RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2016, **6**, 81672

Bio-based polycarbonates derived from the neolignan honokiol†

Kevin T. Wacker, Samantha L. Kristufek, Soon-Mi Lim, Sarosh Kahn and Karen L. Wooley*

Honokiol, a highly functional phenolic- and alkenyl-containing neolignan natural product isolated from several *Magnolia* plant species, is an interesting bio-based resource, which is shown to be useful directly as a monomer for the rapid and scalable synthesis of poly(honokiol carbonate) (PHC). PHC was synthesized in one step from the natural product using condensation polymerization methods. Polymers of number average molecular weight (M_n) ranging from 10–55 kDa were obtained on gram scales in yields up to 80%. Thermal analysis demonstrated high thermal stability, with degradation temperatures in excess of *ca.* 450 °C. Mechanical testing of several PHC polymers indicated a generally increasing storage modulus with increasing M_n and a similar trend with T_g. With an interest toward cardiovascular applications, initial cytotoxicity and fluorescence cell imaging studies were conducted and showed no cytotoxicity toward coronary venular endothelial cells (CVECs), which proliferated on PHC thin films up to a month. Bulk PHC is a robust material, as it underwent slow hydrolytic degradation under basic conditions (*ca.* 0.1% per day under 1 M $NaOH_{(aq)}$), and no observable degradation under acidic and neutral conditions, each at 37 °C over 130 days. These polycarbonates serve as potential specialty engineering- or bio-materials derived from a commercially-available natural product monomer.

Received 2nd August 2016
Accepted 22nd August 2016

DOI: 10.1039/c6ra19568g

www.rsc.org/advances

Introduction

There has been a keen interest in developing renewable resources to supplement or replace petroleum-based feedstocks used in the production of specialty engineering plastics, as well as, materials for biomedical applications.[1–3] This interest stems primarily from suggested dwindling resources of petroleum-based feedstocks[4,5] and the health[6] and environmental[7–9] risks associated with non-degradable polymer waste. On an industrial scale, products such as the Tritan™ line of polymers from the Eastman Chemical Company and Durabio™ from Mitsubishi are being used to replace materials associated with potential endocrine disrupting properties.[10,11] Currently, lignin and polysaccharide-based[12–14] raw materials dominate as renewable sources for the production of small molecules used in sustainable and renewable polymer manufacture processes.[15] These renewable sources yield a variety of monomers that can be used in polyester, polyurethane, polyamide, and polycarbonate – including poly(isosorbide carbonate), (PIC) – production, among others.[16–22] However, renewable polymers constitute less than 1% of the yearly production of polymers worldwide.[1] Of the polymer classes derived from renewable sources, polycarbonates have shown promise as materials that could reduce waste in aqueous environments, as they have potential to yield CO_2 and alcohols or phenols upon hydrolytic degradation. This attractive feature has led to the interest in developing new natural product- and bio-based polycarbonates for a number of applications, such as biomedical applications.[23,24] For example, glucose has been utilized as a monomer, where biocompatibility and biodegradability could be desirable.[25,26] In addition, bio-based polycarbonates for specialty engineering materials such as PIC have potential toward replacing poly(bisphenol A carbonate), BPA-PC, and other bisphenol A-containing materials,[18–20,27] which have been linked to potential carcinogenic and toxic effects.[28,29] Although it is important to develop new, renewable, biocompatible, and potentially (bio) degradable materials, it will be imperative not to compromise performance in comparison to current petrochemical-derived standards. From a performance aspect, yet another aim in utilizing renewable materials is to develop polymers that are unique in having characteristics for which there are no current comparable petrochemically-derived equivalents. The vast chemical and structural diversity of renewable materials is ideal for the development of polymers for new technologies.

Currently, many interesting classes of natural products, such as lignans and neolignans, remain undeveloped for use in material or polymer applications. Lignans and neolignans are structurally diverse secondary plant metabolites produced through oxidative dimerization of a core phenylpropanoid

Departments of Chemistry, Chemical Engineering, Materials Science & Engineering, The Laboratory for Synthetic-Biologic Interactions, Texas A&M University, College Station, Texas 77842-3012, USA. E-mail: wooley@chem.tamu.edu

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c6ra19568g

unit.[30] Of the neolignans, honokiol and its isomers have been widely studied, as they are readily extracted from several *Magnolia* plant species and have shown promise for a number of therapeutic applications in cancer, neuronal disease, inflammation, and cardiovascular disease, among others.[31–36] In addition, honokiol has been used as a therapeutic in several drug delivery and nanoparticle formulations for *in vitro* studies.[37,38] These features, along with the potential to synthesize a number of structurally- and chemically-diverse materials directly from honokiol or its derivatives, prompted our initial investigation into the synthesis and characterization of the linear polymer, poly(honokiol carbonate), PHC. Furthermore, we envisioned that the development of a new monomer from a renewable resource may further the replacement of non-renewable, petroleum-based feedstocks. For applications as a potential natural product-based biomaterial, particularly in cardiovascular diseases, we examined cellular responses to PHC with cytotoxicity assays and fluorescence cell imaging. We anticipate that this initial demonstration of honokiol as a highly functional phenolic- and alkenyl-containing bio-based resource for the direct, rapid, and scalable production of polycarbonates will lead the efforts toward expansion to other (neo)lignans as starting materials/monomers for the synthesis of polymeric materials.

Experimental section

Materials

Pyridine (>99.8%), *N*,*N*-diisopropylethylamine (99.5%), triethylamine (≥99%), poly(bisphenol A carbonate) (M_w = 42.1 kDa, M_n = 21.4 kDa), poly(lactic acid) (M_w = 60 kDa, M_n = 30 kDa), dimethyl sulfoxide, fetal bovine serum (FBS), penicillin–streptomycin, 1.5% bovine gelatin, Phalloidin-Alexa488, and anti-vinculin antibody were purchased from Sigma Aldrich (St. Louis, MO). *N*,*N*-Diisopropylethylamine, triethylamine, and pyridine were distilled over CaH_2 prior to use unless otherwise noted; all other materials was used as received. Diphosgene (98%) was purchased from Alfa Aesar (Ward Hill, MA) and triphosgene (98%) was purchased from Oakwood Chemical (West Columbia, SC), and both were used as received. **Caution:** *Special precautions should be taken when working with phosgene precursors, including diphosgene and triphosgene. They are highly toxic by inhalation and ingestion; use of appropriate personal protective equipment is strongly recommended*. Solvents were purchased from EMD Millipore (Darmstadt, Germany) and used as received. All solvents were of ACS grade or higher. Honokiol (>98%) was purchased from Stanford Chemicals (Irvine, CA) and stored in a desiccator prior to use. Bovine coronary venular endothelial cells (CVECs) were kindly gifted by Professors Cynthia J. Meininger and Andreea Trache at Texas A&M Health Science Center. Glass-bottom cell culture dishes used in PHC thin film coatings were obtained from MatTeck (MatTeck, Ashland, MA) and ninety-six-well plates for cytotoxicity assays were purchased from Argos Technologies (Illinois, USA).

Characterization

NMR spectra (500 MHz for ^{1}H and 125 MHz for ^{13}C) were recorded on a Varian Inova 500 spectrometer. Chemical shifts were referenced to $CHCl_3$ at 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C), respectively. IR spectra were recorded on a Shimadzu IR Prestige attenuated total reflectance Fourier-transform infrared spectrometer (ATR-FTIR) and analyzed using IRsolution v. 1.40 software. Size exclusion chromatography (SEC) measurements were performed on a Waters Chromatography Inc. (Milford, MA) system equipped with an isocratic pump model 1515, a differential refractometer model 2414, and a four-column set of 5 μm Guard (50 × 7.5 mm), Styragel HR 4 5 μm DMF (300 × 7.5 mm), Styragel HR 4E 5 μm DMF (300 × 7.5 mm), and Styragel HR 2 5 μm DMF (300 × 7.5 mm) using DMF (0.05 M LiBr) as the eluent (1.00 mL min^{-1}) at 40 °C. Polymer solutions were prepared at concentrations of 5–10 mg mL^{-1} and an injection volume of 200 μL was used. Analysis was performed using Empower 2 v. 6.10.01.00 software (Waters, Inc.). The system was calibrated with polystyrene standards (Polymer Laboratories, Amherst, MA) ranging from 580 to 1 233 000 Da. Glass transition temperatures (T_g) and the melting transition temperature (T_m) were measured by differential scanning calorimetry (DSC) on a Mettler-Toledo DSC822 (Mettler-Toledo, Inc., Columbus, OH) under N_2 with a heating rate of 10 °C min^{-1} unless otherwise noted. The glass transition and melting transition temperatures of honokiol were measured by heating the sample to 100 °C and immediately transferring the sample into a liquid nitrogen bath before scanning from −50 °C to 100 °C. The measurements were analyzed using Mettler-Toledo STAR^e v.10.00 software. The T_g was taken on the third heating cycle as the extrapolated onset temperature from the intersection of the tangent drawn from the transition curve with the greatest slope with the extrapolated baseline before the transition. Thermogravimetric analysis (TGA) was performed using a Mettler-Toledo model TGA/DSC 1, with a heating rate of 10 °C min^{-1} under an Ar atmosphere. The measurements were analyzed using Mettler-Toledo STAR^e v.10.00 software. Rectangular dynamic mechanical analysis (DMA) samples were prepared using poly(honokiol carbonate) powder, which was ground with a mortar and pestle and pressed into an aluminum mold (2.0 cm × 0.5 cm × 0.2 cm). The powder was then heated in a VWR Symphony Vacuum Oven at ambient pressure using a heating cycle optimized for each number-average molecular weight polymer for a total heating cycle of no more than 48 hours. Samples were then allowed to cool to room temperature before being removed from the mold. Samples were sanded using 100 or 150 grit sandpaper to uniform dimensions. DMA was performed on a Mettler-Toledo TT-DMA system (Mettler-Toledo AG, Schwerzenbach, Switzerland). Sample dimensions were *ca.* 7 × 5 × 0.6 mm. Samples were analyzed in tension *via* thermal scan (3 °C min^{-1}) from 0 °C to 200 °C or 20 °C to 250 °C in autotension mode, with a frequency of 1 Hz, a preload force of 1 N, and a static force of 2 N. DMA data were obtained from Triton Laboratory software and exported to Origin Pro 8.0 for analysis.

Poly(honokiol carbonate) – PHC

The following consist of general procedures for the optimized syntheses of poly(honokiol carbonate) at varying initial molar concentrations of honokiol:

Procedure 1. Honokiol (1.104 g, 4.063 mmol), dry *N*,*N*-diisopropylethylamine (1.00 mL, 5.74 mmol), and pyridine (3.00 mL) were added to a flask, which was then placed in an ice bath and purged with nitrogen. Diphosgene (0.45 mL, 4.5 mmol) was then added to the flask over *ca.* 35 min and the reaction was allowed to proceed for *ca.* 60 h while warming to room temperature before being quenched with saturated aqueous sodium bicarbonate solution. The mixture was then diluted in dichloromethane (40 mL) and washed (deionized water) (40 mL × 2), followed by extraction of the aqueous phase with dichloromethane (10 mL). The combined organic layers were then dried over $MgSO_4$, filtered, and concentrated. The crude polymer was then precipitated into cold methanol (30 mL), centrifuged (10k RPM, 15 °C, 10 min), and decanted twice. The polymer was dried *in vacuo* to afford poly(honokiol carbonate). ATR-FTIR ν_{max} (cm^{-1}) 3085–2820, 1771, 1639, 1608, 1484, 1432, 1234, 1173, 1112; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60–6.60 (br, 6H), 6.10–5.70 (br, 2H), 5.30–4.90 (br, 4H), 3.60–3.10 (br, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.9, 151.8, 151.7, 151.4, 149.0, 148.9, 148.8, 148.7, 146.4, 146.3, 139.0, 138.7, 136.9, 135.5, 133.8, 133.4, 132.1, 131.9, 131.3, 130.9, 129.1, 128.4, 122.1, 116.9, 116.6, 39.6, 34.6, 34.2 ppm.

Procedure 2. Honokiol (1.110 g, 4.086 mmol), dry triethylamine (0.85 mL, 6.1 mmol), and pyridine (4.15 mL) were added to a flask then placed in an ice bath and purged with nitrogen. Diphosgene (0.45 mL, 4.5 mmol) was then added to the flask over *ca.* 35 min and the reaction was allowed to proceed for *ca.* 60 h while warming to room temperature before being quenched with saturated aqueous sodium bicarbonate solution. The mixture was then diluted in dichloromethane (40 mL) and washed (deionized water) (40 mL × 2), followed by extraction of the aqueous phase with dichloromethane (10 mL). The combined organic layers were then dried over $MgSO_4$, filtered, and concentrated. The crude polymer was then precipitated into cold methanol (30 mL), centrifuged (10k RPM, 15 °C, 10 min), and decanted twice. The polymer was dried *in vacuo* to afford poly(honokiol carbonate).

Cell culture

CVECs were cultured in GIBCO® Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) from Invitrogen (Invitrogen, Carlsbad, CA), along with 10% FBS, 100 U mL^{-1} penicillin – 100 U mL^{-1} streptomycin – 0.25 mg mL^{-1} amphotericin B (Lonza, Walkersville, MD), and 20 U mL^{-1} heparin (Midwest Vet Supply, Lakeville, MN). For subculture and experiments, cells were trypsinized and quenched with media. Trypsin was removed after centrifugation at 250 G for 4 minutes. Resuspended cells were plated on 1.5% bovine gelatin-coated flasks for subculture or 50 × 10^3 cells were plated on glass-bottom dishes coated with 1.5% bovine gelatin or polymer coated glass-bottom dishes for experiments.

Polymer thin films for cell culture

A PHC thin film was prepared by solvent casting from polymer solutions in dichloromethane (DCM) on glass-bottom cell culture dishes. A 1% solution was prepared by dissolving 10 mg of PHC in 1.0 mL of DCM, and diluted to 0.1% and 0.01% in DCM. Dried dishes were sterilized under UV for 1 h in the biosafety cabinet and washed with sterile PBS before plating cells. Cells were plated on polymer-coated glass-bottom dishes for experiments. As a control, cells were plated on glass-bottom dishes coated with 1.5% bovine gelatin. Cell media was replaced with fresh media every 2–3 d, and proliferation and morphology of cells were continuously monitored up to one month.

Immunofluorescence and confocal imaging

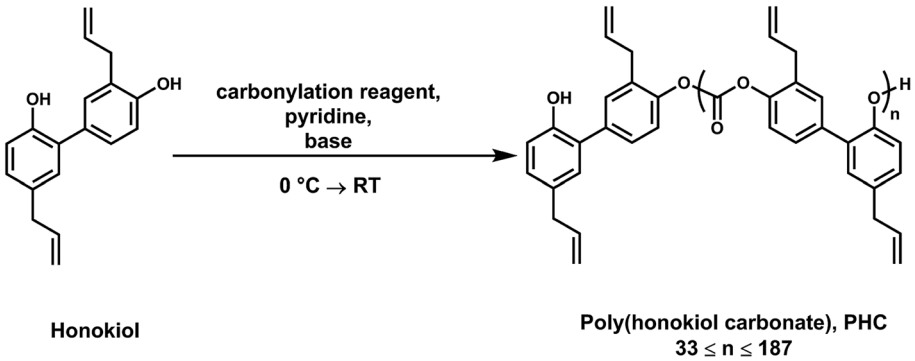
Cells were fixed with 2% paraformaldehyde and stained with Alexa Fluor 488® phalloidin & anti-vinculin with secondary antibody conjugated with Alexa Fluor 647® after 24 h, 1 week, and 1 month from plating to stain actin proteins in cytoskeleton and vinculin protein in focal adhesions, respectively. Laser scanning confocal microscopy was used to acquire fluorescence images. Excitation and emission filter settings were adjusted according to fluorophore spectra provided by the manufacturer. 3D confocal images were acquired as stacks of 15–18 planes with 0.5 μm step size. Overall, cell population was identified from the large filter view with a 10× objective. Details of cell morphology were investigated from fluorescence images acquired with a 20× objective with 2× zoom (40× magnification equivalent).

Degradation of poly(honokiol carbonate)

Samples were prepared from a home-built silicon cylindrical mold and were *ca.* 2.0 mm × 1.5 mm diameter and *ca.* 100 mg. Five to six samples (M_n = 21 kDa) were used for each set of degradation conditions: 1 M $NaOH_{(aq)}$, 1 M $HCl_{(aq)}$, and 1× PBS. All samples were placed in 1 dram vials and set in an incubating shaker at 37 °C at 60 RPM. For the $HCl_{(aq)}$ and $NaOH_{(aq)}$ samples, each solution was replaced every 2–3 days in order to maintain constant concentration of reagent, while 1× PBS was replaced every week. At predetermined times, samples were removed from the shaker, blotted dry, weighed, dried under vacuum at 45 °C to constant weight and reweighed. Swelling percentage was taken as the mass difference between wet and dry sample relative to the dry sample mass.

Results and discussion

The condensation polymerization of honokiol required optimization of the carbonylation agent and the conditions to obtain high molecular weight polycarbonates. The condensation reaction of honokiol was first attempted with relatively mild carbonylation reagents including 1,1′-carbonyldiimidazole (CDI), ethyl chloroformate, and 4-nitrophenyl chloroformate with triethylamine in *N*,*N*-dimethylformamide at 70 °C for 60 h or in bulk at elevated temperatures with diethyl- or diphenylcarbonate under dynamic vacuum. Upon workup, there was no precipitation of polymer from cold methanol and only starting material and small molecules were recovered. Subsequently, the polymerization was attempted with phosgene analogues triphosgene and diphosgene (Scheme 1). **Caution**: *Special precautions should be taken when working with phosgene*



Honokiol

Poly(honokiol carbonate), PHC
33 ≤ n ≤ 187

Scheme 1 General poly(honokiol carbonate) synthesis.

precursors, including diphosgene and triphosgene. They are highly toxic by inhalation and ingestion; use of appropriate personal protective equipment is strongly recommended. Polymers of number average molecular weight, M_n, of up to 82 kDa (DP_n = 280) were obtained using triphosgene in dichloromethane with pyridine, but yields were markedly low – typically less than 10% – and produced, largely, an intractable gel upon workup. Similar results were obtained when switching to diphosgene in pyridine without any additional base at reaction times of 60 h – the reaction mixture became increasingly viscous as the reaction volume decreased, and vigorous stirring was required to prevent gelation.

Conditions were then screened with the aim of reducing the viscosity of the reaction mixture and increasing isolated yield by addition of organic co-bases and reduction in the molarity of the monomer at t_0. *N*,*N*-Diisopropylethylamine (DIPEA) and triethylamine (TEA) proved most successful with an optimal ratio of 0.75 eq. DIPEA or TEA/phenol moiety (Table 1). Reaction conditions utilizing 1 M initial honokiol concentration in pyridine and DIPEA with phosgene precursor (1.0 eq./hydroxyl) and no additional solvent yielded poly(honokiol carbonate) in up to 80% yield.

When reducing the initial concentration of honokiol by dilution with pyridine, high yields were still obtained while the M_n of poly(honokiol carbonate) varied as expected with all other reaction conditions being equal: 0.50–0.80 M, *ca.* 42 kDa; 0.35 M, 27 kDa. The polycondensation was also fairly susceptible to moisture, as using non-distilled base resulted in polymers with lower molecular weights and yields (Table 1, final entry). The M_n and polydispersity, $Đ$, of all polymers were calculated using size exclusion chromatography (SEC) in *N*,*N*-dimethylformamide (0.05 M LiBr) eluent and compared to polystyrene standards (Fig. S1†). (PHC also showed markedly good solubility in many organic solvents, including chloroform, dichloromethane, tetrahydrofuran, dimethylsulfoxide, *etc.*) Confirmation of polymer formation was also noted with ATR-FTIR through disappearance of the O–H stretch of the hydroxyl moieties at 3300 cm^{-1} and appearance of the carbonate moiety with a carbonyl stretch at 1771 cm^{-1} and the symmetric and asymmetric C–O stretches at 1173 and 1234 cm^{-1} (Fig. S2 & S3†).

Table 1 Synthesis of poly(honokiol carbonate) with phosgene analogues

Reagents	Honokiol conc. (M)	M_n (kDa)	$Đ$	Yield (%)
Triphosgene	1.0	82	2.3	5
Diphosgene	1.0	37	3.3	3
Triphosgene/DIPEA[a]	1.0	20	2.0	79
Diphosgene/DIPEA	1.0	55	2.5	80
Diphosgene/DIPEA	0.5	43	2.6	77
Diphosgene/DIPEA	0.3	27	2.8	79
Diphosgene/DIPEA[b]	1.0	15	2.5	44

[a] All polymers were comparable when DIPEA was replaced with TEA. [b] DIPEA not distilled.

Initial thermal analyses had indicated high thermal stability yet relatively low glass transition temperatures, therefore, extensive thermal analyses were conducted for the series of PHCs, with comparisons to honokiol, a poly(bisphenol A carbonate) standard, M_n = 21.4 kDa, $Đ$ = 2.0, literature reports for PIC under similar conditions, and a poly(lactic acid) standard, M_n = 30 kDa, $Đ$ = 2.0. Thermal stabilities were evaluated by thermogravimetric analysis (TGA). PHCs of varying M_n all showed markedly good thermal stability in comparison to the poly(bisphenol A carbonate), poly(lactic acid), and poly(isosorbide carbonate) (Fig. 1), as noted by high onsets of thermal degradation (PHC: 454–462 °C *vs.* BPA-PC: 504 °C, PLA: 338 °C, and PIC: 346 °C (ref. 18 and 39)). The onsets of thermal degradation for the BPA-PC and PLA standards were in good agreement with other reports.[40,41] Interestingly, the side chain alkenyl groups did not cause a significant reduction in thermal stability relative to BPA-PC and no degradation of the PHC carbonate backbone was observed prior to reaching the onset temperature of degradation for the monomer, honokiol (Fig. S8†). While both standards, PLA and BPA-PC, showed one mass loss event, the PHCs exhibited two-stage degradation profiles with a second mass loss event *ca.* 600 °C. Complete degradation of the PHCs, as noted by remaining char mass or total mass loss, was observed only at temperatures above 700 °C. Both PHC and BPA-PC have shown much higher thermal stability than reports for PIC (340–346 °C).[18,39]

Differential scanning calorimetry (DSC) showed one thermal transition for each PHC polymer when using temperature sweeps ranging from 0–200 °C with a single transition and onset

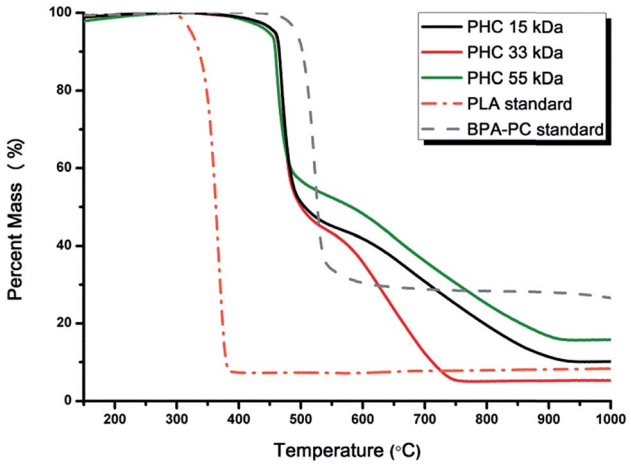


Fig. 1 PHC thermal degradation compared with standards.

in the range of 60–65 °C, corresponding to the glass transition temperature, (T_g) (Table 2). Further analysis up to 400 °C provided no additional thermal transitions corresponding to a melting transition, which suggests PHC to be non-crystalline. The amorphous nature was further confirmed by X-ray powder diffraction (data not shown). We initially predicted the glass transitions of PHC to be lower compared to our BPA-PC standard (T_g = 146 °C) and literature precedence for PIC ($T_g \approx$ 165 °C)[18] as honokiol contains two allylic side chains which are absent in both BPA-PC and PIC and can increase the free volume between the polymer chains of PHC. However, as measured by DSC, the T_g for the original, powder PHC samples were lower than expected, and comparable to current, widely used biomaterial polymers such as poly(lactic acid) (T_g = 57 °C, in agreement with the literature[42]).

Additional thermomechanical properties of the bio-based poly(honokiol carbonate) were determined by dynamic mechanical analysis, DMA, with further comparison to BPA-PC, PIC, and PLA. Samples of PHC with M_n = 23, 31, and 37 kDa were analyzed in tension using temperature sweeps ranging from 0 °C to 200 °C or 20 °C to 250 °C at 1 Hz in triplicate. As shown in Fig. 2, there is an increase in the glass transition temperature as measured by the peak of the loss modulus, E'', for poly(honokiol carbonate) with increasing M_n at room temperature, as well as a general increase in the storage modulus, E'. Most notably, E' of all PHC (1.1–1.7 GPa) were comparable to reported values for PLA (1.6 GPa), PIC (2.7 GPa), and BPA-PC (2.2 GPa), as reported using DMA under similar conditions.[39,43–45] Furthermore, it was intriguing to note the T_g of the highest M_n PHC tested (37 kDa, T_g = 106 °C) was substantially higher than values observed by DSC – all consistently in the range of 60–65 °C – while the T_g of the 23 kDa PHC (75 °C) was in good agreement with the DSC measurements. The DMA samples were then submitted to DSC characterization to compare thermomechanical changes between the original powder samples and the heat processed bars prepared for DMA. By DSC, similar trends were observed for the bar samples as with DMA, wherein PHC of increasing M_n showed steady increases in T_g and higher values than those observed for powder samples (Fig. S18 and S19†). For example, by DSC when

Table 2 Thermal data of honokiol, PHC, and standards, as measured by DSC and TGA

Monomer/Polymer	T_g (°C) – powder	T_g (°C) – bar[a]	T_m (°C)	T_d (°C)
Honokiol	−24	—	86	431
PHC-15 kDa	64	—	—	460
PHC-23 kDa	63	67	—	454
PHC-31 kDa	66	76	—	458
PHC-33 kDa	63	—	—	462
PHC-37 kDa	67	88	—	459
PHC-55 kDa	65	—	—	454
BPA-PC-21 kDa	146	—	—	504
PIC-28 kDa[b]	167	—	—	346
PLA-30 kDa	57	—	177	338

[a] Bar samples were fabricated from powder as in experimental. [b] Ref. 18.

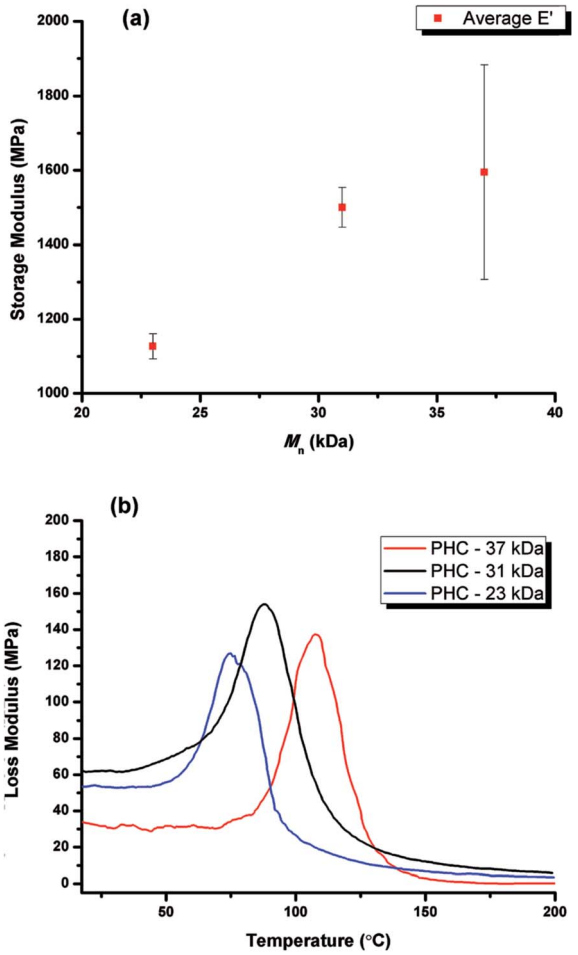


Fig. 2 Preliminary DMA results of PHC. (a) Average E' (25 °C) scaled with M_n. Scale bars represent standard deviation; n = 3. (b) Increasing T_g with M_n as measured by peak of the loss modulus. $T_{g,23\ kDa}$ = 75 °C, $T_{g,31\ kDa}$ = 87 °C, $T_{g,37\ kDa}$ = 106 °C.

bar samples were heated at the same rate as powder samples (10 °C min^{-1}), PHC-31 kDa and PHC-37 kDa displayed T_gs of 76 and 88 °C, respectively. These values are, at least, 10 °C higher than powder samples of any molecular weight. PHC-23 kDa had a T_g similar to the powder sample (67 °C). These effects are more noticeable when bars were heated at a faster rate (40 °C min^{-1}) (Fig. S20†). It is evident that the heat annealing process to form the bars allowed for reorganization of the polymer chains and results in T_gs with higher values than powder samples, more in line with other aromatic and rigid polycarbonates. It is hypothesized that polymers of these M_n and degree of polymerization values, $77 < DP_n < 126$, have not yet reached the theoretical glass transition temperature limit of an infinite molecular weight polymer. Furthermore, difficulties were encountered with processing of uniform sample bars that could survive DMA measurement. In fact, several samples were prone to failure before the full temperature scan completed (Fig. S17†). Future work will expand upon the relationship of these bulk thermomechanical properties, E' and T_g, with M_n,[46,47] as well as modification of synthetic conditions to allow access to higher molecular weight PHC in large quantities and processing conditions to afford uniform DMA samples.

As honokiol has been well studied as a potential therapeutic, particularly in cardiovascular applications,[31,32] several assays were used to assess biocompatibility and cytotoxicity of PHC to coronary venular endothelial cells (CVECs). The endothelium is the inner-most layer of the blood vessel and functions as a barrier between the blood lumen and vessel wall. Malfunction in the endothelium leads to hyperpermeability of the blood vessel that is often associated with cardiovascular diseases and tumor vessel leakage.[48] Therefore, we sought to test adaptability of endothelial cells to PHC, especially for long-term culture, using fluorescence imaging and cytotoxicity assay. Four polymers of $M_n = 20$, 28, 31, 37 kDa were used in MTS assays and showed no cytotoxic effects

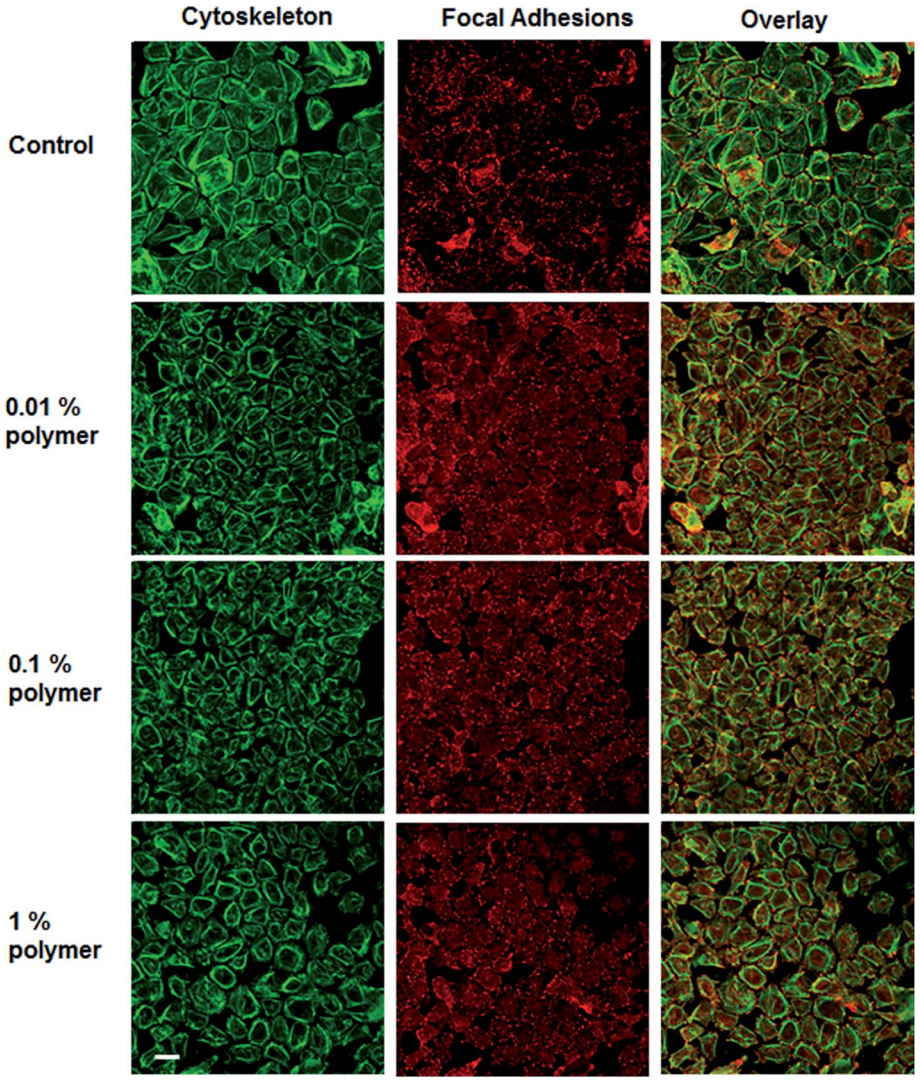


Fig. 3 Bovine coronary venular endothelial cells (CVECs) on poly(honokiol carbonate). Cells were fixed after 1 month of culture on the polymer and stained with phallodin-Alexa488® (green) and vinculin antibody and secondary antibody conjugated with Alexa647® to show cytoskeleton and focal adhesions, respectively. Images were acquired with a laser scanning confocal microscope with 20× objective and 2× magnification. Scale bar 30 μm.

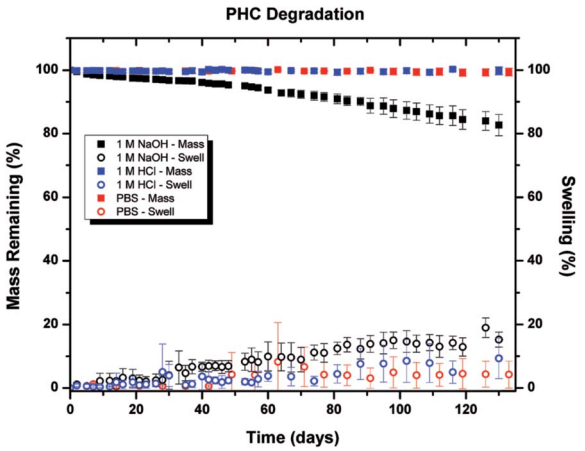


Fig. 4 Stability and swelling of PHC in aqueous media.

to CVECs at all concentrations tested (10–0.0048 μM). IC_{50} values of the polymers could not be determined because high cell-viabilities were observed at the range of the tested concentrations and PHC promoted cell growth at the higher concentrations (Fig. S21†). PHC effect on cell morphology was tracked with fluorescence imaging (Fig. 3). CVECs were plated and cultured up to one month, and actin fibers and focal adhesions were visualized by staining with fluorophores. Cell sensitivity to the extracellular environment and effects on actin fibers and focal adhesion distribution were monitored.[49,50] CVECs plated on PHC-coated cell culture dishes proliferated to reach confluency and maintained the monolayer with refreshed medium every 2–3 days. CVECs cultured on PHC were compared to the cells on gelatin-coated dishes, which is a common substrate for endothelial cell culture. Cells on PHC showed an intact monolayer with well-structured actin fibers and punctate focal adhesions comparable to the cells on the control surface.

To assess the stability and response of bulk PHC to physiological and accelerated degradation conditions, five or six cylindrical samples were prepared and immersed in acidic (1 M $HCl_{(aq)}$), basic (1 M $NaOH_{(aq)}$), and PBS solutions and allowed to stir in an incubator shaker at 37 °C. Degradation of the samples was followed for 130 days (Fig. 4), while the acidic & basic solutions were replaced every 2–3 days and every week for PBS solutions. Weights remained constant for the samples in PBS and acidic solutions, however, the samples swelled gradually to maxima of *ca.* 5 and 10% at 130 days in PBS and $HCl_{(aq)}$ solutions, respectively. In the case of samples subjected to 1 M $NaOH_{(aq)}$, slow, but steady degradation occurred at a mass loss rate *ca.* 0.1% per day. At the completion of the study, 18% average total mass loss was observed under basic environment. Analogous to the acidic and PBS samples, swelling increased with time, yet reached a higher relative percentage, *ca.* 15%. These results suggest PHC could serve as a robust biomaterial in most aqueous environments.

Conclusions

In summary, we have presented a short, straightforward synthesis of a polycarbonate from a renewable resource, honokiol. Unlike other renewable feedstocks, neolignans have been undeveloped until now and additionally do not compete with food production. Thermal characterization of the polymers yielded degradation and glass transition temperatures comparable to industry standards. Thermomechanical characterization has shown generally increasing moduli and T_g as M_n increases and an E' for PHC that is comparable to reported values for BPA-PC, PIC, and PLA at ambient conditions. In addition, the bulk polymers are robust and stable under physiological and acidic conditions yet also biocompatible to endothelial cells *in vitro*. These results suggest potential application of poly(honokiol carbonate) as an engineering or biomedical material where robust features and biocompatibility may be necessary.

Acknowledgements

We kindly thank Profs. Cynthia J. Meininger and Andreea Trache (Texas A&M Health Science Center) for providing coronary venular endothelial cells (CVECs) and gratefully acknowledge financial support from the National Science Foundation (CHE-1410272), and the Welch Foundation through the W. T. Doherty-Welch Chair in Chemistry (A-0001).

References

1 A. L. Holmberg, K. H. Reno, R. P. Wool and T. H. Epps III, *Soft Matter*, 2014, **10**, 7405–7424.
2 S. A. Miller, *Polym. Chem.*, 2014, **5**, 3117–3118.
3 Y. Kanetaka, S. Yamazaki and K. Kimura, *Macromolecules*, 2016, **49**, 1252–1258.
4 L. Yuan, Z. Wang, N. M. Trenor and C. Tang, *Macromolecules*, 2015, **48**, 1320–1328.
5 M. Winkler, T. M. Lacerda, F. Mack and M. A. R. Meier, *Macromolecules*, 2015, **48**, 1398–1403.
6 C. Weinhouse, O. S. Anderson, I. L. Bergin, D. J. Vandenbergh, J. P. Gyekis, M. A. Dingman, J. Y. Yang and D. C. Dolinoy, *Environ. Health Perspect.*, 2014, **122**, 485–491.
7 C. M. Rochman, M. A. Browne, B. S. Halpern, B. T. Hentschel, E. Hoh, H. K. Karapanagioti, L. M. Rios-Mendoza, H. Takada, S. Teh and R. C. Thompson, *Nature*, 2013, **494**, 169–171.
8 S. C. Mauck, S. Wang, W. Ding, B. J. Rohde, C. K. Fortune, G. Yang, S.-K. Ahn and M. L. Robertson, *Macromolecules*, 2016, **49**, 1605–1615.
9 L. Poussard, J. Mariage, B. Grignard, C. Detrembleur, C. Jérôme, C. Calberg, B. Heinrichs, J. De Winter, P. Gerbaux, J. M. Raquez, L. Bonnaud and P. Dubois, *Macromolecules*, 2016, **49**, 2162–2171.
10 F. A. Leibfarth and C. J. Hawker, *J. Polym. Sci., Part A: Polym. Chem.*, 2013, **51**, 3769–3782.
11 M. Fuji, M. Akita and T. Tanaka, US 9193823 B2, 2015.
12 R. Mülhaupt, *Macromol. Chem. Phys.*, 2013, **214**, 159–174.
13 F. H. Isikgor and C. R. Becer, *Polym. Chem.*, 2015, **6**, 4497–4559.

14 C. Japu, A. Martinez de Ilarduya, A. Alla, M. A. G. Garcia-Martin, J. A. Galbis and S. Munoz-Guerra, *Polym. Chem.*, 2014, **5**, 3190–3202.
15 A. J. Ragauskas, C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W. J. Frederick, J. P. Hallett, D. J. Leak, C. L. Liotta, J. R. Mielenz, R. Murphy, R. Templer and T. Tschaplinski, *Science*, 2006, **311**, 484–489.
16 S. Laurichesse and L. Avérous, *Prog. Polym. Sci.*, 2014, **39**, 1266–1290.
17 W. C. Shearouse, L. M. Lillie, T. M. Reineke and W. B. Tolman, *ACS Macro Lett.*, 2015, **4**, 284–288.
18 Q. Li, W. Zhu, C. Li, G. Guan, D. Zhang, Y. Xiao and L. Zheng, *J. Polym. Sci., Part A: Polym. Chem.*, 2013, **51**, 1387–1397.
19 M. Yokoe, K. Aoi and M. Okada, *J. Polym. Sci., Part A: Polym. Chem.*, 2003, **41**, 2312–2321.
20 S. Chatti, H. R. Kricheldorf and G. Schwarz, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **44**, 3616–3628.
21 S. Oliver, O. Vittorio, G. Cirillo and C. Boyer, *Polym. Chem.*, 2016, **7**, 1529–1544.
22 Z. Mou, S. Feng and E. Y. X. Chen, *Polym. Chem.*, 2016, **7**, 1593–1602.
23 J. Xu, E. Feng and J. Song, *J. Appl. Polym. Sci.*, 2014, **131**, 1.
24 T. S. Kristufek, S. L. Kristufek, L. A. Link, A. C. Weems, S. Khan, S.-M. Lim, A. T. Lonnecker, J. E. Raymond, D. J. Maitland and K. L. Wooley, *Polym. Chem.*, 2016, **7**, 2639–2644.
25 K. Mikami, A. T. Lonnecker, T. P. Gustafson, N. F. Zinnel, P. J. Pai, D. H. Russell and K. L. Wooley, *J. Am. Chem. Soc.*, 2013, **135**, 6826–6829.
26 M. Azechi, K. Matsumoto and T. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 2013, **51**, 1651–1655.
27 C. J. Besset, A. T. Lonnecker, J. M. Streff and K. L. Wooley, *Biomacromolecules*, 2011, **12**, 2512–2517.
28 E. A. Kelly, L. A. Opanashuk and A. K. Majewska, *Front. Neuroanat.*, 2014, **8**, 117.
29 D. C. Dolinoy, D. Huang and R. L. Jirtle, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 13056–13061.
30 J.-Y. Pan, S.-L. Chen, M.-H. Yang, J. Wu, J. Sinkkonen and K. Zou, *Nat. Prod. Rep.*, 2009, **26**, 1251–1292.
31 V. B. Pillai, S. Samant, N. R. Sundaresan, H. Raghuraman, G. Kim, M. Y. Bonner, J. L. Arbiser, D. I. Walker, D. P. Jones, D. Gius and M. P. Gupta, *Nat. Commun.*, 2015, **6**, 6656.
32 Y. J. Lee, Y. M. Lee, C. K. Lee, J. K. Jung, S. B. Han and J. T. Hong, *Pharmacol. Ther.*, 2011, **130**, 157–176.
33 D. T. Vavilala, V. K. Ponnaluri, D. Kanjilal and M. Mukherji, *PLoS One*, 2014, **9**, e113717.
34 X. Wang, X. Duan, G. Yang, X. Zhang, L. Deng, H. Zheng, C. Deng, J. Wen, N. Wang, C. Peng, X. Zhao, Y. Wei and L. Chen, *PLoS One*, 2011, **6**, e18490.
35 H. Wang, Z. Liao, X. Sun, Q. Shi, G. Huo, Y. Xie, X. Tang, X. Zhi and Z. Tang, *Neuropharmacology*, 2014, **86**, 9–21.
36 M. E. Munroe, T. R. Businga, J. N. Kline and G. A. Bishop, *J. Immunol.*, 2010, **185**, 5586–5597.
37 N. Qiu, L.-L. Cai, D. Xie, G. Wang, W. Wu, Y. Zhang, H. Song, H. Yin and L. Chen, *Biomed. Mater.*, 2010, **5**, 065006.
38 Y. Xie, Q. Long, Q. J. Wu, S. Shi, M. Dai, Y. Liu, L. Liu, C. Gong, Z. Qian, Y. Wei and X. Zhao, *RSC Adv.*, 2012, **2**, 7759–7771.
39 L. Feng, W. Zhu, C. Li, G. Guan, D. Zhang, Y. Xiao and L. Zheng, *Polym. Chem.*, 2015, **6**, 633–642.
40 C. Puglisi, L. Sturiale and G. Montaudo, *Macromolecules*, 1999, **32**, 2194–2203.
41 T. Maharana, B. Mohanty and Y. S. Negi, *Prog. Polym. Sci.*, 2009, **34**, 99–124.
42 S.-I. Kuroda, K. Terauchi, K. Nogami and I. Mita, *Eur. Polym. J.*, 1989, **25**, 1–7.
43 B. Haidar and T. L. Smith, *Macromolecules*, 1990, **23**, 3710–3712.
44 S. Sinha Ray, K. Yamada, M. Okamoto, A. Ogami and K. Ueda, *Chem. Mater.*, 2003, **15**, 1456–1465.
45 S.-M. Lai, R.-C. Hsu, C.-Y. Hsieh and F.-C. Chiu, *J. Mater. Sci.*, 2015, **50**, 2272–2283.
46 J. H. Gibbs and E. A. DiMarzio, *J. Chem. Phys.*, 1958, **28**, 373–383.
47 G. A. Adam, J. N. Hay, I. W. Parsons and R. N. Haward, *Polymer*, 1976, **17**, 51–57.
48 J. A. Nagy, L. Benjamin, H. Zeng, A. M. Dvorak and H. F. Dvorak, *Angiogenesis*, 2008, **11**, 109–119.
49 A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher, *Cell*, 2006, **126**, 677–689.
50 S.-M. Lim, J. P. Trzeciakowski, H. Sreenivasappa, L. J. Dangott and A. Trache, *Integr. Biol.*, 2012, **4**, 615–627.