick discs in an IR pellet die (International Crystal Laboratories, Garfield, NJ) with a bench-top hydraulic press (Carver model M, Wabash, IN). Pressure of 10,000 psi was applied for 5 min at room temperature. This methodology was preferred as it minimized interferences from external effects (e.g., formulation additives) on polymer degradation. The PBS pH was adjusted to 7.40 using 1 N sodium hydroxide. All pH measurements were performed using an Accumet® AR15 pH meter (Fisher Scientific, Fair Lawn, NJ).

To measure hydrolytic degradation, the polymer (**5**) discs were placed in 20 mL Wheaton glass scintillation vials with 10 mL of PBS and incubated at 37 °C with agitation at 60 rpm using a controlled environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ). The degradation media was collected every 2 days until day 12 and then collected every 4 days until day 30. Media was replaced by fresh PBS (10 mL) and the spent media was analyzed over 30 days by high-performance liquid chromatography (HPLC). The degradation products were analyzed and quantified via HPLC using an XTerra® RP18 5 Vm 4.6×150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual λ Absorbance Detector. All samples were filtered using 0.22 μm poly(vinylidene fluoride) syringe filters and subsequently injected (20 μL) using an autosampler. The mobile phase was comprised of 50 mM KH₂PO₄ with 1 % formic acid in

DI water at pH 2.5 (70 %) and acetonitrile (30 %) run at 1 mL/min flow rate at ambient temperature. Absorbance was monitored at $\lambda = 335$. Amounts were calculated from a calibration curve of known FA standard solutions.

Radical Scavenging (Antioxidant) Activity²⁵

To assess the FA antioxidant activity in degradation media, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was employed. This was evaluated by adding sample (0.1 mL) to a 0.024 mg/mL DPPH solution in methanol (3.9 mL). Day-10 and day-20 polymer degradation media samples (0.1 mL) from the polymer (see *In Vitro* FA Release from Polymer section) were incubated with the 0.024 mg/mL DPPH solution (3.9 mL) at room temperature. After 1 hour, solutions were analyzed via UV/vis with a Perkin-Elmer Lambda XLS spectrophotometer (Waltham, MA) ($\lambda = 517$ nm). A free FA solution was freshly prepared at specific concentrations corresponding to HPLC data gathered on day-10 and -20. These samples were each analyzed identically to the aforementioned degradation media samples. DPPH % radical reduction was calculated by: $[(Abs_{t0} - Abs_t)/Abs_{t0}] \times 100$, where Abs_{t0} is the initial absorbance and Abs_t is the absorbance after a period of time, namely 1 hour. Absorbance values from adding PBS (0.1 mL) to the DPPH solution (3.9 mL) was used as Abs_{t0} . All radical scavenging assays were performed in triplicate. Student's t-tests were used to determine the significant difference of the antioxidant activity between free FA and FA degradation media (significantly different if $p < 0.05$).

In Vitro Cytotoxicity Assay

Evaluation of the polymer cell compatibility was performed by culturing NCTC clone 929 (strain L) mouse areolar fibroblasts in media containing the dissolved polymer. These L929 fibroblasts are a standard cell type for cytocompatibility testing as recommended by ASTM.²⁶ The polymer was dissolved in dimethyl sulfoxide (DMSO; 10 mg/mL) as a stock solution and serially diluted with cell culture media to two concentrations (0.01 mg/mL and 0.10 mg/mL), based on standard cytotoxicity protocols.²⁷⁻³⁰ Cell culture media consisted of Dulbecco's Modified Eagle's Medium, 10 % v/v fetal bovine serum, 1 % L-glutamate, 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 ($n = 3$). The media with dissolved polymer was compared to two controls: DMSO-containing media and media without the polymer or DMSO.

Cellular morphology was observed and documented at 100X original magnification (Olympus, IX81, Center Valley, PA) at 48, 72, and 96 hours post seeding. Cell viability was determined by using a CellTiter 96@AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI). The MTS tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-7 sulfophenyl)-2H-tetrazolium, inner salt; MTS)] is bio-reduced by cells into a colored formazan product that is soluble in the tissue culture medium. Following the appropriate incubation time, 20 μ L of the MTS reagent was added to 100 μ L of culture medium and further incubated for four hours. The absorbance was then recorded with a microplate reader (Model 680; Bio-Rad, Hercules, CA) at $\lambda = 490$ nm. Cell numbers were calculated based upon a standard curve created 24 hours after original cell seeding.

Cell studies were performed in triplicate, and statistical analysis was performed with SPSS software (version 15.0 for Windows; Chicago, IL). ANOVA followed by pairwise comparison with Scheffe's post hoc test allowed for pair-wise comparison of the polymer to the DMSO media control.

***In Vitro* Antibacterial Assays**

A study supplemental to the antioxidant assays was performed to ensure full activity was maintained after polymer synthesis. To further assess FA bioactivity, the antibacterial activity was evaluated by hydrolyzing the polymer with NaOH, degradation products extracted using ethyl acetate, and subsequently dried *in vacuo*. This extracted powder was screened against *Escherichia coli* (*E. coli*) O157:H7 ATCC 43895, a model bacterium, and compared to that of free FA. More detailed methods can be found in Supporting Information.

RESULTS AND DISCUSSION

Polymer Synthesis and Characterization

To overcome FA limitations regarding its poor physicochemical stability, FA was chemically incorporated into a polymer backbone as outlined in Scheme 1. A one-pot Knoevenagel condensation reaction was utilized by reacting *t*-butanol with Meldrum's acid to form a malonic acid monoester, which was immediately reacted with vanillin in the presence of pyridine and piperidine as catalysts to yield the *t*-butyl FA compound (**2**).

To synthesize **4**, various reaction conditions were explored by changing temperature, base, and solvent to directly couple the acyl chloride to the FA phenol, but these conditions resulted in impure products that could not be further purified. For sole reaction at the phenol, various groups were used to protect the carboxylate group, where the *t*-butyl groups yielded pure material without compromising the integrity of the FA structure during protection and deprotection. Ultimately, diacid (**4**) was formed after coupling compound **2** to adipoyl chloride in DMF containing NaH and removing the *t*-butyl ester with TFA. The diacid (**4**) was isolated and the structure confirmed by ¹H and ¹³C NMR, and FTIR spectra. DSC and MS were used for melting point and molecular weight determination, respectively.

¹H NMR spectra confirmed all polymer intermediates and polymer structures shown in Figure 1. Coupling constants between 15.8-16 Hz demonstrated that the double bonds are in the *trans* (*E*) configuration, with signals appearing at 7.84 and 6.83 ppm for **5**. The appearance of signals at 2.67 and 1.92 ppm and the disappearance of the phenol signal (6.06 ppm for **2**) indicated successful acyl chloride coupling to FA in a 1:2 ratio (acyl chloride:FA). The disappearance of signals at 1.54 ppm (Figure 1C) illustrates the complete removal of the protecting *t*-butyl groups. The FTIR spectrum of **4** (Figure 2B) indicates coupling of the acyl chloride via ester bond formation (1766 cm⁻¹) and presence of carboxylic acid bands (at 2586 and 1677 cm⁻¹ illustrating the alcohol and carbonyl groups of the acid).

Due to thermal instability of FA, diacid **4** was used directly for low-temperature solution polymerization using triphosgene as the coupling agent in the presence of triethylamine to form the poly(anhydride-esters) (**5**). The FA structure within the polymer was preserved after polymerization as indicated by the ¹H NMR spectrum (Figure 1D) and FTIR spectrum (Figure 2A), which illustrates the disappearance of the diacid carboxylic acid peaks, presence of the anhydride carbonyls and ester preservation between 1800 and 1720 cm⁻¹, and preservation of the double bonds (1600 and 1630 cm⁻¹). The polymer exhibited a M_w of 21,700 Da with polydispersity index of 1.7 after isolation. Thermal properties of the polymer are favorable as it decomposes at 332 °C and exhibits T_g at 82 °C. The successful FA chemical incorporation into a poly(anhydride-ester) backbone further allows for high drug loading, where 81 % of the polymer is the bioactive, significantly improving upon other researchers' methods.¹³⁻¹⁷

***In Vitro* FA Release from Polymer**

The polymer's *in vitro* degradation was measured by the appearance of FA in degradation media via HPLC to elucidate release rates from **5** to yield **1** and biocompatible adipic acid (not detected by HPLC) as depicted in Scheme 2. This degradation is integral to improving FA stability and delivery, as FA release is controlled via anhydride and ester bond hydrolysis. To ascertain whether the polymer would ultimately hydrolyze and release the covalently attached bioactive, *in vitro* degradation was monitored over 30 days using polymer discs. Hydrolytic degradation of **5** was studied using pressed polymer discs in PBS and analyzed via HPLC. Previous studies have indicated more rapid anhydride bond hydrolysis than that of ester bonds.³¹ Within this study, however, the relative hydrolysis rate was not apparent in the studies performed as diacid (**4**) absorbance (retention time of 8.07 minutes (Figure 3B)), was minimal at the specified wavelength for HPLC studies. Moreover, the diacid exhibits limited solubility in PBS relative to ferulic acid. Although there is likely diacid present due to anhydride bond hydrolysis, minimal amounts were detected under the conditions studied. FA detection with a 3.71 minute retention time (Figure 3B) indicated further hydrolysis of ester bonds. The polymer released 6.2% FA over 30 days as depicted in Figure 3A. It is important to note that no decomposition peaks were observed, suggesting the FA compound remains active. After 30 days, a thinner substrate was observed from the initial 8 mm diameter × 1 mm thick discs suggesting complete degradation for this polymer is > 30 days.

As previous poly(anhydride-ester) studies suggest polymer degradation to be pH-dependent,²⁷ the release medium pH for each sample was monitored to ensure acidic degradation products did not alter the pH. The media pH throughout degradation studies became more acidic, yet only deviated from the initial 7.40 reading by -0.19 ± 0.13 . Due to these low values, it is expected that the slightly acidic media did not affect release rates.

Radical Scavenging (Antioxidant) Activity

To establish whether polymer processing had an effect on antioxidant activity of released FA, a DPPH radical scavenging assay was employed. DPPH is widely used to assess the ability of antioxidants to scavenge or quench free radicals or donate a hydrogen atom, causing a color change from violet to pale yellow and a reduction in absorbance.^{25, 32, 33} The degradation media antioxidant activity from days 10 (containing all products released from days 8-10) and 20 (containing all products released from days 16-20) were analyzed. These were compared to the corresponding free FA (**1**) using freshly prepared solutions with concentrations equal to HPLC data on day-10 (18 ± 1 Ng/mL) and day-20 (52 ± 1 μ g/mL) (Figure 4). Comparable concentrations were chosen because the quenching percentage is dependent on the antioxidant concentration.³²

Student's t-tests comparing degradation media to the free FA solution were performed (significantly different if $p < 0.05$). The observed antioxidant activity showed no statistical differences between degradation media and free FA solution for day 10, whereas day 20 degradation media demonstrated significantly higher radical reduction than FA alone, indicating increased quenching (denoted by asterisk in Figure 4). In both cases, increased antioxidant activity of degradation media relative to free FA was observed. This may be due to potential antioxidant activity of the diacid, as it has the ability to scavenge the free radical similarly to free FA. Additionally, adipic acid was also in the degradation media and not in the free FA solution, which also has shown minimal antioxidant activity.³⁴ Further studies must be performed to elucidate the diacid antioxidant activity. From these results, the polymer degradation products sustain antioxidant activity over time and have not been affected by the synthetic approach utilized.

***In Vitro* Cytotoxicity Assay**

Assessing the polymer toxicity potential is important if this polymer is to be used for *in vivo* applications. Cytotoxicity of **5** was evaluated by culturing fibroblast cells in polymer-containing media at 0.01 and 0.10 mg/mL as these concentrations are well above those seen *in vitro* and can be used to determine dose dependent toxicity. Studies were performed over a 96 hour time period, in which cell proliferation and morphology were observed. For both polymer concentrations (Figure 5), pair-wise comparison with Scheffe's post hoc test indicated no significant statistical difference between the polymer and the DMSO media control at the 48, 72, and 96 hour time points (significantly different if $p < 0.05$).

For all conditions, the cell morphology images demonstrate the typical proliferation expected of healthy fibroblasts. After 96 hours of culture, proliferating viable cells were visible with stellate morphology and extending filopodia. No visible differences were observed in fibroblast morphology for the polymer at the two concentrations and three time points; therefore, under these specific concentrations and conditions, **5** can be considered non-cytotoxic.

***In Vitro* Antibacterial Assays**

To further assess if the polymer processing affect the chemically incorporated FA and remained bioactive, antibacterial tests were performed using *E. coli*, a Gram-negative bacterium that free FA is active against. Degradation media from the *in vitro* release studies yielded FA concentrations too low for antibacterial activity to be observed. Therefore, the polymer was completely hydrolyzed and a fixed concentration was prepared from the extracted degradation products and compared to free FA activity. Samples dissolved in DMSO without added bacteria were first examined to ensure contamination did not occur with the solutions; negligible increase of OD_{630nm} indicated no bacteria growth and thus no contamination.

A fixed FA concentration (3 mg/mL) was selected to compare the bacteria susceptibility to free and extracted FA. The extracted powders from **5** included both FA (82 %) and adipic acid (18 %); free adipic acid was therefore analyzed for antibacterial activity at the concentrations present in extracted powders (0.66 mg/mL). DMSO concentrations were kept lower than 0.5 % to minimize its inhibitory effect.

As depicted in Figure 6, free FA and the extracted powder from **5** demonstrated activity against *E. coli*. Although minimal, adipic acid also demonstrated inhibitory activity. The observed activity showed no statistical differences between free FA and the extracted powder. This assay suggests that the FA retains its antibacterial activity, further demonstrating that the polymer degradation products did not decompose and have not been affected by the synthetic methods utilized. These results also suggest that the polymer may also be useful as preservative agents in formulations due to its antibacterial activity, but additional studies must be performed.

CONCLUSIONS

By incorporating FA into a polymer backbone, the physicochemical stability is improved as FA is protected from premature degradation. The polymer was found to be hydrolytically degradable, where the degradation products exhibited comparable antioxidant and antibacterial activity to that of free FA. The demonstrated controlled release of the chemically incorporated bioactive with no detectable decomposition suggests the frequency of applications of the antioxidant/photoprotective agent can be reduced. The polymer was deemed non-cytotoxic in mouse fibroblast cultures at 0.01 and 0.10 mg/mL concentrations,

demonstrating the potential for use *in vivo*. The polymer design allows for opportunities to develop tunable FA release profiles; future studies will focus on increasing the FA release rate by changing the acyl chloride in polymer synthesis or formulating the polymer into microspheres to further improve their use for skin care formulations and to perform skin permeation experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

^1H NMR spectra of FA-containing polymer and each intermediate step. *t*-Butyl FA, **2** (A), *t*-butyl FA-containing diacid intermediate, **3** (B), FA-containing diacid, **4** (C), and FA-containing polymer, **5** (D) spectra are illustrated above.

Figure 2.
FTIR spectra of FA-containing polymer **5** (A, top) and diacid **4** (B, bottom).

Figure 3.

In vitro FA release from FA-containing poly(anhydride esters) (**5**) (FA $\pm$ standard error) (A). HPLC chromatographs demonstrating appropriate peaks for FA (**1**) and diacid (**4**) for day-10 samples (B).

Figure 4. DPPH reduction results for FA in degradation media and free FA for day-10 and day 20 of the release study. Statistical difference indicated by * ($p < 0.05$).

Figure 5. Cell viability/proliferation after 48, 72, and 96 hours in culture media with the polymer at concentration of (A) 0.01 mg polymer/mL media and (B) 0.10 mg polymer/mL media.

Figure 6.

Growth curve of treated cells at OD 630nm. *E. coli* O157:H7 with DMSO at 0.5 % demonstrating OD readings for FA, extracted powder from **5**, adipic acid, DMSO, and bacterium. Data is presented as an average of triplicate OD measurements from two independent tests where OD directly correlates with cell count.

Scheme 1.

Synthesis of FA-containing poly(anhydride-esters) (**5**) and FA-containing polymer precursors including diacids (**4**).

Scheme 2.

Proposed hydrolytic degradation scheme of FA-containing poly(anhydride-esters) (**5**) to yield diacid (**4**) and FA (**1**).