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Article

# Antimicrobial Active Packaging Prepared by Reactive Extrusion of ε-Poly L-lysine with Polypropylene

Noah A. Doshna, Joshua E. Herskovitz, Halle N. Redfearn, and Julie M. Goddard*

Cite This: https://doi.org/10.1021/acsfoodscitech.1c00280

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**ABSTRACT:** Active packaging offers a unique approach to improving food quality and safety; yet, there remains a need for translatable production methods. We report synthesis of nonmigratory, antimicrobial active packaging. Polylysine (PL) was grafted onto polypropylene (PP) using a free-radical initiator via reactive extrusion, yielding PP-*g*-PL. PP-*g*-PL retained similar hydrophobicity to native PP (native PP 109.2 ± 5.6°; PP-*g*-PL5 116.1 ± 4.8°), important for desirable product-release properties. The surface orientation of polylysine was monitored by the presence of amine groups, with negligible (1.0 ± 0.3 nmol/cm$^2$) amines on control PP and 4.7 ± 1.3, 7.4 ± 2.2, and 10.6 ± 3.8 nmol/cm$^2$ amines on PP-*g*-PL prepared with 1%, 2%, and 3% polylysine. The antimicrobial active packaging material enabled a 1-log reduction in *P. aeruginosa* after 1 h incubation at 37 °C. These results suggest that nonmigratory active packaging can be prepared by reactive extrusion, a scalable technology, with promise in improving food safety and reducing food waste.

**KEYWORDS:** *reactive extrusion, nonmigratory active packaging, food safety, antimicrobial packaging*



## INTRODUCTION

In the United States, there are an estimated 9.4 million instances of food-borne illness every year, resulting in more than 1500 deaths and nearly 56 000 hospitalizations, with an associated economic burden estimated at $15.5 billion annually.[1,2] Microbial contamination by pathogens also contributes to increases in food loss due to large-scale recalls to prevent potential human illness.[3,4] Despite implementation of proper, good manufacturing practices, postprocessing contamination still occurs, and in the case of raw and minimally processed products (e.g., fresh and fresh-cut produce), contamination on farms and during the harvest can lead to foodborne illnesses.[5] In parallel, the impact of microbial contamination on foods and beverages presents a significant challenge to food waste, with up to 25% of food waste attributed to microbial spoilage.[6]

To protect against unwanted microbial growth, a range of natural and synthetic antimicrobials were developed and approved as food additives. Yet, in recent years, a push for "clean label" foods (with reduced additive use) has prompted food manufacturers to seek alternative approaches to traditional preservation through additive use.[7] Active packaging, a technique in which packaging materials perform beyond the traditional functions of food packaging with additional properties like antioxidant or antimicrobial capabilities, offers a unique approach to addressing "clean label" demands.[8] In these systems, the active reagent may intentionally leach into the food matrix over time ("migratory packaging", in which the active agent must be approved as a direct additive thus offering limited benefit over traditional use of additives), or it may be immobilized ("nonmigratory packaging", in which the active agent is covalently bound to the bulk material and thus is approved through the food contact notification program, the process followed by any new food contact material).[9,10] There has been considerable progress in creating *migratory* active packaging materials with antimicrobial properties, but limited research into the creation of antimicrobial active packaging materials intended to be *nonmigratory*.[11,12]

In this work, we describe the synthesis of an antimicrobial, nonmigratory active packaging in which the antimicrobial ε-poly L-lysine is covalently bound to a polypropylene base polymer. E-poly L-lysine is a naturally occurring antimicrobial with efficacy against both Gram-negative and Gram-positive bacteria, including species that are responsible for severe food-borne illness: *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes*.[13] The mechanism of action of ε-poly L-lysine works by disrupting the charge on the headgroup of the phospholipid bilayer of the cell membrane of many bacteria.[14] However, due to the differences in membrane composition between prokaryotic and eukaryotic cells, polylysine does not interfere with the cell membrane of animal cells[14] affording it GRAS (generally regarded as safe) status. This regulatory advantage makes it particularly attractive as an antimicrobial for active packaging applications.[15] Nonmigratory active packaging materials must follow the same approval process as any food contact surface, (i.e., the food contact notification program), and with the lack of significant regulatory precedent, utilizing a GRAS active agent is expected to facilitate this process.

**Special Issue:** Highlights of the AGFD Program at the 2021 ACS Spring Virtual Meeting and Expo

**Received:** July 30, 2021
**Revised:** November 4, 2021
**Accepted:** November 5, 2021

Previous research in preparation of antimicrobial active packaging in which ε-poly L-lysine is the active agent has demonstrated its potential in preventing growth of spoilage and pathogenic microorganisms.[16−18] In one report, Bastarrachea created a nonmigratory active packaging material using both polypropylene and ε-poly lysine, with the use of maleic anhydride as a cross-linker, which showed antimicrobial behavior against both *E. coli* K12 and *Listeria innocua*,[16] resulting in a 5-log reduction of *E. coli* K12 after a 15 min treatment. Against *L. innocua*, the polymer inhibited growth by a factor of $10^4$ CFU/mL in a 21-day storage study in milk. This synthesis made use of a multistep spin-coating process, dissolving styrene maleic anhydride copolymer in acetone and polylysine in water, both with sonication, followed by spin-coating each solution onto a PP/PP-*g*-maleic anhydride blend and thermal curing.[16] A study from Ushimaru et al. showed that compared to untreated polypropylene, the polypropylene coated with a polylysine complex was able to completely inhibit growth of *E. coli* and *Staphylococcus aureus* after a 15 h incubation at 37 °C.[19] They first complexed polylysine with bis(2-ethylhexyl) sulfosuccinate sodium before mixing the complex into molten polypropylene at a 10% w/w concentration.[19] Gao et al. successfully developed an antimicrobial active packaging material from cellulose nanofiber and polylysine, incubating the cellulose in a polylysine solution, followed by a second incubation in a solution of procyanidins to promote cross-linking. Their nanofibers showed antimicrobial efficacy against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria, causing up to a 2-log reduction after a 24 h treatment period, depending on the ratio of cellulose to polylysine in the sample.[17] However, in another study, when polylysine was incorporated into edible whey, alginate and chitosan films, there was little effect on reducing the growth of *E. coli*.[20] The method of incorporating polylysine into active packaging as well as the method used to test its performance in an target application therefore has great influence on its efficacy in antimicrobial active packaging.

While much proof of principle success has been demonstrated in antimicrobial active packaging technologies, the methodology used in their synthesis is neither economical nor scalable for commercial packaging producers. For example, one common process used in the laboratory synthesis of active packaging is solution casting, in which the polymer is dissolved and reacted with the active agent before being cast as a film.[10,21,22] At an industrial scale, this methodology becomes difficult, as the solvents and equipment needed add both direct cost and labor cost to the packaging production process.[10] Another common process is the surface activation of polymers by plasma, ultraviolet light, or a mixture of harsh solvents like hydrogen peroxide and sulfuric acid, which presents similar concerns regarding scale-up.[11] A process that overcomes these translational hurdles and may enable scalable synthesis of active packaging materials is reactive extrusion.[23] Reactive extrusion is commercially employed to create grafted copolymers, enhance polyolefin functionality, and attach more reactive groups, such as maleic anhydride, to unreactive base polymers.[23] In recent years, reactive extrusion has been explored to introduce functional properties through melt graft polymerization of bulk thermoplastic polymers and active agent (e.g., antimicrobial, antioxidant) ligands.[23−26] In this process, the heat and pressure in the extruder barrel permits reactions between a base polymer, the active agent, and an initiator[27] to occur "in the melt", forgoing the need for solvent-based synthesis and lengthy downstream purification steps. Reactive extrusion thus offers a translatable alternative to synthesis of nonmigratory active packaging polymer resins suitable for use as a feedstock in traditional packaging conversion operations.

The goal of this work was to use reactive extrusion to synthesize antimicrobial active packaging in which polypropylene serves as the base polymer, polylysine as the immobilized antimicrobial, and dicumyl peroxide as the free radical initiator and cross-linker (Figure 1). Polylysine's thermostability (120 °C for 20 min) positions it to be a good candidate for use in reactive extrusion processes.[15] Use of a base polymer common in packaging applications, an antimicrobial agent with GRAS status and a cross-linker already approved for use in the synthesis of food packaging materials in a scalable process, further supports the potential translation of this technology as a "clean label" alternative to traditional additive use in reducing bacterial spoilage, food waste, and food-borne illness.[28] To the best of the authors' knowledge this is the first report of using reactive extrusion to prepare a nonmigratory antimicrobial active packaging material, in which the antimicrobial agent in bound by covalent linkages.

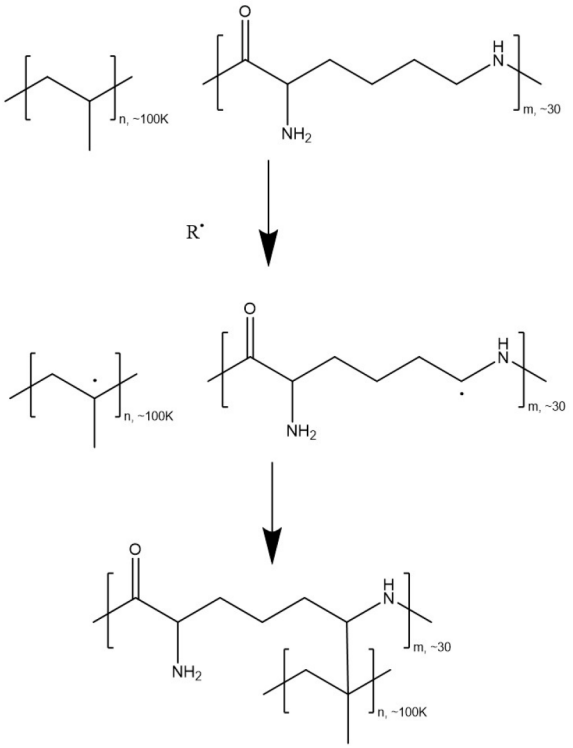


**Figure 1.** Proposed chemistry of reactive extrusion synthesis of PP-*g*-PL with dicumyl peroxide serving as radical initiator (represented here as R·).

## MATERIALS AND METHODS

**Materials.** Dicumyl peroxide, 200 proof ACS reagent grade ethanol, glacial acetic acid, hydrochloric acid (trace mineral grade), and nutrient broth no. 1 were purchased from Millipore Sigma (Burlington, MA, USA). Free-base lyophilized ε-poly L-lysine (polylysine, PL) was purchased from Wilshire Technologies (Princeton, NJ, USA). Isotactic polypropylene pellets were purchased from Scientific Polymer (cat no. 130, CAS no. 9003-07-0, Ontario, NY, USA). Bacteriological agar, bovine serum albumin, orange II dye, and


https://doi.org/10.1021/acsfoodscitech.1c00280
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

0.5 N sodium hydroxide were purchased from Thermo Fisher Scientific (Fairlawn, NJ, USA). Phosphate buffered saline was purchased from VWR Scientific (Radnor, PA, USA). Purge material grades U, E, and EX for the Process 11 extruder were generously donated by Asahi Kasei Asaclean Americas (Parsippany, NJ, USA). Kapton polyimide sheets were sourced from Dupont Chemical (Wilmington, DE, USA).

**Synthesis and Characterization of Antimicrobial Polypropylene (PP-*g*-PL).** *Granularization of Polypropylene.* Isotactic polypropylene (PP) was extruded at 50 rpm in a Process 11 Parallel Twin Screw Extruder (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a 2 mm die and eight heating zones, with the first zone set to 160 °C and the subsequent seven zones set to 180 °C. Extruded polypropylene was cooled in deionized water and pelletized using a Varicut Pelletizer (Thermo Fisher Scientific, Waltham, MA, USA) to form granular polypropylene. Granular polypropylene was stored in covered beakers over calcium sulfate desiccant until further use.

*Synthesis of Antimicrobial Polypropylene-Graft ε-Poly L-Lysine via Reactive Extrusion.* Polypropylene-*graft*-polylysine (PP-*g*-PL) was synthesized by radical graft polymerization of polylysine (PL) to polypropylene in the melt (during extrusion) with dicumyl peroxide (DCP) as a radical initiator. Granular polypropylene was mechanically mixed with 2% w/w dicumyl peroxide and varying concentrations of PL, either 1%, 2%, 3%, or 5% w/w. The mixture was then fed into the extruder using the attached volumetric feeder (11 mm volumetric single screw feeder for process 11 (MK2), Germany by Thermo Electron, Germany) at a rate of 20% the maximum rate. The extruder was set to a temperature of 160 °C in the first zone, and 180 °C in the seven subsequent zones, including the die which was fitted with a 2 mm opening. The extrudate was chilled in deionized water and pelletized using a Varicut pelletizer (Thermo Fisher Scientific, Waltham, MA, USA) to form 0.5 mm pellets. The pelletized polymer was divided into 1.5 g portions, placed between two 5 mil Kapton films, and placed in a Carver hot press (Wabash, IN, USA) at 170 °C for 1 min. The preheated polymer was then pressed at 9000 lbs to form films which were subsequently cooled under a beaker filled with ice and cut into 1 cm × 2 cm coupons for further study. Films were rinsed three times in Milli-Q purified water (18.2 MΩ·cm resistivity and total organic carbon value below 5 ppb, with a final 0.22 μm filter at the dispenser, Milli-Q Advantage A10 Water Purification System, Millipore Sigma, Burlington, MA, USA) to remove unbound polylysine from the surface of the film. Films prepared from granular polypropylene (PP) and polypropylene with 2% w/w dicumyl peroxide (PP.DCP) respectively subjected to the same extrusion process were prepared as process controls.

*Film Thickness Analysis.* Thickness of films prepared from each treatment was determined using a Snapthick 3-way Digital Electronic Thickness Gage (iGaging, San Clemente, CA, USA) with an accuracy of 0.02 mm.

**Surface Chemistry of Antimicrobial Polypropylene.** *Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy.* Changes in the chemistry of the polymer were confirmed by ATR-FTIR Spectroscopy using a IRPrestige FTIR spectrometer equipped with a diamond ATR crystal (Shimadzu Scientific Instruments Inc., Kyoto, Japan). Spectra, averaged from 32 scans, were taken using a Happ-Genzel apodization and a resolution of 4 $cm^{-1}$ with air as the background spectra. Spectra of PP-*g*-PL were compared to spectra of PP and PP.DCP negative and process controls, respectively. Baseline corrections and graphing were conducted using Origin Pro 2019b.

*Surface Dynamic Water Contact Angle Measurements.* Dynamic water contact angle measurements of each film were taken with an Attention Theta Optical Tensiometer (Biolin Scientific, Stockholm, Sweden). Advancing and receding contact angles were recorded. The advancing water contact angle was measured by dispensing a 4 μL droplet of Milli-Q purified water onto the surface of the film, inserting the needle into the droplet, and increasing the size of the droplet at a rate of 0.5 μL/s for 5 s. The advancing contact angle was recorded as the maximum contact angle prior to an increase in the droplet's baseline. Images were recorded at a rate of 14 frames/s (FPS), and the contact angle was measured using the Young−Laplace method. The receding contact angle was measured by removing this droplet at a rate of 0.5 μL/s, and the receding contact angle was defined at the minimum angle achieved before the baseline of the droplet began to recess.

*Quantification of Total Surface Amines.* The density of amine groups (indicating the surface orientation of grafted polylysine groups) on the surface of each film was quantified through a colorimetric dye assay.[29] Film coupons (1 cm × 2 cm) were incubated, with shaking at 180 rpm, for 2 h in 5 mL of a 1 mM solution of Orange II dye in deionized water that had been adjusted to pH 3 using hydrochloric acid. Following incubation, films were rinsed three times in Milli-Q purified water and dried using pressurized air. The films were then placed in 3 mL of deionized water that had been adjusted to pH 12 using sodium hydroxide and incubated for 15 min while shaking at 180 rpm. Absorbance of the desorption solution was read at 455 nm in a Synergy Neo2 Hybrid Multi-Mode Reader (Biotek Instruments, Winooski, VT, USA), and the concentration of amines was determined by comparison to a standard curve created by a dilution scheme of the orange II dye solution in pH 12 deionized water with the assumption of a 1:1 stochiometric relationship between dye and amine groups.

*Migration of the Novel PP-g-PL Polymer in Food Simulants.* The mass of 1 cm × 2 cm films for each treatment was recorded using a Sartorius CPA 225D semimicro balance (Sartorius Co., Goettingen, Germany). Films were then placed in glass vials with 10 mL of a specific food simulant. The simulants used were deionized water ($dH_2O$), 10% v/v ethanol (EtOH) in $dH_2O$, 50% v/v EtOH in $dH_2O$, 3% v/v acetic acid in $dH_2O$, and 95% v/v EtOH in $dH_2O$ in accordance with current European Union regulations.[28] Vials were sealed with a polytetrafluoroethylene-butyl (PTFE-butyl) septum and crimp-top aluminum seal and incubated at 40 °C for 10 days. After incubation, films were removed from their respective simulants, rinsed three times with Milli-Q purified water, and dried for greater than 36 h in a desiccator over calcium sulfate desiccant. The mass of each film was then recorded again and the difference was determined to quantify if migration exceeded 10 mg/100 $cm^2$, the European Union maximum permitted mass of migrating substances.[30]

**Thermal Analysis of Control and Treated Polymers.** Thermal properties of control and treated polymers, along with lyophilized polylysine powder, were analyzed using a modulated differential scanning calorimetry, in a differential scanning calorimeter (DSC 2500, TA Instruments, New Castle, DE, USA). Samples were sealed in aluminum pans, with empty aluminum pans utilized as references. Film samples underwent a heat−cool−heat regiment, heating to 200 °C and cooling to −50 °C. Both heating and cooling steps occurred at a rate of 20 °C/min. Polylysine powder also was subjected to a heat/cool/heat regimen, from 0 to 200 °C, at a rate of 10 °C. Melting temperatures of each film, and the melting temperature of polylysine was recorded and analyzed using TRIOS software (TA Instruments, New Castle, DE, USA). The thermal stability of lyophilized polylysine powder was analyzed using thermogravimetric analysis (TGA Q500, TA Instruments, New Castle, DE, USA). The sample was placed in a platinum crucible and heated at a rate of 10 °C/min to 600 °C under nitrogen. Degradation temperatures of polylysine were recorded and analyzed using Universal Analysis software (TA Instruments, New Castle, DE, USA).

**Antimicrobial Activity of the Functionalized Polymer.** *Preparation of the Microbial Culture.* A culture of *P. aeruginosa* ATCC strain no. 27853 was stored in 20% glycerol at −80 °C until needed. The culture was allowed to melt on ice, before a loopful of suspension was used to streak a single plate of nutrient agar, which was then incubated at 37 °C for 24 h. A single colony from this plate was removed via a sterile loop and used to inoculate 10 mL of nutrient broth, which was then incubated at 37 °C for 18 h. After incubation, 10 μL of this culture was used to inoculate another 10 mL of nutrient broth, which was incubated under the same conditions. Finally, 100 μL of this culture was used to inoculate 100 mL of nutrient broth to be used for the antimicrobial assay.


https://doi.org/10.1021/acsfoodscitech.1c00280
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

*Log-Reduction of an Established Bacterial Colony.* A culture of *P. aeruginosa* ATCC strain no. 27853 was grown in nutrient broth at 37 °C for 12 h to achieve a cell density of $10^9$ CFU/mL. Cell density was estimated by taking the absorbance of the broth at 600 nm in a Synergy Neo2 Hybrid Multi-Mode Reader (Biotek Instruments, Winooski, VT, USA) and confirmed through dilution and plating of the culture on nutrient agar. The culture was diluted to an approximate cell density of $10^6$ CFU/mL with phosphate buffered saline (PBS) solution. Coupons of each film, measuring 1 cm × 2 cm, were placed in 6-well culture plates with 2 mL of the diluted cell culture and incubated at 37 °C, with shaking for 1 h. Solutions of polylysine in PBS were also prepared, with concentrations of 250, 500, 750, and 1250 μg/mL to account for the maximum amount of surface-oriented polylysine in 1%, 2%, 3%, and 5% w/w PP-*g*-PL films, respectively. A 1 mL portion of each polylysine solution was added to a 2 × $10^6$ CFU/mL culture of *P. aeruginosa* and incubated under identical conditions. A solution of 6 μg/mL polylysine was also prepared, and 1 mL of this solution was added to 1 mL of the 2 × $10^6$ CFU/mL culture to achieve a polylysine concentration of 3 μg/mL, the established minimum inhibitory concentration of polylysine against *P. aeruginosa* and incubated under identical conditions.[31] After incubation, dilution schemes were prepared from each well, plated on nutrient agar, and incubated at 37 °C for 18 h. Uninoculated nutrient broth and PBS were each plated in triplicate to confirm sterility.

*Inhibitory effect of PP-g-PL in a Low-Inoculum Food Simulant.* A microbial inhibition assay was conducted using a modified version of the procedure described in the 2019 work of Bastarrachea.[16] A culture of *P. aeruginosa* ATCC strain no. 27853 was grown in nutrient broth at 37 °C for 12 h to achieve a cell density of $10^9$ CFU/mL. Cell density was estimated by taking the absorbance of the broth at 600 nm in a Synergy Neo2 Hybrid Multi-Mode Reader (Biotek Instruments, Winooski, VT, USA) and confirmed through dilution and plating of the culture on nutrient agar. The culture was diluted to an approximate cell density of $10^5$ CFU/mL with PBS. This diluted culture was then placed in 99 mL of nutrient broth to achieve of a final cell density of ∼$10^3$ CFU/mL. Coupons of each treatment, with a total surface area of 10 $cm^2$, were placed in the tube for ratio of 1 $cm^2$ polymer/1 mL nutrient broth. The tubes were then incubated at 37 °C for 16 h, shaking at 200 rpm (setting 4, Cole Parmer ML-51300-00). Over the course of the incubation, aliquots were removed approximately once per hour to measure the optical density at 600 nm. Flasks containing pure media and tubes containing media with 10 μg/mL polylysine were also incubated and measured for optical density as described above.

*Statistics.* Reactive extrusion of the novel polymers, PP.DCP, and PP was carried out in duplicate, on distinct days, utilizing PP that had been granularized the day before extruding each replicate. Film thickness analysis was performed on three locations of four films, for a total of 12 values per treatment. Orange II amine dye assay was performed on six randomly selected films per treatment. mDSC analysis was performed on a single location of a single representative, randomly selected film of each treatment. ATR-FTIR and dynamic water contact angle measurements were each conducted on two locations on three distinct films for a total of six values per treatment. The log-reduction of *P. aeruginosa* experiment was conducted in three wells per treatment, and plated in duplicate, for a total of six CFU/mL values per treatment. The inhibition assay against *P. aeruginosa* was conducted in triplicate, with three flasks of each polymer, three blanks, and three flasks containing 10 μg/mL polylysine. Results of all assays listed above were tested for normality using a Shapiro-Wilks test, followed by an analysis of variance (ANOVA) test, followed by Tukey HSD testing with multiple comparisons ($p < 0.05$). All results are representative of experiments performed on a minimum of two separate days. All statistical analyses were carried out using GraphPad Prism 7.0 software (La Jolla, CA, USA).

## RESULTS AND DISCUSSION

**Synthesis and Characterization of Antimicrobial Polypropylene (PP-*g*-PL).** *Synthesis of Antimicrobial Polypropylene-graft-Polylysine via Reactive Extrusion.* The use of peroxide initiators in the grafting of compounds of note to a polypropylene backbone via reactive extrusion has studied and applied extensively, most commonly in the synthesis of polypropylene-*g*-maleic anhydride.[23,32] Based on work by Shi et al.[32] and our own preliminary investigations, a 2% w/w dicumyl peroxide concentration was selected for all samples. A process control containing 2% DCP with no polylysine was also prepared. Preliminary investigations on films prepared using very low (<0.5%) concentrations of DCP revealed evidence of discrete particles (likely polylysine as this morphology was not observed in PP-DCP, PP, or PP-*g*-PL*x* films) that were blended throughout the material but not covalently bonded to the base polypropylene, further supporting that DCP at 2% w/w is necessary to enable covalent bonding between polylysine and polypropylene, as required for nonmigratory active packaging synthesis by reactive extrusion.

*Film Thickness Analysis.* All films containing DCP were significantly thinner than pure polypropylene films, with average film thicknesses of PP, PP.DCP, PP-*g*-PL1, PP-*g*-PL2, PP-*g*-PL3, and PP-*g*-PL5 being 0.45 ± 0.07, 0.19 ± 0.03, 0.19 ± 0.02, 0.17 ± 0.01, 0.15 ± 0.01, and 0.17 ± 0.02 mm, respectively. Between all samples containing DCP, there was no significant difference in film thickness. This is likely due to the well-documented occurrence of beta-scission in polypropylene treated with radical peroxides such as DCP.[33] Beta-scission creates a smaller average molar mass of the polypropylene molecules and a subsequent lower melting temperature and lower viscosity as equivalent temperatures in the liquid state.[32] Results from DSC (below) support this theory. Nevertheless, uniform film thicknesses were achieved by hot pressing. A comprehensive evaluation of the mechanical properties (e.g., tensile strength and elongation at break) of the films is therefore an important topic for future study, with consideration of the influence of film thickness as thickness (along with material chemistry and other factors) is reported to affect mechanical properties.[34]

**Surface Chemistry of Antimicrobial Polypropylene.** *Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy.* The grafting of surface-oriented polylysine was confirmed via FTIR (Figure 2). A band at ∼1740 $cm^{-1}$ confirmed the presence of a carbon−oxygen double bond, indicative of the ketone group that is prevalent in all proteins, including polylysine. A less substantial peak was observed in the PP.DCP control, which may be due to the formation of a carbon/oxygen double bond within the degraded dicumyl as a termination product of the radical-induced reaction. In the samples containing polylysine, absorbances characteristic of the presence of secondary amines (N−H bend) at ∼1650 and ∼1550 $cm^{-1}$ characteristic of C−N bonds in polylysine and absorbance at ∼1530 $cm^{-1}$ indicative of in-plane deformation of secondary amines, with the strongest and clearest peaks evident in PP-*g*-PL5. The increasing absorbance intensity corresponds with the increasing polylysine concentration of the samples. These results are in agreement with prior reports on the FTIR characterization of polylysine.[30]

*Surface Dynamic Water Contact Angle Measurements.* Dynamic contact angle measurements showed no significant difference in either advancing or receding angles between PP, PP.DCP, PP-*g*-PL1, PP-*g*-PL2, PP-*g*-PL3, and PP-*g*-PL5. The advancing angles were 109.2 ± 5.62, 111.2 ± 4.49, 114.2 ±


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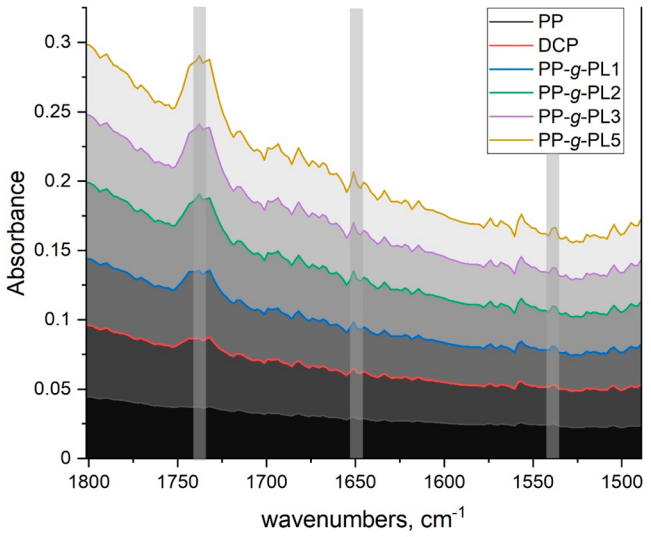


**Figure 2.** FTIR spectra of treated and untreated polymer samples. The most prominent feature is at 1740 $cm^{-1}$, and an absorbance with intensity corresponding to polylysine quantity can be observed. This peak is caused by a carbon−oxygen double bond in a ketone group, a characteristic functional group in polylysine.

4.00, 113.4 ± 4.15, 113.8 ± 3.55, and 116.1 ± 4.81, respectively. Receding contact angles were 85.53 ± 5.15, 77.84 ± 13, 80.07 ± 5.98, 81.93 ± 5.23, 84.94 ± 8.50, and 83.45 ± 6.00, respectively. These results suggest that desirable interfacial properties of polypropylene, such as hydrophobicity, are retained even after grafting up to and including 5% polylysine. This result is significant as many functional ligands of interest in active packaging (antioxidants, antimicrobials) are highly hydrophilic, yet it is important to retain hydrophobicity to ensure product release of packaged goods. In contrast, in a hydrophilic package, viscous liquids would remain on the inner surface of the package, leading to both product waste and consumer dissatisfaction.

*Quantification of Total Surface Amines.* The Orange II dye assay for amine density showed significant difference in amine density between control (both PP and PP.DCP) and polylysine-grafted films (Figure 3). From 0% to 3% polylysine, a linear relationship between polylysine percentage and amine density was confirmed ($R^2$ = 0.998). From 3% to 5% polylysine, there was not a significant increase in amine density, potentially indicating a saturation of amine groups at the polymer's surface, or a limit of the assay. These results suggest that not only was polylysine grafted to the polypropylene base polymer but that sufficient polylysine was surface oriented and thus available for interaction with bacteria in solution.

*Migration of the Novel PP-g-PL Polymer in Food Simulants.* All migration testing data was compared to the European Union's standards for food packaging materials. The standard, devised by the European Food Safety authority, limits the acceptable total migration of plastic packaging material to 10 mg per 100 $cm^2$ of food contact surface area.[35] In this work, double-sided coupons of 1 cm × 2 cm were utilized, for a total surface area of 4 $cm^2$. The overall migration limit per coupon was therefore set at 0.40 mg. As expected, polypropylene met the E.U. criteria in all food simulants. All treated samples had mean weight losses below the E.U. maximum in water, 10% v/v and 50% v/v, and 95% v/v

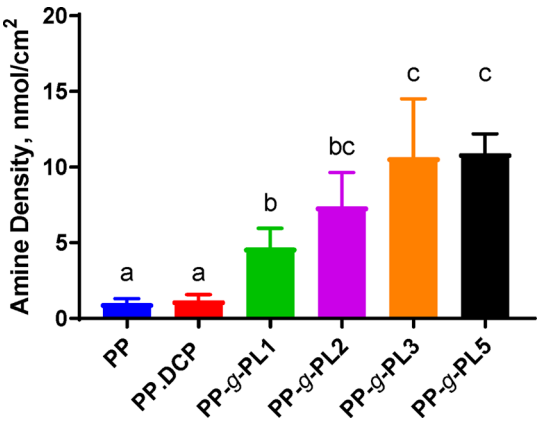


**Figure 3.** Amine density on the surface of control and treatment films, indicating potential for antimicrobial efficacy and the amount of surface-oriented polylysine in each sample. Values represent the mean of $n$ = 6 determinations with error bars depicting the standard deviation. Different letters represent samples that are significantly different from each other as determined by ANOVA and Tukey's test ($p < 0.05$).

ethanol and 3% v/v acetic acid food simulants, designed to reflect hydrophilic foods and nondistilled alcoholic beverages, semilipophillic, and acidic foods, respectively (Figure 4, Table

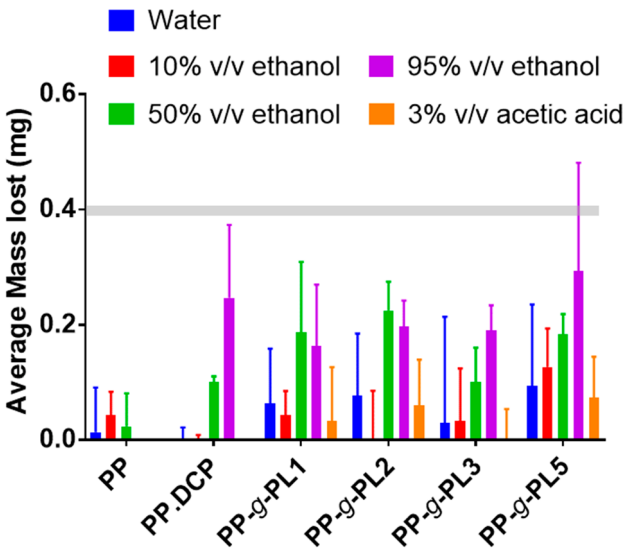


**Figure 4.** Migration of polymer films into food simulants. Bars displays the average ($n$ = 3) mass loss of control and treated films (1 × 2 $cm^2$) after 10 days of submersion in various food simulants at 40 °C. The horizontal line indicates the maximum migration permitted by EU regulations. The error bars display the standard deviation of each data set. The balance used had a sensitivity of 0.01 mg.

1). No statistically significant differences are reported within a film variant (in different simulants) or within a simulant (with different film variants), and both means and deviations are very small values of mass. The observed weight losses, while below regulatory limits, may be due to the creation of polypropylene oligomers of lower molecular mass due to beta scission by the dicumyl peroxide radical initiator. Such beta scission is well reported in the literature and can result in enhanced migration compared to native polypropylene.[33] Nevertheless, these results are promising, as a likely application of these


https://doi.org/10.1021/acsfoodscitech.1c00280
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

**Table 1. Summary of the Potential Approval Status of Each Polymer As a Food Contact Material, As Currently Formulated, under European Union (EU) Food Safety Guidelines[a]**

| polymer → food simulant | PP | PP.DCP | PP-*g*-PL1 | PP-*g*-PL2 | PP-*g*-PL3 | PP-*g*-PL5 |
|---|---|---|---|---|---|---|
| water | 0.01 | 0.00 | 0.06 | 0.08 | 0.03 | 0.09 |
| 10% v/v ethanol | 0.04 | −0.02 | 0.04 | −0.01 | 0.03 | 0.13 |
| 50% v/v ethanol | 0.02 | 0.10 | 0.19 | 0.23 | 0.10 | 0.18 |
| 95% v/v ethanol | −0.19 | 0.25 | 0.16 | 0.20 | 0.19 | 0.29 |
| 3% v/v acetic acid | −0.02 | −0.09 | 0.03 | 0.06 | −0.02 | 0.07 |

[a]Values are mean weight losses of films after 10 days incubation in food simulants, in mg. The EU guidelines limit total migration of a food packaging material to no more than 10 mg/100 $cm^2$ (0.40 mg for the 1 × 2 $cm^2$ coupons used here). Migration levels in all conditions fell within acceptable E.U. limits.

antimicrobial materials would be in aqueous (beverages), acidic (juices and acidified sauces), or slightly lipophilic foods (dressings and sauces). However, more research is needed to explore methods to further reduce migration into food simulants, for example with the addition of plasticizers, reducing dicumyl peroxide content, or exploring alternate initiators with reduced beta scission.

*Differential Scanning Calorimetry.* Normalized heat flow values from −50 to 200 °C were collected for each film and from 0 to 200 °C for polylysine powder (Figure 5, Supplemental Figure 1). The melting temperature of polylysine presented a midpoint of 183.6 °C, higher than the melting temperature of all film samples. Unmodified polypropylene presented the highest melting temperature at 163.1 °C, and all samples extruded with DCP presented lower melting temperatures, supporting the theory explaining the observed reduced film thickness in DCP processed films. PP.DCP had a peak melting temperature of 152.7 °C, and it also displayed a wider and shallower peak compared to the unmodified polypropylene, likely due to the beta scission that occurred, creating oligomers of varying molecular weight. In samples containing 1%, 2%, and 3% PL, two peaks were observed, in each case occurring at approximately 148 and 155.5 °C. In PP-*g*-PL5, only the melting point of 155.5 °C was observed. The mixing of a lower molecular weight polymer, such as polylysine, with a higher weight polymer, such as polypropylene, is reported to produce a more thermodynamically stable composite than a blend of similarly sized polymers, such as PP and polyethylene.[36] Additionally, the melting point depression of samples containing polylysine is a phenomenon that occurs in blended miscible polymers, due to the increased thermodynamic favorability between the two components.[37]

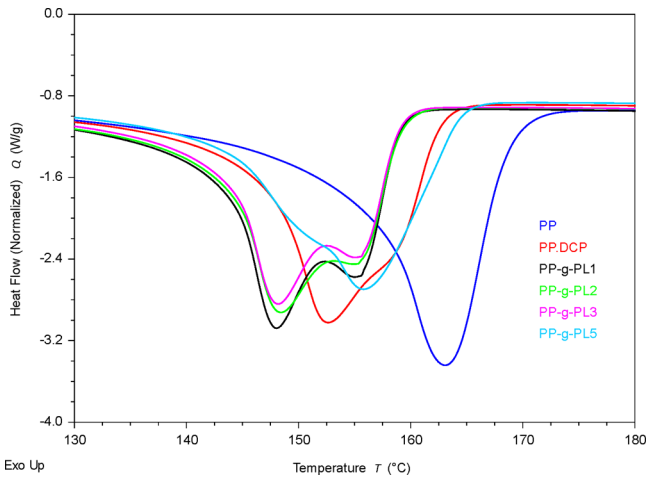


**Figure 5.** Heat flow graph of treated and control films, as determined through differential scanning calorimetry. The curves display overall lower melting temperatures for all samples that include dicumyl peroxide, and dual peaks for PP-g-PL1, PP-g-PL2, and PP-g-PL3, with only the second of those two peaks being present in PP-g-PL5.

**Antimicrobial Activity of the Functionalized Polymer.**

*Log-Reduction of an Established Bacterial Colony.* After 1 h incubation in inoculated PBS, the PP-*g*-PL3 and PP-*g*-PL5 films were as or more effective against a $10^6$ CFU/mL culture of *P. aeruginosa* as the established MIC of polylysine under the same conditions (3 μg/mL) (Figure 6).[31] PP-*g*-PL2 had a statistically significant reduction in microbial populations than the blank control but the practical applications of a reduction of less than 1 log CFU/mL are more limited. Nevertheless, a significant reduction in microbial populations was achieved for all PP-*g*-PL variants, suggesting efficacy of these materials as nonmigratory antimicrobial active packaging films in reducing microbial populations. These results suggest that the heat and pressure of the reactive extrusion process did not inactivate the polylysine. As noted previously, polylysine has been reported to be stable for 20 min at 120 °C, and although our reactive

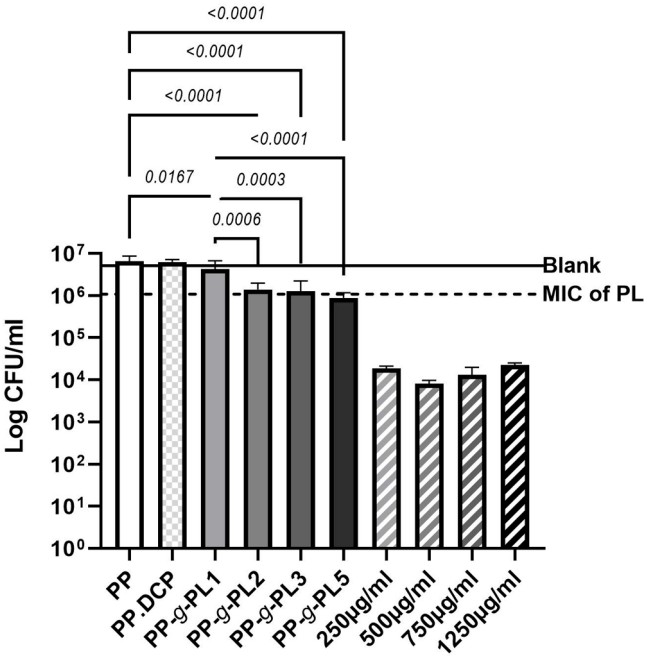


**Figure 6.** Effect of control and treated films on a culture of *P. aeruginosa*. Statistical significance (*p*-values) are provided on film variants (PP → PP-*g*-PL5) where differences exist. Error bars display the standard deviation of each treatment.


https://doi.org/10.1021/acsfoodscitech.1c00280
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

extrusion process takes place at 160−180 °C, the process lasts under 2 min. Because the exposure to high temperatures is short, we expected minimal thermal degradation and thus retention of antimicrobial activity. Thermogravimetric analysis of polylysine at temperatures up to 600 °C indicated a decomposition temperature of ∼260 °C, with ∼90% weight retention at the highest temperature used in our reactive extrusion process (Supplemental Figure 2).

As positive controls, free polylysine was added to PBS inoculated with $10^6$ CFU/mL *P. aeruginosa* at concentrations equivalent to the total theoretical amount of polylysine bound in the PP-*g*-PL samples. Each "free polylysine" control resulted in a ∼3 log reduction in microbial populations. The observed difference in efficacy between free polylysine and immobilized polylysine is likely due to several factors, one of which being the fact that much of the polylysine bound throughout the polymer is not interfacially oriented. Further, it is expected that covalently bound polylysine will have reduced molecular mobility and thus reduced opportunity to interact with cell membranes to disrupt them and cause microbial inactivation, compared to free polylysine. In fact, the efficacy of nonmigratory active packaging technologies hinges on the retention of activity and surface orientation of the covalently bound active ligand, thus demonstrating 1 log reduction in microbial populations in 1 h by a covalently bound polylysine as reported here is an important achievement. Indeed, other active packaging technologies that use polylysine as the antimicrobial ligand achieved greater antimicrobial efficacy, raising an important point about the value of both research which seeks to produce active packaging materials with the highest activity and research which seeks to develop new, more translatable synthesis routes.[16,17,19] We and others have employed multistep, time, and reagent intensive grafting-to approaches which ensure high surface density and orientation of active ligands resulting in higher antimicrobial activity per unit area, with some of the enhanced activity attributed to migration of the antimicrobial from the film surface. In this work, the goal was to demonstrate the potential of a solvent-free, single-step synthesis route for preparing nonmigratory active packaging, using reactive extrusion. We have demonstrated that reactive extrusion can enable retention of some (not all) activity of polylysine after exposure to temperatures above its optimal for stability while incorporating it using covalent linkages (thus qualifying it to be regulated as an indirect additive exempt from labeling). While opportunities remain in improving antimicrobial activity, or in identifying uses (e.g., as part of hurdle technology or as the product contact layer of a multilaminate), our results demonstrate reactive extrusion can be used to develop nonmigratory active packaging with retained activity.

*Inhibitory Effect of PP-g-PL on a Low-Inoculum Food Simulant.* While antimicrobial materials are commonly challenged against high microbial loads, an important potential application of nonmigratory antimicrobial materials is in their ability to control growth of low levels of microbial populations. After exposing PP/PP.DCP (negative controls), PP-*g*-PLx variants, and free polylysine (positive control) to $10^3$ CFU/mL *P. aeruginosa* in nutrient broth for 18 h at 37 °C, there was no observed difference at any time in the optical density of blank, control, or treated films in a culture of *P. aeruginosa* (Figure 7), while the free polylysine effectively controlled microbial growth up until the 15th hour of the study. This lack of efficacy in controlling microbial growth at lower starting inoculum concentration in food simulant is likely a result of surface fouling. Indeed, it has been previously shown that when cationic polymeric antimicrobials, such as polylysine are bound to solid supports, their efficacy in the presence of high levels of organic matter is reduced.[16] This is hypothesized to be due to a fouling effect that blocks the polylysine's amine groups from disrupting the cell membrane of the bacteria, polylysine's antimicrobial mechanism.[14,38] These materials may therefore be better suited to controlling microbial growth in lower organic load conditions. Prior research has demonstrated that it may be possible to retain performance of cationic antimicrobials while controlling surface free energy (and thus mitigating fouling), a strategy that could be employed here.[39]

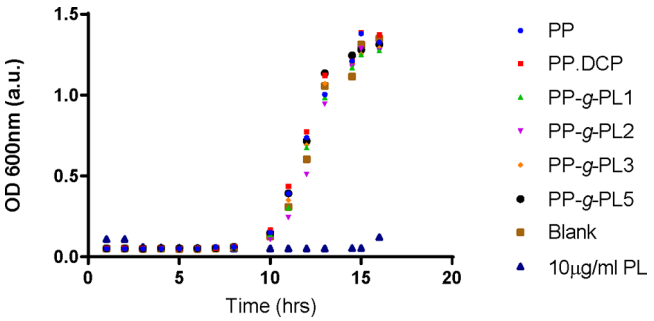


**Figure 7.** No inhibitory effect observed against *P. aeruginosa* in long-term, low-inoculum food simulant studies.

Reactive extrusion is a promising technique for synthesis of nonmigratory active packaging materials with a higher degree of commercial translatability over the state-of-the-art. In particular, antimicrobial active packaging materials can support good manufacturing practices and cleaning and sanitization regimens to reduce the incidence of foodborne illness, and related food waste. In this study, reactive extrusion was utilized to graft polylysine to a polypropylene base polymer with the use of a dicumyl peroxide radical initiator. The presence of polylysine was confirmed via FTIR and Orange II dye assays. The polymer retained desirable traits of polypropylene, such as its hydrophobicity, as confirmed with dynamic contact angle measurements. The novel polymer also showed antimicrobial activity against *P. aeruginosa*, a common food-borne pathogen, achieving a 90% reduction in bacteria after a 1 h treatment. While the polymer did not mitigate microbial growth in a more complex, nutrient rich system, it presents an important proof-of-principle concept study demonstrating that larger antimicrobials that must interact with a cell membrane to be active can be covalently linked to a bulky polymer and retain some activity. Additional studies in modifying the synthesis route to enhance interfacial orientation of antimicrobial polylysine ligands and to demonstrate efficacy against a range of spoilage and pathogenic microorganisms including both Gram-positive and Gram-negative species. On-going research to mitigate the fouling effect of complex food matrices on cationic antimicrobials and in-depth applications studies in true food systems under conditions of intended use are important steps to better demonstrate the potential of this emerging technology in reducing food waste and improving food safety.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.1c00280.



https://doi.org/10.1021/acsfoodscitech.1c00280
ACS Food Sci. Technol. XXXX, XXX, XXX−XXX

Supplemental Figure 1. Dynamic scanning calorimetry of polylysine. Supplemental Figure 2. Thermogravimetric analysis of polylysine (PDF)

## AUTHOR INFORMATION


### Corresponding Author

**Julie M. Goddard** − *Department of Food Science, Cornell University, Ithaca, New York 14853, United States;* orcid.org/0000-0002-3644-0732; Phone: 607-255-8622; Email: goddard@cornell.edu

### Authors

**Noah A. Doshna** − *Department of Food Science, Cornell University, Ithaca, New York 14853, United States*

**Joshua E. Herskovitz** − *Department of Chemical Engineering, Drexel University, Philadelphia, Pennsylvania 19104, United States*

**Halle N. Redfearn** − *Department of Food Science, Cornell University, Ithaca, New York 14853, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsfoodscitech.1c00280



### Funding

This work was supported in part by Hatch Accession number 1016621, and by awards from the U.S. Department of Agriculture National Institute of Food and Agriculture under award numbers 2018-67017-27874 and 2019-68015-29230. This work made use of the Cornell Center for Materials Research Shared Facilities which are supported through the NSF MRSEC program (DMR-1719875).


### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS


The authors gratefully acknowledge Brenda G. Werner for assistance with developing microbiological assays and best practices, and Autumn Rudlong for assistance in data analysis. The authors also gratefully acknowledge Dr. Mohamed Salim, Dr. Darrah Johnson-McDaniel, Dr. John Shu, and Anthony Condo, Jr. of the Cornell Center for Materials Research for their assistance with DSC data collection, and Dr. Jiefu Yin of the Cornell Energy Systems Institute for assistance with and use of a microanalytical balance.


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*ACS Food Sci. Technol.* XXXX, XXX, XXX−XXX