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Protective role of vitamin E to reduce oxidative degradation of soft implantable polyurethanes: *In vitro* study

From mechanical viewpoint

Abstract: Vitamin E (VitE) additives are important in treating osteoarthritis inclusive cartilage regeneration due to their antioxidant and anti-inflammatory properties. The present research study focuses on the ability of biological antioxidant VitE (alpha-tocopherol isoform) to reduce or minimize oxidative degradation of soft implantable polyurethane (PU) elastomers after extended periods of time (5 months) *in vitro*. The effect of the oxidation storage media on the morphology of the segmented PUs was evaluated by mechanical softening, crystallization and melting behavior of both soft and hard segments (SS, HS) using dynamic mechanical analysis (DMA). Bulk mechanical properties of the potential implant materials during ageing were predicted from comprehensive mechanical testing of the biomaterials under tension and compression cyclic loads. 5-months *in vitro* data suggest that the prepared siloxane-poly(carbonate-urethane) formulations have sufficient resistance against degradation to be suitable materials for chondral long-term bio-stable implants. Most importantly, the positive effect of incorporating VitE (0.5 or 1.0% w/w) as bio-antioxidant and lubricant on the bio-stability was observed for all PU-types. VitE-additives protected the surface layer from erosion and cracking during chemical oxidation *in vitro* as well as from thermal oxidation during extrusion re-processing.

Keywords: long-term implants, soft medical-grade polyurethanes, bio-antioxidant, *in vitro* test.

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1 Introduction

Addition of bio-antioxidants inhibits the degradation of polymer-based long-term implants. Vitamin E (VitE) additives are reported to preserve against damage by lipid peroxidation in various cell types including chondrocytes and skeletal muscle cells. This is important to prevent and treat osteoarthritis [1]. Recent pre-clinical research work on the effects of VitE-containing UHMWPE (Ultra high-molecular weight polyethylene), which is commonly used as a material in knee arthroplasty was reviewed by Bracco and Oral [2]. Incorporation of VitE can change the microstructure of the UHMWPE polymer and as a result its tribologic and biomechanical properties, thereby improving the oxidation resistance and fatigue strength of implantable products.

The biocompatibility of UHMWPE samples blended with VitE is studied since 2002 and so far no indications for increased cytotoxic effects of the VitE-stabilized UHMWPE have been found compared to the virgin UHMWPE at low concentrations of alpha-tocopherol. The properties of commercialized antioxidant-stabilized cross-linked UHMWPE liner (Biomet®, Biolox®) are still under clinical investigations [3].

Current implants of polyurethane-(PU) based Bionate-type with cartilage regeneration function in the early stage of osteoarthritis could also be modified with bioantioxidant additives with lubricant properties as well in order to reduce the oxidative degradation of soft implantable PU elastomers.

Although there are a few recent studies available in the literature regarding PU-VitE combinations, there has been no systematic approach to evaluate how product performance is affected by VitE addition. More specifically, there is a significant lack of knowledge about the interplay between thermal re-processing of implantable urethane block-copolymers blended with VitE and their biostability and mechanical fatigue behaviour.

Degradation of commercially available PUs was studied systematically *in vitro* as well as *in vivo* using polyether (PEO

or PTMO) urethanes with an MDI-BD hard segment (HS) like Pellethane 2363 80A(E) and Elastane 80A. These polymers can be regarded as representative model formulations for modern implantable PUs with the same type of HS [4-6]. Since the end of the 1970ies, Pellethane-based cardic insulated pacing leads continue to be implanted in humans. However, enhancing the PU-biostability by tailoring the chemical design of the block-copolymers as well as by incorporating bio-antioxidants is still a challenge. According to the current state of the art, non-degradable PU-materials can be designed from aliphatic polycarbonate urethane (PCU) and silicone-based macrodiols like polydimethylsiloxane and should contain bio-antioxidants and bio-lubricants. Furthermore, they should be properly purified and morphologically stabilized in order to provide reliable *in vitro* testing results and prolong biostability under real life conditions [7]. PCU-based Bionate-55D micro catheter tubes subjected to the aggressive oxidative environment *in vitro* (metal ion oxidation test, MIO) underwent degradation to a much lesser extent than Pellethane-55D-based tubes: they exhibited only minor surface erosion to a depth of up to 1 micrometer and showed no evidence of major cracking after 10 months [8].

Our present study evaluates the biostability of VitE blended- silicon-poly(carbonate urethane) materials (SiPCU_VitE), synthesized in our laboratory and compounded with natural antioxidant VitE, after 5 months under *in vitro* conditions. In order to estimate the effect of incorporating VitE as bio-antioxidant and lubricant on the in vitro biostability of PUs, the following materials were also subjected to accelerated biostability tests: (i) pure SiPCU was used as a reference material. (ii) Commercially available Pellethane 2363-80A of the same hardness was selected as a currently used standard material and was applied either as received (sample a), and (iii) commercial Pellethane after thorough purification (additives-free) (sample b) but without VitE addition, or (iv) blended with 0.5% (sample c) or 1.0% w/w VitE (sample d). After several months of accelerated ageing tests, the mechanical and thermo-mechanical characterization of the tested materials allowed to evaluate the protection efficiency of bio-antioxidant additives in the PU-materials and to compare their performance to earlier materials.

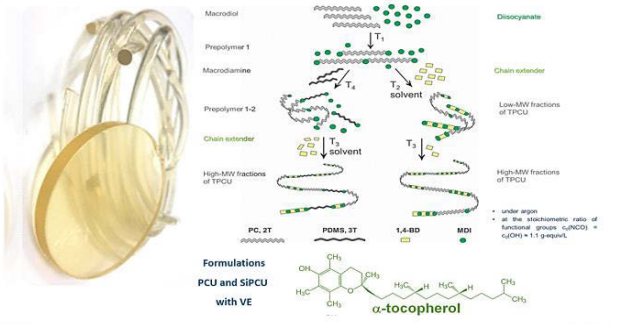

Figure 1: Synthesis and structural design of the bio-stable PCU and SiPCU formulations with VitE. Test specimens had a cylindrical (13x6 mm) or a disk (40x4 mm) geometry.

2 Experimental

2.1 Synthesis and processing

Structural design of the tested bio-stable PU-based formulations (Fig.1) as well as their synthesis and processing techniques were the same as those described previously in [7]. The high molecular weight block-co-polymers with controlled molecular structure were produced using real time analytical monitoring of every step during the polymerization process. The spectroscopic, calorimetric and rheological in line control provided the morphological (and in turn, mechanical) reproducibility of the medical product on the preparative scale.

The commercially available Pellethane 2363 80A granulate was purified using soxhlet ethanol extraction to remove the low molecular weight commercial additives. In order to protect VitE from thermal oxidation and provide the homogeneity of the VitE distribution in the investigated polymer specimens, one part of the purified granulate was compounded with VitE in THF solution. The obtained VitE-saturated polymer mixture was properly dried, cut into small pieces and mixed with two parts of untreated Pellethane by melt extrusion under argon to give a total content of either 0.5 % or 1.0 % w/w VitE in the blend. Thus, before the moulding of the test specimens, granulate of the VitE-containing samples was re-processed. The same approach was applied to the SiPCU to obtain a 0.5 %w/w VitE_SiPCU sample for comparison.

2.2 Mechanical tests

The bulk properties of the PU-based formulations were characterized using application relevant cyclic loading in compression and in tensile tests as described in [7]. Hysteresis ratio at 100% elongation as well as compressive strain under 1200N for the 1st, 4th and 10^{th} cycles were used to evaluate the bio-stability of the bulk material. In addition, E-dynamic moduli (E', MPa) of dried and solution saturated samples were estimated using tensile-mode DMA-temperature scans.

2.3 Accelerated *in vitro* oxidation tests

Specimens were immersed in physiological liquid (PL: 0.9 %w/w NaCl) or oxidative solution (Ox: 0.1M $CoCl_2$/ 20 % H_2O_2) for 3 and 5 months at 37 °C *in vitro* studies. Hydrolytical stability of SiPCU control sample was additionally tested in PBS after 15 months ageing at 37 °C. Stereo microscopic imaging was used to observe the surface quality after storage

and mechanical tests.

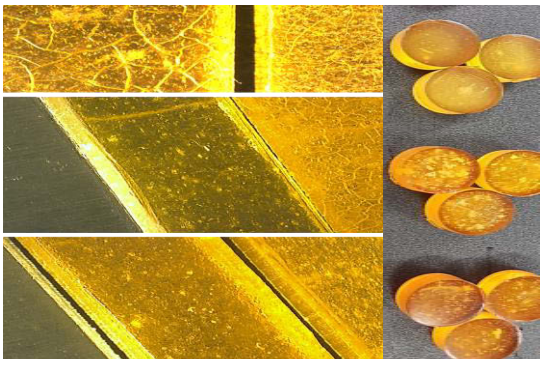

Figure 2: Microscopic images of Pellethane samples (b-d) after oxidative *in vitro* as well as mechanical cyclic ageing (3m PL/ 3m Ox/ 5m Ox)

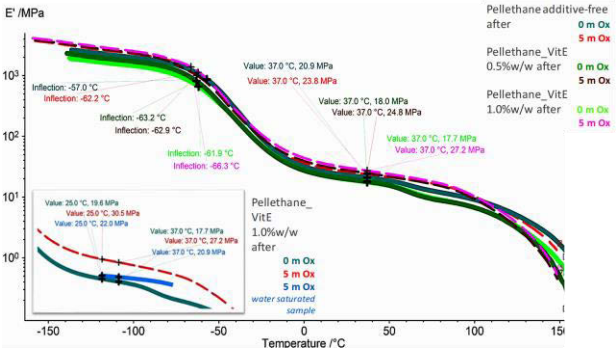

Figure 3: DMA-tensile profiles of VitE-Pellethane samples

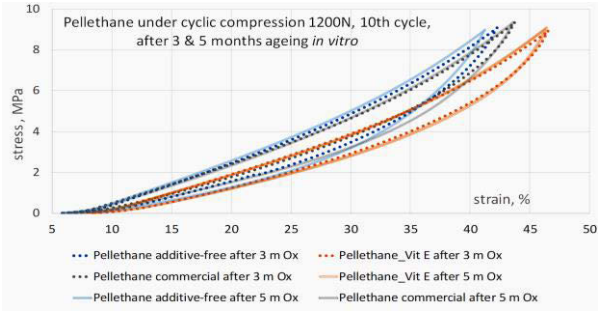

Figure 4: Cyclic compressive response of VitE-Pellethane samples after ageing (saturated samples)

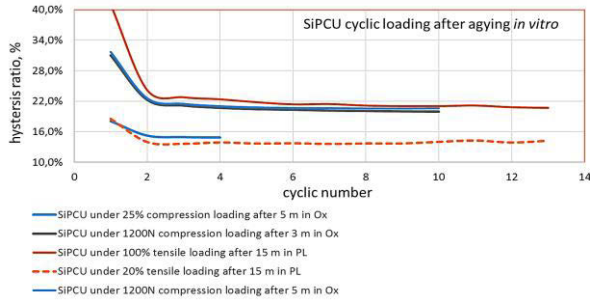

Figure 5: Cyclic hysteresis of SiPCU samples under 100% tensile and1200N compressive after 5m Ox and 15 m PBS at 37°C ageing (saturated samples)

3 Results

Microscopic imaging (Fig. 2) allows qualitative assessment of the extent of tensile cyclic stress and the effects of the medium on the ageing of additive-free Pellethane after 3 and 5 months in Ox (Fig. 2, upper picture), of 0.5%VitE-Pellethane after 3 months PL as well as 3 and 5 months in Ox media (Fig. 2, middle picture), of 1.0%VitE-Pellethane after 3 months in PL as well as 3 and 5 months in Ox (Fig. 2, lower picture). The unmodified sample showed characteristic surface damage already after 3 months of oxidation (Fig. 2). 0.5%w/w of antioxidant protected the polymer material even after 5 months of oxidation, however, under additional cyclic loading in plastic stress-strain range this specimen also showed evidence of surface cracking. Doubling the amount of VitE in Pellethane resulted in significantly better resistance against oxidative degradation following the 5 months exposure period *in vitro* under additional mechanical stress.

Regarding the resistance of the bulk material against oxidative degradation after 5 months, DMA profiles confirmed the presence of VitE as illustrated by the low-modulus curves that indicate a shift in the glass transition temperature by ca. 5 K to lower temperature (Fig.3). In order to estimate the morphological changes of polymer structure, the mechanic cyclic responses of the saturated samples were studied and are presented in Figures 4-6.

4 Discussion

On the one hand, 5 months of MIO-protocol is a cost effective experimental system which corresponds to ca. 2 years *in vivo* and allows comparatively fast assessment of biodegradability [4]. On the other hand, Cobalt-ions can be accumulated within the amide-containing polymer due to the formation of coordinative bonds [9], thereby acting like fillers (light spots

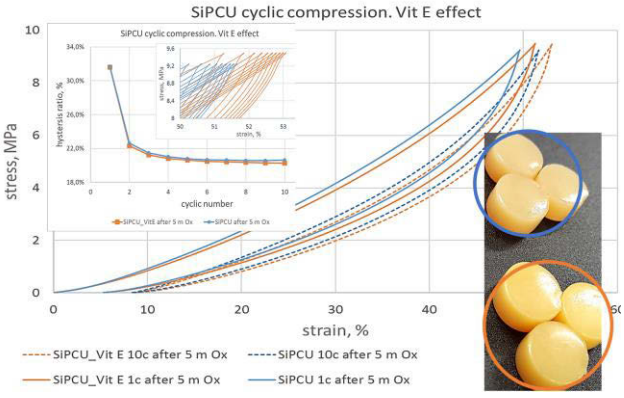

Figure 6: Cyclic compressive responce of VitE-SiPCU samples after 5m Ox ageing (saturated samples)

in the test specimens, Fig. 2). The amount of chelating N-centres increases (a) with the amount of VitE added since the crystalline phase in the HS is disrupted or/and (b) after reprocessing due to hydrolytical and mechanical degradation of PU-chains in the absence of sufficient amounts of compounded antioxidant.

The E`-curves of the aged samples displayed a parallel shift to higher values across the complete temperature range (Fig. 3). The observed effect could be due to either one of two reasons: (1) Cobalt ions accelerate chemical oxidation of the polymer chains (chain scission and crosslinking) leading to morphological changes. This would be the standard interpretation related to the test result using Cobalt ions since they are used in the test for their catalytic effect. (2) However, this effect could be due to a specific ion effect of Cobalt, i.e., the complexing action of the Cobalt ion. Cobalt is one of the d-group metals that are able to form coordination compounds with amide groups [9]. Hence, the observed mechanical increase (Fig. 3) could also be due to the formation of a metal ion containing crystalline phase which displays decreased mechanical stability without necessarily the polymer chains being oxidatively cleaved. If the latter explanation were at least partly true, this could have serious implications for interpreting the results of *in vitro* biostability tests of materials intended as implants: since free Cobalt ions are typically not present in the human body, drawing conclusions about the biostability of a material solely based on the accelerated *in vitro* test could be misleading and the biostability of a potential implant could systematically be underestimated from mechanical viewpoint.

To decide whether polymer degradation or (at least partly) metal complexation had taken place, mechanical testing of the saturated samples after swelling was performed (Fig. 3). Since upon swelling, the original mechanical strength is nearly completely restored, the mechanical tests suggest that explanation 2 is at least partially correct. Upon swelling, the metal ion containing crystalline phase is partially dissolved and the mechanical strength is regained in the application-relevant temperature range. It should be noted, that the T-range from 50 to 90 ºC represents the morphological changes of the Pellethane structure for every sample.

The SiPCU specimens stored for 5 months in the Ox medium showed no significant changes compared to the SiPCU specimens treated only for 3 months oxidized. They were not significantly different from those that had hydrolytically aged for 15 months in terms of tensile or compression hysteresis as well (Fig. 5). Moreover, the mechanical loading of both types demonstrated the same hysteresis ratio values in the elastic (20-25% strain) and in the plastic (100% strain in tensile/ 1200N or ca 50% strain in compression) regions. The protection effect of 0.5 % of VitE in the SiPCU material against oxidation had only a minor deteriorating effect on the mechanical properties (Fig. 6), which can be attributed to sufficient protection of the silicon component in the polymer chains. These results suggest that SiPCU-based formulations may be suitable materials for long-term implant applications, especially in treating osteoarthritis.

Author Statement

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