Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

for

A versatile and straightforward process to turn plastics into antibacterial materials

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I- Experimental section

I.1 Materials.

Methyl iodide (MeI, >99%, Acros), n-butyl methacrylate (BMA, 99%), 2 (dimethylamino)ethyl methacrylate (DMAEMA, 99%), styrene (ReagentPlus®, contains 4-tert-butylcatechol as stabilizer, ≥99%), and acrylonitrile (ACN, ≥99%, contains 35–45 ppm monomethyl ether and hydroquinone as inhibitors) were purchased from Sigma-Aldrich. BlocBuilder (>99%), an alkoxyamine based on the nitroxide SG1 (N-tert-butyl-N-[1-diethylphosphono (2,2-dimethylpropyl)]nitroxide) and the 1-carboxy-1-methylethyl alkyl moiety, and SG1 were kindly provided by Arkema (France). Tetrahydrofuran (≥99% stabilized GPR RECTAPUR) and n-pentane (Technical) were purchased from VWR Chemicals, methanol (laboratory reagent ≥99.6%) and dioxane (anhydrous 99.8%) were purchased from Sigma Aldrich, and *N,N*-dimethylformamide (for analysis-ACS reagent) was purchased from Carlo Erba.

I.2 SEC and NMR Measurements

SEC experiments were performed with a PL120 system (Polymer Laboratories, England), equipped with an injection valve (20 µL loop volume), a column oven and a Refractive Index detector thermostated at 70 °C. The stationary phase was a set of a PL Resipore (50 × 8 mm) guard column and two PL Resipore (300 × 8 mm) columns (Polymer Laboratories, England). The eluent was DMF supplemented with 0.1 M LiBr and delivered at a 0.7 mL min−1 flow rate. Samples were solubilized in a mixture of the eluent and toluene (flow marker) at 0.25 vol% at a concentration of 0.25 wt%. Poly(methyl methacrylate) (PMMA) equivalent number-average and weight-average molar masses (Mn and Mw) and dispersities Đ were calculated by means of the PMMA calibration curve using PMMA standards from 1.86 to 520.0 kg mol−1 (Agilent, USA). ^{1}H NMR spectra in $CDCl_3$ were recorded on a Bruker Avance 400 or 300 spectrometer. Chemical shifts were given in ppm relative to tetramethylsilane.

I.3 Copolymer synthesis

The antibacterial PBMA-b-PDMAEMA B(A10%)Me diblock copolymer was prepared by a three-steps procedure as already described.[1] The diblock copolymer was first prepared in two steps by nitroxide-mediated polymerization (NMP) with the BlocBuilder alkoxyamine as initiator/control agent and in presence of the SG1 nitroxide. Acrylonitrile was used as comonomer to ensure the good control of the polymerization. At the end of the polymerization, the copolymer was recovered by precipitation on cold pentane. The composition in DMAEMA of the copolymer (F_{DMAEMA}) was determined by ^{1}H NMR in $CDCl_3$ (F_{DMAEMA} = 0.64) and the number-average molecular weight (M_n) measured by SEC/DMF (M_n = 18,400 g mol^{-1}). In a third step, the copolymer was quaternized with methyl iodide (MeI) to furnish the antibacterial B(A10%)Me copolymer.

I.4 Elaboration of antibacterial organic materials

Table S1. extrusion conditions

Polymer matrix	Extrusion temperature (°C)	Screw rotating speed (rpm)
PLA	170	80
PETG	220	80
HDPE	170	50
LDPE	150	50

II- Bacterial membrane permeabilization assay

The effect of the matrices on bacterial membrane integrity was evaluated using propidium iodide assay as previously explained.[2,3,4,5] Briefly, pieces of matrices (squares of 4 × 4 mm) were sterilely prepared from extruded films and were placed at the bottom of the wells of sterile polypropylene 96-well microplates (Greiner BioOne). Log phase bacterial suspension of *E. coli* (OD around 0.6) were prepared as explained in the bactericidal assay's section. Bacteria suspensions were centrifuged at 3,000 g for 10 min before to be resuspended in sterile phosphate buffer saline (PBS) to reach a cell density around 10^9 bacteria per ml. Propidium iodide (60 µM) (Sigma-Aldrich) was then added to the bacterial suspension and 10 µl of this suspension were added onto the surface of matrices. Bacteria were incubated at 37 °C in the dark and variation of the fluorescence (excitation at 530 nm and emission at 590 nm) was followed during 60 min using a microplate reader (Biotek, Synergy Mx). Cetyl trimethylammonium bromide (CTAB) at 300 µM (final concentration) was used as positive control giving 100 % bacterial membrane permeabilization. Experiments were conducted in triplicate (n = 3).

III- Innocuity assay

The toxicity of the matrices on human skin cells was evaluated using resazurin assay, as previously described.[6,7] Human normal skin cells used were HaCaT (Creative Bioarray, Shirley, NY 11967, USA) corresponding to normal human epidermal keratinocytes.[8] HaCaT cells were cultured in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% antibiotics (all from Thermo Fisher Scientific, Illkirch–Graffenstaden, France). Cells were routinely grown on 25 cm^2 flasks in a 5% CO_2 incubator at 37 °C. For toxicity assay, cells were detached using a trypsin–EDTA solution (Thermo Fisher Scientific, Illkirch–Graffenstaden, France), counted using Mallasez counting chamber and seeded into 96-well cell culture plates (Greiner bio-one from Dominique Dutscher, Brumath, France) at approximately 10,000 cells per well. The cells were allowed to grow for 48–72 h at 37 °C in a 5% CO_2 incubator up to confluence. The medium of the wells was then aspirated and replaced by 100 µl of fresh complete culture media. Sterile pieces of each matrix (squares of 4 × 4 mm) were added sterilely to wells. After 48 h incubation at 37 °C in a 5% CO_2 incubator, the wells were emptied, and 100 µL of resazurin diluted by 1:10 in PBS containing calcium and magnesium (PBS^{++}, pH 7.4) were added per wells. After 2 h incubation at 37 °C, the fluorescence intensity was measured at excitation wavelength of 530 nm and emission wavelength of 590 nm, using a microplate reader (Biotek, Synergy Mx, Colmar, France). The fluorescence values were normalized by the negative control corresponding to untreated cells, CTAB (300 µM) being used as positive control of cell toxicity. Experiments were conducted in triplicate (n = 3).

For the LDPE films, Mouse fibroblastic lung cells—L929 (C3H/An, Sigma®) were used and were grown as monolayer cultures in DMEM (Sigma®) supplemented with 10% fetal bovine serum (FBS, Sigma®) and 1% antibiotics [100 U/mL penicillin (Sigma®) and 100 µg/mL

streptomycin (Sigma®)]. Cells were maintained at 37°C in a 5% CO_2 humidified atmosphere. Material pieces were deposited first and cells were seeded on them. Cell morphology and spreading was observed using an inverted phase-contrast microscope (Optica XDS2) after 24h, 48h and 72h of contact. MTT assay was then used to assess the viability of the cells. Treatment with phenol (Sigma®; 10 mg/mL in DMEM or RPMI) was used as a positive control (toxic control) as recommended by the standard ISO 10993–5. After L929 seeding into 96-well plates, material pieces were deposited onto cells monolayers and the cells were kept in contact with the materials for 48 h at 37°C − 5% CO_2. The MTT (Sigma®) solution (5 mg/mL in PBS) was then poured in each well and incubated for 3 h at 37°C. Cell supernatant was removed and formazan crystals were dissolved in DMSO (Sigma®). The solution absorbance was read at 570 nm with a microplate reader (ELx800 BioTek); this absorbance was correlated to the mitochondrial activity of the cells and thus to the cell viability by calculating the absorbance ratio between cells exposed to materials or extracts and the non-treated cells

Concerning the extracts, the following process was used. Pieces of material were incubated in DMEM (0.2g of material/ mL of DMEM) for 48 h at 37°C and 5% CO_2. DMEM was supplemented with 10% FBS and 1% antibiotic. After L929 fibroblasts were seeded in 96 well plates for 24h at 37°C, cell supernatant was removed and the extraction solution was poured into each well. MTT test was then realized after 48 h of incubation as previously described.

IV-Mechanical properties

Young Modulus measurement

A Shimadzu servo-electric testing machine was used for tensile tests of $PETG_{0\%}$, $PETG_{2\%}$, $HDPE_{0\%}$, $HDPE_{2\%}$, $PLA_{0\%}$, and $PLA_{2\%}$ specimens. Sample geometries are based on ASTM D638-14 type IV.[9] To evaluate the Young Modulus, a quasi-static tensile test was carried out at a constant cross-head speed of 1 mm/min. A 10 kN load cell, with an uncertainty of ±1% from indicated test force (for 1/1 to 1/500 of load cell rated capacity), was used for measurement of tensile force. Sample images were recorded at 1 Hz by a CCD camera of 2048 x 2048 pixels and synchronized with the force signal. Two-dimensional image correlation software[10] (2D-DIC) was used to calculate the displacements and strain fields in the gage area (12 x 4 mm) as shown in Figure 8(a). For each material condition five samples were tested. Young's modulus was determined from the stress-strain curves on the Hookean region.

Dynamic Mechanical Analysis (DMA)

The glass transition temperatures (Tα) were previously determined by means of differential scanning calorimetry (DSC). They were around 73.1 °C for PETG, 55.7 °C for PLA and Tα<-100 °C for HDPE.

A BOSE tensile test machine was used with a 350 N load cell and equipped with an oven. Temperature can be controlled to -50 °C to 250 °C. DMA tensile tests were performed as a temperature scan ranging from (Tα -50 °C) and (Tα + 50 °C) at 1 Hz frequency to observe the evolution of the behavior around the glass transition. For isotropic material at each temperature (or frequency), a DMA tensile test will give two independent quantities: the storage modulus, (E') related to the elastic energy storage, and the loss modulus (E") related to the internal energy dissipation.

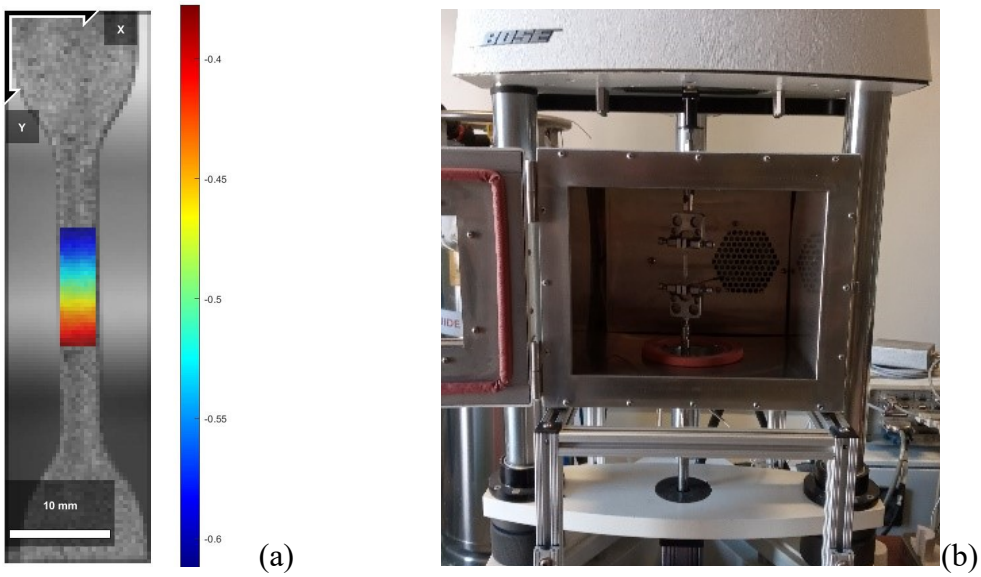

Figure S1. a) Vertical displacement field obtained by 2D-DIC using the 12 x 4mm gage area. b) DMA tensile test machine equipped with an oven.

V- Surface analysis

Contact angle measurements

Hydrophobicity was evaluated by water contact angle measurements using the sessile drop technique. The goniometer (DSA100M, Krüss, Les Ulis, France) deposits a deionized water droplet (300 pL) every mm on a square zone of 7x20 mm^2 (8∗21 drops). Contact angles were monitored for 2 s with a fast CCD camera. Because of the quick evaporation of the small water volumes, only the first acquired image was used to map the hydrophobicity of the surface

AFM

Tapping mode

An Innova AFM (Bruker, Palaiseau, France) was used in tapping mode in air with a NCHV-A probe from Bruker (cantilever resonance frequency around 320 kHz, spring constant around 42 N/m,). Scan rate was 1 Hz. Height images were used to calculate the roughness of the surface (n=6)

NanoIR

NanoIR2 (Anasys Instruments, Santa Barbara, CA) combines an AFM microscope with an infrared (IR) pulsed tunable laser to perform IR analysis under the AFM tip. The beam of a laser (multichip quantum cascade laser QCL from MIRCAT, Daylight solution, San Diego, CA) ranging from 1900 to 900 cm^{-1} was focused from the top side of the sample onto the AFM cantilever that uses a gold coated probe HQ:CSC38/Al-BS-50 from MikroMasch (cantilever resonance frequency around 190 kHz,spring constant around 0.03 N/m).The repetition rate of the laser tuned to match the contact resonant frequency of the AFM cantilever. The spectra were collected with a 1 cm^{-1} spectral resolution.

ToF SIMS

ToF-SIMS data were acquired using an IONTOF M6 instrument (IONTOF GmbH, Münster, Germany) equipped with a reflectron time-of-flight analyser and Bi/Mn primary-ion source. Bi3+ cluster ions were selected from the 30 keV pulsed primary-ion beam for the analysis and the pulses 'bunched' for optimum mass resolution ($m/\Delta m > 8000$). Primary ions were limited to 1×10^{10} ions·cm^{-2}, which is well below that required for static conditions. During data acquisition, the pressure in the analysis chamber was maintained at, or below, 1.5×10^{-9} mbar. Spectra were calibrated on the omnipresent C-, CH-, CH2-, C2-, C3-, C4-, C5H-, C6H- for negative polarity or on the C+, CH+, CH2+, C2H2+, C3H2+, C4H4+, C5H4+ peaks for positive polarity. Based on these datasets the chemical assignments for characteristic fragments were determined. The active compound (amphiphilic copolymer) was drop-casted on a cleaned silicon wafer and employed as a reference. The selected mass ranges (m/z) of the measured polymer samples represent regimes of characteristic peaks with strong intensities.

VI- Tof-SIMS analyses

The specific mass response of the copolymer may be detected in the background of the matrix. For all polymers except PLA, spectra are presented for both secondary-ion polarities (*i.e.*, positive and negative). In the case of PLA, all of the negative-ion peaks characteristic of the copolymer were also present in spectra of the base polymer; therefore, only positive-ion spectra are presented. The selected mass ranges (**m**/**z**) are those in which the spectrum of the copolymer (in orange) contains a characteristic peak of strong intensity. As shown in Figure S2, there is no increased intensity in the spectrum of the organic matrix with the copolymer at **m**/**z** values corresponding to peaks in the copolymer spectrum, for any of the three materials. These results showed that it was not possible to detect the antibacterial additive on the surface samples by ToF-SIMS analysis. These indirect results prove that whatever the matrix, the copolymer should not fully migrate on the surface and stay probably well dispersed in the material.

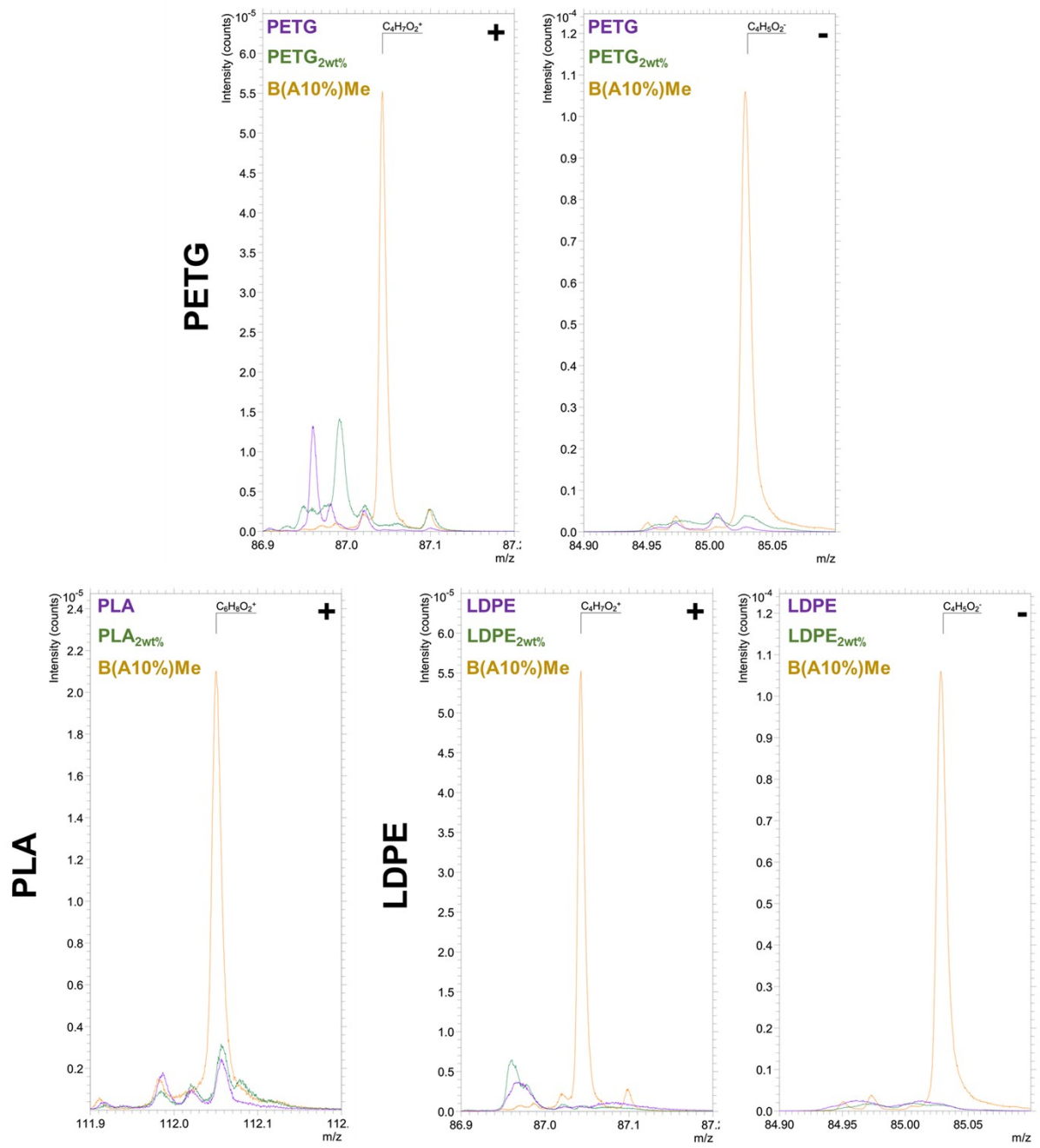

Figure S2. ToF-SIMS analysis (positive and negative secondary-ion polarities) on PETG, PLA and LDPE materials containing 2 wt% of antibacterial copolymer. In orange: copolymer; green: matrix alone and blue: matrix with 2 wt% copolymer.

VII- HPLC chromatogram of the antibacterial copolymer

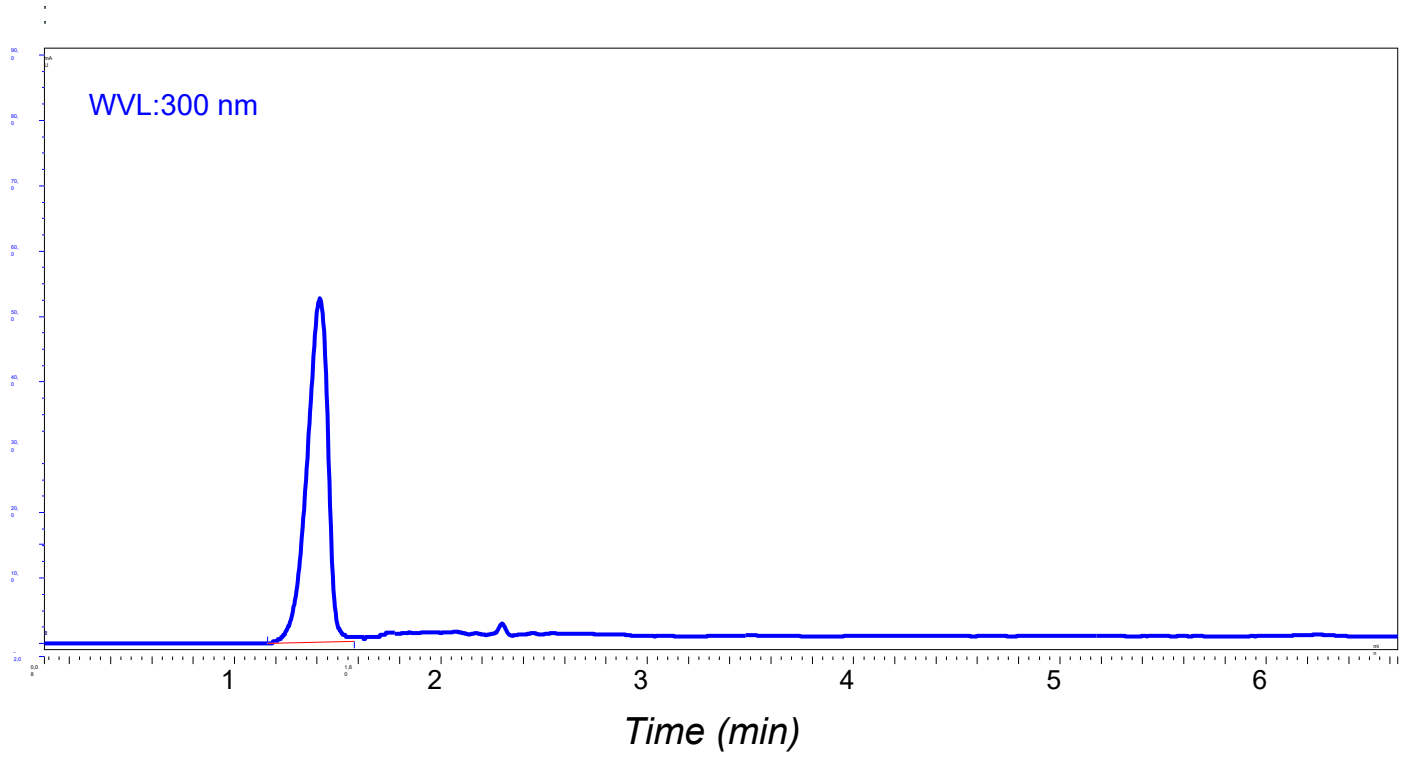

Figure S3. HPLC chromatogram of the copolymer (after heating to remove volatile compounds)

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