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The role of oxidation and enzymatic hydrolysis on the *in vivo* degradation of trimethylene carbonate based photocrosslinkable elastomers

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ABSTRACT

The *in vivo* degradation of trimethylene carbonate (TMC) containing elastomers was investigated, and the mechanism of degradation explored through *in vitro* degradation under enzymatic and oxidative conditions. The elastomers were prepared via UV initiated crosslinking of prepolymers of TMC and equimolar amounts of TMC and ε-caprolactone (CL). The degradation process was followed by investigating the changes in the mechanical properties, mass loss, water uptake, sol content, differential scanning calorimetry, and surface chemistry through attenuated total reflectance infrared (ATR-FTIR) spectroscopy. During *in vivo* degradation, TMC and TMCCL elastomers exhibited surface erosion. The tissue response was of greater intensity in the case of the TMC elastomer. Both elastomers exhibited degradation in cholesterol esterase containing solutions *in vitro*, but no parallels were found between the rate of *in vivo* degradation and the rate of *in vitro* degradation. Only the TMCCL elastomer degraded in lipase. Degradation in a stable superoxide anion *in vitro* medium was consistent with the observed *in vivo* degradation results, indicating a dominant role of oxidation through the secretion of this reactive oxygen species by adherent phagocytic cells in the degradation of these elastomers.

1. Introduction

Synthetic biodegradable elastomers based on trimethylene carbonate (TMC) are potentially useful materials in many biomedical applications, such as tissue engineering scaffolds and drug delivery [1]. Importantly, TMC based polymers degrade by surface erosion without producing acidic products [2], and thus could possibly be used to prepare more effective acid sensitive protein delivery systems. We are interested in the use of photo-cross-linked biodegradable elastomers prepared from ω,ω′,ω″-tri-acrylate star-poly(TMC) and star-poly(TMC-*co*-ε-caprolactone) for this purpose. The *in vivo* mechanism of degradation of, and tissue reaction to, such implants are of great importance, as they provide an idea about the environment in which the therapeutic proteins are retained and eventually released.

Upon implantation, a series of events occurs that results in monocytes moving from the blood to the implanted material surface. The monocytes adhere to the surface in a manner determined by the type, concentration, and conformation of proteins adsorbed to the material surface. Within 48 h, monocytes are the predominant cell type at the biomaterial surface, and ultimately differentiate into monocyte-derived macrophages over a period of several weeks. On the surface, the macrophages spread and, as a result of frustrated phagocytosis, release reactive oxygen species as well as hydrolytic enzymes, and may fuse to form foreign body giant cells (FBGCs) [3]. FBGCs have an increased capacity for enzyme and reactive species secretion, and are longer lived than macrophages. The extent of monocyte adhesion and ultimately FBGC formation, as well as secretion capacity, have been shown to be determined by the chemistry of the material surface [4–7]. Thus, there are multiple possible degradation mechanisms for implanted polycarbonates, including acid–base catalyzed hydrolysis, as well as enzymatically catalyzed hydrolysis and oxidation from reactive oxygen species as a result of the response of phagocytic cells.

The data to date indicate that acid or base catalyzed hydrolysis of the carbonate bonds plays a negligible role in *in vivo* degradation. Mizutani and Matsuda investigated the hydrolytic degradation of photo-cured poly(TMC) and copolymers of poly(TMC-*co*-ε-caprolactone) terminated with coumarin with different ratios of caprolactone (CL) at pH values ranging from 7.4 to 10.2. They found that, irrespective of copolymer composition, hydrolysis occurred on the surface, and the rate of hydrolysis increased as pH and CL content increased [8]. However, when the elastomers were prepared from *star*-poly(trimethylene carbonate) triacrylate, negligible hydrolysis occurred [9]. This difference in degradation is likely due to the photo-reversibility of the [2 + 2] cycloaddition linkage formed upon crosslinking with coumarin. Zhang et al. found that in *in vitro* environments with pH values ranging from 1 to 13, both high and

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low molecular weight uncrosslinked, linear poly(TMC) degraded extremely slowly. This result has been confirmed by others for low molecular weight poly(TMC) degrading in phosphate-buffered saline [10]. Similar results have been reported for poly(ethylene carbonate) immersed in aqueous media with pH ranging from 1 to 12 by Stoll et al. [11].

Zhang et al., on the basis of the negligible *in vitro* degradation, also examined enzymatic degradation and showed that in the presence of lipase the polymer degraded appreciably and in a manner consistent with surface erosion [1]. Moreover, poly(TMC) of greater than about 100 kDa degraded faster *in vivo* and *in vitro* in the presence of lipase than poly(TMC) of lower molecular weight [1]. They attributed this latter result to greater enzyme activity on the surface of the more hydrophobic, higher molecular weight, polymers.

In contrast to these results, Stoll et al. reported that poly(ethylene carbonate) was unaffected by the presence of hydrolytic enzymes in aqueous media [11]. They showed that, *in vitro*, poly(ethylene carbonate) did not degrade in the presence of hydroxyl radicals, but did degrade in a surface erosion manner in the presence of superoxide anion. Acemoglu et al., examined the *in vivo* degradation of both poly(ethylene carbonate) and poly(TMC) and found that poly(ethylene carbonate) degraded much faster [11,12]. On the basis of the results of Stoll et al., it was proposed that the *in vivo* degradation of poly(ethylene carbonate) and poly(TMC) implants proceeds by oxidation via superoxide anion radicals and that the slower oxidation of poly(TMC) was due to the more stable six-membered ring of trimethylene carbonate that is proposed to form as a result of degradation [11,12]. Others have also reported oxidative degradation of carbonate containing polymers in the presence of superoxide anion. For example, Lee et al. treated commercially available Maxon sutures made of a block copolymer of 32.5 wt% of TMC and 67.5 wt% of glycolide with different concentration of KO_2/18-crown-6 ether/THF and found significant mass losses over a 24 h time period [13]. They concluded that the degradation observed had occurred mainly in the glycolide blocks since the carbonate group in the poly(TMC) is less reactive toward nucleophilic attack. Recently, Christenson et al. reported that, although the tested polymers were susceptible to both *in vitro* enzymatic and oxidative degradation, poly(carbonate urethane) degradation *in vivo* also was consistent with an oxidation mechanism [14].

Thus, it is currently unclear as to whether poly(TMC) undergoes degradation *in vivo* primarily through either oxidation or enzyme action. There has been only one study supporting enzymatic degradation, which was performed using *Thermomyces lanuginosus* lipase. Moreover, other esterases, in particular cholesterol esterase, have also been implicated in the degradation of polycarbonate based urethanes. It was reasoned, therefore, that cholesterol esterase may be effective in the degradation of poly(TMC) and its copolymers. Furthermore, more studies support the possibility that poly(TMC), like other polycarbonates, is degraded primarily through oxidative attack. We therefore hypothesized that oxidation also plays a role in the degradation of poly(TMC) elastomers.

TMC is also often co-polymerized with monomers such as ε-caprolactone and D,L-lactide to adjust mechanical properties and degradation rates. The literature indicates the susceptibility of poly(CL) segment of different copolymers to both oxidation and enzymatic hydrolysis [13,15,16]. For example, Gan et al. exposed blends of poly(D,L-lactide) and poly(ε-caprolactone) to *Pseudomonas* lipase and found that only CL regions were prone to enzymatic degradation [15]. Similar results have been reported by Li et al., who found that the 80% weight loss in poly(CL) over 3 days of exposure to *Pseudomonas* lipase was reduced to 10% in a blend of poly(CL) and L-lactide and to 0% in poly (L-lactide) [16]. Moreover, Darwis et al. found that crosslinking poly(CL) via radiation reduced the rate at which lipase AK degraded the samples, and that the rate of reduction increased as the crosslinking density increased [17]. The role of oxidation in the degradation of poly(CL) was demonstrated by Lee and Chu, who showed that poly(CL) was susceptible to superoxide ion-induced degradation [13]. Thus, it was reasoned further that oxidation would play a role in the degradation of elastomers prepared from prepolymers of poly(TMCCL).

Thus, the objectives of this paper were to gain a better understanding of the *in vivo* degradation mechanism of photo-cured TMC and TMCCL elastomers, to determine the rate of change of their mechanical properties during degradation, and to determine the role of oxidation in the overall degradation process. To do this, *in vivo* degradation and *in vitro* enzymatic and oxidative degradation studies were conducted using elastomers made of TMC and equimolar amounts of TMC and CL. For literature comparison purposes, both *Thermomyces lanuginosus* lipase and porcine pancreas cholesterol esterase were used as model enzymes. The degradation process was followed by investigating the changes in the mechanical properties, mass loss, water uptake, sol content, and glass transition temperature. Changes in the surface features of the elastomers were followed by SEM and ATR-FTIR analyses, and the biological response was examined histologically by light microscopy.

2. Materials and methods

1,3-trimethylene carbonate (1,3-dioxan-2-one) was obtained from Boehringer Ingelheim, Germany and used as received. ε-Caprolactone (99%) was obtained from Lancaster Canada, dried over calcium hydride, and distilled under high vacuum. Toluene and dichloromethane were dried over calcium hydride and distilled under argon. Other chemicals were used without further purification. Chemicals used in polymer synthesis include stannous 2-ethylhexanoate (96%) obtained from Aldrich, Canada and glycerol obtained from BDH, USA. Chemicals used in the acrylation process include acryloyl chloride (96%), triethylamine (99.5%), and 4-dimethylaminopyridine (99%) obtained from Aldrich, Canada. 2,2-dimethoxy-2-phenylacetophenone used as a photoinitiator was obtained from Aldrich, Canada. Solvents used for purification of the synthesized polymers include ethyl acetate (99.9%) and methanol (99.8%), obtained from Fisher, Canada. Lipase from *Thermomyces lanuginosus* with a concentration of 10^5 units/g and cholesterol esterase from porcine pancreas with a concentration of 23,600 units/g were purchased from Aldrich, Canada. Materials used for oxidation studies included potassium superoxide, 18-crown-6 ether, iron (II) chloride (98%), cobalt chloride, hydrogen peroxide, and dried THF, all obtained from Sigma, Canada.

2.1. Star-copolymer polymerization

7800 g/mol star-copolymers of TMC and equimolar compositions of ε-caprolactone and TMC were prepared as described elsewhere [18]. Briefly, theoretical amounts of glycerol and monomers were placed in a dried glass ampoule. The ampoule was placed in an oil bath at 90 °C, to melt the monomers. The ampoule was then purged with argon and the required amount of stannous 2-ethylhexanoate calculated on the basis of 3×10^{-3} mol of catalyst per mole of glycerol was added. The ampoule was vacuum-sealed and placed in an oven for 3 days at 130 °C. The termini of the star polymers formed were acrylated using acryloyl chloride. 4-dimethylaminopyridine (DMAP) was used as a catalyst and triethylamine (TEA) was used to scavenge hydrochloric acid formed during the acrylation reaction. Polymers made of TMCCL were purified according to the following procedure: 50 mL of methanol was added to 10 g of the polymer, the mixture was mixed vigorously for 2 h using a magnetic stirrer, after which the mixture was kept at −20 °C for 5 h. The methanol was decanted and the process repeated 3 times. Purification of the TMC polymer was identical with the exception of the addition 1 mL DCM per 10 g of polymer prior to the addition of methanol. The purity of the synthesized prepolymers and their degree of acrylation were determined using a 500 MHz Bruker Avance NMR.

2.2. Preparation of elastomer rods

Prepolymer of a given molecular weight was dissolved in ethyl acetate at a ratio of 1:1 (w:w) and 2,2-dimethoxy-2-phenylacetophenone was added (1.5 wt%) as a photoinitiator. The mixture was poured into Pyrex glass capillary tubes, which had previously been flame sealed at one end, and closed with a rubber septum at the open end. Five tubes were placed horizontally on a Petri dish and exposed for 10 min to long-wave UV radiation at an intensity of 10 mW/cm^2 using a Black-Ray 100 YP UV lamp. The septa were removed, the tubes were placed in a fume hood overnight and then in a vacuum desiccator. Following sol extraction using dichloromethane, the weights and dimensions of the rods were measured. Rods made of TMC had an average diameter of 0.86 ± 0.02 mm, an average length of 14.1 ± 0.9 mm, and an average weight of 10.8 ± 0.7 mg, while rods made of TMCCL had an average diameter of 0.83 ± 0.02 mm, an average length of 15.4 ± 1 mm, and an average weight of 9.7 ± 0.9 mg. The $\pm$ represent standard deviations about the mean.