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1     **Synthesis, Characterization & Cytocompatibility of Poly (diol-co-**  
2            **tricarballylate) Based Thermally Crosslinked Elastomers for**  
3            **Drug Delivery & Tissue Engineering Applications**

4  
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## 26 **Abstract**

27 The aim of this study was to investigate the synthesis and in vitro characterization of  
28 thermoset biodegradable poly (diol-co-tricarballylate) (PDT) elastomeric polymers for the  
29 purpose of their use in implantable drug delivery and tissue engineering applications. The  
30 synthesis was based on thermal crosslinking technique via a polycondensation reaction of  
31 tricarballylic acid with aliphatic diols of varying chain lengths (C6-C12). PDT prepolymers  
32 were synthesized at 140oC for 20 minutes. After purification, the prepolymers were molded  
33 and kept at 120 oC for 18 hours under vacuum to complete the crosslinking process. PDT  
34 prepolymers were characterized by DSC, FT- IR, 1H-NMR and GPC. The PDT elastomers  
35 were also subjected to thermal and structural analysis, as well as sol content, mechanical  
36 testing, in vitro degradation and cytocompatibility studies. The mechanical properties and sol  
37 content were found to be dependent on synthesis conditions and can be controlled by  
38 manipulating the crosslinking density and number of methylene groups in the chain of  
39 precursor aliphatic diol. The family of thermally crosslinked PDT biodegradable polyesters  
40 were successfully prepared and characterized; besides they have promising use in drug  
41 delivery and other biomedical tissue engineering applications.  
42

43 **Keywords:** Poly (diol-tricarballylate), thermal crosslinking, biodegradable elastomer, cytocompatibility,  
44 drug delivery  
45

## 46 **1. Introduction**

47 Polymers possessing rubber-like elasticity, known also as elastomers, have been extensively  
48 studied for their use in the design and development of drug delivery systems [1-6] and other  
49 tissue engineering applications [7-17]. Elastomers possess many advantages over other  
50 synthesized tough polymers. Their mechanical properties can be designed to make them as soft  
51 as body tissues; they have the ability to withstand the mechanical challenges upon implantation  
52 in a moving part of the body and they can also be designed to possess a three-dimensional  
53 structure with uniform degradation pattern which make them well suited for various biomedical  
54 applications [6, 18].

55 Biodegradable elastomers can be classified according to their synthesis and thermo-mechanical  
56 properties into either, thermoplastics [8, 9, 12] or thermosets [1, 7, 10]. Thermoplastics possess  
57 the advantage of being easy to fabricate, but due to their non-amorphous nature they tend to  
58 degrade heterogeneously leading to rapid nonlinear loss of their mechanical properties and  
59 subsequently leading to significant deformations in their structure. On the other hand, thermosets  
60 are not as easy to fabricate but they outperform thermoplastics with uniform biodegradation, better  
61 durability and mechanical properties. This made thermosets as a preferable choice for controlled  
62 drug delivery and tissue engineering applications.

63 Various approaches have been reported in literature to prepare thermoset biodegradable  
64 elastomers. Such approaches depended mainly on the chemical and physical nature of the  
65 monomers utilized and the chemical reaction involved which included but not restricted to  
66 polycondensation [19, 20], polyaddition and the commonly utilized ring opening polymerization  
67 [21, 22]. Polyester based thermoset elastomers are among the most common types of elastomers  
68 synthesized for drug delivery and tissue engineering applications as they are biodegradable,

68 biocompatible and easily prepared. Younes et al reported earlier on the synthesis of a star  
69 copolymers of poly (D, L- lactide) (PDLLA) and poly ( $\epsilon$ -Caprolactone) (PCL) followed by  
70 preparation of a set of biodegradable polyester based elastomers by utilizing ring opening  
71 polymerization initiated by glycerol [23]. Another recently reported approach to synthesize  
72 polyester diol based thermoset elastomers, comprised the reaction of aliphatic diols, which  
73 contain free alcoholic hydroxyl groups, with acids that possess free carboxylic acid groups via  
74 polycondensation reactions. Poly (alkylene-tartrate) (PAT), poly (glycerol-sebacate) (PGS) and  
75 poly (diol-citrate) (PDC) were most reported examples of such elastomers prepared. [8, 9, 12,  
76 24]. Tri-carboxylic acids were favored in the preparation of such elastomers as they result in  
77 formation of star like prepolymers which facilitate the synthesis of thermoset elastomers [9, 11,  
12, 24].

78 Our research laboratory has previously reported on the fabrication and characterization of  
79 photocrosslinked poly (diol-tricarallylate) (PDT) based elastomers utilizing a process that  
80 involved either visible light or UV light photopolymerization and a solvent free strategy of drug  
81 loading [6, 16, 18]. The use of these PDT based elastomers in cardiac tissue engineering  
82 applications was also recently reported [17]. Those elastomers were optically transparent,  
83 exhibited controllable mechanical properties, and proved to be amorphous with glass transition  
84 temperatures below physiological body temperature, making them suitable as elastomeric  
85 implants *in vivo*. Nonetheless, despite their numerous advantages, the process of their  
86 fabrication involved several steps of synthesis and purification to remove the traces of  
87 photoinitiators and catalysts to maintain their excellent reported biocompatibility [16, 25-28].

88 We selected tricarallylic acid (propane-1,2,3-tricarboxylic acid) (TCA) and the aliphatic diols  
89 as building blocks for the fabrication of these elastomers since TCA is one of the simplest  
90 classes of aliphatic acids, it is water soluble, and abundantly present in food products and  
possesses structure

91 similarity to several biological active compounds such as citric acid and amino acid [29, 30]. On  
92 the other hand, aliphatic diols are biocompatible intermediate compounds used in the synthesis  
93 of polymeric systems including, polyesters elastomers, coatings, adhesives and polymeric  
94 plasticizers and their *in vivo* biocompatibility and clearance was extensively reported [18, 31].

95  
96 The aim of this report, is to report on the direct and simple preparation of monodispersed  
97 amorphous, amorphous biocompatible and biodegradable PDT based elastomers via a  
98 polycondensation reaction using catalyst-free thermal crosslinking technique. Various aliphatic  
99 diols with tricarballic acid have been prepared and characterized. The prepolymers and  
100 elastomers prepared have been characterized for their thermal, structural and mechanical  
101 properties. In addition, *in vitro* degradation and long-term cytocompatibility studies were  
102 conducted. The physicochemical nature of these prepared elastomers was modified by varying  
103 the chain length of the aliphatic diol in their structure. As such, these elastomers can be regarded  
104 as viable candidates for drug delivery and other biomedical applications that can offer structural  
integrity and stability over a clinically required period.

## 105 **2. Materials and Methods**

### 106 **2.1 Materials**

107 Tricarballic acid, 1,6-hexanediol, 1, 8-octanediol, 1,10-decanediol, 1,12-dodecanediol,  
108 Penicillin -Streptomycin Solution and Dulbecco's Phosphate Buffered Saline were purchased from  
109 Sigma-Aldrich Chemie GmbH, Germany. Dichloromethane, LiChrosolv<sup>®</sup> acetone, and acetone-d  
110 were purchased from Merck Co., Germany. RPMI ( 1640, Fetal Bovine Serum, L-Glutamine  
111 200mM (100x), 2-Mercaptoethanol (50 mM) and the LIVE/DEAD<sup>®</sup> Viability/Cytotoxicity Kit,

112 for mammalian cells were purchased from Life Technologies Co., Invitrogen, UK. Lonza  
113 Trypsin/EDTA (10x) was purchased from SLS Life Science Co., UK. Vybrant<sup>®</sup> MTT Cell  
114 Proliferation Assay Kit was purchased from ThermoFisher Scientific, Paisley, UK. All  
115 chemicals and solvents were used as received without any further purification.

## 116 **2.2 Synthesis of Poly (diol-co-tricarallylate) (PDT) Prepolymers and Elastomers**

117 TCA was reacted with aliphatic diols of varying chain lengths via a polycondensation reaction. A  
118 representative synthesis process of poly (1,10-decanediol-co-tricarallylate) (PDET) is described  
119 here. Into a glass ampule, an amount of 8.91 g of 1,10-decanediol (0.051 mole) and 6 g of TCA  
120 (0.034 moles) were added and mixed. The mixture was heated at 140 °C for 20 minutes with vortex  
121 mixing until complete melting. The reaction was then continued for 2 hours under 10 inHg  
122 vacuums at 80 °C to prepare the PDT based prepolymers. The prepolymers were poured either into  
123 a glass dog-bone shaped mold or in a glass petri dish and left in the oven at 120 °C for 18 hours  
124 under 5 inHg vacuums to complete the crosslinking process.

## 125 **2.3 Thermal Characterization**

126 The thermal properties of the polymers were characterized using DSC 8000 (Perkin Elmer Co.,  
127 USA) differential scanning calorimeter (DSC) equipped with the intra-cooling system (Intracooler  
128 II). The measurements were carried out at heating rate 10 °C/minute. In order to provide the same  
129 thermal history, 10 mg of each sample was heated from room temperature to 150°C and rapidly  
130 cooled down to -70°C, then DSC scan was recorded by heating from -70 to 150°C.  
131 Thermogravimetric data was obtained using Pyris<sup>®</sup> 6 TGA (Perkin Elmer Co., USA) at a heating  
132 rate of 10 °C/min on 10 mg of a sample. The scan run was recorded from room temperature till  
133 600°C.

## 134 **2.4 Structural Characterization**

### 135 *2.4.1 X-ray Diffraction Analysis*

136 The X-ray diffraction analysis (XRD) of the monomers' powders and the crosslinked fabricated  
137 elastomers was carried out using X-ray diffractometer (D8 Advance, Bruker Co., Germany)  
138 employing CuK $\alpha$  radiation source. A 1° divergence slit was used to analyze between the 2 $\theta$  range  
139 5-40° with a step size of 0.1° and step time of 1 second. The other various components were  
140 assigned through auto-fitting in the instrument using the DIFFRAC.EVA<sup>®</sup> software.

### 141 *2.4.2 Fourier Transform-Infrared*

142 Fourier Transform-Infrared (FT-IR) spectra of the prepared PDT based prepolymers and  
143 elastomers were obtained at room temperature using Jasco<sup>®</sup> FT/IR-4200 (Jasco Inc., Japan)  
144 infrared spectrometer equipped with ATR PRO470-H attenuated total reflection accessory unit,  
145 over the wavelength range of 4000–400 cm<sup>-1</sup>. The spectra were collected with a resolution of 4  
146 cm<sup>-1</sup> and a scan number of 32 using a DLA-TGS detector.

### 147 *2.4.3 Proton Nuclear Magnetic Resonance*

148 Proton Nuclear Magnetic Resonance (<sup>1</sup>H-NMR) spectra for the prepared prepolymers were  
149 recorded at room temperature on a Bruker Ascend<sup>®</sup> 600 MHz NMR spectrometer (Bruker Co.,  
150 Germany). The samples were dissolved in deuterated acetone containing 0.1% (v/v)  
151 tetramethylsilane) in a 5 mm diameter NMR tubes for analysis. The chemical shifts in parts per  
152 million (ppm) for the <sup>1</sup>H-NMR spectra were referenced relative to tetramethylsilane (TMS, 0.00  
153 ppm) as the internal reference.



#### 154 2.4.4 Gel-permeation Chromatography (GPC)

155 Molecular weights and molecular weight distributions of the prepared prepolymers were  
156 determined using a Viscotek GPCmax VE 2001 gel-permeation chromatography (Viscotek,  
157 Malvern, UK) equipped with triple detector array TDA 305 (Light Scattering: RALS 90° angle  
158 and LALS 7° angle, Refractive Index and Viscometer). The column configurations consisted of 4  
159 columns connected in series: T6000M (300 x 8 mm), two T1000 (300 x 8 mm) and FIPA (H100-  
160 3078). The mobile phase consisted of acetone at a flow rate of 1 ml/min at 35°C. The sample  
161 concentration was 20 mg/ml, and the injected volume was 100 µl. Data were collected and handled  
162 using OmniSEC® software package.

#### 163 2.5 Sol Content Measurements

164 Soxhlet extraction was used in determining the sol content of PDT elastomers with  
165 dichloromethane (DCM) as a solvent for 24 hours at 45 °C. Slab samples (20×6×3mm) with weight  
166 ( $W_1$ ) were evaluated. The samples were then dried using two filter papers and weighed ( $W_2$ ). After  
167 that, the samples were dried in vacuum oven at 55°C and maximum vacuum till obtaining constant  
168 weight ( $W_3$ ). Results reported are the mean  $\pm$  SD of triplicate for each elastomeric sample. The sol  
169 content and the swelling degree of the prepared elastomers were calculated using the following  
170 equations:

$$171 \text{ Sol content (\%)} = W_1 - W_3 / W_1 \times 100$$

$$172 \text{ Swelling degree (\%)} = W_2 - W_3 / W_3 \times 100$$

173

## 174 **2.6 Contact Angle Measurements**

175 The contact angles for fabricated elastomers were determined using a goniometer Drop Shape  
176 Analysis System, DSA25, (Krüss GmbH, Hamburg, Germany), equipped with a microsyringe  
177 PTFE needle of 0.5 mm diameter. Using the dynamic sessile drop method, a drop of deionized  
178 water (10  $\mu\text{L}$ ) was dispensed, and then the syringe needle was moved down to the surface of the  
179 elastomeric films. After dispensing, the drop shape was captured with a digital camera within 5 s,  
180 and contact angle, drop diameter were recorded. To determine the contact angle, the drop contour  
181 was mathematically described by the Young–Laplace equation using DSA25, and the contact angle  
182 was determined as the slope of the contour line at the three-phase contact point. Five measurements  
183 were taken for each sample at different sites and were averaged.

## 184 **2.7 Mechanical Properties**

185 Tensile mechanical testing was conducted using Instron tensile 3343 tester with Bluehill<sup>®</sup> software  
186 (Instron Co., USA). The tensile tester was equipped with 1 N load cell. Dog-bone shaped samples  
187 of 28 mm in length, 1.5 mm in thickness, 6 mm in width at the narrow section and 15 mm in width  
188 at the gripping section. The samples were pulled at a rate of 1.0 mm/sec and elongated to failure  
189 at room temperature. Results reported are the mean  $\pm$  SD of triplicate for each elastomeric sample.  
190 Differences were evaluated with One-way ANOVA using SPSS software version 20 and a  $P$  value  
191  $<0.05$  was considered a statistically significant difference. Values were converted to stress-strain  
192 and plotted. Young's modulus was calculated from the initial slope of the stress-strain curve. The  
193 crosslinking density was calculated according to the theory of rubber elasticity following the  
194 equation:  $\rho_c = E / 3RT$ , where  $\rho_c$  represents the number of active network chain segments per unit

195 volume ( $\text{mol/m}^3$ ),  $E$  represents the Young's modulus in Pascal (Pa),  $R$  is the universal gas constant  
196 ( $8.3144 \text{ J/mol K}$ ) and  $T$  is the absolute temperature in kelvin (K).

## 197 **2.8 *In Vitro* Degradation Studies**

198 PDT dog-bone-shaped specimens of known weights ( $W_1$ ), which were 28 mm in length, 3 mm in  
199 thickness and 6 mm and 15 mm in width at the narrow and gripping section, respectively, were  
200 placed in 40 ml vials each containing 35 ml of 0.1 M phosphate buffered saline (PBS, pH 7.4) and  
201 0.01% sodium azide. The vials were placed in a Julabo SW22 (JULABO Labortechnik GmbH,  
202 Seelbach, Germany) shaking water bath at 37 °C and 70 rotations per minute for up to 4 weeks.  
203 The buffer was replaced daily to ensure a constant pH of 7.4. After 1, 2, 3 and 4 weeks, the swollen  
204 weight ( $W_2$ ) and dried weight ( $W_3$ ) were measured after wiping the surface water with filter paper  
205 and after vacuum-drying at 50°C for 2 days, respectively. The tensile properties of the degraded  
206 samples at these intervals were also measured. The results reported as the mean  $\pm$  SD of three  
207 measurements.

208 The water absorption and weight loss calculations were measured as follows:

$$209 \text{Weight loss (\%)} = W_1 - W_3 / W_1 \times 100$$

$$210 \text{Water absorption (\%)} = W_2 - W_3 / W_3 \times 100$$

## 211 **2.9 *In Vitro* Cytocompatibility**

212 A murine renal adenocarcinoma cell-line (RENCA-HA) was grown in T75 tissue-culture treated  
213 flasks using Roswell Park Memorial Institute Medium (RPMI) supplemented with 10% (v/v) fetal  
214 bovine serum, 1% (v/v) L-glutamine, 1% (v/v) penicillin/streptomycin (5000 units), 0.1% (v/v)  $\beta$ -  
215 mercaptoethanol and 0.2% (v/v) geneticin (G418) at 37°C and in a 5%  $\text{CO}_2$ /air atmosphere.

216 The prepared PDT films were cut into circular discs of 7.6 mm in diameter and 4.3 mm in  
217 thickness. The samples were sterilized by soaking in 70% ethanol for 2 minutes followed by  
218 washing with PBS. The cells were plated in Falcon<sup>®</sup> 24-well plates at a density of  $4 \times 10^4$  cells/well  
219 in one ml of medium and allowed to attach for 48 hours. Following cells attachment, scaffolds  
220 were added to the cells in quadruplicates and assessed after 48 hours of incubation with the cells  
221 prior to their staining and measurement. The cells were stained using the LIVE/DEAD<sup>®</sup>  
222 Viability/Cytotoxicity Kit (ETHD-III and Calcein) for mammalian cells. Phase-contrast images  
223 and fluorescent images were taken using Leica AF6000 E wide-field microscope (Leica  
224 Microsystems, Germany) equipped with a high-resolution Hamamatsu Photonics ORCA C4742-  
225 95 CCD camera. Cytotoxicity expressed as relative cell density and cells were assessed visually  
226 and qualitatively compared to the control.

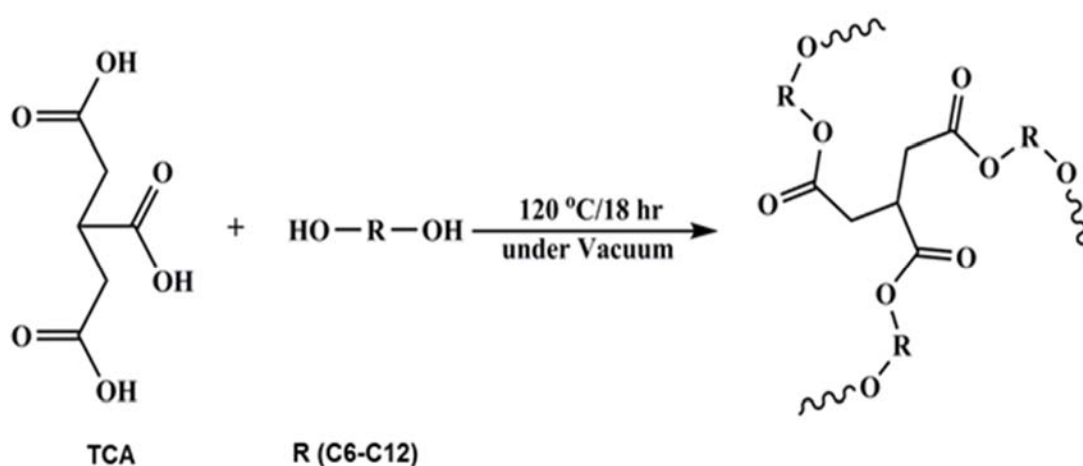
227 PDET scaffolds, as a representative member of the tested PDT based elastomers were subjected  
228 to long-term cytocompatibility study over a period of 3 weeks following the same above  
229 protocol with the exception that the incubation medium was replaced every 48 hours with a  
230 fresh medium. Cells viability was quantified following the MTT 3-(4,5-dimethylthiazol-2-  
231 yl)-2,5- diphenyltetrazolium bromide assay protocol as reported by us earlier [18]. Results were  
232 recorded as percentage absorbance relative to the incubated control cells only. The cytotoxicity  
233 assay results were used to calculate cell viability after incubation with elastomer as follows:

$$234 \quad \text{Cell viability (\%)} = [A] / [A]_c \times 100$$

235 Where [A] is the absorbance in a well containing the elastomer sample and [A]<sub>c</sub> is the mean  
236 absorbance for control cells. Results reported as the mean  $\pm$  SD from three replicates of the PDET  
237 elastomer preparation.

### 238 3. Results & Discussion

239 The synthesis of the elastomers (Fig. 1) was achieved through utilization of a polycondensation  
240 reaction that was accompanied by a loss of water molecule to form a polyester. The synthesis was  
241 carried out at different temperatures according to the stage of the preparation. In the prepolymer  
242 preparation stage; TCA was added to the aliphatic diol in a glass ampule and heated at 140 °C for  
243 20 minutes until complete melting of the mix. The use of this relatively high temperature was to  
244 ensure complete melting of the acids and the diols, to initiate and facilitate the polycondensation  
245 reaction. The prepolymer synthesis continued later at 80 °C and 10 inHg vacuum for two hours.



246

247 **Fig. 1.** Schematic illustration of the chemical synthesis of PDT based elastomers.

248 At this stage, the prepolymer (i.e. polymerized but un-crosslinked) obtained was viscous but  
249 pourable, transparent, clear and colorless to faint-yellow in color. Those PDT prepolymers were  
250 capable to be processed to various shapes by melting or dissolving in organic solvents.

251 During the elastomer preparation stage, the prepolymers were poured in the desired molds and left  
252 in a vacuum oven at 120 °C and 5 inHg vacuums for 18 hours to undergo the thermal crosslinking

process. The molds used were either glass petri dishes to prepare elastomeric films, which were cut afterwards into circular discs, or customized glass dog-bone shaped molds to prepare dog-bone shaped elastomeric specimens, which were subjected to mechanical testing. The prepared crosslinked thermoset PDT based elastomers (Table 1), as with other chemically crosslinked polymers, were neither soluble nor meltable. They were also stretchable, rubbery, and they tend to swell rather than dissolve when placed in organic solvents.

**Table 1**  
The monomers used in PDT prepolymers and elastomers synthesis.

Diol used	Acid used	Molar ratio diol: acid	Name	Code of elastomer
1,6-hexanediol	TCA	3:2	Poly(1,6-hexanediol-co-tricarballylate)	PHT
1,8-octanediol	TCA	3:2	Poly(1,8-octanediol-co-tricarballylate)	POT
1,10-decanediol	TCA	3:2	Poly(1,10-decanediol-co-tricarballylate)	PDET
1,12-dodecanediol	TCA	3:2	Poly(1,12-dodecanediol-co-tricarballylate)	PDDT

### 3.1 Thermal Characterization

#### 3.1.1 Differential Scanning Calorimetry

The thermal analysis of the prepolymers showed that PHT and POT were amorphous while PDET and PDDT were crystalline. The T<sub>g</sub> of the prepared prepolymers are listed in Table 2. After ~~complete~~ the crosslinking process took place, the elastomers converted to the amorphous state with no endothermic peaks detected. As reported, all the prepared elastomers possessed T<sub>g</sub>

267 temperatures below 37°C which indicated that they will be in their rubbery state at body  
 268 temperature.

269 **Table 2**  
 270 Thermal data of PDT prepolymers and elastomers using DSC analysis.

	Prepolymer			Elastomer		
	<i>T<sub>g</sub></i> (°C)	<i>T<sub>m</sub></i> (°C)	$\Delta H$ (J/g)	<i>T<sub>g</sub></i> (°C)	<i>T<sub>m</sub></i> (°C)	$\Delta H$ (J/g)
PHT	-63	-	-	-28	-	-
POT	-59	-	-	-26	-	-
PDET	-41	-8	34	-24	-	-
PDDT	-31	30	46	-10	-	-

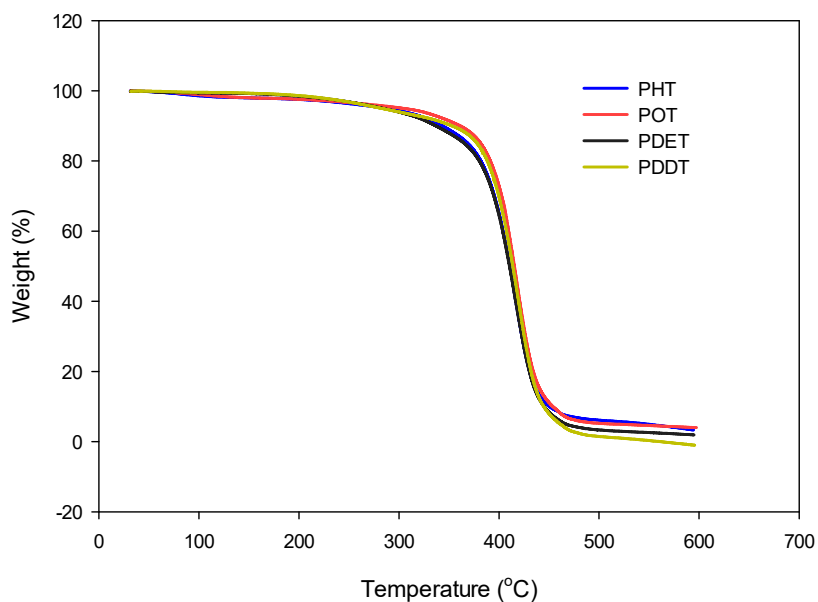
271 The thermal behavior of the PDT prepolymers can be explained as follows: as the number of the  
 272 methylene groups in the polymer chain increases, the molecular weight also increases, resulting in  
 273 an increase in both the *T<sub>g</sub>* and the degree of the polymer crystallinity. This is consistent with what  
 274 was reported earlier concerning the increase in *T<sub>g</sub>* and crystallinity for an aliphatic polyester upon  
 275 the increase in the number of methylene groups in their backbone chain length [32].

276 Following crosslinking and the formation of the elastomer, the crystallinity of the prepolymer  
 277 disappears. This can be explained by the fact that at the prepolymer state, there exists some loose  
 278 uncrosslinked chains that could have rearranged to form the crystal lattice pattern. Though, after  
 279 complete crosslinking, to form the elastomers, the network of the loose chains was minimized and  
 280 disappeared which in turn is reflected on the amorphous state of the elastomer [33]. Thus, the  
 281 crosslinking suppressed the mobility of the molecular chains and prevented chains rearrangement  
 282 as a result of which, an obstruction of crystal formation took place [34]. The *T<sub>g</sub>* of the PDT  
 283 elastomers increased as the elastomer's molecular weight increased. Hence, PHT possessed the  
 284 lowest *T<sub>g</sub>* at -28 °C, while the PDDT possessed the highest *T<sub>g</sub>* at -10 °C. This is contributed to the

285 effect of the increase of the molecular weight of the aliphatic polyester on the Tg of the elastomers  
286 which are fully crosslinked.

### 287 3.1.2 Thermogravimetric Analysis

288 This technique provides complimentary and supplementary characterization information to DSC.  
289 The TGA thermograms of PDT elastomers (Fig. 2) demonstrated their stability at high  
290 temperatures. Elastomers started to lose their weight at 371 °C and the weight loss increased with  
291 the increase in the temperature while maximum degradation took place at 480 °C. These results  
292 for PDT were similar to the prepared PDC based elastomers reported by Yang *et. al.* They have  
293 observed that the weight loss ranged between 229°C to 274°C [24]. This indicated that TCA was  
294 more stable than citric acid as PDT elastomers started their thermal degradation at higher  
295 temperatures.



296

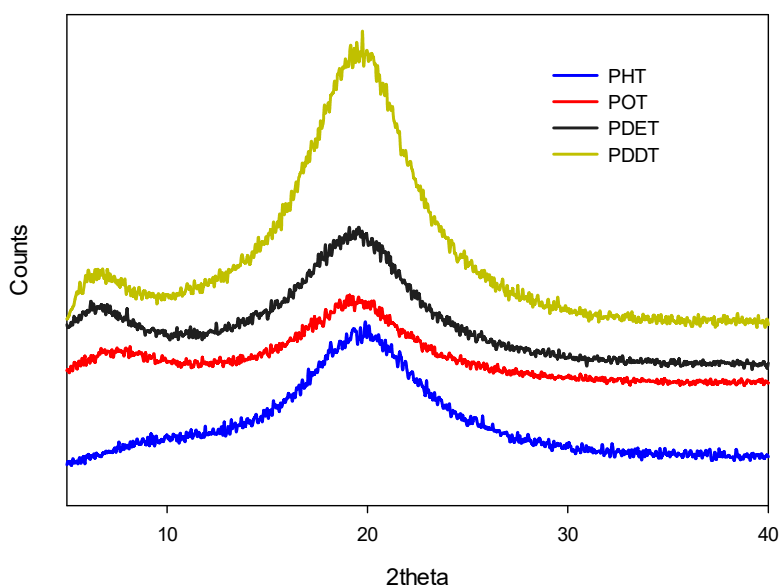
297 **Fig. 2.** TGA thermograms of PDT elastomers.



## 298 3.2 Structural Characterization

### 299 3.2.1 X-ray Diffraction Analysis

300 The XRD patterns of TCA, (1,6-Hexanediol), (1,-8-octanediol), (1,10-decanediol) and (1,12-  
301 dodecanediol) powders produced various and distinctive sharp peaks. Conversely, upon  
302 crosslinking process and preparation of PHT, POT, PDET and PDDT elastomers, their XRD  
303 patterns showed an amorphous-like pattern with no distinctive peaks. This is an indication for the  
304 formation of amorphous elastomers and the crosslinking of their monomers. This confirms the  
305 results of the DSC, where all the PDT prepared elastomers were found to be amorphous (Fig. 3)  
306 with no endothermic peaks.

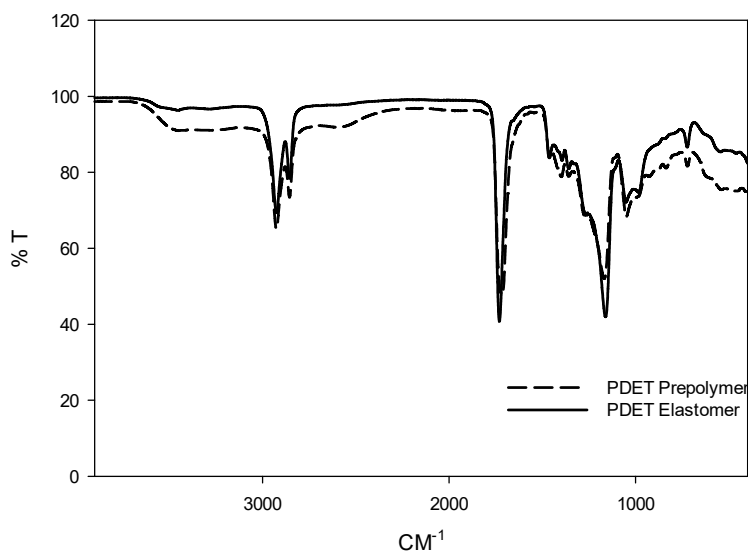


307  
308 **Fig. 3.** XRD pattern of PDT elastomers.

### 309 3.2.2 Fourier Transform-Infrared Spectroscopy (FT-IR)

310 The PDT prepolymers and elastomers with different chain lengths possessed almost the same  
311 spectra. The FT-IR spectra of PDET prepolymer and elastomer as a representative example are

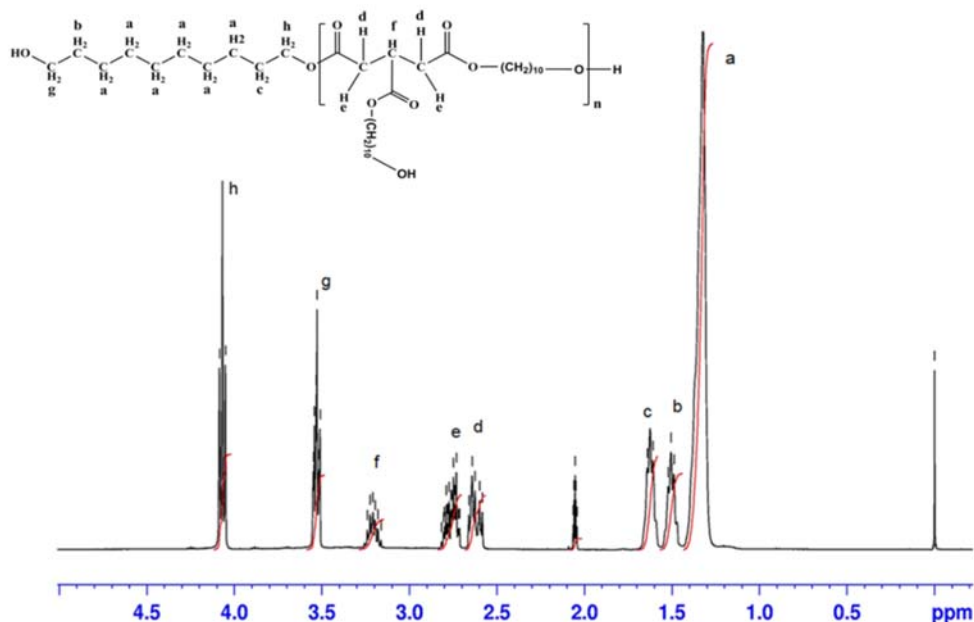
312 shown in Figure 4. The prepolymer possessed three distinctive bands. The first is a broad  
313 absorption band at 3600-3400  $\text{cm}^{-1}$  which corresponds to the hydroxyl stretching vibrations for the  
314 free OH. The broadening of the band was attributed to the intermolecular hydrogen bond  
315 formation. The second absorption bands at about 2938  $\text{cm}^{-1}$  and 2825  $\text{cm}^{-1}$  were attributed to the  
316 C-H stretching vibrations of the methylene group. The third absorption band at 1730  $\text{cm}^{-1}$   
317 represented the carbonyl group of the formed ester. The bands at 1300-1000  $\text{cm}^{-1}$  were attributed  
318 to C-O stretching vibrations. All these absorptions bands remained the same in the elastomer  
319 except for the broad peak at 3600-3400  $\text{cm}^{-1}$  which either disappeared or significantly reduced as  
320 a result of the consumption of the OH in the polycondensation reaction to form the ester. These  
321 results demonstrated the purity of the structure of the prepared prepolymers and elastomers; as all  
322 the peaks in the spectra were corresponding to a certain function group in the samples prepared  
323 and there was complete absence of any unexpected peaks in the spectra.



324  
325 **Fig. 4.** FT-IR spectra of PDET prepolymer and elastomer.  
326

327 3.2.3 Proton Nuclear Magnetic Resonance ( $^1\text{H-NMR}$ )

328 The  $^1\text{H-NMR}$  spectra (Fig. 5) of PDET prepolymer is used here as a representative example. The  
329 peak at 2 ppm represents the solvent used to dissolve the prepolymer (acetone-d). The peak  
330 assigned letter (a) that appears at 1.35 ppm was attributed to the protons of the methylene group  
331 positioned in the middle of the structure of the 1,10-decanediol. The (b) and (c) (1.5 and 1.65 ppm  
332 respectively) represents the protons positioned in the pre-terminal carbon atoms of the diol. The  
333 (d) and (e) (2.65 and 2.75 ppm respectively) are the protons located on carbons adjacent to the  
334 prochiral center of the TCA. The (f) appears at 3.3 ppm represents the protons of the prochiral  
335 center placed just in the middle of the TCA. The (g) and (h) (3.55 and 4.1 ppm respectively)  
336 represents the protons of the terminal carbons of the diol which is directly attached to the OH and  
337 the ester bond with the acid respectively.



338  
339 **Fig. 5.**  $^1\text{H-NMR}$  spectra of PDET prepolymer.  
340

### 341 3.2.4 Gel-permeation Chromatography (GPC)

342 The molecular weights of the PDT prepolymers as measured *via* GPC are listed in Table 3. As  
343 expected, the molecular weights of the prepared prepolymers increased upon increasing the  
344 number of the methylene groups in the backbone of the used diol. The GPC analysis also showed  
345 that the prepared prepolymers demonstrated narrow distribution of their molecular weights with  
346 polydispersity indices approaching unity (1.16 - 1.39). The molecular weights of the prepolymers  
347 prepared here were in close alignment with the published GPC data of Younes *et al.* who used the  
348 visible light photo-crosslinking technique for the elastomers fabrication [18].

349 **Table 3**  
350 GPC results of the PDT prepolymers.

Prepolymer	$M_n$ (g/mol)	$M_w$ (g/mol)	$M_w / M_n$
PHT	835	1049	1.26
POT	934	1131	1.21
PDET	1046	1221	1.16
PDDT	1332	1863	1.39

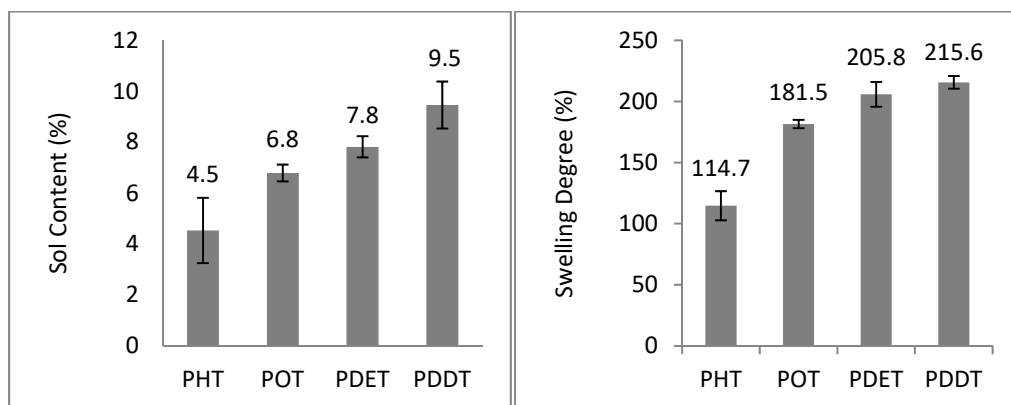
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### 352 3.3 Sol Content & Swelling Degree

353 A direct proportional relationship (Fig. 6) between the diol chain lengths to the percentage of sol  
354 content and swelling degree was observed. The percentages increased upon increasing the diol  
355 chain length from 1,6-hexanediol to 1,12-dodecanediol. The later possessed the highest sol content  
356 of 9.5 % and a swelling degree of 215.6%. These results may be attributed to the fact that the  
357 crosslinking density decreased upon increasing in the aliphatic diol chain length. As the chain  
358 length is a function for the molecular weight; thus, the increase in the molecular weight of the  
359 sample, will result in a decrease in the crosslinking which will consequently be translated into an

360 increase in both the sol content and the elasticity which will be interpreted into the elongation of  
361 the elastomers when tested for their mechanical properties.

362 These results matched what was reported earlier with other elastomeric scaffolds prepared the  
363 photocrosslinking methods and using diverse monomers. The photo-crosslinked elastomers using  
364 the same monomers as have been reported by our research group; possessed nearly the same range  
365 of sol content. The measurements for the photocrosslinked varied from 8-11%. There is a small  
366 difference, as the range expressed here in the thermal crosslinked was between 4.5-9.5%. This is  
367 due to the fact that the thermal energy produced much greater crosslinked elastomers with higher  
368 crosslinking density than those of the photocrosslinked version of the elastomers [15, 16].



369  
370 **Fig. 6.** Sol content and swelling degree of PDT elastomers using Soxhlet extraction.

371

### 372 3.4 Contact Angle Measurement

373 The contact angles of the PDT based elastomers increased with increasing the number of  
374 methylene groups in the monomers' diol. As reported in Table 4, all the PDT elastomers possessed  
375 contact angles below 90° and as such, tend to be more hydrophilic in nature. This has an impact  
376 on the elastomers' cytocompatibility, cell attachment, and growth. Many studies proved that cells

377 attach, spread and prefers growing on moderately hydrophilic substances than on hydrophobic or  
378 very hydrophilic ones [18, 35]. PHT with the least number of methylene groups in the diol chain  
379 lengths possessed the least contact angle of 72° while PDDT with 12 Carbons in the diol chain  
380 length possessed the highest contact angle of 85°. The values of the PDT contact angles followed  
381 the same range for different elastomers being prepared using different monomers and crosslinking  
382 techniques for tissue engineering and drug delivery applications [36-38].

383 **Table 4**  
384 The water-in-air contact angles of PDT elastomers.

Scaffold	Contact angle (°)
PHT	72 ± 1.10
POT	76 ± 1.86
PDET	82 ± 1.15
PDDT	85 ± 1.18

385

### 386 **3.5 Mechanical Properties**

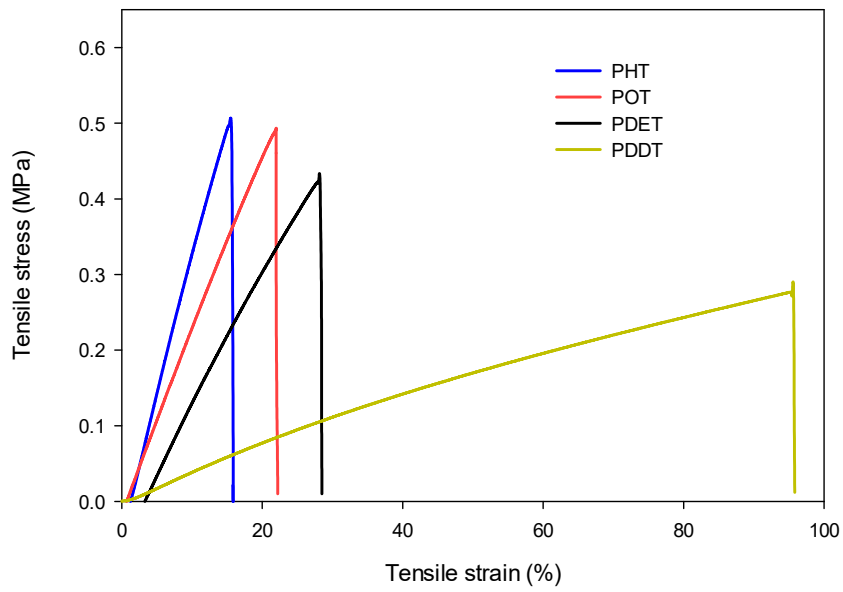
387 The PDT elastomers were subjected to tensile testing to evaluate the effect of the aliphatic diol  
388 chain length on their mechanical properties. As shown in Figure 7, tensile testing of the thermally  
389 crosslinked PDT based elastomers produced representative uniaxial tensile-strain curves which are  
390 characteristics of typical elastomeric materials. Representative images of PDDT elastomer before  
391 and after being tested are shown in Figure 7b. As shown, 100% recovery was obtained for the  
392 PDDT elastomer after being stretched to break. Average values of the ultimate tensile stress ( $\sigma$   
393 (MPa)), maximum strain ( $\epsilon$  (%)), the young's modulus (E (MPa)) and the crosslinking density ( $\rho_c$ )  
394 are summarized in Table 5.

395 As the chain length of the elastomer decreases, the crosslinking density increases, resulting in a  
 396 decrease in  $\epsilon$  accompanied by an increase in  $E$ . PHT elastomer showed the highest  $\sigma$  and  $E$  values  
 397 which was attributed to the fact that PHT possessed the lowest number of methylene groups in the  
 398 chain of the diol used in their preparation. As the diol chain length decreased, the  $\rho_x$  of the polymer  
 399 increased, which resulted in the formation of a more crosslinked elastomer that was stiffer and less  
 400 extensible. On the other hand, increasing the aliphatic diol chain length decreased  $\rho_x$  and, therefore,  
 401 increased  $\epsilon$  of the elastomer as in PDDT elastomer which possessed the highest chain length of the  
 402 diol. It also showed a significant difference in its mechanical properties compared to the other  
 403 elastomers. This could be attributed to the elastomers' crosslinking density. The differences  
 404 between the crosslinking densities of the other diols were relevantly minimal with increasing of  
 405 the diol chain length. However, the PDDT was found to be less than that of the PDET by 80%.  
 406 The sol content of the PDDT elastomer was also higher than the other diols which further proves  
 407 the effect of the unreacted prepolymer chains within the elastomeric structure which contributes  
 408 with the long chain length of the PDDT elastomer in having significant results in their mechanical  
 409 properties.

410 **Table 5**  
 411 Mechanical properties of PDT elastomers.

Elastomer	$\sigma$ (MPa)	$\epsilon$ (%)	$E$ (MPa)	$\rho_x$ (mol/m <sup>3</sup> )
PHT	0.498 ± 0.02	16.43 ± 1.11	3.57 ± 0.25 <sup>+</sup>	476.84 ± 33.39
POT	0.454 ± 0.06	20.71 ± 1.89	2.5 ± 0.46	333.92 ± 61.44
PDET	0.424 ± 0.02	28 ± 3.89	1.88 ± 0.23	251.11 ± 30.72
PDDT	0.248 ± 0.07 <sup>*</sup>	97.72 ± 12.64 <sup>*</sup>	0.377 ± 0.07 <sup>*</sup>	50.35 ± 9.34

412 Values are reported as (mean ± SD) of triplicates of each sample. The analysis was conducted using One-way ANOVA  
 413 followed by Tukey's HSD and p value < 0.05. (\*) is significant over PHT, POT, and PDET. (+) is significant over  
 414 POT and PDET.



(a)

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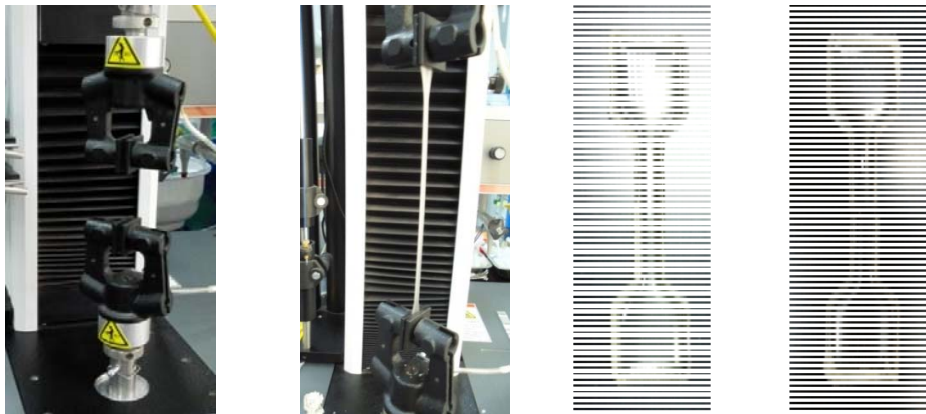
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(b)

422

423

424 **Fig. 7.** (a) Stress-strain curves of different PDT elastomers. (b) PDDT elastomer shows 100% recovery after being  
 425 stretched to break.



426 The obtained mechanical properties were in agreement with reports on other researched thermally-  
427 crosslinked elastomers such as those based on star copolymers of DLLA-PCL, PAT, PGS and  
428 PDC [8, 9, 12, 23, 24]. For example, in a recent work carried out by Khademhosseini and his  
429 group, they observed the decrease in tensile strength and increase in tensile strain of PGS from 0.5  
430 MPa and 38% to less than 0.1 MPa and 98% by tuning the length of polyethylene glycol block  
431 attached to the PGS [39].

432 Many studies have also reported that elastomers' mechanical properties and degradation pattern,  
433 as in case of our PDT based elastomers, were found to be heavily dependent on parameters such  
434 as polymerization reaction time, reaction temperature, monomers molar ratios and time of curing  
435 [9, 24, 31, 37]. For example, in case of PGS, the E was reported to be in the range of 0.056-1.5  
436 MPa, and its elongation at break ranges from 40 to 450 % depending on the synthesis conditions  
437 and length of diol chain [31, 37]. On the other hand, poly (diol citrate) (POC), which has raised  
438 the most interest of the four reported PDC based elastomers, because of its desirable mechanical  
439 properties, was found to have an E ranging from 0.92–16.4 MPa,  $\sigma$  of 6.1 MPa and  $\epsilon$  of 117-265  
440 % [9, 24]. Various studies including this one, which reported on the mechanical and degradation  
441 properties of PDT based elastomers have shown comparable mechanical properties to those  
442 reported for PGS and PDC elastomers. The E of PDT based elastomers ranged from 0.012-3.5  
443 MPa while  $\epsilon$  ranged from 16- 300% depending on many factors related to synthesis conditions.  
444 The above values of E for PDT based elastomers cover those of many soft tissues, such as muscle  
445 (0.01–0.5 MPa) [40, 41], skin (0.7–16 MPa)[40, 42] and ligament (0.5–1.5 MPa) [43, 44].

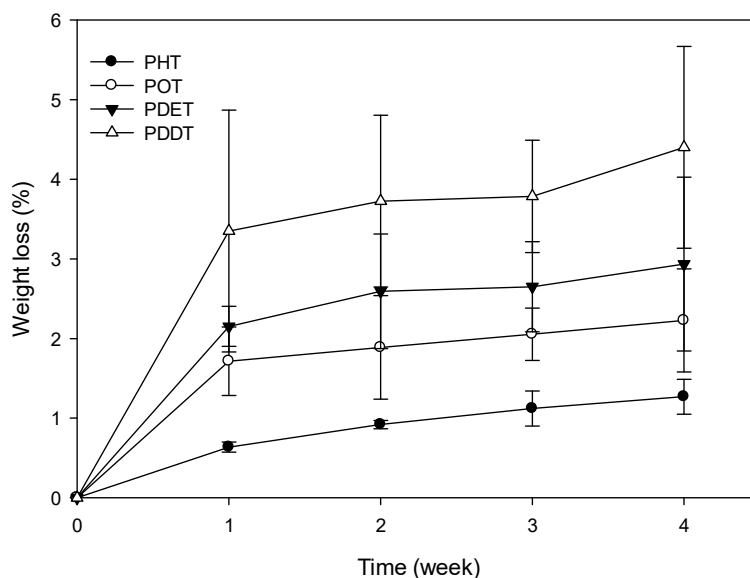
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448 **3.6 In Vitro Degradation**

449 **3.6.1 Influence of Chain Length on in Vitro Degradation**

450 In order to investigate the influence of chain length on the degradation rate and the changes in the  
451 mechanical properties of the elastomer during *in vitro* degradation, four different thermally  
452 crosslinked PDT elastomers, based on varying the chain lengths of aliphatic diol were prepared  
453 and tested. The water absorption and weight loss (Fig. 8 and Fig. 9) of the elastomers were directly  
454 proportional to the chain length of the aliphatic diol used and inversely proportional to the  
455 elastomers crosslinking density. PHT elastomer possessed the lowest number of methylene groups  
456 in its chain and the highest crosslinking density  $476 \text{ mol/m}^3$ ; demonstrated the lowest weight loss  
457 with minimal water uptake rate.



458

459 **Fig. 8.** Percentage weight loss versus time of PDT elastomers degradation studies in PBS at 37°C. Error bars  
460 represent the standard deviation of the mean of measurements from three samples.

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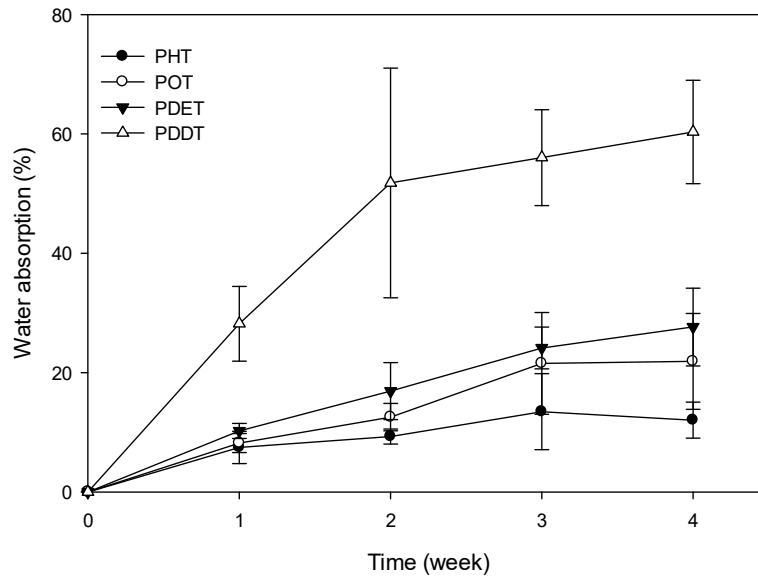
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478 **Fig. 9.** Percentage water absorption versus time of PDT elastomers degradation studies in PBS at 37°C. Error  
479 bars represent the standard deviation of the mean of measurements from three samples.  
480

481 On the other hand, PDDT elastomer, which possessed the highest number of methylene groups in  
482 its chain and the lowest crosslinking density ( $50 \text{ mol/m}^3$ ); showed the highest weight loss with  
483 maximal water uptake rate. This can be contributed to the high amount of the sol content in the  
484 PDDT elastomer which facilitated its degradation. These findings were consistent with PDT  
485 elastomers prepared using the photo-crosslinking technique with regards to water diffusion into  
486 the bulk of elastomeric polyesters at temperatures above their glass transition [16]. Also, the results  
487 are in accordance with the fact that water diffusion and mass loss are inversely proportional to the  
488 polymers crosslinking density [45].

### 489 3.6.2 Degradation Behavior

490 As with other reported photocured PDT based elastomers [6, 16, 18, 29, 30], some morphological  
491 changes of the elastomers' shapes (Fig. 10) were observed during the degradation study. PDDT

492 was used here as a representative example as the other PDT elastomers behaved similarly. The  
493 elastomers retained their dimensions, but they increased in thickness. After the immersion of the  
494 elastomeric samples in the PBS; water diffusion and absorption into the elastomer mass took place  
495 and resulted in the hydrolysis of the polymer chains. This process wasn't limited to the surface  
496 only, but mainly happened to the bulk of the elastomer. The degradation was accelerated by the  
497 diffuse out of hydrolysis products from the sol phase of the polymer which further contributed to  
498 the formation of oligo carboxylic acids within the polymer mass which autocatalyzed the  
499 degradation rate further and increased the hydrophilic character of the polymer due to the  
500 formation of free COOH and OH moieties within the elastomer bulk. As such, the elastomers  
501 became more susceptible to water absorption. By the end of the fourth week, the samples were  
502 swollen and changed from flat shape to bloated convex shape and their surface became smoother  
503 and translucent.

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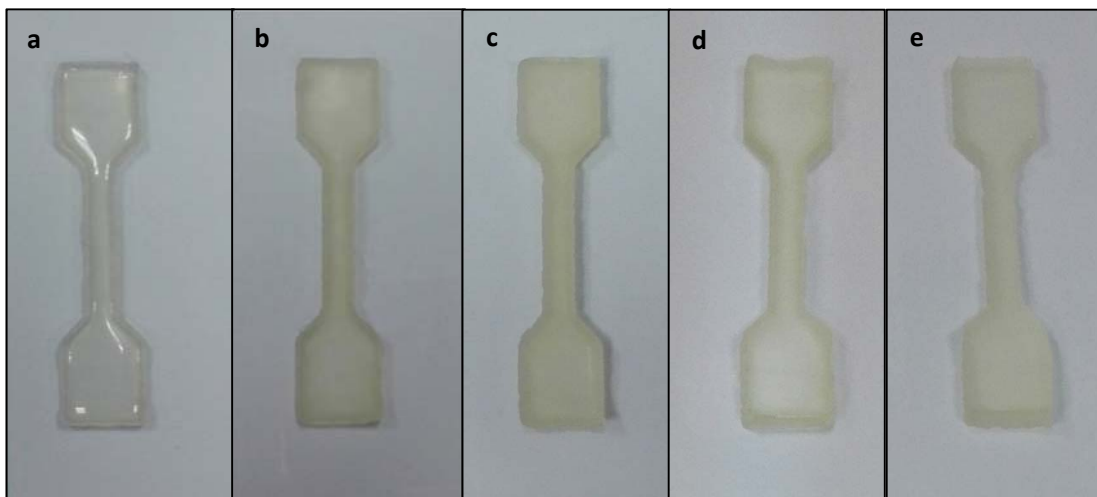
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511

512 **Fig. 10.** Images of the PDDT elastomers during *in vitro* degradation after (a) 0, (b) 1, (c) 2, (d) 3 and (e) 4 weeks.

513

### 514 3.6.3 Changes in the Mechanical Properties during *in Vitro* Degradation

515 The changes in the mechanical properties (Fig. 11-13) of the elastomers with respect to time during  
516 *in vitro* hydrolytic degradation study. Although the elastomers showed a decrease in their  
517 mechanical strength with time, they maintained their shape and extensibility over the testing  
518 period. Both young's modulus and ultimate tensile stress decreased in a linear fashion with time,  
519 indicative of zero-order degradation mechanism. This linear decrease was observed regardless of  
520 the network composition, the crosslinking density and the initial young's modulus of the  
521 elastomers. The change in the tensile strain (Fig. 13) was less sensitive to the degradation of the  
522 PDT elastomers. No significant change in the elongation throughout the four weeks of the *in vitro*  
523 degradation study. These results confirmed that the hydrolytic degradation of these elastomers  
524 followed a bulk erosion mechanism. It is only with surface erosion degradation pattern that the  
525 elastomers can maintain their mechanical properties unchanged [46]. Moreover, young's modulus  
526 (Fig. 11) and ultimate tensile stress (Fig. 12) for all the PDT elastomers linearly decreased with  
527 time.

528 Through a linear regression of the zero-order degradation kinetics of the data (Fig. 11 and Fig. 12)  
529 using equations (1) and (2), the rate constants were calculated and listed in Table 6.

$$530 \quad E_t = E_0 - K_E t, \quad (1)$$

$$531 \quad \sigma_t = \sigma_0 - K_\sigma t. \quad (2)$$

532

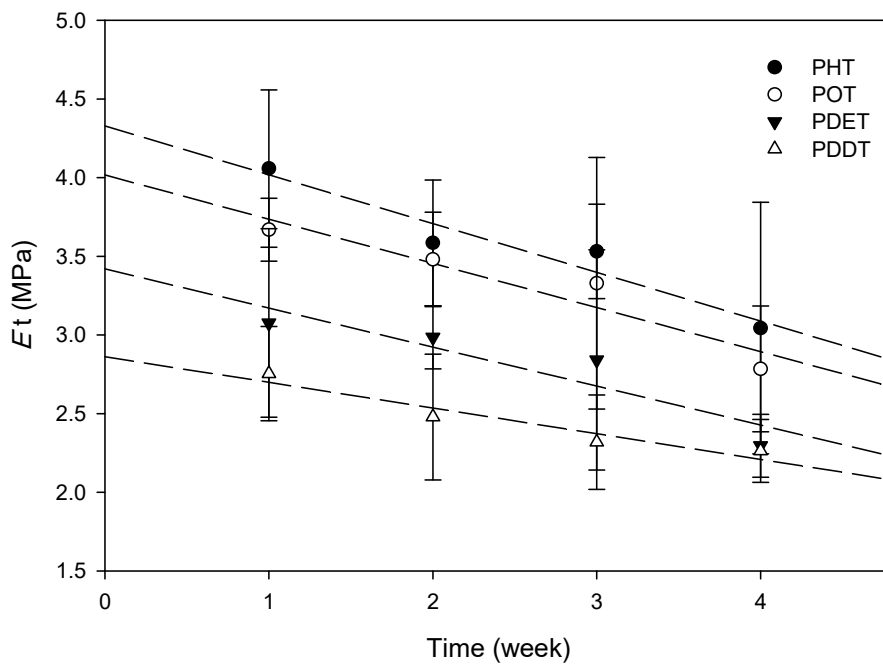
533 The (t) in the above equations donates to the immersion time (in weeks) in PBS. The values of  $E_0$   
534 and  $\sigma_0$  correspond to the intercepts obtained from extrapolating the zero-order fitted line. While,  
535  $K_E$  and  $K_\sigma$  represents the zero-order degradation constants for young's modulus and the ultimate  
536 tensile stress respectively. The decrease in the aliphatic diol chain length in the elastomer was

537 accompanied by an increase in  $K_E$  and  $K_\sigma$ . As reported in Table 6, PHT which possess the shortest  
 538 aliphatic in the chain length had  $K_E$  and  $K_\sigma$  of 0.30984 and 0.43763 MPa/week respectively, while  
 539 PDDT with the longest aliphatic chain length possessed 0.16345 and 0.12655 MPa/week for  $K_E$   
 540 and  $K_\sigma$  respectively.

541 As described earlier in the mechanical testing of the elastomers,  $E$  was depending mainly on the  
 542 crosslinking density of the elastomers and the ultimate tensile stress depends on the distribution of  
 543 end to end distances between the crosslinks [45, 46]. As such, lower molecular weight PDT  
 544 elastomers (shorter chain lengths) demonstrated faster decline in their mechanical strength  
 545 compared to the higher molecular weight (longer chain lengths) PDT elastomers. These results  
 546 came with complete agreement with other reported PDT elastomers using photocrosslinking  
 547 technique, where the degradation rate was inversely proportional to the molecular weight of the  
 548 elastomers [18]. Thus, the mechanical parameters decrease in a much faster rate as the molecular  
 549 weight between the crosslinks decreases [16, 46]. By the end of the 4<sup>th</sup> week study period in PBS,  
 550 the elastomers maintained their original shape with minor degradation.

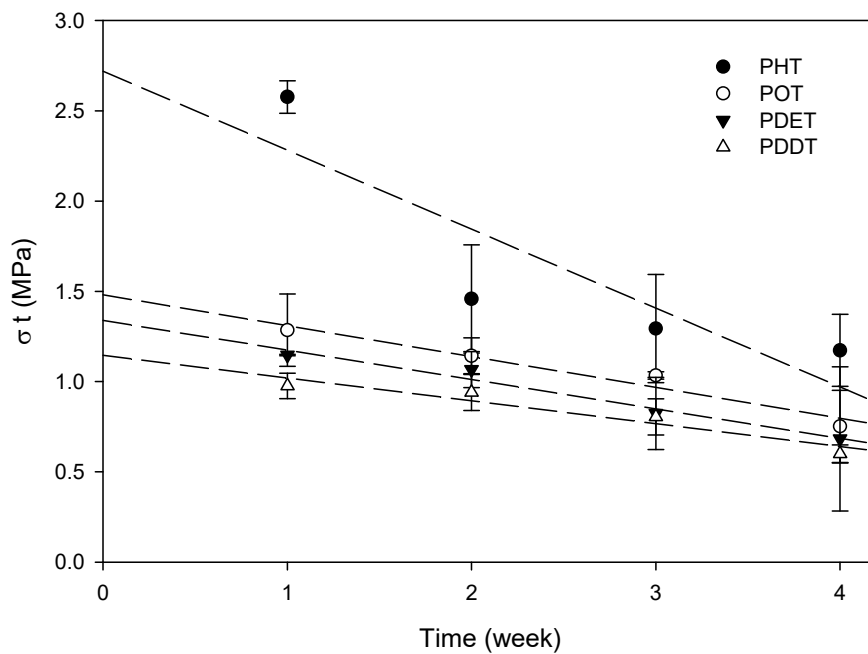
551 **Table 6**  
 552 Linear regression coefficients values for PDT elastomers during *in vitro* degradation in PBS (pH 7.4).

Elastomer	$E_0$ (MPa)	$K_E$ (MPa/week)	$\sigma_0$ (MPa)	$K_\sigma$ (MPa/week)
PHT	4.32916	0.30984	2.7198	0.43763
POT	4.01743	0.28078	1.4815	0.17116
PDET	3.42091	0.24859	1.33955	0.16375
PDDT	2.86227	0.16345	1.1462	0.12655



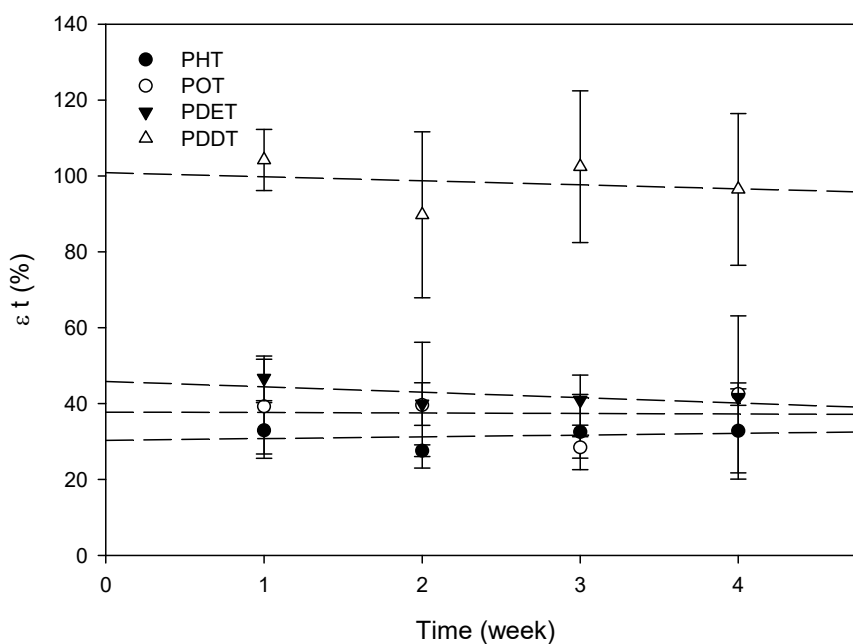
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554 **Fig. 11.** Change in young's modulus of PDT elastomers during degradation in PBS at 37°C. Error bars represent  
 555 the standard deviation of the mean of measurements from three samples.



556

557 **Fig. 12.** Change in ultimate stress of PDT elastomers during degradation in PBS at 37°C. Error bars represent the  
 558 standard deviation of the mean of measurements from three samples.  
 559



560

561 **Fig. 13.** Change in ultimate strain of PDT elastomers during degradation in PBS at 37°C. Error bars represent the  
 562 standard deviation of the mean of measurements from three samples.

563

564 **3.7 *In Vitro* Cytocompatibility**

565 The different chain lengths of the PDT elastomers were tested for their cytocompatibility using  
 566 RENCA-HA cells. Firstly, the scaffolds were tested to see if they have any impact on the pH of  
 567 the media, which has direct effect on the cells proliferation and growth. The PDT elastomeric discs  
 568 were added in a 24-well plate with 1 ml serum free medium (i.e. no cells were used) and kept  
 569 overnight in an incubator. The pH of the media was measured using a litmus paper and the change  
 570 in its color was compared visually to a color pH standard, as well as a control well plate which  
 571 holds media only. As presented in Table 7, not all the scaffolds showed the same effect on pH of  
 572 the media. PHT scaffold possessed the least pH indicating more acidity. This may be attributed to  
 573 the faster degradation of PHT than the other PDT elastomers as have been presented earlier in

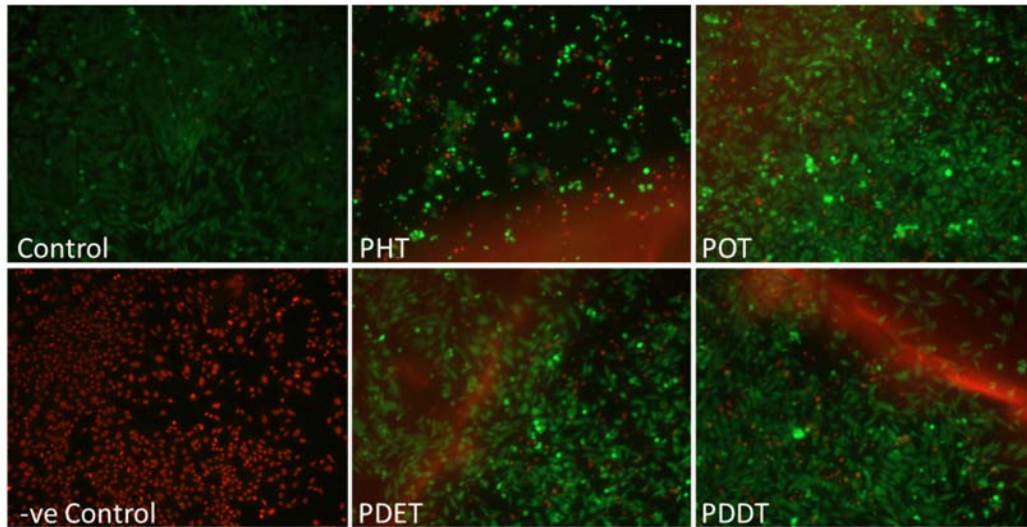


574 Table 6. Upon degradation, the ester bond is hydrolyzed, releasing tricarboxylic acid which is  
575 responsible for the drop in the pH of the medium.

576 **Table 7**  
577 The effect of the different scaffolds prepared on the pH of the media.

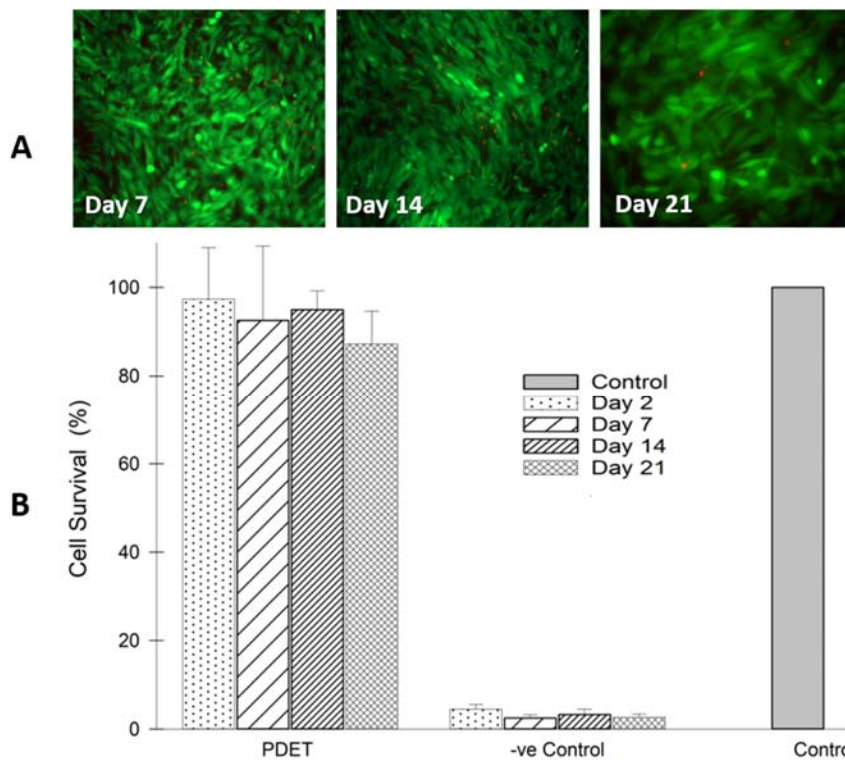
Scaffold	pH
Control	8.5
PHT	7
POT	7-7.5
PDET	8
PDDT	8.5

578  
579 Secondly, RENCA-HA cells were incubated with the PDT elastomers, and then the cells were  
580 stained using ETHD-III and Calcein fluorescent dyes. Representative images (Fig. 14) for each  
581 PDT scaffolds incubated with the cells, where they were compared to a control well, which  
582 possessed the cells only and a negative control well, in which 2 drops of ethanol were added to the  
583 control to induce cell death. POT, PDET and PDDT elastomers observed to be more compatible  
584 with cells than PHT, possibly due to its faster degradation as reported earlier [18]. The cells were  
585 healthy growing, maintaining their spindle shape especially with POT, PDET and PDDT  
586 elastomers where the green fluorescence is dominant, which is an indication for living cells that  
587 resembled the cells in the control.



588

589 **Fig. 14.** Fluorescent images of RENCA-HA cells incubated with the scaffolds for 48 hours without replacing the  
 590 media.  
 591



592

593 **Fig. 15.** Effect of PDET elastomeric scaffolds on RENCA-HA viability. (A) showing the fluorescent images of cells  
 594 incubated with the PDET scaffolds over a period of 21 days (25x magnification) and (B) cell viability estimated by  
 595 MTT assay after 7, 14, and 21 days of PDET elastomer incubation with cells. Results are expressed as the percentage  
 596 of viable cells compared with controls (mean  $\pm$  SD, n = 3). The significance of the results was determined by  
 597 comparison with control value using 1-way ANOVA; \*p < 0.05.  
 598

599 Following the long-term cytocompatibility studies on PDET elastomers and as seen in Figure 15a,  
600 cells attached were very healthy and maintained their viability over 21 days of incubation. Cells  
601 appeared spindle-shaped which is their standard appearance on plastic as undifferentiated cells.  
602 There is however at day 21 some change of phenotype (presence of cuboidal cells) suggesting  
603 minor differentiation.

604 The MTT assay results of the PDET elastomers are shown in Figure 15b. The differences in  
605 mitochondrial function associated with PDET elastomer were expressed as a percentage relative  
606 to the control cells (set at 100%), where higher absorbance values indicated increased metabolic  
607 activity of viable cells. The exposure of the cells to the PDET elastomer degradation products did  
608 not cause any significant effect on the high metabolic rate. This observation confirmed the  
609 biocompatible nature of these elastomers. The above results are in full alignment with the *in vivo*  
610 biocompatibility studies in rats conducted on various PDT photocrosslinked based elastomers  
611 which exhibited better *in vivo* biocompatibility than PLGA, evidenced by mild acute inflammatory  
612 reaction and less fibrous capsules of chronic inflammatory response [18].

## 613 **4. Conclusion**

614 We have reported on the successful syntheses and characterization of different biodegradable PDT  
615 elastomers using thermal crosslinking. The elastomers proved to be biocompatible with linear and  
616 homogenous degradation. The elastomers can be designed with different crosslinking density and  
617 degradation time which make them easily tailored to achieve the desired implantation and drug  
618 release rates in the design of controlled drug delivery systems and other biomedical and tissue  
619 engineering applications. Full long term *in vivo* biocompatibility and degradation studies in animal

620 model are needed to further report on the actual immune response and degradation behavior of  
621 various fabricated versions of the PDT thermally crosslinked elastomers.

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626 herein are solely the responsibility of the authors.

## 627 **Authors Contributions**

628 Ms. Youmna M. Hassouna, the MSc student and Dr. Somayeh Zamani (Postdoc) performed the  
629 research experiments, analysis and collected data. Dr. Wael Kafienah guided and helped Youmna  
630 Hassouna in performing the *in vitro* cytocompatibility studies. Dr. Husam M. Younes designed the  
631 research question and project, designed the performed experiments, analyzed data and wrote the  
632 final manuscript.

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