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PREVENTION OF POLYURETHANE OXIDATIVE DEGRADATION WITH PHENOLIC-ANTIOXIDANTS COVALENTLY ATTACHED TO THE HARD SEGMENTS: STRUCTURE FUNCTION RELATIONSHIPS

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Abstract

Oxidative degradation of the polyurethane elastomeric (PU) components greatly reduces the efficacy of PU containing cardiovascular devices. Covalently appending the phenol-based antioxidant, 4-substituted 2,6-di-*tert*-butylphenol (DBP), to PU hard segments effectively reduced oxidative degradation of the PU *in vivo* and *in vitro* in prior studies by our group. In these experiments we analyze the contribution of the tethering molecule to the antioxidant capabilities of the DBP modified PU. Bromoalkylation chemistry was used to link DBP to the hard segment of the polyether polyurethane, Tecothane, via our original linker (PU-DBP), or variants containing side chains with 1 (PU-C-DBP) or 3 (PU-3C-DBP) carbons. Two additional DBP variants were fabricated in which the DBP group was appended to the alkyl chain via an oxygen atom (PU-O-DBP) or an amide linkage in the middle of the tether (PU-NHCO-DBP). All DBP variant films and unmodified control films were subject to oxidative degradation via 15 day immersion in a solution of 20% H₂O₂ + 0.1 M CoCl₂. At the end of the oxidation protocol films were analyzed for the presence of oxidation related endpoints via scanning electron microscopy, contact angle measurements and Fourier transformation infrared spectroscopy (FTIR). All DBP containing variants resisted oxidation damage significantly better than the unmodified control PU. SEM analysis of oxidized PU-C-DBP and PU-O-DBP showed evidence of surface cracking consistent with oxidative degradation of the PU surfaces. Similarly there was a trend in increased ether cross-linking, a marker for oxidative degradation, in PU-C-DBP and PU-NHCO-DBP films. Consistent with these FTIR results, both PU-C-DBP and PU-NHCO-DBP had significant reductions in measured surface hydrophobicity as a result of oxidation. These data show for the first time that the choice of linker molecule significantly affects the efficiency of the linked phenolic antioxidant.

INTRODUCTION

Polyurethane elastomer (PU) components of implantable vascular devices, such as pace maker lead insulation, are subject to oxidative degradation caused by the release of reactive oxygen species from adherent monocyte derived macrophages. Given the cellular mechanisms for oxidative degradation of PU, it was often regarded as a surface event¹. However, in the case of pacemaker lead insulation, others have shown that metal ion oxidation is responsible for degradation of the inner polyurethane insulation that is not exposed to tissue, but is in direct contact with the conductor coils². Coblending antioxidants,

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such as vitamin E, to bulk preparations of polyurethane has been shown to reduce oxidative damage of PU films both *in vitro* and *in vivo*^{3,4}. Unfortunately, the coblending strategy is limited by the relatively low concentrations of antioxidants, which can be added to PU preparations. This is a concern when one considers that the antioxidant capacity is depleted after reacting with hydroxyl radicals.

We have previously characterized a chemical synthesis in which bromoalkyl groups are covalently appended to activated nitrogen molecules of the PU hard segments⁵. This bulk chemical modification allows for the subsequent reaction of thiolated compounds to the bromoalkylated site, thus yielding a PU covalently configured with therapeutic molecules. Our group has shown that mercapto-cholesterol, appended to bulk preparations of PU via bromoalkylation chemistry, provides a high affinity surface for endothelial precursor cells under high shear flow conditions *in vivo* and *in vitro*^{6,7}. In addition, bisphosphonate groups appended to PU via bromoalkylation can prevent calcification of PU heart valve leaflets in a juvenile sheep model⁸. Thus, bromoalkylation chemical synthesis allows for the covalent addition of a broad variety of therapeutic agents to bulk preparations of PU.

More recently we used this bromoalkylation chemistry to append 4-substituted 2,6-di-*tert*-butylphenol (DBP), a phenolic-based antioxidant, to PU^{9,10}. Analysis of the DBP configured PU (PU-DBP) showed that the addition of DBP to PU inhibited oxidative degradation as assessed by an *in vitro* assay using a solution of hydrogen peroxide and cobalt chloride and assessing for markers of oxidative degradation^{9,10}. Of note, was that the concentration of antioxidant loaded via bromoalkylation was approximately 30-fold higher than the co-blending strategy^{9,10}. Subsequent studies showed that the level of DBP antioxidant bound to PU, via bromoalkylation chemistry, can be further increased with minimal loss of the PU film's physical properties¹⁰.

Although these prior studies with PU-DBP showed that the bromoalkylation chemical synthesis is a useful strategy for covalently attaching relatively high levels of antioxidant to confer resistance to oxidative degradation upon PU films, the contribution of the tethering molecule's structure with respect to oxidation resistance was not investigated. Such information could prove invaluable for designing second-generation PU variants that could allow for multiple therapeutic molecules capable of resisting oxidative degradation as well as other pathological conditions affecting implantable PU containing devices. In addition, knowing the propensity of certain chemical linkers for susceptibility to oxidative damage may prove beneficial for designing biodegradable PUs. Thus, we investigated the hypothesis that the chemical structure of the linker molecule contributes directly to the antioxidant properties of DBP configured PUs. The goal of this study was to compare, with respect to well-established oxidative related endpoints, our original PU configured with 4-substituted 2,6-di-*tert*-butylphenol (DBP) to 4 additional PUs which differed in the chemical structure of the thiol containing linker molecule and the nature of atom directly attached to the DBP aromatic ring.

MATERIALS AND METHODS

The PU used for these studies was Tecothane[®] TT1074A, an aromatic polyether polyurethane, which was obtained from Thermedics Inc. (Waltham, MA), purified and characterized by ¹H NMR as reported previously¹¹. The percentage of soft segments as determined via ¹H NMR was 65%. Chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

Synthesis of DBP-containing PU

Table 1 summarizes the 4 additional variations from our original DBP-modified PU (PU-DBP). The chemical scheme of the original PU-DBP and the 4 variants are shown in Figure 1. PU-DBP contains a sulfur atom attached directly to the benzene ring in *p*-position to the phenolic OH. Theoretically, replacement of the sulfur in this position with a carbon atom could influence the behavior of the hindered phenolic radical trap, and thus, affect the stability of the modified PU. To examine the effects of such replacement on antioxidant capacity, we synthesized two variants of HS-DBP with carbon atoms attached directly to the benzene ring in *p*-position to the OH. Correspondingly, two variants of the DBP-modified PU were fabricated: with bridges between the aromatic ring and the sulfur atom consisting of 1 (PU-C-DBP) or 3 (PU-3C-DBP) carbons.

Two additional variants were formulated. The first tested the hypothesis that a group more prone to conjugation with the aromatic ring, such as an oxygen atom, would stabilize the phenoxyl radical during inhibition of the radical-induced oxidation, rendering the antioxidant moiety more efficient. Thus we linked the DBP group to the alkyl chain *via* an oxygen atom (PU-O-DBP). An additional variant (PU-NHCO-DBP) contained a carbon atom in *p*-position to the OH (similarly to PU-C-DBP and PU-3C-DBP), but differed by the presence of amide linkage in the middle of molecular tether.

Schemes 1–6 (displayed in the supplemental data) show the chemical synthesis of the five thiolated 4-substituted DBP (HS-DBP) required for the subsequent modifications of PU. Scheme 6 illustrates the covalent binding of different HS-DPP to the bromobutylated PU. The antioxidant loading for all variants tested corresponded to approximately 2.4% of the total polymer weight. Details of the chemistry used are as follows.

Synthesis of 4-mercapto-2,6-di-*tert*-butylphenol (Scheme 1, 1)

The synthesis of the simplest HS-DBP (**1**) was disclosed previously⁹ Briefly, **1** was prepared using a modification of described¹² procedure, by direct sulfuration of DBP followed by reduction of the resulting polysulfides and vacuum-distillation of the reduction product.

Synthesis of 4-mercaptomethyl-2,6-di-*tert*-butylphenol (Scheme 2, 6)

The compound **6** was prepared from the commercially available 2,6-di-*tert*-butyl-4-hydroxymethylphenol (**2**, TCI America, Portland, OR), which was transformed into 2,6-di-*tert*-butyl-4-chloromethylphenol (**3**) using a known procedure¹³ and reacted with an excess of thiourea in EtOH at 20–40 °C, forming the corresponding thiuronium salt (**4**) in a nearly quantitative yield. A MeOH-solution of **4** was reacted with AcSH in the presence of Et₃N for 5 min. at 20–35 °C. After drying *in vacuo*, dilution with water, acidification and extraction with hexane, 4-acetylthiomethyl-2,6-di-*tert*-butylphenol (**5**) was obtained in a 89% yield. For the purification, **5** was dissolved in a mixture of CHCl₃ and hexane (2:1 by volume), filtered through SiO₂ and recrystallized from cold pentane. NH₂OH was added to an argon-protected solution of purified **5** in THF and EtOH. After 1 h at room temperature, the reaction solution was poured into 1.5 M HCOOH, the organic solvents were distilled off *in vacuo* and the residue was extracted with hexane. The organic phase was washed with water, filtered through SiO₂, the sorbent was washed with hexane–CHCl₃ (10:1 by volume), and the filtrate was dried *in vacuo*. The crude thiol **6** was recrystallized from cold pentane, yield of the pure compound was 86%. An attempt to obtain the thiol **6** *via* the standard cleavage of **4** with methylamine was unsuccessful, affording the amine **7** instead of the expected thiol.

Synthesis of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanethiol (Scheme 3, 12)

The key compound for preparations of thiols **12** and **20** – methyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate (Scheme 3, **8**) – was prepared *via* nucleophilic addition of DBP to methyl acrylate, as described in the literature¹⁴. The ester **8** was reduced quantitatively with LiAlH₄ in boiling Et₂O to 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanol (**9**). The latter was transformed into the corresponding mesylate **10** according to the described¹⁵ general procedure. The mesylate was reacted with thiourea in EtOH at reflux, and the resulting thiuronium salt **11** was cleaved with aqueous MeNH₂ in MeOH to the aim product **12**. The thiol **12** was further purified by filtration through SiO₂ in a hexane–CHCl₃ solution (4:1 by volume) and recrystallized from cold (–20 °C) pentane. The overall yield of the pure thiol **12** was 87% (calculated for **8**).

Synthesis of 4-(3,5-di-*tert*-butyl-4-hydroxyphenoxy)butanethiol (Scheme 4, 17)

Commercial 2,6-di-*tert*-butyl-1,4-benzoquinone (**13**) was quantitatively reduced to 2,6-di-*tert*-butylhydroquinone (**14**) with Zn dust in a mixture of PhMe, EtOH and diluted HCl. O-Alkylation of the hydroquinone **14** tetramethylammonium salt with a large excess of 1,4-dibromobutane in DMSO proceeded sufficiently regioselectively, forming mostly 2,6-di-*tert*-butyl-4-(4-bromobutoxy)phenol (**15**) and no noticeable amounts of the undesired 1-substituted isomer. The compound **15** was purified by vacuum distillation and transformed into the corresponding thiuronium salt (**16**) *via* refluxing with an excess of thiourea in EtOH. The thiol **17** was then obtained by cleavage of **16** with BuNH₂ in i-PrOH. Filtration through SiO₂ in a hexane solution and crystallization from i-PrOH–water (1:1) at 0 °C yielded 48% (calculated for the starting **13**) of the pure thiol **17**.

Synthesis of N-(2-mercaptoethyl)-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionamide (Scheme 5, 20)

The methyl ester **8** dissolved in MeOH was hydrolyzed with NaOH at reflux for 0.5 h. in an argon atmosphere. The alkaline mixture was neutralized with CO₂, dried *in vacuo*, extracted with hexane and acidified with HCOOH, giving 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionic acid (**18**). After recrystallization from CHCl₃–hexane, the yield of pure acid **18** was 78%. The acid was transformed into its N-succinimidyl ester **19** (yield 90%) and reacted in i-PrOH–CH₂Cl₂ with an excess of cystamine base, prepared as described previously¹⁶. The resulting mixture of disulfides was purified from the unreacted cystamine and reduced to the thiol **20** with Me₃P in a mixture of CH₂Cl₂ and i-PrOH containing AcOH and water. After filtration through SiO₂ in a CHCl₃ solution and crystallization from CH₂Cl₂–hexane, the pure compound **20** was obtained in a 92% yield. We have found that a successful cleavage of disulfide bond with Me₃P in organic solvents requires not only slightly acidic conditions (as we noted previously¹⁶) but also a presence of water or alcohols. In CH₂Cl₂–AcOH solutions lacking those hydroxyl-containing compounds, the resulting thiol **10** undergoes a significant (up to 35%) acetylation.

Attachments of the HS-DBP to bromobutylated PU (Scheme 6, 21 – 25)

Covalent attachments of HS-DBP (Schemes 1–5, **1**, **6**, **12**, **17**, **20**) to the bromobutylated PU were carried out according to the standard procedure described previously^{6,8,17} for the analogous attachments of other thiolated compounds to polyurethanes. Bromobutylated PU was prepared from Tecothane TT1074A as described previously¹¹, but with the amount of lithium *tert*-butoxide decreased to 2.1 mmol. ¹H NMR spectral analysis of the bromobutylated PU found ca. 5% (0.12 mmol/g) of the urethane sites modified with pendant 4-bromobutyl groups. The bromobutylated PU was dissolved in N,N-dimethylacetamide (DMAc) under argon flow and cooled to –5 °C. The antioxidant thiol was dissolved in DMAc and added into the stirred mixture followed by a freshly prepared DMAc-solution of

(Bu₄N)₂B₄O₇. The mixture was stirred for 1.5 h at 0 °C and acidified with AcOH. The polymers **21** – **25** (PU-DBP, PU-C-DBP, PU-3C-DBP, PU-O-DBP, and PU-NHCO-DBP) were precipitated with cold MeOH, washed and dried as described for the cholesterol-derivatized PU.⁶ ¹H NMR of **21** – **25** clearly demonstrated in every case a complete replacement of the bromine atoms with the corresponding antioxidant moieties (Table 2). PU films were cast from THF solutions as described previously¹⁸.

Uniaxial stress-strain measurements on PU samples

Uniaxial stress-strain measurements were performed on PU and DBP containing PU variants. Samples for analysis were cut into 1 cm² pieces and two semicircular pieces were cut out from opposite sides yielding a typical "dog-bone" shape. The sample was mounted in a uniaxial tensile tester (Instron Model 4206, Norwood, MA) outfitted with a 100-N load cell. Once mounted, the actuator stretched the sample at a constant rate of 2.54 cm/min until failure. Failure data was not attainable for some groups because the limits of the tensile tester were exceeded. Data was expressed as Langrangian Stress (MPa) plotted versus applied strain (as a percentage of deformation).

in vitro oxidative degradation study

Control PU films and DBP containing variants were exposed to oxidative degradation conditions using previously described methodologies^{9,20}. Briefly, films (150 μm × 1 × 3 cm) of Tecothane, or the five variants detailed above were immersed for 15 days in 20% H₂O₂ + 0.1 M CoCl₂ or a control solution of distilled water. Films were then incubated for 15 days at 37°C. Solutions were changed every third day. At the conclusion of the study, films were rinsed in dH₂O and vacuum dried at room temperature and stored in a desiccator at –20°C, until further analysis.

Fourier transform infrared spectra (FTIR)

FTIR spectra of the samples were measured by attenuated total reflectance spectroscopy (FTIR-ATR), as previously described^{9,20}, using a Nicolet 5-Protégé 460 spectrophotometer E.S.P. (Nicolet, Madison, WI). All spectra were the result of 200 scans collected at a resolution of 2 cm^{–1} at a 45° angle of incidence. All spectra were recorded under identical conditions and adjusted for atmospheric water vapor and carbon dioxide transmittance by subtraction of the appropriate reference spectrum using the Omnic software package (Nicolet).

Scanning Electron Microscopy (SEM)

Surface morphology of the control and oxidized polyurethane configurations was analyzed using SEM. Samples were sputter coated with platinum (thickness = 1–2 nm) and examined with a JEOL 6300 FV SEM (Peabody, MA) at a 5 keV acceleration voltage.

Contact Angle Analysis

Water contact angle measurements were obtained on oxidized and control samples of unmodified and PU variants containing DBP using a custom built imaging goniometer system using established methodology⁹. The sessile drops were immediately visualized using a CCD camera (Edmund Scientific Co., Barrington NJ) and contact angle measurements were analyzed using Scion Image analysis software package (Scion Inc., Frederick, MD). The average of eight measurements of each sample was recorded.

Statistical analysis

Data were calculated as means ± standard error (SE). Student's *t*-test was used to determine the significance of differences. Statistical significance was noted with *p* ≤ 0.05

RESULTS

Uniaxial mechanical testing (Figure 2) showed that with the exception of PU-C-DBP, all DBP containing variants demonstrated similar elastomeric properties, comparable to unmodified PU. However these variants were shown to be less deformable than unmodified PU under comparable stress. Of interest is that PU-C-DBP films were markedly more deformable than even unmodified PU under comparable stress.

SEM (Figure 3) provided qualitative assessment of the extent of oxidative degradation of unmodified PU and the DBP loaded PU variants. Unmodified PU films showed widespread pitting on the surface following oxidation. Our original DBP modified PU, PU-DBP, had only minimal surface changes, following oxidation. In contrast, of all the DBP modified PUs following oxidation, PU-C-DBP showed the most extensive changes in the surface morphology. Oxidized samples from PU-C-DBP films showed characteristic surface cracking typical of oxidative damage. Following oxidation, PU-3C-DBP films exhibited slight evidence of surface damage in the form of abrasion and pitting. PU-O-DBP also showed evidence of surface cracking, although not to the extent seen in the PU-C-DBP films. Similar to the PU-3C-DBP oxidized films, oxidized PU-NHCO-DBP also showed signs of oxidative damage in the form of pitting and surface abrasion.

Surface changes elicited by oxidative degradation were assessed by FTIR analysis. Figure 4 shows characteristic ATR-FTIR of oxidized and control spectra for solvent cast PU films configured with the above described DBP containing variants. In addition, FTIR spectra showed an increase in peak intensity at 1700 cm^{-1} and an appearance of a new shoulder peak at 1512 cm^{-1} . These peak changes were noted in all antioxidant-containing variants and were reported previously by others and by us^{9,10,19}. The 1700 cm^{-1} spectral peak most likely represents changes in the hydrogen bonding of the hard segment¹⁹. The appearance of the 1512 cm^{-1} peak in both the control and oxidized samples of the DBP modified variants has been previously reported by our group to be consistent with changes in the urethane's nitrogens as a result of the DBP modification^{9,10}. The appearance of a new peak at 1174 cm^{-1} was noted in spectra from oxidized unmodified PU films, and to a lesser extent oxidized PU-C-DBP and PU-NHCO-DBP films. The 1174 cm^{-1} peak has been assigned to the $\nu(\text{C-O-C})$ of a branched ether created by crosslinking the polyether soft segment²⁰. Additional changes, in the form of decreasing peak intensity in the 1110 cm^{-1} peak of the spectra from unmodified PU as a result of oxidation. This change in the 1110 cm^{-1} spectral peak was attributed to oxidation of the soft-segment ethers resulting in chain scission^{20,21}. Others and we have used the loss of the 1110 cm^{-1} peak and the appearance of the 1174 cm^{-1} as markers of oxidative degradation of PU. Quantitative analysis of changes in the 1110 cm^{-1} and 1174 cm^{-1} peak intensity is detailed below in Figure 5.

Spectral peak intensity for the 1110 cm^{-1} peak (Figure 5A) and the 1170 cm^{-1} peak (Figure 5B), representing soft segment retention and ether cross-linking respectively, were normalized to that of the 1590 cm^{-1} peak height. The 1590 cm^{-1} peak is identified as the aromatic ring structure of the PU hard segment and is not susceptible to oxidative degradation^{2,9,10,21}. There was significantly ($p < 0.05$) more soft segment loss (1110 cm^{-1}) in the unmodified PU films following oxidation compared to the DBP modified variants. Soft segment loss was not observed in the PU-3C-DBP and PU-NHCO-DBP modified films, and only a slight decrease was noted in the PU-DBP variants. PU-C-DBP and PU-O-DBP showed more soft segment loss than the others variants, but this was not statistically significant. Representative, deconvoluted, isolated 1174 cm^{-1} spectral peaks from oxidized DBP-modified and unmodified films are shown in Figure 5C. As expected, the most intense 1174 cm^{-1} spectral peak was seen in the unmodified PU and ether cross-linking was significantly ($p < 0.05$) reduced in all DBP modified PUs compared to unmodified PU. PU-

C-DBP, PU-3C-DBP, PU-O-DBP, and PU-NHCO-DBP had slightly noticeable spectral peaks of varying intensity, in this region.

Changes in the surface free energy, as quantified by contact angle measurements, were used to assess the inhibitory effects of the tested DBP containing variants to oxidative degradation. Table 3 shows that all DBP configured PUs had contact angle measurements pre-oxidation that did not significantly differ from each other. Upon oxidation, contact angle measurements from unmodified PU films were significantly decreased. Likewise PU-NHCO-DBP and PU-C-DBP also exhibited a significant decrease in contact angle measurements as a result of oxidation. These results are consistent with the changes in the FTIR spectra showing more ether cross-linking in the PU-C-DBP and PU-NHCO-DBP variants compared with the other DBP tested variants.

DISCUSSION

Oxidative degradation of the PU components remains one of the largest obstacles in long-term survival of vascular devices ¹. Previous work by our group has shown that PU covalently appended with phenolic antioxidants, via our bromoalkylation chemistry, significantly resist oxidative degradation *in vitro* and *in vivo* ^{9,10}. The five variants we tested herein represented a relatively broad, although non-exhaustive, selection of linker chemistries. We compared the effectiveness of three different atoms (sulfur, carbon, and oxygen) in appending the hindered phenol to the N-alkylated region of PU. In addition the influence of the spacer length between the aromatic ring and the sulfur atom on resistance to oxidative degradation was also compared. These results show for the first time that the choice of chemical structure linking the pendant molecule to the PU backbone significantly affects the efficacy of the therapeutic molecule attached.

Previous work by others showed that coblending of phenol-containing antioxidants could effectively reduce the effects of oxidative degradation *in vitro* and *in vivo*. Using a similar strategy we showed that covalently appending antioxidants directly to the urethane hard segments, via bromoalkylation chemistry, also showed comparable levels of protection against oxidative degradation ^{9,10}. Of note, is that our chemistry allowed for the addition of 30-fold more antioxidant than coblending ^{3,4,9,10}. This is important when considering the chemical capacity of antioxidant protection is finite and that chronic inflammation, which coincides with the release of oxygen radicals from MDMs and foreign body giant cells, could persist for the entire lifetime of the implanted vascular device, which may require proper functioning for decades in a patient. Thus it is necessary to maximize the polyurethane's antioxidant capacity. We hypothesized that the efficiency of the hindered phenolic group in trapping oxygen-generated radicals might be significantly affected by the nature of the 4-substituent. With this purpose, we synthesized other variants of HS-DBP: with either an oxygen atom (**17**) or with a carbon atom (**6**, **12** and **20**) directly attached to the aromatic ring and tested these DBP-containing variants for the capacity to confer oxidation resistance to polyether films *in vitro*. The *in vitro* model that we used is a well established and cost effective experimental system previously used by us and others to approximate *in vivo* oxidation in an accelerated manner ^{9,10,21}. In addition it has been shown that 15 days of exposure to the oxidative degradation protocol corresponds to approximately 10 weeks *in vivo* ^{9,10,21}.

Our original design, PU-DBP, was the only variant containing a sulfur atom attached to the 4-position of aromatic ring ^{9,10}. Consistent with our previously reported results, this variant proved the most efficient in preventing oxidative degradation of the polyether polyurethane, as assessed quantitatively by the absence of ether cross-linking, measured via FTIR, and retention of surface energy as determined via contact angle measurements. In our previous

studies we noted that DBP loaded PU (PU-DBP) resisted oxidative degradation with minimal loss of mechanical properties following two week exposure to H_2O_2 - CoCl_2 solution. In addition, based on our prior uniaxial testing and gross observations, there was no evidence of phase separation in unmodified Tecothane or the DBP containing variant films. Likewise, the mechanical properties of modified PU **21**–**25** were virtually identical to those of non-modified PU Tecothane TT1074A, as it should be expected taking into account that only ca.5% of the urethane sites were affected.

With a single carbon atom attached to the 4-position of the aromatic ring, PU-C-DBP represented the shortest linker of all the carbon-attached variants. Mechanical analysis showed that this DBP containing variant had greater deformability than even the unmodified PU. Surprisingly, PU-C-DBP was the least effective in preventing oxidative degradation. It is possible to hypothesize that the phenoxyl radicals formed from the pendant stabilizers can be spontaneously cleaved eliminating 2,6-di-*tert*-butylbenzoquinone methide and thus, transferring the radical center onto the macromolecule (Figure 6A). A possible elimination of 2,6-di-*tert*-butylbenzoquinone methide from the related compound **4** in reaction with methylamine is in agreement with our observations, when the amine **7** was formed instead of the expected thiol **6** (Scheme 2). Of note, the resulting sulfur radical in PU-C-DBP is less energetic than the carbon radical resulting from the cleavage product in PU-O-DBP thereby enabling the release of cleavage product more rapidly in PU-C-DBP than in PU-O-DBP. This would explain why PU-C-DBP was even less effective than PU-O-DBP in preventing oxidative degradation.

Compared to PU-DBP, PU-3C-DBP was the next effective in preventing oxidative degradation. Oxidized PU-3C-DBP films showed no significant changes in contact angle measurements and had only slightly more ether crosslinking compared to PU-DBP. Similar to PU-DBP, SEM comparisons of pre and post oxidized films showed only minimal differences in surface morphology. Considering the comparable resistance to oxidative degradation, these results suggest that PU-3C-DBP may be a suitable alternative to PU-DBP for certain applications.

PU-O-DBP is the only antioxidant variant that contains an oxygen atom attached to the 4-position of aromatic ring. In addition, the spacer molecule was much longer compared to that used in PU-DBP. Compared to PU-DBP, PU-O-DBP was not as effective in resisting oxidative degradation. Although contact angle measurements showed no significant changes in the surface energy of oxidized variant films compared to control treated films, the FTIR results of oxidized PU-O-DBP films show a two-fold increase in ether cross-linking compared to identically treated PU-DBP samples. In addition, SEM studies revealed evidence of surface cracking. Similar to PU-C-DBP, the poor efficiency observed probably results from the instability of the linkers arising when the radical trap is in action. We hypothesize that the phenoxyl radicals formed from the pendant stabilizers can be spontaneously cleaved eliminating 2,6-di-*tert*-butyl-1,4-benzoquinone and thus, transferring the radical center onto the macromolecule (Figure 6B).

PU-NHCO-DBP which has the longest linker also had significant reduction in contact angle measurements post oxidation as well as a three-fold increase in ether cross-linking, compared to PU-DBP. A possible explanation is found in the chemical nature of the linker. The amide bond present in PU-NHCO-DBP confers a more hydrophilic chemical environment, which may make it more amenable to H_2O_2 and Co^{2+} permeation. These data therefore suggest that the hydrophilicity of the linker may play a role in its antioxidant capacity. These results are somewhat in agreement with a recent report showing that PUs containing more hydrophobic chain extenders prevented degradation of biodegradable PUs²². However, further investigation regarding this possibility is required.

We have shown herein that the chemical linker significantly alters the antioxidant capacity of PU films covalently modified, via hard segment bromoalkylation, with hindered phenolic antioxidants. These five linkage variants differ in their attachment atom, linking the DBP to the PU and their reactivity towards the bromoalkylated PU. Although beyond the scope of these current investigations, the data shown here provide important insight into choosing specific chemical linkages for designing second-generation PUs configured with multiple therapeutic moieties, or polymeric linkages capable of attaching multiple DBP molecules to the same urethane hard segment. In addition, the data shown here provides evidence for designing degradable PUs for biomedical applications based on the linker's susceptibility to form phenoxyl radicals. These possible applications of alternative linker chemistries remain an active focus of research in our laboratory.

CONCLUSION

The above data show that linker chemistry plays a significant role in the oxidation resistance of PU covalently modified, via bromoalkylation chemistry, with pendant DBP moieties. Our original DBP configured PU (PU-DBP) was the most effective at reducing oxidative damage *in vitro*. Based on FTIR, contact angle analysis, and SEM studies, it appears that chemical stability of the linker, with respect to its reactivity with the radical trap is the single biggest contributor to antioxidant efficiency. In addition, there appears to be no direct correlation in the length of pendant molecule and the resistance conferred on the PU to oxidative degradation. These studies presented here also suggest that the hydrophilic properties of the linker may play a role in the antioxidant capacity of the attached radical trap.

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Figure 1.
Schematic diagram of the chemical distinctions of the 5 different DBP containing variants.

Figure 2. Uniaxial testing results, stress-strain curves for Tecothane TT-1074A (PU) and DBP modified PU variants. PU-C-DBP showed greater deformability. Other DBP containing variants had similar mechanical properties that were slightly reduced compared to unmodified PU films. (n = 3 tests for each film).

Figure 3. Scanning electron micrographs of Tecothane TT-1074A (PU) and DBP modified variants. Unmodified PU films or films covalently linked to DBP antioxidants, via 1 of 5 linker chemistry variations, were exposed to water or an oxidative solution of $\text{H}_2\text{O}_2/\text{CoCl}_2$ as detailed in Methods for 15 days. Representative micrographs show surface damage to unmodified PU and the DBP containing variants, PU-C-DBP and PU-O-DBP, following oxidation. In contrast, there was little evidence of oxidative induced surface damage on the surfaces of oxidized PU-DBP, PU-3C-DBP, and PU-NHCO-DBP films. Insets are untreated films of the indicated formulation. Bar = 50 μm at 250 X and Bar = 5 μm at 2500 X.

Figure 4.

FTIR Spectra of the polyurethane elastomer (PU), Tecothane TT-1074A, and DBP containing PU variants. PU covalently modified, post polymerization, with one of five thiolated DBP variants, which differed from each other in length and/or chemical composition of the linker molecule, were oxidized (oxi) as detailed in *Methods*. Peaks of interest are labeled with arrows. There are noted changes, denoted with arrow, in the peak distribution of 1056 cm⁻¹ and 1099 cm⁻¹ which have been previously attributed to nitrogen and the adjacent carboxylic acid groups as a result of bromoalkylation chemistry. In addition differences were noted in the 1517 cm⁻¹ spectra from DBP containing films, which corresponds to changes in the urethane's nitrogen bond. Similarly, the 1700 cm⁻¹ peak intensity, corresponding to the urethane's C=O bond is altered following oxidation.

Figure 5.

Quantitative analysis of oxidative degradation as assessed by soft segment retention (A) or ether crosslinking (B) in Tecothane TT-1074A (PU) and PU modified phenolic ring antioxidant (DBP) with covalently linked with 1 of 5 pendant molecules as detailed in *Methods*. (A) Soft segment retention was significantly improved in all antioxidant containing films compared to unmodified PU. Soft segment retention calculated as normalized intensity of the 1110 cm⁻¹ peak from oxidized samples compared to untreated controls. (B) All antioxidant containing films were significantly better than the unmodified PU in resisting oxidative degradation following two week exposure to CoCl₂/20% H₂O₂. Ether cross-linking was assessed from 1170 cm⁻¹ peak heights measured from FTIR spectra (C). Ether cross-linking = A_{1170}/A_{1590} . * $p < 0.05$ versus DBP modified PU variants. Data presented as average $\pm$ standard error of the mean ($n = 3$)

A. **B.** **Figure 6.**

Schematic diagram showing molecular cleavage of the DBP antioxidant from (A) PU-C-DBP and (B) PU-O-DBP.

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; Su = N-succinimidyl

Scheme 5.

21 (PU-DBP): Z = none; **22** (PU-C-DBP): Z = CH_2 ; **23** (PU-3C-DBP): Z = $(\text{CH}_2)_3$;
24 (PU-O-DBP): Z = $(\text{CH}_2)_4\text{O}$; **25** (PU-NHCO-DBP): Z = $(\text{CH}_2)_2\text{NHCO}(\text{CH}_2)_2$

Scheme 6.

Table 1

DBP containing PU variants and their chemical properties

Polyurethane (PU)	Rationale for Modification	4-position on DBP	Intervening Atoms between the sulfur atom and the Aromatic Ring	Number of Bonds between the sulfur atom and the Aromatic Ring	Scheme
PU-DBP		Sulfur	None	One	1
PU-C-DBP	Assess the effectiveness of the phenoxyl radical trap in the presence of carbon connected to the phenol instead of sulfur	Carbon	1 Carbon	Two	2
PU-3C-DBP	Increase the distance between the sulfur atom and the aromatic ring to improve the stability of the phenoxyl radical trap	Carbon	3 Carbons	Four	3
PU-O-DBP	Assess the stability and efficiency of the phenoxyl radical trap having an oxygen atom conjugated with the ring	Oxygen	1 Oxygen, 4 Carbons (ether bond)	Six	4
PU-NHCO-DBP	Assess the stability and efficiency of the phenoxyl radical trap in more hydrophilic environment with the presence of an amide bond	Carbon	1 Nitrogen, 1 Oxygen, 5 Carbons (amide bond)	Seven	5

TABLE 2¹H NMR (400 MHz, DMF-d₇) data of the antioxidant-modified PU, δ ppm.

Polymer	(CH ₃) ₃ C ^a	OH ^a	Ar-H ^a	Other characteristic signals
PU-DBP	1.41 ^b	7.07	7.17 ^c	2.86 (CH ₂ S)
PU-C-DBP	1.42 ^b	6.87 ^b	7.13 ^b	2.45 (CH ₂ SCH ₂ Ar); 3.68 ^b (CH ₂ Ar)
PU-3C-DBP	1.41 ^b	6.70 ^b	7.00 ^b	2.45 – 2.55 (2CH ₂) ^d ; 2.60 (CH ₂) ^d
PU-O-DBP	1.41	6.57	6.73	2.49 – 2.60 (2CH ₂ S); 3.95 (CH ₂ O)
PU-NHCO-DBP	1.41	6.71	7.02	2.43, 2.53, 2.57, 2.82 (4CH ₂) ^d ; 7.95 (NH)

^a Of 4-hydroxy-3,5-di-tert-butylphenyl.^b Doubled.^c Overlapping with the PU aromatic protons.^d CH₂ of the bridge, with no clear assignment.

Table 3

Contact angle analysis of oxidized and control DBP containing PE variants.

Polyurethane (PU)	Treatment	Contact angle (°) ± SD	Notes
Unmodified	Untreated	66.7 ± 0.6*1	*1 $p < 0.05$
	Water	65.6 ± 2.2	
	Oxidized	57.6 ± 2.0*1	
PU-DBP	Untreated	75.7 ± 0.9	N/S
	Water	76.1 ± 1.3	
	Oxidized	74.5 ± 0.9	
PU-C-DBP	Untreated	73.1 ± 1.5*2	*2 $p = 0.017$
	Water	72.6 ± 1.5	
	Oxidized	59.3 ± 0.5*2	
PU-3C-DBP	Untreated	72.4 ± 0.9	N/S
	Water	71.8 ± 0.7	
	Oxidized	66.4 ± 1.8	
PU-O-DBP	Untreated	69.9 ± 4.8	N/S
	Water	72.2 ± 1.3	
	Oxidized	68.8 ± 2.5	
PU-NHCO-DBP	Untreated	70.4 ± 0.1*3	* 3 $p = 0.01$
	Water	72.5 ± 3.0	
	Oxidized	59.9 ± 0.6*3	

Averages $n = 5 \pm$ standard error of the means