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Shape memory polyurethanes with oxidation-induced degradation: *In vivo* and *in vitro* correlations for endovascular material applications

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Abstract

The synthesis of thermoset shape memory polymer (SMP) polyurethanes from symmetric, aliphatic alcohols and diisocyanates has previously demonstrated excellent biocompatibility in short term *in vitro* and *in vivo* studies, although long term stability has not been investigated. Here we demonstrate that while rapid oxidation occurs in these thermoset SMPs, facilitated by the incorporation of multi-functional, branching amino groups, byproduct analysis does not indicate toxicological concern for these materials. Through complex multi-step chemical reactions, chain scission begins from the amines in the monomeric repeat units, and results, ultimately, in the formation of carboxylic acids, secondary and primary amines; the degradation rate and product concentrations were confirmed using liquid chromatography mass spectrometry, in model compound studies, yielding a previously unexamined degradation mechanism for these biomaterials. The rate of degradation is dependent on the hydrogen peroxide concentration, and comparison of explanted samples reveals a much slower rate *in vivo* compared to the widely accepted literature *in vitro* real-time equivalent of 3% H₂O₂. Cytotoxicity studies of the material surface, and examination of the degradation product accumulations, indicate that degradation has negligible impact on cytotoxicity of these materials.

Keywords

Biomaterials; Polyurethane; Shape memory; Surface characterization; Oxidation

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Author contributions

Andrew C Weems worked on the experimental design, performed all bulk and model compound studies, analyzed and processed the data, and produced the manuscripts. Kevin T Wacker worked on the model compound studies, provided input and expertise on the proposed mechanisms, and worked on the manuscript. James K Carrow performed the cytocompatibility studies, and Anthony J Boyle performed the surface area compilation and analysis. Duncan J Maitland worked on the experimental design, oversaw the experimental work and analysis, and worked on the manuscript.

Competing interests

Regarding conflicts, Andrew C Weems and Anthony J Boyle hold stock options in Shape Memory Medical, Inc. (SMM), which holds a license for the shape memory polymers developed in Maitland's laboratory. Duncan Maitland owns stock and is on the board of directors at SMM.

1. Introduction

The translation of novel biomedical polymeric materials has historically lacked a rigorous understanding of the impact of material degradation for long-term implanted devices. Regulatory agencies often have to evaluate device submissions without a thorough understanding of degradation products, degradation kinetics and product toxicity [1–3]. However, *in vivo* studies, especially for slowly degrading materials, are often too costly, complicated and time consuming for a thorough evaluation of material degradation risk [1]. Alternatively, *in vitro* studies often fail to accurately reproduce the *in vivo* environment and are rarely conducted to quantify degradation product species and kinetics [1–3]. We propose a rigorous methodology for *in vitro* quantification of degradation products and kinetics on a novel polyurethane biomaterial. Further, this study validates the *in vitro* projections with materials degraded *in vivo* and provides a preliminary evaluation of the degradation product toxicity.

Polyurethanes have been in use for over fifty years as medical materials [1–7]. During this time, several widespread failures of these materials have been reported in both literature and the clinic, with two of the most notable being porous polyurethane coatings for silicone-based implants, and polyurethane coatings for pacemaker wire leads. By 1993 it was estimated that the failure of polyurethane pacemaker leads alone would result in above \$55 million dollars for device replacement [8]. A more recent 2016 study found that for pacemaker/defibrillator lead damage within the first year of device implantation results in approximately \$1.1 billion costs, with damage to the leads occurring in 0.47% to 1.94% of implanted devices, depending on specific application [9]. In a smaller case study, 27 pacemaker leads were returned to the manufacturer and analyzed, with 8 of the devices having undergone material failure that lead to 4 high voltage failures and one death, with an average implantation time of approximately of 25.5 months [10]. Based upon the study being performed and the device selected for analysis, this failure rate may range from 0.47% over two and half years to 2.71% per year or higher [9,10]. Failures for commercially available medical devices result in patient morbidity and mortality, and a major cause of this is the lack of analysis that is performed prior to implantation of the devices [1–10]. Correlations between the *in vivo* and *in vitro* responses for the selected materials must be performed to not only characterize the rates of degradation, but also to determine the possible mechanisms, products, and associated patient risks.

Some of the analysis performed on the porous polyurethane systems has led to contradictory results; lab methodology used for testing was not supported by *in vivo* data [11–16]. The urethane coatings were reported to undergo hydrolysis in the body, resulting in the presence of aromatic amines formed after hydrolysis of the carbamate, but the hydrolysis was a result of testing conditions that did not align with what the material would have experienced *in vivo*. This ultimately yielded data that does not accurately predict toxicity and stability over time [1–3,17–21]. Additional long term studies of these polyurethane foams have further indicated a reduced rate of tumorigenesis, no association between cancer incidence and the use of the aromatic diisocyanates, and no long term risk of cancer in both animal and human studies. However, a few studies still point to possible risks within the first few years after

implantation [11–16]. These conflicting outcomes present the need for better analyses of degradation and toxicity [1,3,7].

For many polyurethane systems, oxidation is also known to occur in addition to hydrolysis, but is typically limited to the chain extenders in the soft segment, such as ether linkages [1–3,5,18–20]. Despite these degradation concerns, tissue engineered scaffolds, drug delivery therapeutics, and devices continue to utilize poly(urethane urea) chemistries due to the versatility of the compositions and the ease with which traditional polymerization yields the final products [1–6]. However, there are still a lack of studies that provide adequate correlations between *in vivo* and *in vitro* behaviors as well as degradation mechanisms and toxicity assessments.

In fact, polyurethanes have gained even greater interest as medical materials due to inherent shape memory properties [22–27]. Ideal vascular materials will incorporate stimuli-responsive polymers (for minimally invasive procedures) with a controlled biostability that do not suffer from the limitations of thermoplastic systems [22]. An example of a series of stimuli-responsive polyurethanes intended for minimally-invasive vascular medical devices was initially developed by Wilson *et al.*, with the intent to overcome toxicity limitations while also improving on the shape-responsiveness of the polymers [22]. The developed shape memory polymers (SMPs) are amorphous, highly crosslinked, and have ultra-low density, and envisioned for use in vascular occlusion applications [22–26]. These SMPs were derived from monomers that are symmetric, multi-functional, and aliphatic, based upon the use of polyaddition reactions between diisocyanates and amino tri- or tetra-ols. This approach allowed for the formation of highly crosslinked networks, yielding high-strain-low-stress shape recovery, glass transition temperatures tunable around body temperature, and excellent biocompatibility, confirmed using both *in vitro* cellular studies as well as histological analysis of implants [22–26]. However, given that the alcohols used in the synthesis of these SMPs are also polyamines, it was important to consider their ultimate fate, and to conduct material degradation studies. Similar amino alcohol species have also found use in gene and drug delivery [27–35].

2. Methods and materials

2.1. Materials

N,N,N',N'-Tetrakis(2-hydroxypropyl)ethylenediamine (HPED, 99%, Sigma Aldrich), triethanolamine (TEA, 98%, Sigma Aldrich), hexamethylene diisocyanate (HDI, TCI America, >98%), 2,2,4-trimethyl hexamethylene diisocyanate (TMHDI, TCI America, a mixture of 2,2,4 and 2,4,4 monomers, >97%), isophorone diisocyanate (IPDI, Sigma Aldrich, 98%) were the monomers used in the synthesis of bulk SMPs. Hexyl isocyanate (TCI America, >98%) was used without purification. Hydrogen peroxide (50%, H₂O₂), sodium hydroxide (NaOH pellets, Sigma, >97%), phosphate buffered saline (PBS, Sigma, pH=7.4), cobalt chloride (CoCl₂, anhydrous, Alfa Aesar, 98%) were used for degradation solutions, in reverse osmosis (RO) water. Ethanol (EtOH, 195 proof, Sigma), isopropyl alcohol (99%, IPA, Sigma Aldrich), acetone (99%, Sigma) were used for cleaning. Phloxine B (PhB, 99%, Sigma) was used for confocal microscopy.

Bis(2-hydroxypropyl)amine (>98%), 3-amino-1-propanol (>99%), ethanolamine (>99%), diethanolamine (>99%), oxalic acid (>99%), glycolic acid (99%), glyoxal (40% in H₂O), and allyl alcohol (>99%) were purchased from Sigma Aldrich, and were used without further modification. Di-*tert*-butyl dicarbonate (99%), molecular iodine (99.99%, trace metals basis), sodium thiosulfate (99%), and hydrogen chloride solution (4 M in dioxane) were obtained from Sigma Aldrich, as well. Acetonitrile (99.93%, HPLC grade, Sigma), methanol (50% in H₂O with 0.1% v/v formic acid, LCMS grade, Sigma), methanol (99.9%, LCMS grade, Sigma), and water solution (0.5 % v/v formic acid, HPLC grade, Sigma) were used with liquid chromatography. Pancreatin, collagenase, and trypsin were purchased from Sigma, and used without modification.

2.2. Synthesis of SMPs

Porous SMP foams were synthesized using a two-premix method, consisting of an isocyanate premix and an alcohol premix (Fig. 1). Detailed synthetic information is provided in Supplemental Materials Synthesis, and previous publications [23–26]. The isocyanate (NCO) index of the premix is 2.5–3.0 (ratio of [NCO]:[OH]), adjusted to achieve a variety of pore sizes and densities (compositions and nomenclature are presented in Table 1; the composition is defined by diisocyanate species and the percentage of alcohol groups contributed by HPED, ie. HH60 is HDI-based with 60% of hydroxyls from HPED and the remaining 40% from TEA) [23–26]. Non-porous SMP films were synthesized using the same polyaddition, with stoichiometric amounts of diisocyanates added to an alcohol mixture of HPED and TEA [22]. Theoretical crosslink density of films was determined from the theoretical functionality of a repeat unit (ie HPED with a diisocyanate has a functionality of 4, TEA with diisocyanate has a functionality of 3) [22].

Samples were cleaned using water and IPA rinses at 35 °C (2 × water for 15 min under sonication, followed by 2× IPA for 15 min under sonication, with tumbling steps in between sonication, followed by 4× water for 15 min with sonication and 30 s tumbling in between) and dried at 50 °C under vacuum overnight.

2.3. General procedures

Samples were characterized using differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA) (further experimental details presented in Supplemental Materials: Thermal Characterization). For DSC, approximately 5 mg samples were heated from –40 °C to 120 °C at a rate of 10 °C/min, with the glass transition temperature (T_g) determined from the half height transition of the third heating cycle. For DMA, a single temperature sweep from 0 °C to 100 °C at 2 °C/min was used, with the peak tan δ value being taken as the T_g .

Morphological characterization of SMPs is presented in greater detail in Supplemental Materials (Morphological Characterization). Briefly, SMP foam cell structure and film surfaces were examined using scanning electron microscopy (SEM). Foams and films for SEM imaging were sputter-coated prior to imaging at 10 kV with 11× and 110× magnifications. Surface area was determined using confocal microscopy. Z-stacks were used for 3D construction of pores. Image J was used to approximate the surface area of the pores,

which was compared to average pore size of the samples. Porosity and density were calculated using the mass and total volume of SMP foam cubes of approximately 1 cm³ [5]. Contact angle experiments were performed using a KSV CMA-2008 Contact Angle Analyzer (KSV Instruments, Ltd., Helsinki, Finland), with a 5 μ L droplet was allowed to come to equilibrium on select polymer film surfaces over a five-minute period. The stationary water droplet angle was calculated ($n = 5$) for each sample composition. Gel fraction was determined using protocols presented by JR Martin et al. [5]. In short, mass before and after solvent extractions in EtOH at 50 °C for 24 hr were compared to obtain the residual SMP mass.

Shape memory testing was performed using previously reported methods.²⁷ In short, SMP cylinders 6 mm in diameter were compressed on a wire, stored in the compressed state overnight, and submerged in H₂O heated to 37 °C. This was repeated with 50 °C solutions. More detailed methodology is presented in Supplemental Materials.

Attenuated total reflectance Fourier transform infrared (ATRFTIR) spectroscopy was performed using a Bruker ALPHA infrared spectrometer (Bruker, Billerica, MA). Spectra data was collected in absorption mode with a resolution of 1 cm⁻¹. OPUS software was used to examine spectra, identify peaks, and perform baseline and atmospheric corrections. Examinations were performed in triplicate to confirm results. The location of the relevant peaks (carbonyl peak corresponding to the urethane ranging from 1686 to 1699 cm⁻¹ and the tertiary amine peak at *ca* 1051 cm⁻¹), as well as the intensities relative to the carbon skeletal peak at 1243 cm⁻¹ (found to be constant), were quantified and used to characterize the extent of degradation, with the peak shift of the carbonyl proportional to the degradation time.

Solution state nuclear magnetic resonance (¹³C 125 MHz and ¹H 500 MHz) was performed on an INOVA 500 spectrometer and spectra were referenced to solvent, CDCl₃ or DMSO-*d*₆. Solid state CP (cross-polarization) MAS NMR (¹³C 100 MHz) was performed using a 4.0 mm ¹³C probe on an Avance-400 Solids spectrometer (Bruker). A frequency of 123.63 MHz was used, with a spin speed of 5 kHz and 90° pulse lengths of 4.5 μ s. A recycle delay of 5 s and a contact time of 1.6 ms were the used measurement parameters. Edited CP MAS NMR experiments were performed as well, to observe positively-phased signals for C and CH carbons, negatively-phased CH₂, and zero-intensity signals for CH carbons to assist in peak assignments, with an external TMS standard.

2.4. Degradation

Phosphate buffered saline (PBS, pH=7.4) was made by adding RO water to packets of PBS solution, mixed well, and stored at 4 °C (producing 3 L of solution). 0.1 M NaOH solutions were made by adding 12 g of NaOH to 3 L of RO water, mixed well, and stored at 4 °C. 3% H₂O₂ solution was made by diluting 180 mL of 50% H₂O₂ with 2820 mL of RO water, mixed well, and stored at 4 °C. 20% H₂O₂ with CoCl₂ was made by first weighing 7.77 g of CoCl₂ into a beaker, which was dissolved in 400 mL of RO water. 400 mL of 50% H₂O₂ was diluted with 200 mL of RO water, which was then poured into the CoCl₂ solution. The H₂O₂ solution was kept cold prior to dilution and addition. It sat for 10 min to warm to room temperature prior to use. The 20% H₂O₂ (accelerated) solution and 3% H₂O₂ (real time)

were used for oxidative solutions, based upon literature review [1]. PBS was the real time hydrolytic and NaOH solution was the accelerated hydrolytic condition.

Sample cubes were then cut (2 cm × 2 cm × 2 cm) from the dried blocks using a hot wire cutter. Sections were taken from the center of the blocks to ensure that minimal changes to the foam pores had occurred due to cleaning. Masses were then recorded for each cube, and each cube was placed in a labeled, sealed vial.

SMP cubes were submerged in 30 mL of solution in a sealed vial. The vials were placed in a 37 °C oven (±0.1 °C, measured with a thermocouple over 3 days). Samples were incubated for 3 days before replenishing the solution, checked daily to monitor solution levels, and were weighed every week for real time testing, and every 3 days for accelerated testing. Samples were then removed, rinsed thoroughly in ethanol, blotted with a Kim Wipe and placed on a second Kim Wipe in a 50 °C oven. Vacuum was pulled overnight, at 30 mm Hg. After removal from the oven, samples were weighed and returned to the vials, with freshly replenished solution. Sample analysis is detailed below. Films were tested using the same protocol, but were cut into DMA bars with thicknesses of ~3 mm. The mass was converted to remaining mass percentage, and the average change and standard deviation was recorded and plotted.

2.5. Tissue Share program

Previous work by Rodriguez et al. with these materials (HH60 composition) included the implantation and histopathological evaluation after 0, 30, and 90 days in porcine aneurysms [38]. The SMPs were incubated in enzyme baths of pancreatin, collagenase and trypsin (initially tested with control SMPs to determine no degradation would occur to the polymer, only to the tissue) in order to remove tissue using a modified version of Zhang's tissue removal protocol [39]. After removal, SMPs were repeatedly washed in DI H₂O at 37 °C with sonication. The SMPs were then dried and characterized morphologically and spectroscopically.

2.6. Cytocompatibility

Green fluorescent protein (GFP)-expressing fibroblasts (NIH3T3/GFP, Cell Biolabs Inc., USA) were expanded then seeded onto control (unmodified) and degraded films at a density of 20,000 cells/film in normal growth media, which contained high glucose Dulbecco's Modified Eagle Medium (DMEM, GE Biosciences, USA) with 10% FBS (Atlanta Biologicals, USA), and 1% penicillin/streptomycin (100 U/μg/mL, Invitrogen, USA). For foam studies, foams were soaked overnight in cell-binding media, which contained normal growth media with 1% dissolved gelatin, prior to cell seeding. After initial cell seeding, films and foams were transferred to new well-plates to ensure cell growth was limited to the polymeric biomaterial.

To investigate cell proliferation, an Alamar Blue assay (Thermo Fisher, USA) was utilized per the manufacturer's instructions to calculate percent reduction of resazurin (blue) to resorufin (pink). Proliferation within foam samples was normalized to accessible surface area via considerations of foam volume and percent porosity. At the respective time points (Day 0, Day 1, Day 3, and Day 7), cell morphology was evaluated using fluorescence

microscopy with an excitation of 488 nm (Nikon Ti-U Inverted Microscope, USA); for Day 0 time point, cells were allowed to adhere for three hours prior to imaging. Representative images were selected over the course of imaging three replicates for each film or foam.

2.7. Degradation model compounds

TEA and HPED were added to respective 20 mL round bottom flasks equipped with stir bars, and sealed. The flasks were charged with a slight excess of hexyl isocyanate, and the flasks were purged with nitrogen. The reactions were allowed to continue overnight at 50 °C. TEA-hexyl produced a waxy solid, and HPED-hexyl produced a viscous liquid. Both products were purified using silica column chromatography (90% ethyl acetate in hexanes).

Samples were incubated at 37 °C with 50% hydrogen peroxide (uncatalyzed). Aliquots were separated, filtered through activated carbon, and characterized; the remainder of the sample was left in solution. Degradation rates were determined and products were characterized using NMR (^1H , ^{13}C), LC-MS, and FTIR characterization.

2.8. LCMS standard compounds

A general protocol for the synthesis of LCMS standards is presented here for bis(2-hydroxypropyl)amine. Di-*tert*-butyl carbonate (10 mmol) was added in stoichiometric amounts to bis(2-hydroxypropyl) amine in a 10 mL round bottom flask with a stir bar, along with 1 mmol molecular iodine, as a catalyst, and was allowed to react over night at 50 °C. Stoichiometric amounts of hexyl isocyanate were then added to the solution and allowed to react for 24 hours, under nitrogen at 50 °C. Characterization confirmed the presence of carbamates, and deprotection was performed using 4 M HCl in dioxane for 3 hours. LCMS standards were washed with sodium thiosulfate (aq.) to remove iodine, and then the organic phase was collected and dried at 50 °C under vacuum (30 in Hg). Samples were diluted to 0.1 mg/mL, and then diluted by 50×6 additional times to produce 6 calibration standards. Samples were dissolved in 50% methanol with 0.5% formic acid, the same solvent used for LCMS characterization. This procedure was followed for 3-amino-1-propanol, ethanolamine, and diethanolamine. Lactic acid and glycolic acid were purified and reacted with hexyl-isocyanate. Glyoxal and oxalic acid were used as received.

LCMS was performed using a ThermoFisher Orbitrap with a Hewlett Packard LC attachment, and a C₁₈ normal phase silica gel column. The chromatography profile was 23 minutes long, altering from 95% methanol to 90% acetonitrile over 18 minutes with a linear gradient, with the remaining time at 95% acetonitrile to remove contaminants. A blank of 50% methanol with 0.5% formic acid was used to clean the column.

2.9. Statistics

Gravimetric and cytocompatibility results were analyzed for statistical significance using a Student's two tailed T-test. The certainty was assessed at 95% (p-value <0.05). Average and standard deviation were calculated for all test sets, and sample sizes are noted in figures where relevant.

3. Results

3.1. Synthesis and thermal characterization

The reactions of the alcohols with the diisocyanates produces the urethane bonds, and water with isocyanate produces urea [22–26]. Substitution of the diisocyanates or alcohols does not affect gel fraction of the materials (all greater than 98%). Porosity and pore size of the SMPs can be partially controlled through a combination of NCO index, premix viscosity, catalyst concentrations, and concentration of physical blowing agents that are used, as demonstrated by Singhal et al. [25]. By removing the catalysts and blowing agents, non-porous films were produced; decreasing premix viscosity and NCO index results in larger pore sizes and reduced densities of the SMPs [22].

Increasing TEA percentage of the material decrease the T_g , while increasing HPED percentage results in increased T_g , along with greater crosslink density, higher elastic modulus, and greater hydrophobicity (HH100 contact angle of $77.0 \pm 0.6^\circ$, HH0 contact angle of $61.3 \pm 1.2^\circ$, TMH0 contact angle of $84.5 \pm 0.5^\circ$, and IP/TMH0 $97.3 \pm 0.4^\circ$), results which are better detailed elsewhere [22,25]. These same trends will occur as the diisocyanate is changed from HDI to THMDI to IPDI, in increasing order of modulus, thermal transition, and hydrophobicity. The shape memory effect is impacted by the transition, as well. HDI containing SMPs (HH60) will undergo shape recovery at 37°C in H_2O , while both the TMHDI and IPDI do not (Supplemental Materials Fig. 1). At 50°C , TMHDI SMPs will undergo rapid shape recovery (all compositions), while IPDI-containing SMPs maintain the secondary shape. This trend in recovery is also demonstrated in strain fixity at room temperatures; below T_g all SMP compositions exhibited nearly 100% strain fixity.

3.2. Gravimetric and microscopic analysis

For porous SMPs incubated in accelerated hydrolytic solution (0.1 M NaOH), no changes in the pore structure were found (no strut erosion, membrane rupture, pore collapse) after 36 weeks. This is contrasted with mass loss occurring across oxidative solutions with varied concentrations of H_2O_2 (Fig. 2A). There was no statistical difference between the 50% H_2O_2 and metal catalyzed 20% H_2O_2 rates of degradation; while all degradation rates were not different within the initial 3 days for any concentration, with 10% or greater H_2O_2 concentrations resulting in statistically significant rates of degradation. SMPs in accelerated oxidative solution (20% H_2O_2 with 0.1 M CoCl_2) underwent complete mass loss in less than 30 days, with HDI degrading in 9 days, TMHDI in 20 days, and 50% IPDI/50% TMHDI in approximately 28 days; these differences were found to be statistically significant (Fig. 2B). Furthermore, increasing theoretical crosslink density as calculated from monomer functionality, which was achieved by increasing the concentration of HPED relative to TEA, was found to increase oxidative stability (Fig. 2C). Using the literature equivalent of real time oxidative solution, 3% H_2O_2 , all mass loss rates regardless of surface area were similar over the first 40 days, but became statistically significant after this initiation time (Fig. 2D) for surface area differences greater than $10\text{ mm}^2/\text{mm}^3$. By 80 days smaller differences in surface area per volume are statistically significant, although a visible trend began to develop at *ca* 60 days. The discrepancy in porosity, as determined from surface area to

volume ratios, appears to dominate compositional differences (alcohol ratios) and thermal variation in accelerated studies (Fig. 2D insert).

These results are supported by gravimetric analysis in 3% H₂O₂ solution. Porous SMPs incubated in this solution displayed similar trends based on T_g and diisocyanate species (Supplemental Materials Fig. 5). The time for 50% mass loss of the porous SMPs in 3% H₂O₂ is approximately 100 days for HDI, and 150 days for TMHDI using extrapolation. Mechanical changes in the SMPs were also readily apparent within weeks of immersion in 3% H₂O₂.

A lack of mass loss over the examined period was also observed, in agreement with SEM results. These results contrasted with the behavior in real time oxidation: pore collapse and membrane rupture/erosion were found to occur within 4–6 weeks (Fig. 3). For non-porous materials, surface erosion appeared as pitting and the formation of surface rippling, culminating in total material collapse.

SEM of non-porous SMP displayed no significant changes for hydrolytically degraded films. For oxidatively-degraded films, the hydrophobicity (and thermal transition) had distinct differences on the degradation and the topology of the film after incubation.

3.3. Spectroscopic characterization

Gravimetric analysis was related to the morphological changes and the network behavior through examination of spectroscopic changes by FTIR and solid state ¹³C nuclear magnetic resonance (¹³C NMR). The scission of the C-N bond in the tertiary amine was indicated through both the shift in the urethane peak (1688 cm⁻¹ to 1699 cm⁻¹) relative to the urea peak (at 1640 cm⁻¹) in FTIR, as well as the formation of new peaks in the ¹³C NMR spectra within the amine region (60 ppm and 67 ppm converging into a single peak at 65 ppm that shifts to 68 ppm) indicative of primary amine formation (Supplemental Materials Fig. 7 through Fig. 9). Characterization of urea content in similar materials is presented in greater depth by Singhal et al. [25]. Solid state ¹³C NMR showed no change in the urethane peak resonance (155 ppm); and any potential formation of an aldehyde may be obscured by a spinning side band. Neither FTIR, nor solid state ¹³C NMR, spectroscopic signals indicated distinct change during the more mild hydrolytic testing, under both real time and accelerated conditions (Supplemental Materials Fig. 10).

3.4. In vivo-In vitro degradation correlations

Spectroscopic analysis of *in vivo* samples indicates that oxidation occurs, but at a slower rate than what is indicated as real time by previous (*in vitro*) studies [1,5,11,12]. Surface analysis was performed using FTIR and SEM (Fig. 4) [1,5]. SEM images reveal that the material underwent collapse similar to what is found for *in vitro* samples: membranes are completely destroyed by 90 days, with some fragments still seen at 30 days. Surface pitting is also greater over time. Spectroscopic comparison indicates similar changes in peak location and relative height compared with *in vitro* testing, albeit at a slower rate. This is supported by C1 s analysis *via* XPS, confirming oxidation of the material surface (Supplemental Materials).

Comparison of the *in vivo* and *in vitro* surfaces indicates, again, the loss of membranes over the course of degradation. Additionally, the struts of the SMP are thinner, with surface pits forming and residue being clearly visible. The initial SMPs do not contain these issues.

3.5. Cytocompatibility

The relationship between cytocompatibility and degradation of the SMPs was examined using direct contact studies, with no differences between surfaces found. Cell morphology and proliferation were found to be equivalent across the control, oxidative, and hydrolytically treated surfaces (Supplemental Materials Fig. 11).

3.6. Model compounds

Due to the observations of material breakdown macroscopically and gravimetrically, yet the limited solid-state FTIR and ^{13}C NMR evidence for the chemistry occurring during degradation of the thermosets, model studies were undertaken. Although the harsh conditions of the oxidatively-degraded samples showed distinct spectroscopic changes to the amine (C-N bond), indicating the scission of the tertiary amine, limitations in the detailed analysis of the thermoset networks (limited analysis *via* NMR, mass spectrometry, *etc.*, as a result of insolubility), required the examination of degradation products using small molecule analogs and LC-MS [28]. The small molecule structures include urethane and amino linkages to simulate the chemical moieties in the SMP thermosets.

Figs. 5 and 6 indicates the major amine degradation products observed by LCMS. Both alcohols will oxidize to form the respective amine oxides (N-oxides) or aminium radical cations [28,29,35–37]. The tetra-ol (HPED, starting material **7**) will have steps analogous to the triol TEA to form N-oxide(s) or radical cation(s) (product **8**), prior to any fragmentation occurring. One of the N-oxides fragments by C-N cleavage to form an aldehyde (eventually a carboxylic acid) and a secondary amine, which may further fragment to form a primary amine endgroup (product **10**). Further fragmentation at this amine (C-N), could produce either an aldehyde and subsequent acid end group after concomitant release of ammonia (product **13**). Alternatively, the N-oxide (product **9**) may fragment to form another secondary amine (product **14**). Internal C-N fragmentation (product **13** $\rightarrow$ **18** or **17** $\rightarrow$ **19**) would result in the formation of small molecule aldehydes (glyoxal), glycolic acid, and carboxylic acids (oxalic acid) from cleavage of the ethylene chain linking the amine groups. These small molecules will form through a glyoxylic acid intermediate (product **15**) before formation of oxalic acid (product **16**). Oxalic acid formation appears more likely than dialdehyde (glyoxal) due to consumption of aldehyde in the highly oxidative environment.

Carboxylic acids are formed rapidly during the degradation process in 50% H_2O_2 . ^1H NMR indicates the formation of carboxylic acid with significant presence of aldehyde (relative to the carboxylic acid peak). ^{13}C also confirms presence of aldehyde and acid resonances at *ca.* δ 207 & 173 ppm. The NMR spectra support the degradation products as presented in Figs. 5 and 6 (aldehyde and carboxylic acid formation), as does the carbonyl peak shift from 1686 cm^{-1} , indicative of free carbonyl compared with hydrogen bonded carbonyl [40,41]. At any time during the degradation of the bulk material, sufficiently small network fragments may be solubilized and interact directly with or be taken up by cells present at the material

interface. Other products are indicated by literature and were found, but accounted for less than 0.05% (by mass) of the total.

4. Discussion

The variation in T_g associated with moisture is due to the plasticization of the urethane bonds *via* interruption of the hydrogen bonding by water molecules. Without a solvent, sufficient temperature is required for movement of the polymer backbone, which allows for shape change associated with the return the entropic maximum that determines the primary shape of the material. In the presence of a solvent that can interrupt the hydrogen bonding, such as water or ethanol, the required temperature for the shape to change to occur is dramatically reduced, and shape recovery can be achieved at lower temperatures through the use of solvent as opposed to a purely thermally-driven recovery mechanism, due to the increased mobility of the crosslinking segments with disruption of the hydrogen bonded urethane and urea segments [40,41,48,49].

Gravimetric analysis of the non-porous SMPs revealed rapid mass loss when incubated in oxidative solutions, with the rate of degradation appearing to be dependent on the T_g [1,50]. TMH0 (TEA as the alcohol monomer, T_g of approximately $-11\text{ }^{\circ}\text{C}$) films degraded significantly faster compared to the TMH100 (HPED as the alcohol monomer, T_g of approximately $29.5\text{ }^{\circ}\text{C}$). This dependence on T_g is also demonstrated by the substitution of the diisocyanate species in films containing only HPED as the alcohol monomer (Supplemental Materials, Fig. 5). In gravimetric studies using 3% H_2O_2 , HDI films degraded more rapidly, and IPDI films were found to be extremely oxidatively resistant, comparatively. These behaviors agree with examinations of the porous SMPs, such as with the delay in the onset of mass loss found in real time oxidative testing. However, it is hypothesized that the surface area is a greater contributor to the rate of degradation compared with the more moderate backbone rigidity or hydrophobicity, due to the increased rate of mass loss for samples with comparable T_g s. Accelerated oxidative testing (20% H_2O_2 , 0.1 M CoCl_2) resulted in rapid oxidation and total mass loss within days for materials with T_g s ranging from $20\text{ }^{\circ}\text{C}$ to $130\text{ }^{\circ}\text{C}$; degradation rate was T_g dependent, as well as H_2O_2 concentration and surface-area dependent (Fig. 2). In the more oxidatively resistant films, the role of crosslink density and composition became more apparent. Increasing the crosslink density resulted in greater stability, which may be related further to increased hydrophobicity and thermal transitions. This is not an unexpected result, as the greater crosslink density allows for more extensive chain scission prior to erosion and gravimetric loss of the material. However, further work is needed to decouple crosslink density from thermal and hydrophobic properties as well as directly measure crosslink density to confirm the correlation to the polyol functionality noted in this manuscript.

For the porous SMPs, the larger pores (lower surface area) result in a slower mass loss rate compared with smaller pores. The nonporous films have lower T_g s, but slower degradation rates, further indicating the importance of available surface area in the degradation process. This behavior is due to the presence of membranes in the porous materials, which may allow for the formation of concentration gradients prior to the membrane perforation and removal. Simultaneously, the hydroxyl radicals of degradation solutions are also degrading the struts,

which eventually results in the complete material collapse as well as surface pitting and cracking.

The morphological, compositional, and gravimetric changes also factor into changes in shape memory behavior, although the change in functionality is less important for many applications. Examination of the shape memory behavior indicated that there is a rapid loss in shape memory as a result of oxidative degradation, and that such changes are not displayed for hydrolytically-incubated samples. However, the presented changes in the material may be the result of a number of factors, including increased modulus, decreased crosslink density, and new surface functional groups. These changes are compounded by the heating of samples for shape setting. Further work is required to fully characterize change in shape memory behavior. Surface analysis indicates that the time implanted at the aneurysm site differs from the literature standard 3% H₂O₂ solution as a real-time approximation [1]. This difference is such that 45 ± 3 days *in vitro* is the equivalent of 90 days *in vivo*. The urethane peak shift in FTIR (1686 to 1693 cm⁻¹) and change in the percentage of C1 s determined by XPS indicated a surface aged to the equivalent of 48 ± 2 days and 32 ± 1 days, respectively. This suggests that a 1% or 1.5% H₂O₂ solution would be a more appropriate solution for modeling degradation for these highly porous SMP scaffolds when they are used in endovascular applications, with the comparison between the H₂O₂ concentration-dependent rates displayed in Fig. 2; the lower-than-anticipated degradation rate is most likely due to the combination of surface chemistry, high surface area, and the porosity [5,31,32]. As compared to the silk and polypropylene present at the aneurysm sites, these SMPs display reduced inflammatory response and higher neovascularization, resulting in lower numbers of macrophages and neutrophils at the implant site, which in turn results in lower levels of peroxides and superoxides, and will ultimately decrease the oxidation rate at the material surface [31–33].

Analysis of the degradation products and interpolation of the concentration vs degradation rate allowed for determination of the concentration and species of degradation products based upon the now determined rate of material degradation (Fig. 2). Spectroscopically, the shift in the carbonyl peak indicates the formation of free carbonyls in the presence of the urethane and urea bonds [40,41]. The change in the peaks around 1100–1000 cm⁻¹ indicate changes in the amines, which corresponds well with the model compound studies. It is interesting to note that the gravimetric changes can be correlated with the model compound studies, in that the time to amine oxide loss, or scission of the polymer backbone. The time to the formation of amine oxides, which are then consumed during scission, may be the initial period without mass loss during these studies, and only after this is sufficient scission occurring to allow for soluble products to move into solution from the bulk SMP. This was beneficial in relating the model study with the bulk behavior, and may be of further use in developing toxicological profiles for individual degradation products when scaled appropriately.

This analysis allows for the identification of molecular species that are known to be toxic, carcinogenic, or have other risks associated with them, as well as to determine the formation rate of these products. Through this analysis, it is possible to further understand the long-term behavior of these implants and the toxicological risks associated with potential

exposure to degradation products. While a complete toxicological risk assessment is ultimately necessary prior to commercialization, the most immediately concerning products of SMP fragmentation are glycolic acid and oxalic acid, which could limit the maximum mass of implanted materials if the daily dose rate exceeded the human safe exposure limit of 0.54 mg/kg body weight/day, determined from reproductive effects in animal studies; for an average 70 kg human this threshold is 37.8 mg/day for oxalic acid, which has a lower threshold compared with glycolic acid [45,46]. The analysis of SMP degradation indicates that the levels of exposure for 10 cm³ of SMP foam that has a total mass of approximately 400 mg (approximately 32.0 to 36.6 mg total as a maximum possible dose) are well below what would be considered a patient protective safe exposure limit per day [45,46]. For an average sized aneurysm occlusion device, the total dose of oxalic acid is closer to 1.5 mg (porous SMP mass of approximately 16.0 mg).

The rate of mass loss at 50% H₂O₂ is the equivalent of the 20% H₂O₂ catalyzed by CoCl₂, which allowed for the comparison of the model compounds with the bulk SMP degradation. Under these conditions, mass loss of the polymer, which occurs by 2 weeks, appears to happen after the consumption of the tertiary amines and the initial formations of the secondary and primary amines, respectively (Fig. 7). Additionally, the delay in mass loss found over the initial few days and concomitant mass increase is most likely due to the formation of amine oxides from the available amines. Only after remaining amines have been oxidized will significant chain scission begin to occur, and subsequent mass loss observed. Furthermore, the majority of the oxidation results in the generation of small molecule degradation products (primary amine end groups rather than longer chains containing repeating secondary amines).

It is also possible that these products will not completely oxidize. The roles of primary amines and aldehydes in the adhesion of biomaterials with tissue has been the subject of recent study, with aldehydes in particular being used in adhesives; increasing concentration of aldehydes increased the adhesion of the biomaterial to the tissue [47,50]. In the case of the presented SMPs, oxidation of the material produces aldehydes along with lower amines. This increased adhesion, in addition to the high porosity of the SMP, may explain previous results that demonstrate excellent biocompatibility of the starting SMP as well as the obtained results for oxidized material surfaces here [42–44,47,50]. While the presented results do not indicate that all degradation will be tolerable *in vivo*, they do provide evidence to suggest that long-term implantation of these SMP materials is possible. Further examination of the extractable or soluble degradation products, as well as fully degraded SMPs with cytocompatibility assays is necessary to fully confirm this. These experiments should also include mutagenicity testing, to determine that no adverse events will occur as a result of long term exposure over the life of the biomaterial.

5. Conclusions

In conclusion, we have demonstrated the degradation analysis of porous and non-porous SMPs intended for implantable vascular medical devices, and correlated the *in vivo* and *in vitro* behaviors. The use of amino-alcohols allows for macrophage-mediated degradation of the SMPs *via* an oxidative mechanism that results in the formation of lower amines and

aldehydes, determined through model compound studies and analytical characterization. The degraded materials still possess excellent cytocompatibility, indicating their utility for long-term usage. Characterization of *in vivo* SMP samples was used to determine the rate of degradation and the relationship of the macrophage presence to the material oxidation observed. The *in vitro* degradation was used to present a potential mechanism of degradation in the bulk SMP, as well as to assess the rate of degradation product formation and examine the potential toxic risk these products pose to the host.

It is our belief that while many applications propose the use of amine-containing materials and similar structures, there is insufficient information currently available to accurately and appropriately assess the risks associated with using such materials for medical applications. The presented work provides results that support the use of these SMPs for vascular device, specifically for cerebrovascular aneurysm occlusions, and introduces the methodology required for accurate assessment of long-term stability and safety. Wide-spread use of such methods will allow for more accurate, safer modeling of implantable biomaterials and increase the translation of more reliable devices with fewer patient risks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.actbio.2017.06.030>.

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Statement of Significance

This paper presents an in-depth analysis on the degradation of porous, shape memory polyurethanes (SMPs), including traditional surface characterization as well as model degradation compounds with absolute quantification. This combination of techniques allows for determination of rates of degradation as well as accumulation of individual degradation products. These behaviors are used for *in vivo-in vitro* comparisons for determination of real time degradation rates. Previous studies have primarily been limited to surface characterization without examination of degradation products and accumulation rates. To our knowledge, our work presents a unique example where a range of material scales (atomistic-scale model compounds along with macroscopic porous SMPs) are used in conjunction with *ex planted* samples for calculation of degradation rates and toxicological risk.

Fig. 1.

Overview of starting materials and generalized network of the shape memory polyurethanes.

Fig. 2.

Gravimetric analysis of HH60 SMPs (A) in varied concentrations of H_2O_2 , (B) comparison of diisocyanate species (with an alcohol ratio of 60% HPED to 40% TEA) on the rate of degradation in accelerated oxidative conditions (right, HH60, TH60, IP/TH60 in 20% H_2O_2 with 0.1 M CoCl_2 at 37 °C), (C) accelerated oxidative degradation of TMHDI films with varying composition (crosslink density), and (D) 3% H_2O_2 solution's effect on varied surface area to volume ratios for TMH60 SMPs. (D inset) The effect of composition compared with surface area per volume in 20% H_2O_2 with 0.1 M CoCl_2 at 37 °C. Statistical difference is denoted above time points ($p < 0.05$); (*) denotes statistical differences between 3% and 0.5% H_2O_2 , ** denotes statistical difference between 3% and 50% H_2O_2 , *** denotes differences between 10% and 50% H_2O_2 ; **** denotes differences between 10% and 20% H_2O_2 ; § denotes differences between all three compositions; θ denotes TMH25 and TMH50 are not statistically different from TMH100; ϕ denotes statistical differences between TMH100, TMH50, and TMH25; α denotes statistical differences between surface area to volume ratio, as determined from pore size). (n = 14).

Fig. 3. SEM images and constructed 3-D pore renderings of porous SMPs (HH60), and the effect of hydrolytic (0.1 M NaOH for 15 weeks, top left) and oxidative (3% H_2O_2 for 8 weeks, top right) conditions on the morphology. All testing occurred at 37 °C.

Fig. 4. Microscopic and spectroscopic analysis of implanted, porous, HDI SMPs (HH60). (A) displays FTIR comparison (between 1800 and 800 cm^{-1}) for an untreated sample (day 0), a sample degraded in 3% H_2O_2 for 90 days (90 day *in vitro*), and a sample explanted from the porcine sidewall aneurysm after 90 days (90 day implant). (B) C1s XPS peak intensities for comparisons of *in vivo* and *in vitro* foam samples. SEM images of day 0 (C), day 30 (D), and day 90 (E); it is apparent that the membranes are degraded over the course of the implantation, with residual membranes shown in (C) compared with (B) (complete membranes are present) and (D) (no membranes remain). ($n = 3$).

Fig. 5.

Proposed degradation pathway for TEA in an oxidative environment. The observed species are the TEA-hexyl starting material (**1**), which oxidizes to the N-oxide (amine oxide, **2**). Fragmentation combined with oxidation and reduction will lead to the formation of the secondary amine (**3**), and an aldehyde (**4**), which further oxidizes to a carboxylic acid (**5**). The secondary amine fragments to form the primary amine (**6**), which will also lead to carboxylic acid (**5**); the byproduct of the primary amine fragmentation is ammonia.

Fig. 6.

Proposed degradation pathway for HPED in an oxidative environment. The starting compound (7) can oxidize to the N-oxide (8). Fragmentation will lead to a secondary amine (9), or an N-oxide and a primary amine (10) which will further degrade to a single N-oxide (13). The formation of (9) also yields an aldehyde (11), which further oxidizes to a common final degradation product, (12). The degradation of (13) with competing asymmetric C-N fragmentation pathways is governed by sterics and electronics *e.g.* product (17) versus (18) from (13). Degradation to form (18) will yield a small molecule glyoxylic acid (15) that oxidizes to oxalic acid (16). Analogous competitive cleavage is also possible with other degradation intermediates such as (9). The byproduct of the primary amine (19) degradation is ammonia.

Fig. 7.
Abundances of major degradation products for the model compound study, determined from LCMS, displaying abundance of degradation products for HPED-hexyl (B) and TEA-hexyl (A). (n = 3).

Table 1

Nomenclature and compositions (in grams) of the examined SMP formulations for 32 g scale synthesis, with species names denoted by the diisocyanate used and the percentage of HPED comprising the alcohol composition.

Species	HDI (g)	TMHDI (g)	IPDI (g)	TEA (g)	HPED (g)	H ₂ O (g)
HH100	25.036	–	–	–	11.317	–
HH80	25.389	–	–	0.961	9.182	1.115
HH60	25.727	–	–	3.168	6.985	1.131
HH40	23.126	–	–	4.821	4.724	1.148
HH20	26.511	–	–	6.522	2.397	1.165
HH0	26.908	–	–	8.275	–	1.182
TMH100	–	26.716	–	–	9.464	–
TMH80	–	26.484	–	1.303	7.661	0.931
TMH60	–	26.800	–	2.637	5.814	0.942
TMH50	–	26.960	–	3.316	4.874	–
TMH40	–	27.123	–	4.003	3.923	0.953
TMH20	–	27.454	–	5.402	1.985	0.965
TMH0	–	27.793	–	6.836	–	–
IP/TMH100	–	12.789	13.520	–	9.248	–
IP/TMH80	–	12.936	13.675	1.273	7.484	0.909
IP/TMH60	–	13.087	13.834	2.575	5.678	0.920
IP/TMH50	–	13.163	13.915	3.238	4.759	0.925
IP/TMH40	–	13.241	13.997	3.908	3.830	0.930
IP/TMH20	–	13.339	14.164	5.273	1.938	0.942
IP/TMH0	–	13.560	14.335	6.671	–	–