Respirometry and Cell Viability Studies for Sustainable Polyesters and their Hydrolysis Products

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Abstract

Significant research effort has been directed towards the development of sustainable plastics that are high performance, bioderived and/or degrade into nontoxic byproducts under natural or engineered environments (i.e., industrial composting facilities). We report the low cytotoxicity of poly(γ-methyl-ε-caprolactone) (PMCL) based materials and a hydrolysis product of PMCL, sodium 6-hydroxy-4-methylcaproate. The concentration of sodium 6-hydroxy-4-methylcaproate that leads to 50% cell death (TD_{50}) is 179 mM, a value that is higher than for the hydrolysis products of the more common polycaprolactone and polylactide. We also report the degradability of two PMCL materials with different architectures (cross-linked and linear triblock polymers) under simulated industrial composting conditions. These materials reached high degrees of carbon mineralization (>85%) over the course of 120 days as monitored by CO_2 evolution. Lastly, we examined the industrial compostability of a new aromatic polyester, poly(salicylic methyl glycolide) (PSMG). This material reached 89% degradation after 120 days, an important finding given the recalcitrance towards degradation of ubiquitous aromatic polyesters.

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Introduction

Plastics have become increasingly prevalent in society and are essential to our everyday lives. However, with the increased use of plastics, concerns over their sustainability have become apparent. Two major issues associated with plastics are primarily related to the reliance of fossil fuel feedstocks and environmental pollution. Fossil fuel feedstocks were used in the production of almost 360 million metric tons of plastics in 2018.[1] While a small percentage of these plastics are recycled or incinerated for energy recovery, most of them either accumulate in landfills or end up in the environment.[1-2] Most conventional plastics are not biodegradable in natural environments or are industrially compostable, and can persist for hundreds of years.[3-4] Over time with exposure to UV light, oxidative conditions, and abrasive forces, mismanaged plastic pollution fragments into micro- and nano-plastics, distributing ultra-small plastic particles across the environment negatively impacting ecosystems and food sources.[3, 5-9] Moreover, microplastics, small plastic particles that are less than 5 mm in length,[10] can act as a vector for other pollutants and demonstrate ecotoxicological effects dependent on the particle size, dose, and interaction with organisms.[11-12]

These issues have led to significant research efforts in degradable polymers. A major issue with materials labeled as degradable is the often-mistaken interpretation that these materials are biodegradable and/or compostable over reasonable time frames. Polylactide (PLA) is one example: while PLA can be synthesized from sustainable resources and is known to be industrially compostable, it can be labeled as biodegradable in a misleading manner.[13] Biodegradable plastics degrade under the action of natural microorganism metabolism, whereas compostable plastics undergo degradation in an engineered environment, with specific microorganisms, temperature, humidity, and other conditions to yield CO_2, water, inorganic compounds, and biomass. For a material to be labeled compostable, it must typically also degrade at a rate consistent with other

known compostable materials such as cellulose.[14-15] Studies have shown that when exposed to natural environmental conditions, versions of PLA do not actually decompose at a detectable rate.[16] Although not biodegradable in natural environments at appreciable rates, PLA degrades under aerobic industrial composting conditions that are typically maintained at temperatures above 50 °C.[17]

Promising new candidates to address the challenges in sustainability are other related synthetic polyesters.[18-24] Specifically, we have focused research efforts toward polymers based on γ-methyl-ε-caprolactone (MCL).[18, 20, 25-27] MCL-based polymers have potential to be both bioderived[25] and biodegradable/compostable.[26] While PMCL as a homopolymer has a low T_g and limited applications,[27] unlike PLA and or PHA homopolymers, it's use in multicomponent materials is well established. For example, PLLA-PMCL-PLLA triblock polymers perform well as thermoplastic elastomers and have high ultimate tensile strengths and elongations at break that are comparable to SBS and SIS.[27] PMCL has also been utilized in crosslinked materials.[26, 28] These crosslinked materials have been shown to have relatively strengths and elongation at break.[26, 28] Additionally, these crosslinked materials have been shown to hydrolytically degrade under basic[28] and enzymatic conditions.[26] Under these enzymatic conditions, MCL polymers undergo surface erosion hydrolysis, producing 6-hydroxy-4-methyl caproic acid (or the corresponding carboxylate depending on pH).

To determine potential adverse toxicity, we examined the viability of mammalian fibroblasts cultured both on MCL-based polymers and in the presence of the hydrolysis product, sodium 6-hydroxy-4-methylcaproate. We view this type of cell viability study important to connect microorganism viability in natural environment degradation and in engineered environments. Because biodegradability is highly dependent upon many factors including environmental

conditions (e.g., pH and temperature) and polymer properties (e.g., polarity, glass transition temperature, and morphology),[29-30] we examined the degradation of MCL based materials under industrial composting conditions through model respirometry experiments.

In addition to aliphatic polyesters, such as PMCL, aromatic polyesters are also important materials.[31] However, most aromatic polyesters are nondegradable.[32] Poly(salicylic methyl glycolide) (PSMG) is a new, sustainable, aromatic polyester.[33] This polymer has potential in a wide range of applications due to its comparable glass transition temperature ($T_g \approx 85$ °C) and Young's modulus ($E \approx 2.3$ GPa) to poly(ethylene terephthalate). Furthermore, reports have shown that PSMG undergoes complete degradation in seawater at 50 °C in 60 days.[33] In addition to our studies on the aliphatic polyester, PMCL, in this work, we also examined the compostability of the aromatic polyester, PSMG.

Materials and Methods

All reagents were used as received unless otherwise indicated. Dichloromethane (DCM), ethyl acetate, hexanes, and anhydrous tetrahydrofuran (THF) were purchased from Fisher Scientific, whereas methanol was purchased from Sigma-Aldrich. THF was purified by passing through two neutral alumina-packed columns followed by a third column packed with activated 4 Å molecular sieves under nitrogen pressure, and was degassed by three freeze−pump−thaw cycles prior to use. All other solvents were reagent grade or better and used as received. Lactide was generously provided by Altasorb (a subsidiary of Ortec, Inc.). Lactide and 1,4-benzenedimethanol were recrystallized from toluene (×3), dried under vacuum for 48 h, and stored under inert atmosphere. Anhydrous toluene was obtained through a JC Meyer solvent drying system. Trifluoroacetic acid (TFA, Millipore-Sigma), 4-dimethylaminopyridine (DMAP, Millipore-

Sigma), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, Millipore-Sigma), 1,2,4-trichlorobenzene (TCB, Millipore-Sigma), and acetone (HPLC grade, Fisher Scientific) were purchased and used without any purification. Diphenyl phosphate (DPP) was purchased from Sigma-Aldrich, dried under vacuum for 120 h and stored in a glove box under inert atmosphere. 1,1,2,2,-tetrachloroethane (TCE, Anhydrous, MilliporeSigma) was distilled over calcium hydride prior to use. $SnOct_2$ used for polymerization was purified by triple distillation under high vacuum and argon (50−100 mTorr and 130−150 °C) and was stored under a nitrogen atmosphere. The $SnOct_2$ used for elastomer synthesis was purchased from Alfa Aesar (96%) and was stored in a refrigerator after being placed under a vacuum for 1 week to remove residual 2-ethylhexanoic acid. The deuterium-labeled solvents used for NMR spectroscopy, $CDCl_3$ (99.8% with 0.05 vol % tetramethylsilane (TMS) as reference standard) and D_2O (99.9% with 0.75 wt % 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid, sodium salt (TSP) as reference standard), were purchased from Cambridge Isotope Laboratories and Sigma-Aldrich, respectively. All monomers were synthesized as previously reported.[26, 33]

Synthesis of PLLA-PMCL-PLLA triblock polymers. PLLA-PMCL-PLLA triblock polymers were synthesized according to the literature.[18] In a glove box under nitrogen atmosphere, MCL (30.0 g, 234 mmol) and 1,4-benzenedimethanol (BDM, 50.0 mg, 0.361 mmol) were added to a pressure vessel and mixed. Once the BDM was dissolved, diphenyl phosphate (DPP, 600 mg, 0.240 mmol) was added, the pressure vessel was sealed, and mixing was continued. Once the DPP was dissolved, the pressure vessel was placed into a preheated sand bath at 100 °C until conversion reached ≥ 95% (as determined by ^{1}H NMR spectroscopy). The polymer was purified by dissolving in chloroform with 5 mg/mL of pyridine and precipitated into cold methanol (3x), hexanes (1x), and dried *in vacuo*.

10.0 g of the dried PMCL was dissolved in 8 mL of dry toluene in a glove box under nitrogen atmosphere. Once the PMCL was completely dissolved, L-lactide (7.00 g, 48.6 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 600 μL, 4.02 mmol) was added and mixed at room temperature. After 1 h, 1.00 g of benzoic acid was added and the mixture was dissolved in chloroform and precipitated into cold methanol (3x), hexanes (1x), and dried *in vacuo*. ^{1}H NMR data is given in Figure S1, and SEC data is given in Figure S5.

Synthesis of PMCL crosslinked elastomers. PMCL crosslinked elastomers were synthesized according to the literature.[26] In a typical polymerization, a 50 mL pressure vessel was loaded with MCL (20.0 g, 156 mmol) and pentaerythritol (137 mg, 1.01 mmol) under an inert atmosphere. A stock solution of $SnOct_2$ was prepared in toluene and added (90 μL stock solution, 12.7 mg, 31.2 μmol of $SnOct_2$) to the pressure vessel. A Teflon-coated magnetic stir bar was added to the pressure vessel, which was subsequently sealed, removed from inert atmosphere, and placed in a preheated silicone oil bath. The polymerization was allowed to proceed for 2 h at 160 °C; by the end of the reaction, the contents were still clear but had a slight yellow tinge, and the viscosity had increased drastically such that the stir bar was not stirring effectively. DCM was added to approximately double the volume in the pressure vessel, and the crude PMCL was dissolved overnight. PMCL was then precipitated from DCM twice–first into methanol, then using hexanes–and consolidated into a tared jar. The pure polymer was then dried under a stream of nitrogen gas for 24 h before being placed in a vacuum oven, where it was dried under a vacuum for 2 days. The temperature in the vacuum oven was then elevated to 60 °C, and the polymer was dried under a vacuum for 2 more days. Typical conversions of monomer were greater than 98%, and typical yields were greater than or equal to 90%.

To produce the crosslinked elastomers, star-shaped PMCL (4.0 g) of M_n = 11, 22, or 32 kg/mol was dissolved in DCM (4 mL) in a 20 mL vial using a small Teflon-coated magnetic stir bar. To each vial, bis(β-lactone) cross-linker was added such that the PMCL end group to β-lactone ratio was 1.0 to 1.5 (180, 91, and 65 mg, respectively). Next, $SnOct_2$ was added (100 mg/mL stock solution in DCM) in amounts corresponding to 2.5 mol % with respect to the PMCL end groups (15, 7, and 5 mg, respectively). After stirring for 30 s, the homogeneous mixture was poured into aluminum weigh pans (7 cm diameter). A small amount of DCM (1−2 mL) was used to finish the transfer, and the solvent cast mixtures were put under a stream of nitrogen gas for 24 h to evaporate solvent. After drying, the pans were put in a preheated oven (120 °C) under a nitrogen atmosphere for 24 h.

Synthesis of poly(salicylic methyl glycolide) (PSMG). PSMG was synthesized according to the literature.[33] In a typical bulk polymerization, salicylic methyl glycolide, 1,4-benzenedimethanol, and 4-(dimethylamino)pyridine (DMAP) were added to a pressure vessel under an argon atmosphere in a glove box. A Teflon-coated magnetic stir bar was added to the pressure vessel, which was subsequently sealed, taken out of the glove box, and placed in a preheated oil bath at 140 °C. After a certain time, the vessel was opened and cooled in an ice bath to stop the reaction. The crude solution was added dropwise to cold methanol. The white powder product was precipitated out. The collected polymer was dissolved in chloroform and precipitated into cold methanol twice more. The final polymer powder was dried *in vacuo*. For the ^{1}H NMR data see Figure S2, for SEC data see Figure S5.

Synthesis of sodium 6-hydroxy-4-methylcaproate. Sodium 6-hydroxy-4-methylcaproate was synthesized according to the literature.[34] Aqueous NaOH (0.7 mL, 7.75 mmol) was added dropwise to MCL (990 mg, 7.75 mmol) and the reaction was stirred for 20 h open to air. After 20 h, the pH of the reaction mixture was 7. The aqueous solution was extracted with DCM (3 x 5 mL). The H_2O was removed *in vacuo* to yield a white powder (1.27 g, 86.2 %). ^{1}H NMR (500 MHz, D_2O): δ 0.90 (d, 3H, C***H₃***), 1.50 (m, 5H, C***H₂***-C***H***-C***H₂***), 2.19 (m, 2H, C***H₂***-COONa), 3.66 (m, 2H, C***H₂***-OH) (See Figure S3). ^{13}C NMR (125 MHz, D_2O): δ 18.52, 28.84, 33.04, 35.22, 38.23, 59.88, 184.17 (See Figure S4).

Synthesis of sodium 6-hydroxycaproate. Sodium 6-hydroxycaproate was synthesized according to the literature.[34] Aqueous NaOH (0.7 mL, 8.72 mmol) was added dropwise to ε-caprolactone (CL) (1.00 g, 8.72 mmol) and the reaction was stirred for 3 h open to air. After 3 h, the pH of the reaction mixture was 7. The aqueous solution was extracted with DCM (3 x 5 mL) and the organic layer discarded. The H_2O was removed *in vacuo* to yield a white powder (1.40 g, 96.1 %). All characterization data matched the literature. ^{1}H NMR (500 MHz, D_2O): δ 1.34 (m, 2H, CH_2-C***H₂***-CH_2), 1.57 (m, 4H, C***H₂***-CH_2-C***H₂***), 2.19 (m, 2H, C***H₂***-COONa), 3.60 (m, 2H, C***H₂***-OH). ^{13}C NMR (125 MHz, D_2O): δ 25.00, 25.60, 31.10, 37.53, 61.67, 183.90.

Preparation of PLLA-PMCL-PLLA films. PLLA-PMCL-PLLA films were processed using a Carver® hydraulic press. The polymer (1 g) was placed on a Teflon sheet and pressed at 180 °C with 700 lbs of pressure for 3 min followed by pressing with 3000 lbs of pressure for 5 min. The film was then rapidly cooled in a water-injected press. Circular samples were cut from this film

using a biopsy punch. Polycaprolactone (PCL, Sigma-Aldrich, average M_n = 45 kDa) control films were fabricated in a similar manner.

Live/dead staining for cells cultured on polymer films. Polymer films (0.3 mm thick and 8 mm in diameter) were each attached to a cover glass through a thin layer of vacuum grease (the cover glass and vacuum grease were autoclaved) and then placed in a 24-well tissue culture plate. The films were soaked in Penn-Strep (5%) for 2 h for sterilization, followed by washing with phosphate-buffered saline (PBS) 3 times. NIH 3T3 fibroblasts were seeded on the polymer films at a density of 50,000 cells per well, and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% Penn-Strep in a tissue incubator (37 °C, 5% carbon dioxide, 100% relative humidity). At 24 h post seeding, the cells were stained with the live/dead reagents, ethidium homodimer and calcein AM (0.1% v/v), for 30 min in the dark. Each sample was then flipped upside down onto a cover glass slide and imaged on a Zeiss Axio Observer inverted microscope with a 5× objective. Cells seeded directly on the bottom surface in the culture plate (tissue culture polystyrene (TCPS)) and cells seeded on PCL films were examined as controls.

AlamarBlue assay for cells cultured on polymer films. Polymer films (0.3 mm thick and 6 mm in diameter) were each attached to the bottom of a well in a 96-well tissue culture by applying a thin layer of autoclaved vacuum grease underneath the films. The polymer films were soaked in Penn-Strep (5%) for 2 h for sterilization and washed with PBS 3 times followed by incubation in the cell culture medium overnight prior to cell seeding. Fibroblasts were seeded at a density of 5000 cells per well and cultured for 24 h, followed by the AlamarBlue assay to determine cell

viability. Fibroblasts seeded on TCPS and PCL films were examined as controls. To perform the AlamarBlue assay, the cell culture medium was replaced with phenol red free DMEM containing 10% v/v AlamarBlue reagent (Bio-Rad), followed by incubation for 4 h. The medium of each sample (100 μL) was transferred to a new 96-well plate and the fluorescence signal with excitation/emission at 560/590 nm was measured using a BioTek Cytation 3 Cell Imaging Multi-Mode plate reader. Since the AlamarBlue signal is dependent on the total cell number, the differences in cell adhesion properties between PLLA-PMCL-PLLA and the controls were expected to cause errors in evaluating their relative cytotoxicity. To address this issue, the AlamarBlue assay was also performed for samples cultured for 4 h post seeding as a normalization reference, and a medium change was conducted at 4 h for the samples to be evaluated at 24 h (the cells not adhered to material surfaces at 4 h would be removed during medium change). The AlamarBlue signal at 24 h normalized to that at 4 h for each material reveals the increase in cell number between 4 and 24 h. A higher value of cell number increase represents a lower level of material cytotoxicity. The experiments were performed in triplicate, and statistical analysis was conducted by unpaired t-tests.

Cytotoxicity evaluation of polymer hydrolysis products. The cytotoxicity of sodium 6-hydroxy-4-methylcaproate (the hydrolysis product of PMCL) and sodium 6-hydroxycaproate (the hydrolysis product of PCL) was evaluated by determining the median toxic dose, TD_{50} (the concentration resulting in 50% cell viability), from a dose-response curve of cell viability as previously reported.[35] Fibroblasts were seeded in a 96-well cell culture plate at a density of 5,000 cells per well and cultured for 24 h. The culture medium was then replaced with serum-free DMEM containing the test compound at various concentrations, followed by an additional incubation in

the tissue culture incubator for 24 h. A control in which no compound was added in serum-free DMEM was performed. Cell viability was evaluated using the AlamarBlue assay, and the fluorescence intensity of each sample containing a test compound was normalized to that of the control. A dose–response curve of normalized cell viability was plotted and the TD_{50} was determined using GraphPad Prism 8. The experiments were performed in triplicate.

Polymers for Industrial Composting. The polymers used for industrial composting studies were prepared according to the descriptions above.[18, 27, 33] PSMG samples were 40 kDa with a dispersity of 1.12 as determined by SEC light scattering. The PMCL based samples studied in composting were mixed molar masses and had compositions according to Table 1. The crosslinked PMCL sample contained < 0.7 wt% of Sn and 2-7 wt% crosslinker. PMCL samples were ground into small pieces (< 5 mm × < 5 mm × 1 mm) and PSMG samples were fine powders.

Table 1. Polymer compositions for PMCL containing samples studied in composting.

Polymer	M_n **(kDa)**[a]	**Volume Fraction of PLLA (**f_{PLLA}**)**	**Percentage of Polymer in respirometry experiments (%)**
Crosslinked PMCL	11[b] 22[b] 32[b]	NA	43 23 34
PLLA-PMCL-PLLA	33 67	0.37 0.32	33 67

[a] M_n determined by ^{1}H NMR spectroscopy end-group analysis. [b] Prepolymer M_n before cross-linking.

Respirometry. 4-5 month-old raw compost was collected from an industrial composting plant located in Athens, Georgia. The feedstocks for this material were forest residue, food waste, and livestock manure. The temperature in the composting pile (30 cm depth) was 40 °C at the time of

collection. The raw compost was sieved using a 4.76 mm sieve to produce the fine compost inoculum. See Tables S1 and S2 for compost details. Compost analysis was performed at the New Materials Institute at the University of Georgia.

The sample environment in the respirometer was maintained under modified ASTM D5338-15 conditions. Approximately 6 g of each sample was prepared with 125 g of total solids compost (by dry weight basis). During the study, the incubators were held at 58 °C and the mixture of compost and samples in each reactor was stirred once a week to avoid channeling and to control compost moisture. DI water was added to the compost as needed to maintain moisture between 45 and 60%. The amount of water used in the rehydration varied depending on the sample.

Polymer degradation was determined by biogas evolution and monitored using an ECHO respirometer (Slovenske Konjice, Slovenia). An air flow of 200 mL min^{-1} was pumped into each reactor, and the composition of exhausted gas was analyzed by the built-in gas sensors. All samples were performed in triplicate for 120 days and compared against 20 μm α-cellulose powder as the positive control. Methane concentrations were monitored to ensure aerobic conditions were maintained throughout the experiment and were found to be negligible. The evolved biogas of each sample was calculated daily by the difference in CO_2 production (mass in mg) of each sample compared to the daily average CO_2 contribution from blank controls as described by equation 1.

$$Sample_{CO_2} = Reactor_{CO_2} - Blank_{CO_2} \quad (1)$$

The sample mass and the percent organic carbon content (Table 2) was used to determine the carbon contributions to CO_2 for each sample. The organic carbon and nitrogen contents of the polymers were determined with a Vario Elementar EL/max elemental analyzer using a TCD detector (Elementar Analysensysteme GmbH, Hanau, Germany). The dimensionless ratio of the

molar mass of carbon dioxide to carbon 44/12 was used to account for the carbon in the CO_2 generated from each reactor for each day. The calculated evolved CO_2 for each sample was then used to calculate the daily absolute biodegradation according to equation 2.

$$Absolute\ Biodegradation\ (\%) = \left(\frac{Sample_{CO_2}}{Sample\ Mass\ X\ Carbon\ Content\ (\%) X\ 44/12}\right) * 100 \qquad (2)$$

Table 2. Carbon and nitrogen content of samples.

Sample	**Carbon (%)**	**Nitrogen (%)**
α-Cellulose	42.51	0.01
PLLA-PMCL-PLLA	63.11	0.037
Crosslinked PMCL	64.99	0.036
PSMG	66.34	0.020

Results and Discussion

Polymer and small molecule synthesis

PLLA-PMCL-PLLA, crosslinked PMCL, and PSMG were synthesized according to the literature (chemical structures shown in Figure 1).[18, 26, 33]

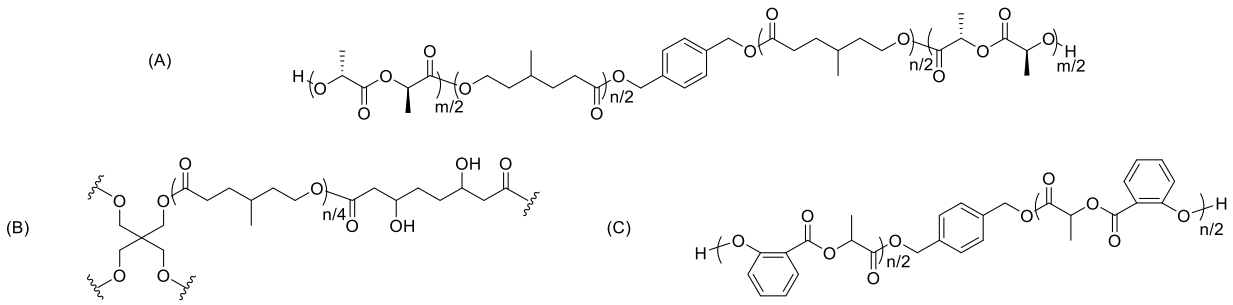
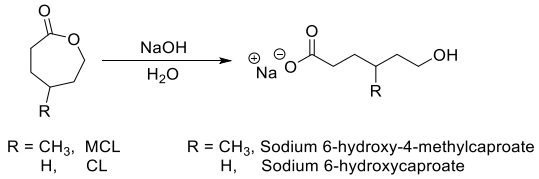


Figure 1. Structures of (A) PLLA-PMCL-PLLA, (B) crosslinked PMCL, and (C) PSMG.

At physiological pH, containing sodium, the hydrolysis products of PCL and PMCL are sodium 6-hydroxycaproate and sodium 6-hydroxy-4-methylcaproate, respectively. The cytotoxicity of sodium 6-hydroxycaproate has been well studied given that PCL is a widely used biocompatible polymer;[36-37] however, the cytotoxicity of sodium 6-hydroxy-4-methylcaproate remains unknown. We therefore sought to synthesize sodium 6-hydroxycaproate and sodium 6-hydroxy-4-methylcaproate and compare them in this regard. We hydrolyzed the respective lactone monomers following literature procedures (Scheme 1).[34] The sodium salts produced were afforded as white powders in high yields (>86%) and high purities (>95%).



Scheme 1. Hydrolysis of MCL and CL.

Cytotoxicity of polymer films

The cytotoxicity of PLLA-PMCL-PLLA was first evaluated using a qualitative live/dead staining assay for fibroblasts cultured on polymer films for 24 h. Two well-known non-cytotoxic materials, PCL[38] and standard tissue culture polystyrene (TCPS),[39] were investigated as controls. Fluorescent images revealed that the cells cultured on PLLA-PMCL-PLLA films were highly viable, as many green cells were observed and the ratio of green to red cells was large (Figure 2a). In addition, the spread morphology was indicative of healthy cells and good cell adhesion,[40] similar to the cells cultured on TCPS (Figure 2c). The cells cultured on PCL films showed a slightly rounded morphology (Figure 2b). These results suggest that PLLA-PMCL-PLLA is non-cytotoxic.

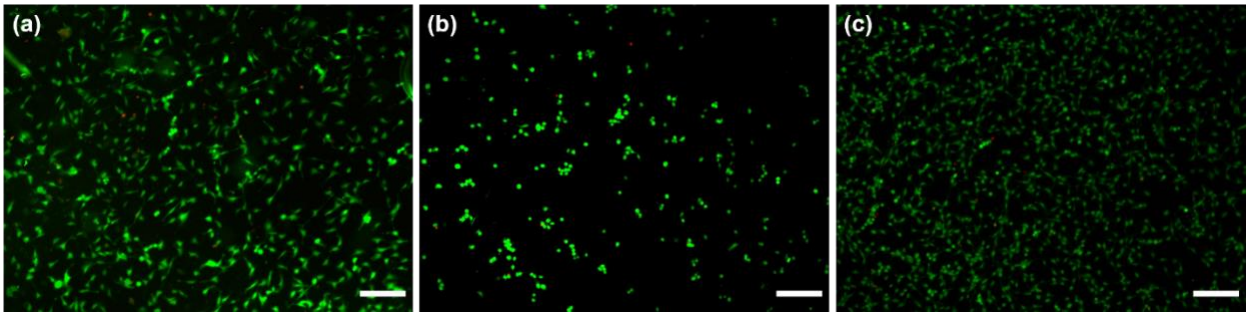


Figure 2. Live/dead assay for NIH 3T3 fibroblasts cultured for 24 h on (**a**) PLLA-PMCL-PLLA, (**b**) PCL, and (**c**) TCPS. Green represents live cells and red represents dead cells. The scale bar is 200 μm.

The cytotoxicity of PLLA-PMCL-PLLA was further quantitatively evaluated using the AlamarBlue assay for fibroblasts cultured on polymer films for 24 h, with PCL and TCPS as controls. To reveal different cell adhesion properties between the three materials and eliminate their effects on cytotoxicity evaluation, the AlamarBlue assay was also performed for cells cultured for 4 h post seeding on each material. For the samples evaluated at 24 h, a medium change was performed at 4 h to remove non-adherent cells, so that the AlamarBlue signal at 24 h

normalized to that at 4 h represents the increase in cell number between 4 and 24 h. The number of cells adhered on PLLA-PMCL-PLLA at 4 h is smaller than that on PCL or TCPS, but the increase in cell number between 4 and 24 h is greater than that on PCL and similar to that on TCPS (Figure 3). Since a greater increase in cell number indicates a lower level of cytotoxicity, these results suggest that PLLA-PMCL-PLLA is similar to TCPS and better than PCL in terms of cytocompatibility. This increase in cell proliferation on PMCL materials has also been observed in PMCL-PCL scaffolds[41] and other crosslinked PMCL samples.[28]

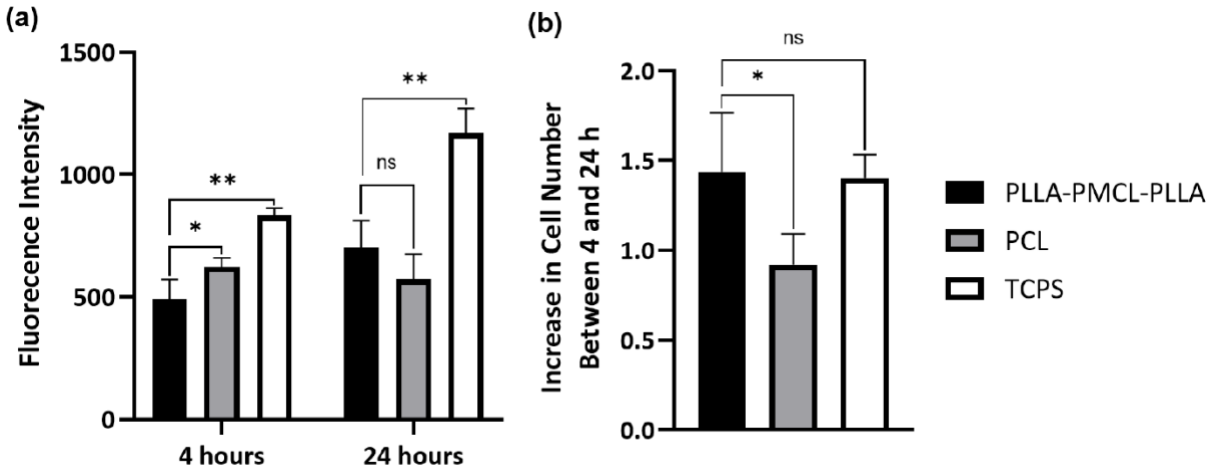


Figure 3. AlamarBlue assay for fibroblasts cultured on PLLA-PMCL-PLLA, PCL, and TCPS. (**a**) AlamarBlue fluorescence intensity measured at 4 and 24 h post cell seeding. (**b**) Increase in cell number between 4 and 24 h as calculated by normalizing the AlamarBlue signal at 24 h to that at 4 h. Error bars represent standard deviations, n = 3. ** p-value <0.01, * p <0.1, ns = not significant.

Cytotoxicity of polymer hydrolysis products

The dose-response curves of cell viability when exposed to sodium 6-hydroxy-4-methylcaproate and sodium 6-hydroxycaproate, the respective hydrolysis products of PMCL and

PCL, were similar (Figure 4). The TD_{50} (median toxic dose) values determined from these curves were 179.1±2.2 mM for sodium 6-hydroxy-4-methylcaproate and 171.7±1.9 mM for sodium 6-hydroxycaproate. PCL has been used in many FDA-approved, implantable medical devices.[42] The hydrolysis product is sodium 6-hydroxycaproate[38] and has been reported to be non-cytotoxic.[37] Sodium 6-hydroxy-4-methylcaproate has a slightly higher TD_{50} than that of sodium 6-hydroxycaproate suggesting that the PMCL hydrolysis product is also non-cytotoxic and MCL-based polymers could potentially be used for implantable medical devices. Importantly, the TD_{50} of sodium 6-hydroxy-4-methylcaproate is approximately four times higher than that of lactic acid (46.18 mM),[43] the hydrolysis product of PLA. PLA is a widely recognized sustainable and degradable plastic, and it has been used in FDA-approved implantable medical devices.[44] The significantly higher TD_{50} of sodium 6-hydroxy-4-methylcaproate as compared with the hydrolysis product of PLA further confirms the excellent cytocompatibility of PMCL.

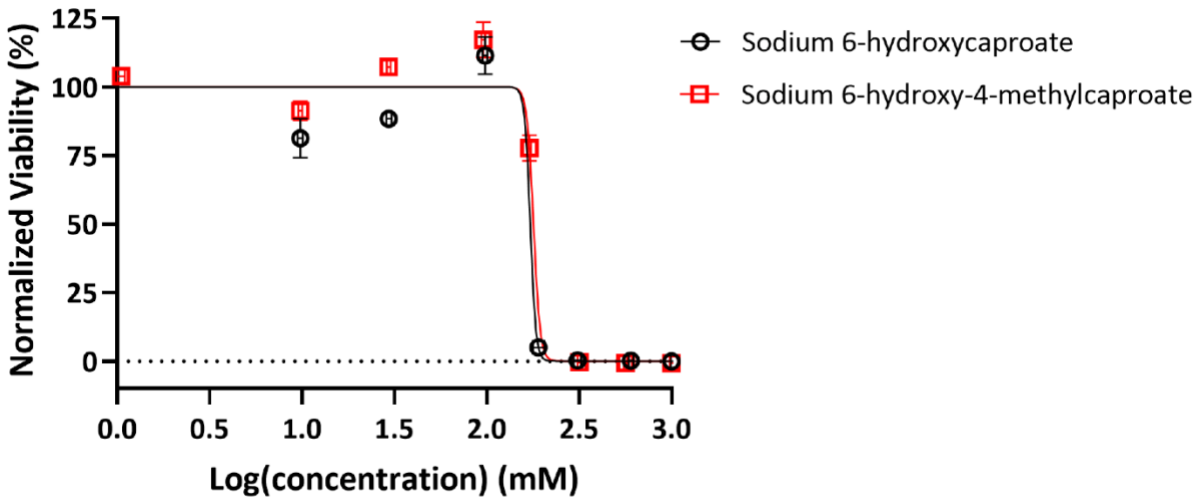


Figure 4. Dose-response curves of cell viability when exposed to the hydrolysis products of PMCL (red squares) and PCL (black circles). The cell viability is normalized to a non-treated control. The

curves represent the nonlinear regression analysis by GraphPad Prism 8 to determine the TD_{50}. Error bars represent standard deviation, n = 3.

While the hydrolysis products of the aliphatic polyesters studied in this work are shown to have good cytocompatibility, the hydrolysis products of the aromatic polyester (PSMG) are salicylic acid and lactic acid. Salicylic acid has been previously reported to have a TD_{50} of 11.80 mM.[43] While this value suggests a lower cytocompatibility compared to the hydrolysis product of PMCL, exogenous salicylic acid has been shown to be beneficial for plant growth in soil.[45-49]

Respirometry experiments with polymer samples

Although PLA is a well-known degradable plastic under industrial composting, it has been reported that composting facilities have refused acceptance of PLA into composting streams because of the high temperatures (> 60 °C) required to degrade over reasonable time frames.[50] Hydrolysis of PLA into low molar mass oligomers can be facilitated at these conditions given its glass transition temperature ($T_g \approx 60$ °C). These low molar mass oligomers and monomers can be more rapidly digested by the microbial consortia.[51] Therefore, the overall rate of carbon mineralization of high molar mass polymers is largely dependent on abiotic hydrolysis at early stages. This characteristic is related to the "hydrolysis lag period" of CO_2 formation measured in respirometry experiments.[52] The PMCL-based materials showed a hydrolysis lag period of ~15 days under industrial composting conditions (Figure 5), suggesting limited microbial metabolism during this early phase. Both PMCL materials demonstrated a similar hydrolysis lag profile as PLA under related hydrolytic conditions.[17] On the contrary, PSMG showed no induction period and immediate conversion to CO_2, which is attributed to rapid hydrolytic fragmentation under

these conditions.[33] This rapid hydrolysis may be partially attributed to the increased surface area of the PSMG, which was introduced to the compost as a fine powder in contrast to the PMCL materials that were cut into small pieces.[53-54] Attempts were made to grind all materials into the finest possible powder due to the parameters of ASTM D6400, however, the PMCL based materials have a low T_g (-60 °C) and are rubbery and difficult to grind. The short amount of time required for PSMG to reach the maximum conversion of polymer to CO_2 (< 30 days) is an important finding, as many compostable polymers take extended periods of time to fully compost.[17, 55]

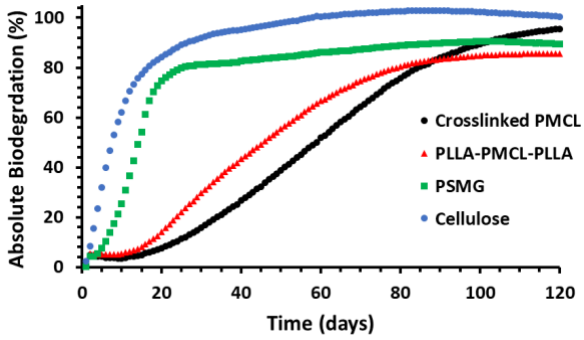


Figure 5. Absolute biodegradation profiles of polyesters and the positive control cellulose revealed by CO_2 respirometry. Each individual point is the cumulative amount of CO_2 produced referenced to the control.

The absolute biodegradation profiles reveal that PSMG demonstrated the most rapid conversion to CO_2, suggesting a complete metabolism of available organic carbon to biogas and biomass by day 120. Notably, the final absolute biodegradation value at 120 days is approximately 89%, which is close to the 90% minimum organic carbon mineralization, relative to cellulose controls, required for composting standards (ASTM D6400-19/EN 13432). However, under the

industrial composting conditions followed in this work (ASTM D5338), 180 days are allocated for a material to mineralize to the 90% threshold (due to the COVID-19 pandemic, the composting studies were halted at 120 days). Similarly, the absolute biodegradation of PLLA-PMCL-PLLA demonstrated approximately 86% by the end of the experiment and appears to have plateaued. The crosslinked PMCL did indeed surpass 90% absolute biodegradation. The differences in the degree of mineralization of the two different PMCL materials may be attributable to the crystalline PLLA end blocks in the triblock polymer materials, as studies have shown that crystalline materials degrade at slower rates under composting conditions.[56] While crosslinked PMCL showed the slowest overall rate of biodegradation, as evident by the increased amount of time required to reach the maximum conversion of polymer to CO_2, the slow rate of biodegradation for both PMCL materials could ultimately translate into more undigested polymer in some composting facilities. The U.S. EPA only requires operating temperatures of ≥40 °C for five days, with temperatures exceeding 55 °C for at least four hours in order to kill any pathogens.[57] In compost piles where thermophilic temperatures (40–60 °C) may only exist for a few weeks before distribution or use, some PMCL may not be mineralized. Conversely, PSMG may provide better composting outcomes in practice given its higher rate of mineralization, achieving near-complete carbon metabolism by about 30 days.

Although first order kinetic models have been used in previous work,[55] both samples containing PMCL deviate strongly from first order kinetics due to the contributions from both abiotic hydrolysis and biological metabolism processes. Use of a modified Gompertz growth model adopted from anerobic digestion,[55, 58-60] equation 3, more closely represents the cumulative CO_2 evolution for all samples, including the cellulose positive control (Figure S6).

$$V_{nCO_2} = P_m \times \exp\left(-\exp\left[\frac{R_m \times e}{P_m}(\lambda - t) + 1\right]\right) \quad (3)$$

Where V_{nCO_2} is the net cumulative CO_2 production from tested samples on the n^{th} day (mL); P_m is the total CO_2 production potential (mg); R_m is the maximum specific CO_2 production rate ($mg \cdot day^{-1}$); e is Euler's number; λ is the lag phase time (days); and t is the n^{th} day of operation (days). A comparison of the rates of absolute biodegradation by fitting the data using the modified Gompertz model are collated in Table 3.

Table 3. Modified Gompertz model parameters of CO_2 production and gaseous carbon loss from samples.

Parameters	**Cellulose**	**Crosslinked PMCL**	**PLLA-PMCL-PLLA**	**PSMG**
P_m (mL $CO_2 \cdot g^{-1}$ sample)	842	1360	1110	1140
R_m (mL $CO_2 \cdot d^{-1}$)	43.6	17.6	19.6	74.1
λ (day)	-1	22	13	5
CO_2 yield (mL $CO_2 \cdot g^{-1}$ sample)	845	1230	1070	1170
Absolute biodegradation at day 120 (%)	101	95.5	85.5	89.4
Biodegradation relative to cellulose at day 120 (%)	N/A	95.0	85.1	89.0

The rate of mineralization for each sample may be compared directly using the CO_2 production rate coefficient, R_m. Notably, PSMG returned a higher rate of biodegradation than the cellulose control, displayed by the shorter time required to reach the maximum amount of CO_2 produced. However, the cellulose control demonstrated complete mineralization to CO_2 (absolute biodegradation) while PSMG was not converted entirely to CO_2, suggesting that the remaining carbon was either converted to biomass or unassimilated by the microorganisms.[61] The physical properties of PSMG, such as high glass transition temperature (T_g ~ 85 °C) and competitive

Young's modulus (E ~2.3 GPa)[33] with polyethylene terephthalate (PET), makes its practical compost-degradation rate an important finding, as PET is non-biodegradable and used in many single-use applications,[32] which contributes to increased waste and potential environmental pollution.[62]

Because of the challenges associated with monitoring carbon conversion to biomass, it is common practice to compare the biodegradation of samples relative to that of cellulose control data, as shown in Figure 6. This method is more useful when the cellulose controls demonstrate absolute mineralization values significantly below 100%; however, in our experiments, the absolute biodegradation of the cellulose controls averaged approximately 100% by day 120.

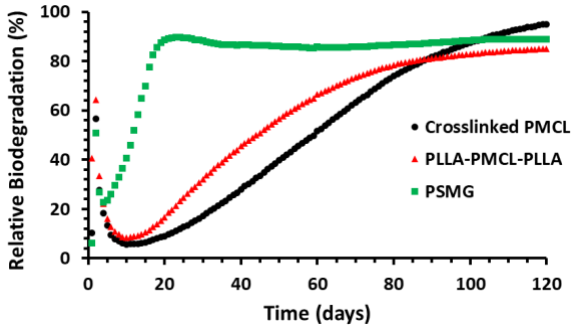


Figure 6. Biodegradation of tested polyesters relative to cellulose controls.

Inspection of each time point on this plot reveals information on the relative rate of biodegradation for each day when compared to the cellulose. The colonization phase shortly following day 0 often results in the observed high variability. By day 20, the relative biodegradation values for both the PMCL materials are just beginning to increase when compared to PSMG that has nearly completed the mineralization process. This indicates that PSMG degraded at a rate similar with the cellulose control and the composting process is complete by three weeks at 58 °C, which is a reasonable timeline and temperature for conventional composting operations.

Likely, the remaining 11% of carbon for PSMG and 15% of carbon for PLLA-PMCL-PLLA that did not mineralize was converted into biomass; however, further examination of the residues or experimentation with heavy isotopes would be necessary to verify this hypothesis.

Conclusion

We have shown that both MCL-based triblock polymers and the hydrolysis product for PMCL repeat units have excellent cytocompatibility. This makes PMCL elastomers a suitable and sustainable candidate for applications in medical devices. Additionally, CO_2 respirometry studies of MCL-based polymers showed that these materials reach high degrees of mineralization (>85%) over the course of 120 days under simulated industrial composting conditions. While previously noted to be enzymatically hydrolyzable, the results of this work allow for the addition of PMCL to the list of other important sustainable, compostable materials (e.g., PLA). Finally, we have demonstrated that PSMG, a sustainable aromatic polyester, is rapidly degradable under industrial composting conditions. Based on these findings, PSMG provides a promising compostable alternative to PET. Although the PSMG and PLLA-PMCL-PLLA samples reached 86 and 89% organic carbon mineralization, just shy of the 90% target set by ASTM D5338, these materials had reached a plateau in biodegradation by 120 days. However, these are single experiments performed on unformulated, neat polymers, and fully formulated materials could yield different results depending on additive packages.

Associated Content

The Supporting Information is available free of charge on the ACS Publications website at DOI: xx.

NMR spectra of polymers studies and their hydrolysis products. SEC traces of the polymers studied. Elemental analysis, CO_2 production, and hi resolution images of the compost. (PDF)

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Author Contributions

LR: lead author and project manager, synthesis of materials for cell viability studies; AS: cell viability studies, edited manuscript; JH: composting studies, edited manuscript; EMW: composting studies, edited manuscript; DCB: synthesis of triblock polymers, edited manuscript; HJK: synthesis of PSMG, edited manuscript; GXD: synthesis of crosslinked elastomers, edited manuscript; CJE: led and conceived of project, co-designed experiments and edited manuscript; WS: led cell viability studies, co-designed experiments, and edited manuscript; MAH: led and conceived of project, co-designed experiments, analyzed data, edited manuscript, and organized research team.

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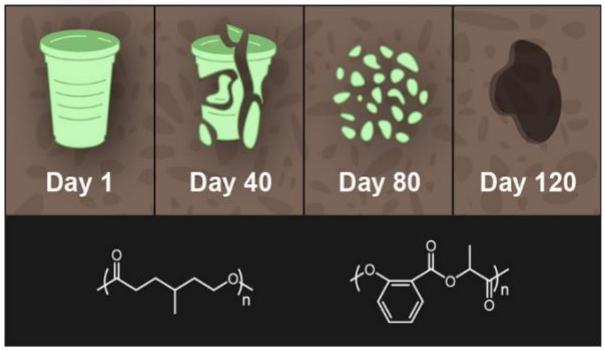
TOC



Sustainable polyesters decompose under industrial composting conditions.