Biostability of Polyether–Urethane Scaffolds: A Comparison of Two Novel Processing Methods and the Effect of Higher Gamma-Irradiation Dose

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Abstract: This article deals with enzyme-induced biodegradation behavior of thermoplastic polyether–urethane (TPU). Porous scaffolds were processed by a new foaming method applied in hot pressing and injection molding. The scaffolds were subsequently γ sterilized. The samples were incubated with cholesterol esterase (CE) for 28 days to simulate an enzymatic degradation order to assess polymer biostability. The main focus of degradation products was the most toxic one: methylene dianiline (MDA). LC/MS was used to separate the breakdown products and to identify possible MDA amounts. The results showed that (a) the hot-pressed sample released an MDA amount almost twice as large (0.26 ng ± 0.008) as that of the injection-molded samples (0.15 ng ± 0.003) after incubation with enzyme activity in the physiological range, and (b) a tenfold increase in CE activity revealed considerably higher MDA amounts (7540.0 ng ± 0.004). This enzyme concentration is physiologically unlikely, however, but may occur for extreme high inflammation behavior. Even for extremely high levels of CE enzyme, the scaffold will not discharge MDA above toxic levels. The injection-molded samples sterilized at 25 kGy seem to represent the most promising processing method. Therefore, the new injection-molding foaming process of polyether–urethane can be considered appropriate for use as a biomaterial.

Keywords: polyurethane(s); porosity; scaffolds; enzyme(s); biodegradation; degradation; sterilization; FTIR; in vitro; molecular weight; radiation

INTRODUCTION

Two main criteria—biocompatibility and biostability—determine the successfulness of a biomaterial for permanent implantation. Biocompatibility consists of both surface and structure compatibility.1 Biocompatibility of implants can generally be investigated by cytotoxicity tests according to ISO 10993-5 by observing the biomaterial susceptibility of recipient tissues to different biomaterials.2 Additionally, the implant must remain inert within the aggressive body environment.3,4 Some of these body fluids may initiate a degradation of the implant material.5 The degradation of an implant may release substances into the body. Some of these compounds may be toxic, and cannot, therefore, allow for cell proliferation and attachment. The latter point is known as biostability,6 and is an essential point that must be considered.

A new foaming process has been established for hot pressing and injection molding.7,8 The material used in these two processes is a thermoplastic polyether–urethane, which is widely employed in medical applications like catheters, pacemaker leads, intraaortic balloons, and vascular grafts, because of its good biocompatibility.9,10 However, there are concerns about their biodegradation products in vivo.9,11,12 The main problem from a medical viewpoint is that their degradation products are suspected to be carcinogens. Particularly, animal experiments revealed a high occurrence of several carcinomas after feeding rats with one of the degradation products, MDA.13 Several mechanisms can initiate decomposition processes such as hydrolysis, oxidation, as well as chemical and enzymatic degradation.14

In order to compare the two different foam-processing methods, an in vitro degradation study was performed. Thus, this report deals with enzyme-induced degradation, which is considered the most aggressive mechanism against polyurethanes.15,16 The experiment was conducted with the hydrolytic enzyme cholesterol esterase (CE), which is considered to be the most destructive of the enzymes present in the body.15–17 This enzymatic test was, therefore, chosen to sim-
ulate a worst-case-scenario in vivo degradation process. If toxic substances were being released, the material or processing method would have to be changed. It is synthesized in monocyte-derived macrophages (MDMs) and has caused thus far the most biomaterial degradation with in vitro enzyme incubations. The biodegradation products from polyether–urethane scaffolds were isolated and investigated with specific attention to the toxic decomposition product MDA. Release of methylene dianiline, MDA, the most toxic of the degradation products from polyether–urethane, has been monitored over an incubation time of 28 days. Molecular weight changes and surface chemical structural differences were also measured.

In addition, a higher gamma sterilization radiation dose was applied to investigate whether this would increase biostability. Previous studies on the porous polyether–urethane scaffolds has showed that the biocompatibility was increased by higher gamma-irradiation doses.

### MATERIALS AND METHODS

#### Scaffold Production

The porous molded thermoplastic polyether–urethane (Texin 986®, Bayer Polymers, Pittsburgh, PA) scaffold (diameter of 9 mm and a height of 3 mm) were manufactured on an injection-molding machine (KM 125 C2, Krauss Maffei GmbH, Munich, Germany) with a 30-cm screw and on a heated press (250P, Dr. Collin GmbH, Ebersberg, Germany) with the use of a new foaming technique. The technique uses water as a foaming agent combined with salt leaching to ensure interconnective pores. Absorbed water in the polymer prior to processing expands during plasticization and generates a porous structure. The mean pore size in the samples was 270 ± 60 μm and range between 30 and 450 μm. The porosity was 65 ± 3%.

#### Reagents and Chemicals

Enzyme activity measurements were conducted with 4-nitrophenyl acetate (Sigma Catalogue No. N8130, Sigma Aldrich Chemie GmbH, Munich, Germany) as a substrate cholesterol esterase from bovine pancreas (EC 3.1.1.13, Catalogue No. C3766, Sigma Aldrich Chemie GmbH, Munich, Germany) and sodium-phosphate buffer (50 mM, pH 7.0, Catalogue No. 82636, Sigma Aldrich Chemie GmbH, Munich, Germany). Additionally, sodium azide (Sigma Catalogue No. 19,993-1, Sigma Aldrich Chemie GmbH, Munich, Germany) was added to avoid microbial contamination. HPLC was calibrated with pure methylene dianiline standards (Catalogue No. 13,245-4, Sigma Aldrich Chemie GmbH, Munich, Germany), HPLC-grade water (LiChrosolv, VWR, Darmstadt, Germany), acetonitrile (Sigma Catalogue No. 34998, Sigma Aldrich Chemie GmbH, Munich, Germany), and ammonium hydrogencarbonate buffer (100 mM, pH 10.0) were used as mobile phase compounds. The latter was manufactured by dissolving ammonium hydrogencarbonate (Catalogue No. 1.01131.5000, VWR, Darmstadt, Germany) in HPLC-grade water and adjusting the pH with ammonium hydroxide (Alfa Catalogue No. ALFA035614.K2, Sigma Aldrich Chemie GmbH, Munich, Germany), in accordance with Waters GmbH laboratory guidelines.

#### Test Specimens

Both processing methods were analyzed in terms of potential breakdown products after cholesterol esterase incubation. In addition to the physiologically defined enzyme activity, a tenfold increase of cholesterol esterase was used to simulate a very strong chronic inflammation and an abnormally strong enzymatic attack. A total of four different samples were incubated with cholesterol esterase. Likewise, positive-control (embedding medium with CE solution) and negative-control measurements (embedding medium only with buffer) were conducted to investigate the influence of specimen containers. Precursors of the scaffold, which were 10 wt % of the entire scaffold size, were used as test samples (n = 1). Four different samples were tested for MDA released because of enzymatic degradation. One was produced by hot pressing and subsequently γ sterilized at 25 kGy. The three other samples were injection molded. Two of them were γ sterilized at 60 kGy and the last was γ sterilized at 25 kGy. One of the injection-molded samples was sterilized at 60 kGy and was exposed to a 10-times-greater enzyme concentration than the one described below. This was to simulate an extremely strong chronic inflammation. The testing chamber had to be tested for inertness. Thus, two controls, one positive and one negative, were monitored along with the scaffold samples. The positive control contained embedding media (tube, wax, etc.) and CE solution. The negative control had embedding mediums but no CE enzyme added. The difference in chromatographs from the controls would reveal possible influences of specimen holders. An overview of all tested polymer samples and test parameters is given below (Table 1). Sample preparation for the experiment was carried out at 37°C.

Prior to enzyme incubation the scaffolds were subjected to γ sterilization (Isotron, Allershausen, Germany) carried out with radiation doses of 25 kGy and 60 kGy. The samples (n = 1) were placed in sterilized silicone tubes to achieve a uniform working surface for cholesterol esterase. Afterwards, the tubes containing the specimens were inserted into 15 mL centrifuge tubes, which were half-filled with a paraffin embedding medium. The specimens were attached with silicone grease (Wacker-Chemie, Munich, Germany) to the paraffin layer before the enzyme solutions were added.

#### Incubation Procedure

The incubation procedure was closely adapted to biodegradation experiments conducted by Tang, Labow, and Santerre. Physiologically relevant enzyme activity was obtained based on data from Labow, Meek, and Santerre. Labow et al. have simulated the amount of CE that can be released through a chronic inflammation (Figure 1). The duration of the degradation study was 28 days.
The physiological enzyme activity was obtained from the release of cholesterol esterase (CE) due to a chronic inflammation on a polyether, according to Labow et al.21 The human body reacts to biomaterials with an inflammatory defence mechanism. This foreign-body reaction is principally described through the two phases of acute and chronic inflammation. Polymorphonuclear leukocytes (PMNs) are activated during the acute phase. Here, foreign bodies are normally encapsulated, digested, and withdrawn by oxidative compounds and enzymes of giant cells, such as serine proteases like cathepsin G and elastase.22 In contrast, the leukocytes’ response to adhesion and spreading on a biomaterial is termed frustrated phagocytosis because of the lack of an enclosed phagosome.23 When the inflammation response becomes chronic, monocyte-derived macrophages (MDMs) are the predominant adherent cells in the implant sites. These cells exhibit a considerably greater degradative potential than PMNs.21 MDMs synthesize the hydrolytic enzyme cholesterol esterase.24 Labow et al.21 has measured the CE concentration released from these mechanisms based on a given scaffold surface area. This value, adjusted only to the surface area of the scaffold, was also taken to be physiologically normal.

Cholesterol esterase incubation and replenishing solutions were prepared by dissolving enzyme powder in a sodium-phosphate buffer (50 mM, pH 7.0, Sigma Aldrich Chemie GmbH, Munich, Germany). The surface area of the scaffold was measured at 1.27 cm². The polyether–urethane samples were then incubated with 2–3 mL of enzyme solution (EC 3.1.1.13, Sigma Aldrich Chemie GmbH, Munich, Germany). The polymer specimens were placed on a tumbling table (Polymax 1040, Heidolph Instruments, Schwabach, Germany) and incubated at 37°C (Binder FD, Binder GmbH; Tüttlingen, Germany). In order to prevent the microbial contamination, 0.1% w/w sodium azide (Sigma Aldrich Chemie GmbH, Munich, Germany) was added to the solutions once a week. As enzyme concentration decreased over time, a fresh quantity of CE was added to the test tubes every second day. The CE amount was monitored every second day. During the incubation period, 1-mL aliquots were removed from the polymer incubation solutions every second day for analysis. The extracts were stored at 4°C prior to preparation for LC-MS analysis.

### Spectroscopy

Efficiency of an enzyme does not only depend on its weight or volume, but also on its activity grade and purity. Enzymes are quantified according to their activity, namely, with activity units (U). In order to assess and determine the cholesterol esterase activity, photometric analysis was conducted with the use of an ultraviolet-visible (UV-Vis) spectral photometer (Specord-210, Jena Analytik AG, Jena, Germany). The generated data were evaluated with the WinASPECT software. A unit of enzyme activity was defined according to Labow et al.21 and Tang, Labow, and Santerre,25 “one unit of cholesterol esterase, CE, was defined as the amount of enzyme required to generate 1 nmol/min of p-nitrophenol from the hydrolysis of p-nitrophenyl acetate at pH 7.0 and 25°C as determined by a spectrophotometric assay at 410 nm.” The initial substrate conversion of 1 nmol/min is, thereby, directly proportional to CE activity. The chemical pathway of the hydrolysis can be viewed below (Figure 2).

During the hydrolysis phase of nitrophenyl acetate, changes in absorption/extinction were recorded by UV-Vis spectrometry. The initial test solution turned from transparent to a deep neon-yellow color. The activity of one unit can be found by applying the modified Lambert-Beer formula (Eq.1)
and backcounting the enzyme weight from the dissolved solution. The reaction rate of nitrophenyl acetate to nitrophenol and acetic acid can be calculated as follows:

$$\frac{\Delta c}{\Delta t} = \frac{\Delta A_{410}}{\Delta t \cdot d \cdot \varepsilon_{410}} \frac{V_{\text{int}}}{V_{\text{cuvette}}}$$

where $A$ is the measured absorbance at 410 nm within a certain time interval, $\varepsilon_{410}$ is the wavelength-dependent molar extinction coefficient of nitrophenol (6,100 L*mol$^{-1}$*cm$^{-1}$), $d$ is the cuvette thickness, and $c$ is the analyte concentration. The ratio $V_{\text{tot}}$ and $V_{\text{cuvette}}$ was used to determine the enzyme concentration. The substrate has to be in excess in order to achieve complete reaction.

**Degradation Product**

Diphenylmethane diisocyanate (MDI) is utilized as an aromatic monomer during polymer synthesis and connects the polyether with the urethane linkage. This linkage can, however, be cracked hydrolytically through an enzymatic attack with cholesterol esterase as well as through thermal oxidation at elevated temperatures at approximately 150°C. This leads to release of MDA. Other degradation products may also be produced. However, this study has focused on the detection of MDA, as it might be the most toxic of the degradation products from polyether-urethane. The release of the toxic breakdown product methylene dianiline (MDA) from the material was considered to be possible.

**Isolation of Degradation Products**

Prior to MDA detection by LC-MS analysis, the extracted polymer solutions from the degradation experiment had to be cleaned. The solution contained enzymes and enzyme breakdown products in addition to polymer derived from the test-chamber and TPU-derived products, which made the MDA detection difficult. The isolation procedure is described below. First, the extracts (1 mL) were centrifuged for 50 min at 4300 rpm to remove high-molecular-weight protein with the use of centrifugal filter devices (Centricon YM-10, 10,000 MW cutoff, Millipore). The filtrate was then freeze-dried overnight in a lyophilisator with the use of a double vacuum chamber with dry flask and ice condenser (Alpha 1-2, Christ, Munich, Germany). The solid residue remaining after this process was extracted with 2 mL acetonitrile and shaken for 90 min in an electronic shaking/vibrating unit (Sieve shaker AS 2000 basic, Retsch GmbH & Co. KG, Haan, Germany). Accordingly, the organic polymer degradation products were separated from the salt and residual enzyme protein. Subsequent evaporation of the acetonitrile solution was carried out at 50°C in a drying cabinet overnight. The solid residue after this process was dissolved by using 2 mL of HPLC grade water (WVR, Darmstadt, Germany) and was filtered through a 0.45-µm hydrophilic polypropylene membrane filter (Acrodisc 13 GHP, Waters, Eschborn, Germany). The resulting solutions were stored at 4°C in a refrigerator until LC-MS analysis was carried out. The products from positive and negative control samples were processed identically. The extracts were subsequently analyzed in HPLC, where the MDA amount was recorded. An accumulated value was obtained by summing up the single MDA values of respective extraction points over the entire incubation period. The samples were 10 wt % of the entire scaffold, and the incubation volume was about 2 mL. Based on these values a total accumulated MDA release could be estimated.

**Separation of Degradation Products by Liquid Chromatography**

The solutions were analyzed on a high-performance liquid chromatography (HPLC) system (600 E LC, Waters GmbH, Eschborn, Germany) with a 717plus Autosampler, Jetstream column oven, and a 2487 Dual UV Absorbance detector (Waters GmbH, Eschborn, Germany). The Waters Empower chromatography software was used to acquire and process data. The polymer extracts were separated with a Waters X-Terra MS C18 column (4.6 * 100 mm, 3.5 µm) with a flow rate of 1.5 mL/min and detected by a UV detector at a wavelength of 254 nm. The HPLC solvent gradient used is given in Table II

The testing cycle time for one injection was 22 min, with 3-min delays between each measurement. Prior to use, all the mobile phase components (except HPLC-grade water) were filtered through a 0.45-µm hydrophilic polypropylene membrane filter (Acrodisc 13 GHP, Waters). The calibration took place by dissolving 0.1 mg/mL of MDA (Catalogue No. YM-10, Ammonium Hydrogen Carbonate Buffer, pH 10.0)

<table>
<thead>
<tr>
<th>Time</th>
<th>Flow (mL/min)</th>
<th>%A</th>
<th>%B</th>
<th>%C</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.5</td>
<td>80</td>
<td>10</td>
<td>10</td>
<td>Linear</td>
</tr>
<tr>
<td>20</td>
<td>1.5</td>
<td>50</td>
<td>40</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** Chemical pathway for enzyme cholesterol esterase (CE) activity determination. The CE concentration cannot be measured, and its concentration is thus indirectly determined through the hydrolysis of nitrophenyl acetate and measurements of the end product nitrophenol.
The MDA amounts were determined by the UV response with a retention time ranging from 2.0 to 2.2 min. The detector response (signal height in microvolts) was compared to the initial calibration curves and, subsequently, the released MDA concentration was calculated. The sample runs were repeated three times and compared to the calibration curve.

Degradation-Product Identification by Mass Spectroscopy

Following UV detection, the isolated polymer extracts were applied to electrospray ionization mass spectrometry (ESI-MS) for identification, which were carried out on a quadruple mass spectrometer (Micromass ZQ 2000, Waters, Milford). The data were also processed by using the Empower operating software. The isolated polymer extracts were delivered with a delay of 0.6 ± 0.05 min (n = 20) into the ionization source, with the use of the HPLC pump. The voltage applied to the tip of the ESI needle was 4.0 kV, and the voltage applied to the cone was 27 V. Nitrogen was used as collision gas. The ESI⁻ mode was used in order to identify the components in the injected solution. A signal for the protonated MDA at a mass-to-charge ratio (m/z) of 199.3 (m + 1/z) and a scan of the masses between 50 and 250 Da (m/z = 50–250) was recorded. Mass spectrum results were then processed with Empower software and plotted as relative ion intensity versus mass-to-charge (m/z) ratio.

Molecular-Weight Analysis

Molecular-weight analysis of the polymers was carried out with the use of a gel-permeation chromatography (GPC) system (600 E, Waters GmbH, Eschborn, Germany) with a refractive index detector, Waters 410, a column oven Jettstream, 717plus Autosampler, four Waters Styragel columns HT no. 3, 4, and 6 were delivered in dimethylformamide, DMF. Again, the Waters Empower chromatography software was used to acquire and process data. The solution for the analysis was HPLC Grade DMF with an additional 0.05 m LiBr to circumvent the hydrophobic interactions between the solvent and the polymer. The columns were calibrated with 12 polystyrene standards (VWR, Darmstadt, Germany) (n = 5). All measurements were done at 85 °C to ensure a high viscosity of the test fluid. All samples were filtered through a 0.45-µm hydrophilic polypropylene membrane filter (Acrodisc 13 GHP, Waters) and analyzed three times.

Results

Structural Surface Chemical Analysis

Attenuated total reflection Fourier transform infrared spectroscopy ATR FTIR (Spectrum one, Perkin Elmer Instruments, Rodgau-Juegesheim, Germany) was used to analyze the chemical structure of the samples after the production process. This analysis provided information regarding the structural differences. All samples were shredded into small pieces (0.5 ± 0.1 mm) and placed into a powder funnel where pressure was applied. This procedure allowed the system to avoid measuring the air gaps in the porous polymer.

Gel-Permeation Chromatography

A molecular-weight analysis revealed that the hot-pressed scaffold, which was attacked by enzymes lost 8.5 ± 1.3% of its \( M_n \) and 13.1 ± 1.9% of its \( M_w \) (Figure 4). The sample, which was attacked at a 10-fold enzyme concentration, did not lose more of its molecular weight compared to the other samples. Generally, a 20% decrease in molecular weight was observed compared to the control.

LC Calibration

A standard dilution series of MDA was prepared and subsequently injected (50 µL) into the HPLC column. The retention time of pure MDA was determined to be 2.1 min (Figure 5). MDA calibration curves were generated by recording the UV detector’s signal height. For data evaluation, two different calibration curves were calculated on the basis of linear regression. Slight MDA amounts were determined with a calibration curve ranging from 0–100 ppb MDA (n = 50, \( R^2 = 0.987 \)). One nanogram of MDA per milliliter of solvent and HPLC-grade water, respectively, is equivalent to 1 ppb.
Separation of Degradation Products by LC

The extracts from the incubation media were separated by HPLC in order to isolate individual biodegradation products prior to detection of released MDA amounts. Figure 6 shows selected UV chromatograms for the degradation products of an injection-molded sample and control samples, following a 7-day incubation period. The figure shows a distinctive and characterizing MDA peak at a retention time of 2.1 min. The positive and negative control samples do not display any peaks at the same time. Hence, the control samples do not release MDA into the extract. This ensures the inertness of the specimen holder as well as the embedding medium for MDA detection.

The peak at retention time 2.1 was identified as MDA by mass spectroscopy (Figure 7). MDA has a molecular weight of 198.3 Da. Figure 7 was recorded 0.7 min after a peak was observed by the UV detector. The time delay between the UV and MS detector has been measured to be 0.7 min. Therefore, this diagram was the true MS scan of the UV peak at 2.1 min.

This diagram proves that the UV peak at 2.1 min was, indeed, MDA. Because this signal hit its peak at 199.3 Daltons, the compound detected by the UV detector can only be MDA.

The recorded chromatograms for the entire retention time display several peaks, and not only for MDA. Figure 8 shows a chromatograph with peaks at retention times of 0.75, 1.0 and 1.4 min. These peaks were suspected to be other enzyme-catalyzed, degradation-related products. Peaks were also generated in the positive and negative control sample. As the positive control has identical peaks, compared to the 25-kGy sterilized injection-molded sample, it is clear that the degradation products come from the testing chamber. The peaks cannot arise from the polyurethane sample, as the positive control had none. Hence, the degradation products must arise from the embedding medium, which was the silicone tube, paraffin wax, or polystyrene test tube. Interestingly, the negative control (neither polyurethane nor enzyme, only silicone tube, paraffin wax, and polystyrene test tube) also shows releases of degradation products. However, they are less distinctive. This means that substances are being released from the embedding medium without enzymes. For the peaks at 0.75 and 1.0 min, the peak heights are identical to the positive control and scaffold material. If the polyether–ure-
thane had degradation products for these retention times, one would expect that their peaks would be higher. Because this was not the case, one can conclude that the scaffolds did not have additional degradation products at 0.75 and 1.0 min. The peak at 1.4 min is even higher for the positive control than the scaffold material, and additional degradative products from the polyether–urethane are thus not possible.

Figure 9 illustrates the progressive MDA release of the polymer samples during cholesterol esterase incubation with a physiologically relevant enzyme activity. All specimens released MDA increasingly over a period of 28 days. No constant level was achieved during this period. This characterizing decomposition behavior was also observed in the injection-molded samples, which were incubated with a 10-fold increased CE activity. There, a mean constant level of 377 ppb ± 2.2 · 10⁻⁴ MDA (Figure 10) was obtained after 20 days. The latter accumulated amount of 377 ppb is almost 30,000 to 55,000 times larger when compared to the physiologically incubated scaffold samples, 0.013 ppb ± 3.9 · 10⁻⁴ and 0.007 ppb ± 1.5 · 10⁻⁴.

Table III shows the accumulated MDA release (n = 3) of the entire scaffold after a 28-day incubation period with cholesterol esterase based on the released MDA amounts per milliliter of extract (Figure 9).

**DISCUSSION**

The lowest MDA concentration was released from the scaffold that was manufactured by injection molding. The progressive MDA levels and the accumulated value seem to be independent of the γ-irradiation dose (± 0.01 ng). This fact was contradictory to another study, where an increase in γ-irradiation dose improved the biocompatibility.¹⁹ Almost double the MDA amount, 0.26 ng ± 0.004, was detected in the polymer extracts from the hot-pressed samples. The considerably elevated processing time (30 min in contrast to 45 s with injection molding) is a potential reason. The optical density from a WST-1 assay has already shown that hot-pressed scaffolds do not perform as well as the injection-molded ones.⁷,⁸ These MDA amounts are still considerably lower (factor of 30,000) in contrast to the sample that was incubated with 10 times the increased CE activity. Here (10-fold CE concentration) a total of 7540.0 ± 0.008 ng MDA was released after 28 days. This enzyme activity was initially defined to simulate an extremely strong inflammation and an abnormal cholesterol esterase attack. However, this value is still more than eight times lower than LOAFL (54.3 mg).²⁹ Table III summarizes the measured MDA releases from the scaffold based on biodegradation test conditions.

From a medical point of view, MDA must be thoroughly monitored because of its carcinogenic potential, which was observed in Weinberger’s animal study.¹³ The released MDA doses in this study are harmless compared with data from the literature.²⁹ The half-life of MDA-hydrolyzed urine is reported to be 70 to 80 h and 21 days in serum.³⁰,³¹ In the calculation, the half time was not considered and MDA was assumed to accumulate constantly. Hence, the calculated accumulated values represent a worst-case scenario.
TABLE III. Test Samples for Biodegradation Experiment (n = 3)

<table>
<thead>
<tr>
<th>Polymer Processing Method</th>
<th>Accumulated MDA Release (SD) (ng)</th>
<th>Sterilization γ Dose (kGy)</th>
<th>Incubation Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot pressing</td>
<td>0.26 ± 0.008</td>
<td>25</td>
<td>Physiological</td>
</tr>
<tr>
<td>Injection molding</td>
<td>0.15 ± 0.003</td>
<td>25</td>
<td>Physiological</td>
</tr>
<tr>
<td>Injection molding</td>
<td>0.14 ± 0.003</td>
<td>60</td>
<td>Physiological</td>
</tr>
<tr>
<td>Injection molding</td>
<td>7540.0 ± 0.004</td>
<td>60</td>
<td>Tenfold increase</td>
</tr>
</tbody>
</table>

Lowest observed adverse effect level: 5.43 × 10¹ ng⁻¹

The MDA release from the 10-fold CE activity compared to the physiological value shows that the CE enzyme is indeed extremely aggressive toward the polyether–urethane, which has already been described by several scientists. Several authors have used techniques such as radiolabeling and MS to detect the small quantities released through degradation studies. This study has shown that the detection of MDA is possible for low quantities (less than picogram) by means of modern columns and HPLC chromatographic techniques.

CONCLUSION

The goal was to compare two different processing methods for the scaffolds, as well as the effect of high γ-sterilization dosage as it relates to resistance against degradation. Degradation may release compounds at toxic levels. Biodegradation tests were conducted with four different polyether–urethane samples, following incubation with an inflammation enzyme. Cholesterol esterase (CE) attack over 28 days caused MDA release from all test specimens. Very low amounts of MDA were released, and their concentrations were well below toxic levels (< 50,000), according to LOAFEL. The mean released MDA amounts were lowest for injection-molded samples. The different γ-sterilized doses had minor impact. Double the amount of MDA was found for scaffolds processed by hot pressing (0.256 ng ± 0.008). Incubation with a 10-fold increased CE enzyme activity revealed a considerably elevated MDA release of 7540.0 ng ± 0.004 (n = 3). This enzyme activity was initially employed to simulate an extremely strong inflammation and an abnormal cholesterol esterase attack. However, this value is still more than eight times lower than the lowest observed adverse effect level (54.3 mg). The amount of MDA released at normal physiological enzyme levels did not reach a plateau during the test period, which was the case with the 10-fold increased CE concentration.

The test chamber was found to be unsuitable for the detection of other degradation products, as the chamber itself was not inert. Two peaks were detected by HPLC from the embedding materials. In order to quantify and analyze degradation products other than MDA, another testing chamber has to be used.

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