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In vitro bio-stability screening of novel implantable polyurethane elastomers

Morphological design and mechanical aspects

Abstract: A series of novel biomedical TPCUs with different percentages of hard segment and a silicone component in the soft segment were synthesized in a multi-stage one-pot method. The kinetic profiles of the urethane formation in TPCU-based copolymer systems were monitored by rheological, in line FTIR spectroscopic (React IR) and real-time calorimetric (RC1) methods. This process-analytically monitored multi step synthesis was successfully used to optimize the production of medical-grade TPCU elastomers on preparative scale (in lots of several kg) with controlled molecular structure and mechanical properties. Various surface and bulk analytical methods as well as systematic studies of the mechanic response of the elastomer end-products towards compression and tensile loading were used to estimate the bio-stability of the prepared TPCUs *in vitro* after 3 months. The tests suggested that high bio-stability of all polyurethane formulations using accelerating *in vitro* test can be attributed to the synthetic design as well as to the specific techniques used for specimen preparation, namely: (i) the annealing for reducing residual polymer surface stress and preventing IES, (ii) stabilization of the morphology by long-time storage of the specimens after processing bevor being immersed in the test liquids, (iii) purification by extraction to remove the shot chain oligomers which are the most susceptible to degradation. All mechanical tests were performed on cylindrical and circular disc specimens for modelling the thickness of the meniscus implants under application-relevant stress conditions.

Keywords: *in vitro*, long-term implants, fatigue, bio-stable polyurethane, morphological design, large scale production.

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1 Introduction. Designing bio-stable elastomers

The development of novel bio-stable thermoplastic polycarbonate-urethane (TPCU) is currently of great interest for fabricating the next generation of long-term implants [1-3]. Such materials combine rubber elasticity at body temperature and thermoplastic processability on heating. This makes it possible to produce a tailor-made, patient-specific implant form design, using injection moulding, vacuum pressure and 3D-printing technologies.

The biomechanical behaviour of the segmented polyurethane block co-polymer elastomers, as well as the hydrolytic and oxidative stability, and the biocompatibility of the potential medical materials are strongly dependent on (i) nature and concentrations of the two segmental blocks; (ii) variations in block lengths, polarity and coupling order of the hard (HS) and soft (SS) segments in the polymer chain within one specific formulation [1]. Obviously, undefined variations in the synthesis protocol for high molecular weight TPCU block-copolymers are among the most critical factors that influence the reproducibility of the final properties of the medical product. Optimizing the large scale production of TPCU-elastomers with controlled molecular structure of the polymer chains and properties via establishing reliable catalyst-free, multi-step synthesis procedures was the major objective of this project. Another important goal was to estimate the *in vitro* bio-stability of novel bio-medical polyurethane formulations from a mechanical point of view.

In accordance with modern approaches to enhancing oxidative stability of PU-based materials, our samples were designed using: (i) modifying the soft segment with a hydrophobic silicone (PDMS) component; (ii) increasing the crystallinity via incorporating a diisocyanate of symmetrical structure without conformational isomers as well as an aliphatic polycarbonate in SS (additional crystallisation capability); (iii) blending of the end product with antioxidants (Vitamin E as a natural hydrophobic antioxidant).

Recently, the mechanical response and bio-stability of the commercially available MDI-based TPCUs such as

Corethane 80A, Bionate 80A, CarboSil 20 80A were studied and compared with the biomechanical performance of the polyether-based urethane Pellethane 2363-80A at the same test conditions [3]. Metal ion-induced accelerated oxidative (MIO) degradation testing was used to predict clinical performance of bio-medical elastomers. Samples which were aged for 36 days at 37 ºC *in vitro* showed distinct structural and morphological changes at the surface, significant mass loss, as well as increased surface roughness and number of cavities. Although some changes at the surface had occurred, the bulk properties of the TPCUs remained unchanged and displayed, e.g, the same tensile response after one month *in vitro*. The Bionate II 80A and CarboSil (silicone-polycarbonate)-urethane (TSiPCU) are considered to be the most promising materials for orthopedical long-term implants [3].

The aims of our work were (a) to produce novel softer TPCU- and TSiPCU-based materials with a controlled segmental distribution and length in the polymer chain on a kg-scale; (b) to test the new materials in vitro in model meniscus implants under application-relevant conditions; and (c) to estimate the biostability of the soft elastomers from a mechanical point of view under long-term oxidative treatment.

2 Materials and methods

2.1 Production of elastomers

Synthetic design of bio-elastomers. Compositional variations of PC-PDMS-MDI-BD block copolymers were synthesized using the multi-step approach which gives good control of polymer architecture in catalyst free systems. The prepolymer formation (end-capping of the macrodiol) was carried out in bulk, while the chain extending of the oligomers with a low molecular weight diol took place in dimethylacetamide as a solvent. The synthetic protocol and structural design of the new biopolymers are illustrated schematically in [4].

Experimental setup of the real-time monitoring polyurethane formation. Segmental constitution during catalyst free polyurethane synthesis was controlled *in situ* using three sensors: FTIR spectroscopic ReactIR probe, real-time calorimetric RT-Cal, and in-line rheological measurement integrated in the computer-controlled automatic reaction system RC1 from Mettler Toledo (Fig. 1). The kinetic profiles of the last steps were analysed and used to optimize the reaction parameters (concentration, flow rate of reagent addition, mixing rate, reaction temperature and time), which allowed to produce polyurethane chains with well-controlled structure und distribution of HS length.

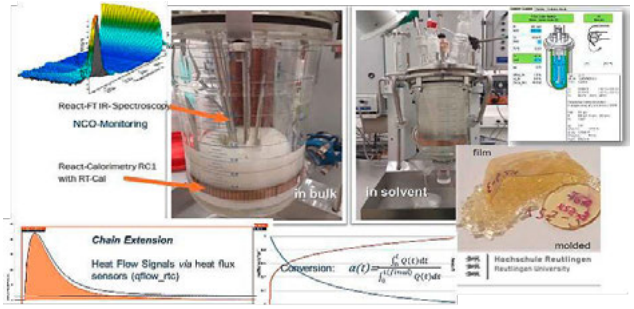

Figure 1: Experimental setup of the *real-time* monitoring polyurethane formation in TSiPCU production.

Processing. Prior to melt extrusion, the elastomers were dried for several days to remove residual solvent. The materials selected for bio-testing were extracted for 4 days in ethanol, in order to eliminate the effect of any residual additives as well as leaching during the long-term oxidative storage. The purified granulate was compounded with 0.5%w/w of the natural antioxidant Vitamin E (VE). Test specimens were moulded into cylindrical or disk geometry of 13 or 40 mm diameter and 6 or 4 mm thickness, correspondently. The structure and molecular weight of the samples before and after processing were controlled using FTIR, DSC and GPC methods.

2.2 Mechanical tests. Characterization of bulk properties

The tensile dog-bone specimens were cut from injection moulded disks (test length of 20 mm). The cylindrical specimens were used for compression tests. The fatigue behaviour of each material was investigated under cyclic loading in an elastic range and under physiological load: 100% of tensile elongation and 1200N of compressive load. The ultimate tensile strength (UTS, MPa), failure strain (FS, %), Young's Modulus (E_{20}, MPa), hysteresis ratio at 100% elongation (hr_{100}, %) for 4th and 10th loading cycles, compressive strain under 1200N (CS_{1200}, %) as well as hr (%) for 1st, 4th and 10th cycles were used to evaluate bio-stability of the bulk material.

2.3 Accelerated *in vitro* oxidation tests

Specimens were stored at ambient temperature for at least 3 weeks after processing before being immersed in physiological liquid (PL) 0.9%w/w NaCl or oxidative solution (OS) 0.1M $CoCl_2$/20%H_2O_2 for 3 months at 37 ºC *in vitro* studies. Degradation testing was performed on Pellethane 2363-80A as a reference and on the new TPCU-formulations. SEM was used to control the surface quality after storage tests.

3 Results and discussion

A new synthesis protocol made it possible to reproducibly prepare up to 1 kg of the compositionally homogenous bio-elastomer with Mw 130-140 kDa and MWD in step-growth polyaddition in the range of 2.2 - 2.5

3.1 Materials of choice for *in vitro* test

The mechanic response of the elastomer end-products to compression and tensile loading was systematically studied for controlling the reproducibility of the synthetic procedure as well as for selecting the best material specimens for the biological tests. UTS, hardness and CS_{1200N} of the elastomer products synthesized in 0.5L and 2.0L reactors were highly dependent on the composition, but, were not significantly affected by the selected up-scaling strategy (Fig. 2). Moreover, the fatigue behaviour of the specimens after 100 tensile cycles (Fig. 3) was also considered and the samples F1 and F4 were selected as the basis for bio-stable formulations.

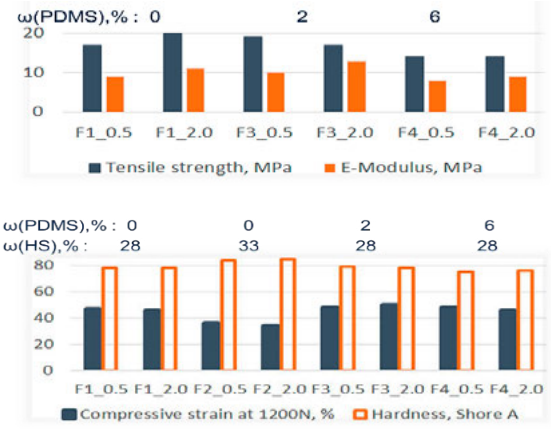

Figure 2: Mechanical properties of the dried F 1 - 4: (a) tensile test (*sd = 0.2-0.6*); (b) compression test under application-relevant loading (*sd = 0.1-0.4*)

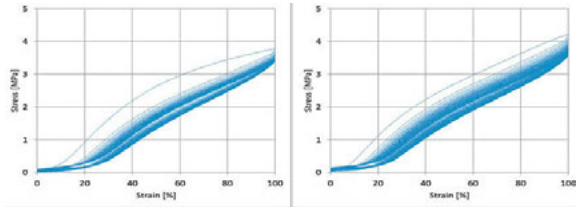

Figure 3: Cyclic tensile testing the sample F1 dried (right) and after 1 month of storage at 37 oC in PL solution (left). For fatigue life estimation of the test-specimens the hysteresis ratios (hr, %) of 4th, 10th and 100th cycles were calculated.

3.2 Evaluation of biostability

The resistance of the novel materials against oxidative degradation *in vitro* was evaluated using qualitative comparison of the surface aged in the PL and OS as well as quantitative estimation of the aged bulk material via mechanical response of the specimens under application-relevant stress.

Surface quality after aging. The SEM images for Pellethane and TSiPCU aged in both PL and OS media do not show any significant evidence of surface damage or erosion after long-term storage tests (Fig.4).

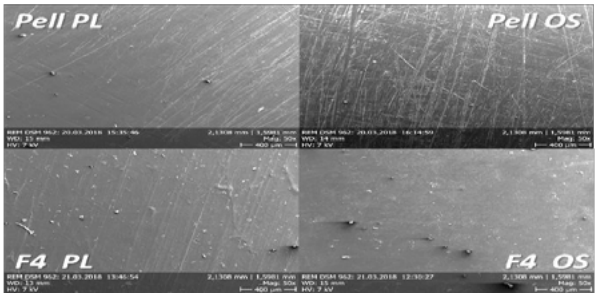

Figure 4: SEM micrographs (50X) of Pellethane 2363-80A and sample F4 after 3 months of storage at 37 °C in PL and in OS.

Bulk properties after aging. All mechanical tests were performed on the samples saturated with a liquid to simulate a *sinovial* environment. In order to eliminate the softening effect by water and the stiffening effect caused by the essential reorganization of the microdomain structure during aging, but not due to oxidative degradation, [5-6], the samples aged in OS were compared to those submersed in PL as the references. The data on tensile and compression characteristics of the hydrated samples are summarized in Tables 1-2.

Table 1: Tensile properties of TPU-based formulations after 3 months treatment in PL and OS at 37 °C, (*sd = 0.2-0.6*)

Sample		UTS	FS	E_{20}_4	$hr_{100}4$	$hr_{100}10$
Pell	PL	15.5±1.2	464±38	17.7±0.4	17.4	15.5
	OS	15.2±1.3	522±33	16.8±0.1	16.9	15.0
F1	PL	19.4±0.9	462±18	11.8±0.8	19.3	18.3
	OS	18.0±0.6	472±8	11.6±0.1	21.2	20.0
F1-VE	PL	19.2±1.1	448±23	11.3±0.2	19.2	18.3
	OS	17.0±1.0	458±17	11.4±0.4	20.4	19.4
F4	PL	15.2±0.7	427±27	11.8±0.2	19.9	18.6
	OS	16.5±1.4	477±27	11.5±0.4	19.9	18.4
F4-VE	PL	15.6±0.6	407±20	12.7±0.3	19.7	18.4
	OS	15.6±0.4	446±10	11.7±0.7	20.3	18.4

No sample showed noticeable changes in the mechanical properties for both storage media suggesting good stability of the bulk material after 3 months using an accelerated *in vitro* method.

Table 2: Compression properties of TPUs after 3 months treatment in PL and OS at 37 °C (*sd = 0.1-0.3)*

Sample		CS_{1200}	$hr_{25\%}\_1$	$hr_{25\%}\_4$	hr_{1200N}_4	hr_{1200N}_10
Pell	PL	40.8	17.6	12.5	15.9	15.4
	OS	41.7	17.4	11.6	15.8	15.4
F1	PL	47.1	16.0	12.6	19.2	18.9
	OS	48.0	17.8	14.2	19.1	18.3
F1-VE	PL	46.8	14.6	11.3	17.9	17.6
	OS	47.7	16.4	13.1	18.7	18.5
F4	PL	48.2	17.0	13.8	17.9	17.6
	OS	47.8	18.0	14.0	17.9	17.2
F4-VE	PL	48.8	16.7	13.1	18.1	17.7
	OS	49.0	17.2	13.5	18.5	18.1

4 Conclusion

Bio-stability of the novel implantable materials was evaluated by (i) characterization of the aged surfaces and by (ii) a systematic study of the mechanical response of the moulded cylindrical and circular disc specimens modelling meniscus implant thickness under application-relevant testing conditions. The unexpected bio-stability of all formulations after 3 months using the accelerated *in vitro* testing can only partly be explained by the chemical design. It is to a certain extent also due to the specific procedure used for specimen preparation which included annealing, long-term storage before testing, and additional purification of granulate: (i) Annealing. During fabrication of the test specimen, a residual polymer surface stress may have been introduced which results in ESC during bio-stability tests [2]. This effect was prevented in the present work by sufficient annealing of the produced testing materials and thereby reducing potentially deleterious residual polymer surface stresses, (ii) Long time storage before testing. Although after annealing the long chain block-copolymer material tends to reach the equilibrium morphology, however, this happens very slowly. The softer the materials are (the less HS is incorporated) the slower the kinetics of completing formation of the H-bonded network gets. After 60 h of storage the material still does not exibit the maximum degree of H- bonding of carbonate and urethane groups, thereby leaving the polymer more susceptible to oxidative degradation and hydrolysis [5-6]. Hence, in many studies observation of inferior bio-stabilities of the tested materials may not reflect the true potential of the produced polymer but may rather be attributed to sub-optimal specimen preparation procedures. In the present study this effect was avoided and an increased morphological stability with the highest degree of hard-soft segment phase separation was obtained by long periods of storage after processing of the test specimens before they were immersed in the test liquids, (iii) Extraction. Shot chain oligomers can migrate into the surface of the specimens where they are the most susceptible to degradation. By purification of granulate before processing these oligomers were practically completely removed. Nevertheless, the eventual leaching of vitamin E after long-term storage and reorganisation of the bulk microstructure caused by it still need to be addressed in further studies.

Author Statement

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