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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% H_2O_2. Cytotoxicity studies of the material surface, and examination of the degradation product accumulations, indicate that degradation has negligible impact on cytotoxicity of these materials.

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.

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 devices 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

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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 tumorogenesis, 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%, H_2O_2), sodium hydroxide (NaOH pellets, Sigma, >97%), phosphate buffered saline (PBS, Sigma, pH=7.4), cobalt chloride ($CoCl_2$, 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 H_2O), 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 H_20 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 sonica-