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Formation and Elution of Toxic Compounds from γ -Ray Sterilized Medical Products: Toxic Compound Formation and Ames test of Eluted Components

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Abstract

No formation of MDA was observed in chain-extended thermoplastic polyurethane (PU) when sterilized by autoclave or γ-ray irradiation. No formation of MDA was observed in nonchain-extended thermoplastic PU when sterilized by γ-ray irradiation. Less than 1 ppm of MDA was produced in nonchain-extended thermoplastic PU sterilized by autoclave sterilization. Autoclave sterilization did not produce MDA in thermosetting PU potting material. MDA formation in potting material was promoted by γ-ray irradiation and increased with increasing irradiation doses at a quadratic equation of regression. MDA formation at 100 kGy irradiation is a few ppm and less than one ppm at 25 kGy irradiation, therefore the potential risk to human recipients was not significant. The elution of compounds other than MDA from potting material was more problematic. Solvent extracts from potting material presented mutagenicity in the absence of metabolic activity (S9Mix). MDA presented mutagenicity in the presence of metabolic activity; therefore MDA was not the major mutagenic candidate. The chemical and biological characteristics of the specific mutagens required to identify in a further study. Negative promotion of MDA formation and a lesser presence of mutagen in autoclave sterilized potting material indicated that autoclave sterilization was preferable if the material is tolerable to heating.

Keywords: Sterilization techniques; Toxic compounds

Introduction

Polyurethane (PU) is widely used for its good biocompatibility and biostability [1-5]. Standard chain-extended thermoplastic PU is synthesized by the reaction of diisocyanate and polyol such as 4,4' -diphenylmethane diisocyanate (MDI) and polytetramethyleneglycol (PTMG). 1,4-Butanediol (BU) or 1,4-buthylenediamine is used as a chain extending agent (Figure 1, -O-$(CH_2)_4$-O-). When PU attains an appropriate molecular weight, n-butanol is added to terminate the polymerization reaction, thus the properties of chain-extended and segmented PU render it appropriate for the construction of medical equipment such as intra-aortic balloons, ventricular assist devices, vascular grafts etc. Hard and soft segment separation is discussed to be a reason of PU's good biocompatibility. Pellethane® and Biomer® both commercially available PUs are fabricated using BU or 1,4-buthylenediamine, respectively [6]. Thermosetting PU potting material is fabricated by reacting MDI with castor oil in place of PTMG [7]. No terminating reagent and chain-extending reagent are added. Potting material is used to connect fibers in artificial dialysis devices, plasma separators and other medical devices. Thermosetting PU potting material is more complex due to the use of a partially hydrolyzed castor oil exhibiting a complicated chemical structure, therefore more rigid than thermoplastic PU.

Accepted sterilization procedures for medical devices are γ-ray irradiation, β-ray irradiation, steam autoclaving, or ethylene oxide or formaldehyde gaseous chemical sterilization [8-13]. The Delany clause in the U.S.A. prohibits the manufacture or sterilization of medical devices exhibiting a potential for the production of toxic compounds, therefore the formation and elution of N,N-methylenedianiline (MDA, Figure 1) and other unidentified toxic compounds require evaluation. The author has previously reported that during γ-ray or autoclave sterilization, toxic and low molecular weight compounds have been formed in PU as a result of degradation [9,14,15]. These compounds as well as gaseous chemical residue, i.e. ethylene oxide and

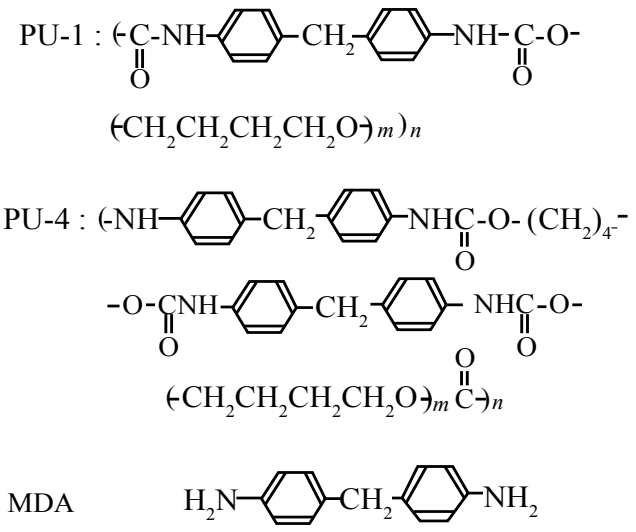

Figure 1: The structure of nonchain-extended and chain-extended thermoplastic polyurethane (PU) and MDA. The upper and the middle figures are the chemical structure of nonchain-extended and chain-extended thermoplastic PU, respectively.

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formaldehyde, require careful evaluation to estimate the risk factor to patient and recipients exposed [10,11]. β-ray irradiation sterilization is an alternative, but does not provide an effective depth of sterilization compared with γ-ray (Bruck and Muller, 1988; Gardner, 1986).

It has been reported that MDA (Figure 1 lowest), a carcinogen and mutagen is formed in autoclave-sterilized chain-extended thermoplastic PU and is detectable at a level of a few ppb to a few hundred ppb in aqueous extract, depending on the period of autoclaving or material [16-18]. Excepting for the author's report, there has been an absence of reports describing the formation of MDA and other toxic and mutagenic compounds in sterilized thermoplastic and thermosetting PUs when subjected to gamma-ray irradiation or autoclave sterilization so far [9,14]. The carcinogenicity and toxicity of MDA has previously been reported [2-4,19-30]. The MDA content was determined using a high performance liquid chromatography (HPLC) combined with an electrochemical detector (ECD) and ultraviolet (UV) detection simultaneously. Detection order is UV and ECD and drain to prevent damage of ECD cell window by backpressure.

The mechanism of MDA formation in γ-ray irradiated potting material was studied [9]. Urethane linkage, soft segment C-C linkage and so on were found to be cleaved by γ-ray irradiation in chain-extended thermoplastic PU and in a model compound of diethyl 4,4' -methylenebis (N-phenyl.carbamate) [9,31].

Gel permeation chromatography (GPC) combined with UV detection at 290 nm as well as refractive index (RI) detection was used for molecular weight changes of PU before and after γ-ray irradiation. Thermoplastic PUs dissolved in N,N-dimethylformamide (DMF) or tetrahydrofuran (THF) were used for the GPC sample [15]. GPC analysis combined with UV (290 nm) and ECD (900 mV) detections was used for the analysis of serum and methanol extracts from thermoplastic PU as well as from thermosetting PU potting material [8,15]. The solvent extracts were subjected to mutagenicity testing (Ames test) both in the presence and in the absence of metabolic activity, S9Mix, to ascertain whether in fact MDA is the predominant mutagen in the solvent extract of both PUs [9]. Cytotoxicity testing of methanol extract from thermoplastic PU was also examined [15].

Physical tests such as tensile strength and elongation testing were performed to identify and confirm crosslinking and degradation by γ-ray irradiation [15]. Residual radicals were determined to clarify whether these radicals caused further degradation [15].

Experimental

Materials

Two kinds of thermoplastic PU were synthesized. One, nonchain-extended thermoplastic PU (Figure 1 upper) and the other, chain-extended PU (Figure 1 middle) Chain-extended PU was fabricated by reacting MDI with an excess of PTMG over MDI. BU was added (Figure 1 middle $-O-(CH_2)_4-O-$), and the polymerization reaction terminated by the addition of n-butanol. The resulting chain-extended and segmented PU was similar in chemical structure to commercially available Pellethane® 2363. Nonchain-extended thermoplastic PU was fabricated in an identical manner, omitting BU (Figure 1 upper). No additives such as initiator, stabilizer and plasticizer and so on were added during fabrication of both thermoplastic PUs. Due to its fragility, nonchain-extended thermoplastic PU is inappropriate for medical use.

Prepared thermoplastic PUs were dissolved in N,N-dimethylformamide (DMF) at a concentration of 30%. A DMF solution of PU was placed on glass Petri dishes to form uniform layers. A PU film of approx. 1 mm was obtained after complete DMF evaporation for a period of two consecutive months at room temperature. The covered dishes were subjected to γ-ray irradiation [8].

Thermosetting PU potting material was prepared by reacting MDI with partially hydrolyzed caster oil in place of PTMG [7]. The fabrication of commercial potting material is a single step without addition of chain-extending or terminating reagent [32]. The setting process time does not allow sufficiently hardening, furthermore a greater amount of MDI than castor oil is used, causing more unreacted MDI to be retained in potting material than in thermoplastic PU [8,9]. MDI has a potential for conversion to MDA in an aqueous atmosphere.

Sterilization

γ-ray irradiation sterilization was carried out at a commercial irradiation plant at a rate of 25 kGy/25 h in air from 0 to 100 kGy at 25 kGy intervals using cobalt-60 (^{60}Co).

Autoclave sterilization was performed at 121.1°C for 30-60min.

Determination of residual radicals after γ-ray irradiation

Residual radicals were quantified by a chemiluminescence method [15]. Irradiated and nonirradiated thermoplastic PU samples of 3×3 cm (thickness is 0.1 cm) were placed in a thermostatically controlled chamber, maintained at 40°C. Light generated by radical recombination reactions (stable radicals) was determined with a photocounter at 2 weeks following irradiation. The result from nonirradiated PU was estimated as blank value. The chemiluminescence detector, CLD-110, was supplied by Tohokudenshi Co. Ltd, Tokyo, Japan.

MDA analysis with high performance liquid chromatography (HPLC)

Analytical column: Toso ODS-120T-1251, (4.6×250 mm), eluent: acetonitrile/50 mM ammonium acetate (1/3), detector: ECD (applied voltage 900 mV *vs.* Ag/ AgCI, glassy carbon working electrode) and UV at 245 nm.

Gel permeation chromatography (GPC) for the analysis of the molecular weight of PU

A Toso-GPC analytical column was packed with TSK gel GMH (molecular weight exclusion limit 400,000,000) for the analysis of PU molecular weight. PU was wholly dissolved in either DMF or in THF to measure the number average molecular weight (Mn) and the weight average molecular weight (Mw) in order to compare Mn and Mw before and after γ-ray irradiation or autoclave sterilization.

GPC was performed using a DMF eluent containing 10 mM LiBr at a flow rate of 1.0 ml/min. Column and injection temperature was maintained at 40°C. The eluate was monitored simultaneously by UV at 290 nm and by RI. ECD was appropriate in the presence of LiBr of DMF eluent due to necessity of ion charge of ECD detection. GPC sample should be dissolved in a GPC eluent. The concentration of GPC sample is 0.05-0.1%. Polystyrene was used as a reference standard compound.

GPC analysis for solvent extracts of PU

TSK gel G4000H (molecular weight exclusion limit 400,000) combined with TSK gel G3000H (molecular weight exclusion limit 60,000) and TSK gel G2000H (molecular weight exclusion limit 10,000) were used for GPC analysis of extractable PU oligomers, i.e. extracted oligomers with serum, methanol or acetone (Shintani, 1989b, 1990).

The GPC condition was identical to Mn and Mw analysis for whole PU. In PU extract analysis, samples were redissolved in a GPC eluent at a concentration of 0.05-0.1% after evaporation.

Tensile strength, elongation and breaking stress testing

Chain-extended and nonchain-extended thermoplastic PU strips were prepared using a Danbel cutter, JIS K-71 13-2 specification. The test was performed according to JIS K-7113 [15,33].

Nonaqueous titration method for primary amine and secondary amine (NH) in urethane linkage

For the determination of PUs exhibiting primary amine, 2 g of PU samples were dissolved in 50 ml of DMF containing 0.1% LiCI, 2 ml of acetic acid was added and the solution titrated with 0.01N perchloric acid in dioxane, observing microburette recording potential values after each portion of the titrant. The blank sample was simultaneously titrated.

For the determination of NH group in urethane linkage in PU, the procedure was as follows: 2 g of PU samples were dissolved in 50 ml of DMF containing 0.1% LiCI. The titrant was 0.01 N NaOH in DMF containing 0.1% LiCI. 0.4 g of NaOH was dissolved in a trace of water; thereafter DMF containing 0.1% LiCI was volumed to 1 liter. Other procedure was identical to that used in the determination of PUs exhibiting primary amine ($-NH_2$).

Results and Discussion

Determination of MDA in chain-extended and nonchain-extended thermoplastic PUs when sterilized by autoclave or by γ-ray irradiation and the change in physical parameters before and after sterilization.

The author's current experimental data indicated that MDA was not detected in γ-ray or autoclave sterilized chain-extended thermoplastic PU [8]. Similarly MDA was not detected in γ-ray sterilized nonchain-extended thermoplastic PU (Shintani, 1989a). MDA was detected at less than 1 ppm in nonchain-extended thermoplastic PU when autoclave sterilized at 121.1°C for 60min [8]. The detection limit of HPLC is 3 ppb [8].

It has been reported that MDA was detected at a few hundred ppb to a few ppb in aqueous extract from chain-extended thermoplastic PU when autoclave sterilized [18]. Mw was reported to decrease linearly with increasing autoclaving period [18]. However, the author's data using chain-extended thermoplastic PU showed no significant Mw change in bulk PU either before or after autoclave sterilization, suggesting that the PU sample was not significantly hydrolyzed or deteriorated by autoclave sterilization [8]. The difference of both data is thought to be due to the difference of sample thickness, thus it is thought that only the immediate surface area at nm to μm level is hydrolyzed as is the case in implanted PU. This must be confirmed by using SEM (scanning electron microscopy) in future study, which depth file is identical gas plasma sterilization [34]. In that sense, Mw in bulk PU is thought to be unchanged [8]. Pellethane® 2363-550, widely used for biomedical devices, has a chemical structure identical to chain-extended thermoplastic PU in the author's experiment. Pellethane^R is evaluated hydrolytic stability over a 6 month period [4,35]. Other papers concerning *in vivo* implantation testing of chain-extended thermoplastic PU in animals and humans reported no Mw change in bulk PU (Griesser, 1991), correlating with the author's results [8].

When autoclaving procedures at 121.1°C for 1 h were applied to nonchain-extended thermoplastic PU, MDA formed at <1 ppm [8]. MDA was produced, however Mw in bulk PU was unchanged by autoclaving due to shallow penetration depth file [8]. Although the author has not identified the trigger for MDA production in nonchain-extended thermoplastic PU during autoclaving, it is believed to be due to a higher degree of fragility and pliancy, therefore water more easily penetrating the interior to hydrolyze, access and extract interior MDA in nonchain-extended thermoplastic PU [4,8]. Hardness, swelling capacity, and penetration depth are critical factors for MDA extraction.

Mw from irradiated chain-extended thermoplastic PU decreases linearly with increasing irradiation doses [15]. Superimposed GPC chromatograms indicate that initial elution times are almost identical and that completion and peak top times of GPC elution increase with increasing irradiation doses. Mw decreases with increasing irradiation doses due to degradation in chain-extended PU by γ-ray irradiation [15]. The G value of degradation by γ-ray irradiation was 1-1.3 [15], identical to that in the paper presented by Okamura [36]. G value indicates the number of molecules increasing or decreasing when 100 eV is absorbed to materials. The greater the G value is, the greater the crosslinking or degradation [9,15,36]. The breaking stress of γ-ray irradiated chain-extended PU decreased linearly with increasing irradiation doses, indicating degradation [15]. This correlates well with Mw change in PU.

On the contrary, Mw from irradiated nonchain extended thermoplastic PU increased linearly with increasing irradiation doses [15]. Superimposed GPC chromatograms indicate that the initial elution time decreased and that completion and peak top times of GPC elution increased with increasing irradiation doses, indicating that both crosslinking and degradation occurred simultaneously. Mw increased with increasing irradiation doses, indicating a predominance of crosslinking. The G value of crosslinking by irradiation was 0.2 [15], identical to the amount presented in the paper by Okamura [36]. The breaking stress of γ-ray irradiated nonchain-extended thermoplastic PU increased with increasing irradiation doses, indicating crosslinking [15]. This correlates well with Mw change in PU [15].

Mw decreased when subjected to γ-ray irradiation in chain-extended thermoplastic PU, but MDA was not produced. This was thought to be due to rare possibilities of simultaneous cleavage at two successive urethane linkages forming MDA [9,31].

It has been reported that PU was cross-linked by γ-ray irradiation [12,37], however the authors have no evidence of cross-linking in chain-extended PU such as Pellethane® 2363 when subjected to γ-ray irradiation [15]. The author's data, however, indicated a clear evidence of degradation in γ-ray irradiated chain-extended thermoplastic PU and predominant crosslinking in nonchain-extended thermoplastic PU [15].

Tensile strength testing

The stress-strain (S-S) curves for chain-extended and nonchain-extended thermoplastic PU samples were studied [15].

Nonchain-extended PU showed a clear yield point and exhibited a significant ductility. Breaking stress increased with increasing irradiation [15]. The S-S curve for nonchain-extended PU showed a significant change with increasing irradiation, presumably due to increased crosslinking and the degree of crystallinity. The tensile strength increased with increasing irradiation doses.

In contrast, chain-extended PU did not show a clear yield point and was brittle rather than ductile. Breaking stress decreased

with increasing irradiation, indicating degradation. The degree of crystallinity decreased with increasing irradiation due to degradation. These results correspond to those of Mw changes.

The breaking stress in chain-extended PU is a few hundred kg/cm^2 and that of nonchain-extended PU is around 10 kg/cm^2, indicating the latter is more pliant.

Residual radical (safety radical) determination

After the 14th day of irradiation, residual radicals in PUs were determined [15]. Residual radicals increased linearly with increasing irradiation in a first order equation of regression.

Mw at the 14th day and at the 6th month after irradiation did not significantly differ, indicating that residual radicals have little effect on further degradation after irradiation, contrary to the result in the paper [38]. Residual radicals are thought to effect on the shallow depth of PU surface, therefore Mw in bulk PU is unchanged. PU samples in the author's experiment were 1 mm in thickness, greater than the sample in the paper [38]. This difference of thickness is thought to lead to the difference of both data.

UV determination of methanol extracts from nonchain-extended and chain -extended thermoplastic PUs.

Methanol extracts from irradiated and nonirradiated thermoplastic PUs were determined for UV absorbance at 245 nm [15], the maximum wavelength for PU oligomers [9]. UV Absorption spectra of methanol extracts from PU are identical to those from PU oligomers, thus elution is for the most part PU oligomers and monomers [9]. UV absorption spectrum of MDA is also identical to PU oligomer. Urethane linkage in PU (hard segment) indicates UV absorption at 245 nm; however PTMG soft segments do not. UV absorbance at 245 nm increased with increasing irradiation doses, indicating that elution of components such as PU oligomers and monomers from irradiated thermoplastic PUs increased with increasing irradiation doses [15]. The total UV absorbance of methanol extract from nonchain-extended thermoplastic PU is, greater than that from chain-extended thermoplastic PU, indicating the former to be more pliant [15]. The smaller the molecular weight in thermoplastic PU is the greater elution to methanol. Elution of GPC peak with a greater peak top molecular weight (MGPC), specifically 22,000 of MGPC, was greater from a smaller molecular weight PU (Figure 2) [15].

GPC analysis of methanol extracts from irradiated and non-irradiated thermoplastic PUs

Methanol extracts from irradiated or from nonirradiated nonchain-extended and chain-extended thermoplastic PUs were subjected to GPC analysis combined with UV detection at 290 nm. The GPC chromatogram from chain-extended thermoplastic PU irradiated at 50 kGy is presented in figure 2. The peak top molecular weight (MGPC), specifically 22,000, 12,800, 9,500, 5,400 and 1,600, increased with increasing irradiation doses [15]. The ratio of peak heights is presented with irradiation (Table 1). The elution of PU oligomers with a greater MGPC, i.e. 22,000, more significantly increased with increasing irradiation (Table 1). Monomer MGPC is 1,600.

Interestingly a MGPC of 5,400 is the predominant peak eluted from nonchain-extended thermoplastic PU and that of 9,500 is the predominant peak from chain-extended thermoplastic PU [9]. PU oligomers were mostly PTMG combined with MDI [9], the PTMG soft segment proving more abundant [4,39-41]. This is due to a greater PTMG in the PU surface than in PU interior. The components in the surface area are more extractable. The reason for the difference in predominant peak in each PU is unclear. As they were detectable by UV at 290 nm, MDI was present in each peak. The quantity of MDI was the greatest in MGPC of 9500 [9], therefore the molar absorption coefficient and biological characteristics of each peak differ.

Additional study by refractive index (RI) detection revealed the elution of PTMG, 1,4-butanediol (BU) and n-butanol in this elution order [9]. BU was not eluted from nonchain-extended PU.

In the paper presented by Marchant et al., PU oligomers of Mw from 17,000-200 are reported to be eluted to methanol by GPC analysis [42], which coincides with the author's results [8]. In the papers presented by Ratner, methanol elution of PU oligomers of Mw <30,000 is reported [39,43], this confirming the author's findings [8,9,15].

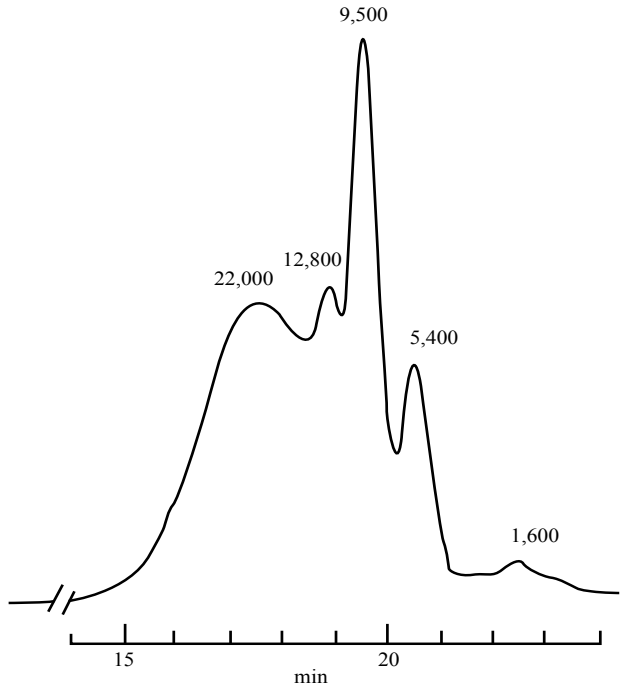

Figure 2: GPC chromatogram of methanol extract from chain-extended thermoplastic PU detected by UV (290 nm).

	Peak top molecular weight, MGPC				
PU-kGy	22,000	12,800	9500	5400	1600
PUI)-0	17.4	6.6	32.8	100.0	4.6
PUI)-25	20.8	5.7	23.6	100.0	4.7
PUI)-50	41.2	7.9	29.4	100.0	4.4
PUI)-75	62.5	5.0	36.3	100.0	17.5
PUI)-100	112.6	11.5	43.7	100.0	4.6
PU2)-0	72.1	63.6	281.8	100.0	13.6
PU2)-25	125.0	35.7	207.1	100.0	10.7
PU2)-50	324.1	41.4	248.3	100.0	12.1
PU2)-75	348.0	52.0	232.0	100.0	7.2
PU2)-100	465.9	63.6	281.8	100.0	13.6

PUI) Nonchain-extended thermoplastic PU
PU2) Chain-extended thermoplastic PU

Table 1: Relative peak height of MGPC of methanol extract from y-ray irradiated nonchain-extended and chain-extended thermoplastic Pus.

Determination of MDA in γ-ray and autoclave sterilized potting material from thermosetting PU

MDA was detectable in nonsterilized potting material due to the fact that MDI, a starting reagent, is utilized in greater quantity than castor oil during PU fabrication [8,9]. MDI is rapidly converted to MDA in an aqueous atmosphere.

No increase in MDA was observed either before or after autoclave sterilization, indicating that autoclave sterilization (121.1°C for 30 min and 1 h) did not produce MDA in thermosetting PU potting material [8,9]. The formation of compounds other than MDA was also not significantly increased after autoclave sterilization. This indicated by the difference in HPLC peak heights before and after autoclave sterilization.

On the contrary, MDA formation increased with increasing irradiation doses by a second order equation of regression [8,9]. The amount of MDA was a few ppm at 100 kGy irradiation [8,9]. The more pliant the potting materials, the greater the MDA formation [9], indicating that a relationship exists between hardness of potting material and the formation and elution of MDA [9]. One exceptional sample, however, showed less MDA elution from irradiated potting material in the early stage of time course elution, i.e. around 1 week from the beginning of elution [9]. The total MDA elution, however, from irradiated potting materials was greater than that from non-irradiated samples without exception [9]. Although the author has not established the rationale for the diminished MDA at an early stage of elution in one thermosetting PU, the author considers this to be a favorable material due to the less elution when irradiated by γ-ray. One speculation is that crosslinking occur at the surface and crosslinling may prevent interior MDA elution. This must be further confirmed by SEM (scanning electron microscopy). The less MDA elution in the early stage, the more favorable the material for artificial dialyzers and plasma separators. These devices are for the most part used for short periods of time around 3-4 h and are not reused, therefore the less amount of elution at the early stage is the most critical and favorable benefit to the patients. Cytotoxicity test of methanol extract from thermoplastic PU indicated that the early stage elution contained more cytotoxic compounds in greater quantity [15]. Further clarification is required to study for the diminished MDA elution. The author's speculation is due to an increased crosslinking in the surface matrix by γ-ray irradiation [9], restricting methanol penetration of the interior of potting material to extract interior MDA.

Mechanism of MDA formation and degradation by γ-ray irradiation

Simultaneous cleavage at two successive urethane linkages forming MDA is quite rare [9,31]. This is confirmed by the fact that MDA was not formed in irradiated thermoplastic PU and in the model compound as being shown in Figure 1 [9,31]. MDA will be formed from compound d, not from a to c.

If γ-ray irradiation cleaves urethane linkage, PU oligomers with terminal amino groups will increase with increasing irradiation. If urethane linkage proximal to the terminal amino group, i.e compound d in figure 3, is cleaved, MDA is produced. The author's experimental data showed that the amount of PU oligomers with terminal amino groups increased with increasing irradiation doses and that the regression line between irradiation dose and the amount of PU oligomers with terminal amino groups was a first order equation [9]. The quantity of PU or PU oligomers with terminal amino groups (R-NH_2) was determined by non-aqueous titration method [9].

The author speculated that MDA formation by irradiation was due to cleavage at urethane linkage proximal to the terminal amino groups (R-NH_2, [9]). More abundant PU with terminal amino group was evident in thermosetting PU potting material than thermoplastic PU due to insufficient hardening and the addition of a greater amount of MDI than castor oil during fabrication [8,9]. Residual MDI was converted to MDA in aqueous circumstances, thus MDA was detectable in nonsterilized thermosetting PU potting material.

Cleavage at urethane linkage by γ-ray irradiation was calculated to be around 10-20% of the total cleavage [9]. The remainder was cleaved at PTMG, soft segment and other C-C linkages [9], indicating that cleavage at urethane linkages by γ-ray irradiation was not predominant [9,31]. Oxidative cleavage by peroxjde is reported to occur predominantly at the soft segment and C-C linkages due to radicals produced from peroxide [4,44-46]. Their results coincided with the author's current findings on γ-ray irradiation cleavage. γ-ray irradiation cleavage in air results in oxidative radical cleavage. It has been reported that MDA and other primary amines, i.e. compound d in figure 3, were detected by enzymatic cleavage (lipase and esterase, [4,42]). It was reported that elution of compounds other than MDA were more abundant in nonsterilized chain-extended thermoplastic PU as well as from enzymatic catalyzed PU. The reported PU material has an identical structure to Pellethane® [42]. HPLC was detected by

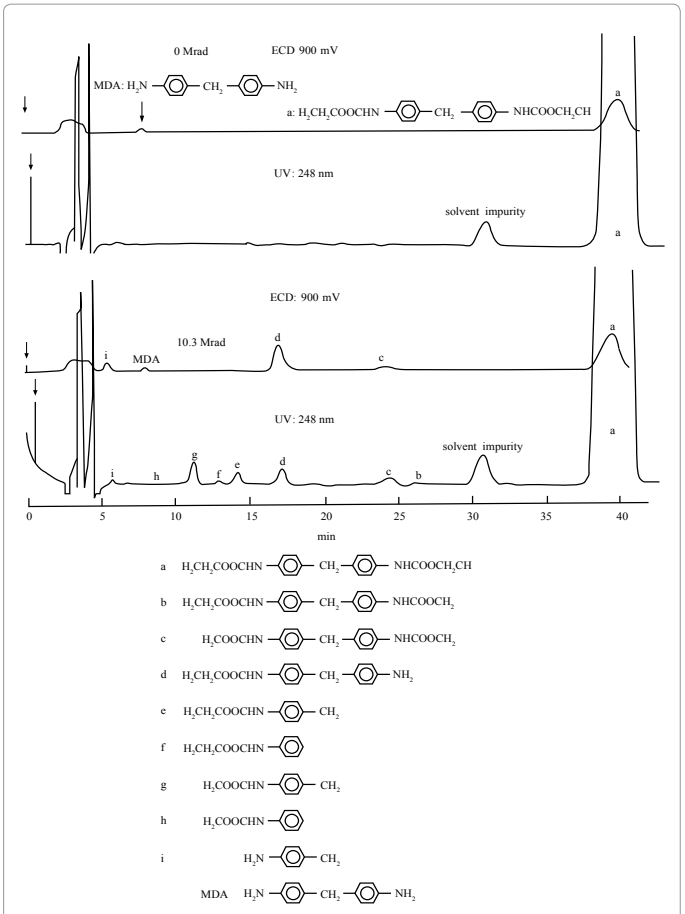

Figure 3: HPLC chromatogram of irradiated and nonirradiated diethyl 4,4' – methylenebis (N -phenylcarbamate). Detection was carried out by UV (248 nm) and ECD (900 mY). The structure of peaks (a)-{i} are determined as indicated in the footnote; (a) is a starting compound. They are produced predominantly by the cleavage at C-C linkage.

UV at 254 nm [42].

In order to establish predominant cleavage at C-C linkage by γ-ray irradiation, the author carried out an experiment using a model compound of diethyl 4,4' –methylenebis (N-phenylcarbamate) both nonirradiated and irradiated at 100 kGy [31]. When irradiated, MDA formation in the model compound was negligible by UV or ECD detection because simultaneous cleavage at two successive urethane linkages was rare (Figures 1 and 3) [31]. Elution of compounds exhibiting one terminal amino group was detected simultaneously by UV (245 nm, compound d in Figure 3) and ECD (900 mV [31]. ECD detection is more sensitive to aromatic amine or phenol than UV at 254 nm and selective for detecting compounds exhibiting aromatic amino groups [8,9,31]. The cleavage rate at one urethane linkage to form a compound exhibiting one terminal amino group is calculated to be around 20% of total cleavage [31]. The calculation using peak height by UV detection was carried out as follows: the peak area detected by ECD (peaks (d) and (i) in Figure 3) was divided by the total peak area from (b) to (i) in figure 3. Peak (a) is a starting compound, thus omitted. Peaks detected by UV, but not by ECD, were speculated to be produced by cleavage at linkages other than urethane linkage. On the contrary, peaks detected by ECD as well as by UV were speculated to be produced by cleavage at urethane linkage (Figure 3). The chemical structure of these peaks is presented in the footnote of figure 3. As mentioned, formation of these peaks was mostly due to cleavage at linkages other than urethane linkages. Cleavages at urethane linkage at most around 20% of total cleavage, which was not predominant. This coincided with the data from irradiated chain-extended thermoplastic PU.

Mechanism of cross/inking in nonchain-extended thermoplastic PU by γ-ray irradiation

To clarify the mechanism of crosslinking in γ-ray irradiated nonchain-extended thermoplastic PU, nonchain-extended PU was fabricated using PTMG with various molecular weights. Identical MDI and n-butanol are used in the fabrication of all nonchain-extended thermoplastic PU. γ-ray irradiation sterilization was applied to the prepared PUs. Mw in nonchain-extended thermoplastic PU increased with increasing irradiation due to crosslinking [15].

As there is a linear relationship between PTMG molecular weight and Mw in irradiated nonchain-extended thermoplastic PU, it is speculated that crosslinking occurs predominantly at PTMG (soft segment) rather than at urethane linkage (hard segment, [9]). The quantity of crosslinking occurring at PTMG is calculated to be more than 90% of the total crosslinking, the remainder occurring at urethane linkage [9]. Calculation was carried out using a non-aqueous titration method [9]. If urethane linkage is crosslinked at NH in urethane linkage by γ-ray irradiation, acidity will decrease with increasing irradiation as a result of increased crosslinking at NH because NH in urethane linkage indicates acidity [9]. Decreased acidity is thought to be due to N-N linkage by crosslinking at NH. The amount of decreased NH in urethane linkage is calculated to be 6% of total crosslinking, indicating that N-N crosslinking is around 6%, the remainder occurring at the PTMG soft segment, i.e. C-C crosslinking.

Crosslinking as well as degradation occurred predominantly at PTMG soft segment [9].

Risk factor estimation for MDA formation from γ-ray irradiated thermosetting PU potting material

The author subjected MDA in thermosetting PU potting material at 25 kGy irradiation to evaluate the risk factor to human beings. Less than one ppm of MDA was formed by 25 kGy irradiation [8]. The author's evaluation at the formation and elution level indicated "not significant". The estimated cancer causing risk factor of MDA to human beings is 0.29 (29 persons per 100 persons) when absorbing 1 mg of MDA/kg body weight/day. This is significant when compared with the amount of MDA eluted from 25 kGy irradiated potting material.

The author speculates MDA from irradiated potting material may not be a critical compound. Compounds other than MDA are more problematic due to greater quantity formation with a higher level of toxicity. The solvent extract confirmed mutagenicity. It is discussed in the next section on mutagenicity testing (Ames test). In the paper presented by Marchant et al., unidentified compounds other than MDA were eluted in greater quantity from untreated thermoplastic PU and from enzymatic catalyzed thermoplastic PU [42].

Mutagenicity testing (Ames test) of solvent extracts from γ-ray irradiated or autoclave sterilized thermosetting PU potting material

Methanol extract as well as serum extracts from sterilized potting material were subjected to Ames mutation testing [9]. Extracts from potting material irradiated at 105 kGy indicated positive in the absence of metabolic activity, S9Mix, but less positive in the presence of metabolic activity (Table 2), indicating the predominant mutagen in the extract to be compounds other than MDA as MDA indicates mutagenicity in the presence of metabolic activity, S9Mix, [47]. Non-irradiated potting material indicated negative mutagenicity both in the presence and the absence of metabolic activity (Table 2), indicating that mutagens increase with irradiation doses.

The GPC chromatogram of methanol extract from potting material by UV detection (290 nm) exhibit several peaks; 160,000, 11,000, 8,700, 5,400, 3,200 and 1,600 as MGPC vs polystyrene reference (Figure 4) [8]. Those by ECD detection (900 mV) exhibits peaks; 11,000, 8,700, 5,400, 3,200 and 1,600 as MGPC, for the most part PU oligomers exhibiting OH, SH, NH and NHOH terminal groups. These peaks are thought

	TA 100		
Materials	**ComcCone (μg/plate)**	**-S9Mix**	**+ S9Mix**
Methanol extract	0	151	114
from nonirradiated	500	150	115
potting material	1000	173	115
	2500	182	145
	5000	171	154
Methanol extract	0	109	100
from 105 kGy	500	175	105
irradiated	1000	214	136
potting material	2500	511	157
	5000	1014	191
Acetone extract	0	151	114
from nonirradiated	500	120	103
potting material	1000	105	106
	2500	134	119
	5000	134	106
Acetone extract	0	127	116
from 105kGy	500	128	107
irradiated	1000	147	107
potting material	2500	155	145
	5000	249	126

Table 2: Ames test of base substitution mutation detection using methanol and acetone extracts from irradiated and nonirradiated thermosetting PU potting material.

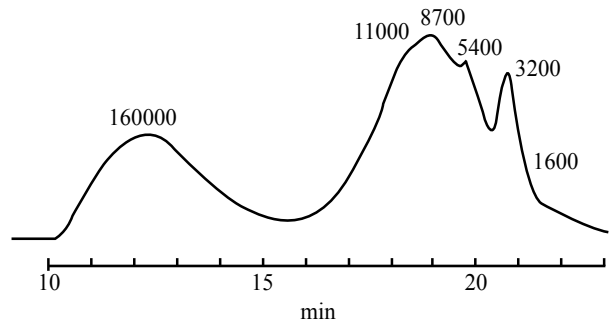

Figure 4: GPC chromatogram of methanol extract from irradiated thermosetting PU potting material. Relative MGPC peaks are 160,000, 11,000, 8,700, 5,400, 3,200 and 1,600 (monomer) by UV detection (290 nm). Peaks of 11,000, 8,700, 5,400, 3,200 and 1,600 were detectable by ECD detection (900 mY). Those of real MGPC by light-scattering detection are, respectively, 2,700, 1,740, 880, 540 and 160.

to contain compounds indicating mutagenicity in the absence of metabolic activity (S9Mix). MGPC of 160,000 peak was not detected by ECD, indicating portion of urethane linkage is minor. Further study is suggested in order to identify the specific mutagen in irradiated potting material. Serum extract presented an identical GPC chromatogram.

In Table 2, mutagenicity testing of methanol and acetone extracts from thermosetting PU potting material are presented. Methanol extract showed around 4 times greater mutagenicity than acetone extract. The quantity of acetone extract was around 1.6 times greater than methanol extract, however the mutagenicity result was contrary to the quantity of the extract, indicating that quality rather than quantity of components in the extract should be seriously considered.

There have been reports concerning the influence of solvent effect used for extraction in mutagenicity testing [48]. In the report, methanol used for extraction reacted with an original mutagen, 2,4 diaminotoluene (2,4-DAT), producing bis (2,4-diamino-5-tolyl) methane, a mutagen 5 times greater than the original compound 2,4-DAT, indicating a false positive mutagenicity data. Ethyl acetate used for extraction is likely to produce acetylation of the compound of interest, thus greater mutagenicity. The author's data established an absence of mutagen in nonirradiated potting material and the fact that mutagenicity increased with increasing ir.radiation doses, specifically in the absence of metabolic activation (S9Mix, Table 2). The author cannot deny that a greater mutagenicity from methanol extract than acetone extract in the absence of metabolic activity has a false positive potential, as the author has not yet identified mutagens because mutagenicity did not increase with increasing the concentration of methanol extract from nonirradiated potting materials, indicating that the reaction products with methanol may not be a predominant mutagen. γ-ray irradiation may be the predominant factor producing mutagen.

Autoclave sterilization had no apparent effect on the formation of MDA or other compounds in potting material. Data was obtained by HPLC and by GPC chromatograms from the difference in peak heights before and after autoclaving [8,9]. Potting material being rigid and complex is able to tolerate hydrolysis during autoclave sterilization. The construction matrix of potting material is complex, therefore hot water does not easily penetrate the inner area to hydrolyze and extract interior MDA.

Comparison of autoclave and γ-ray irradiation sterilization

No MDA was formed in chain-extended thermoplastic PU when sterilized by autoclave or by γ-ray irradiation and no MDA in nonchain-extended thermoplastic PU when sterilized by γ-ray irradiation. Less than 1 ppm of MDA was produced in nonchain.extended thermoplastic PU when sterilized by autoclaving. Nonchain-extended thermoplastic PU is inappropriate for medical use.

Autoclave sterilization at 121.1°C for 1 h did not produce MDA in thermosetting PU potting material. MDA, however, was formed in potting material subjected to γ-ray irradiation. MDA increased with increasing irradiation doses. MDA at 100 kGy irradiation was around a few ppm [8,9].

Methanol and serum extracts from autoclave sterilized potting material were quantitatively less than those sterilized with γ-ray irradiation. The extracts from sterilized potting material indicated mutagenicity in the absence of metabolic activity (S9Mix, [9]). The quantity of mutagenicity from autoclave sterilized potting material is less than that from γ-ray irradiated potting material, confirming that autoclave sterilization is more favorable, providing the material can withstand the process. The disadvantage of autoclave sterilization is that a few medical polymers, i.e. polysulfone and cellulose, are tolerant to heat. An alternative and safer sterilization procedure [13] and a more rigid potting material tolerable of γ-ray irradiation sterilization are keenly required [32]. As an alternative safer sterilization procedure, the author recommends gas plasma sterilization [34,49,50].

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