



Biocompatible photocrosslinked poly(ester anhydride) based on functionalized poly(ϵ -caprolactone) prepolymer shows surface erosion controlled drug release *in vitro* and *in vivo*

J. Mönkäre ^{a,*}, R.A. Hakala ^{b,1}, M.A. Vlasova ^a, A. Huotari ^a, M. Kilpeläinen ^{a,c}, A. Kiviniemi ^a, V. Meretoja ^d, K.H. Herzig ^{c,e}, H. Korhonen ^b, J.V. Seppälä ^b, K. Järvinen ^a

^a School of Pharmacy, Pharmaceutical Technology, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland

^b Laboratory of Polymer Technology, Department of Biotechnology and Chemical Technology, School of Science and Technology, Aalto University, P.O. Box 16100, FI-00076 Aalto, Finland

^c Institute of Biomedicine and Biocenter of Oulu, University of Oulu, P.O. Box 5000, FI-90014 Oulu, Finland

^d Department of Prosthetic Dentistry and Biomaterials Science, Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, FI-20520 Turku, Finland

^e Department Psychiatry, Kuopio University Hospital, Box 1627, FI-70211 Kuopio, Finland

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ABSTRACT

Star-shaped poly(ϵ -caprolactone) oligomers functionalized with succinic anhydride were used as prepolymers to prepare photocrosslinked poly(ester anhydride) to evaluate their *in vivo* drug delivery functionality and biocompatibility. Thus, in this work, erosion, drug release and safety of the photocrosslinked poly(ester anhydride) were examined *in vitro* and *in vivo*. A small water-soluble drug, propranolol HCl (M_w 296 g/mol, solubility 50 mg/ml), was used as the model drug in an evaluation of the erosion controlled release. Drug-free and drug-loaded (10–60% w/w) poly(ester anhydride) discoids eroded *in vitro* (pH 7.4 buffer, +37 °C) linearly within 24–48 h. A strong correlation between the polymer erosion and the linear drug release *in vitro* was observed, indicating that the release had been controlled by the erosion of the polymer. Similarly, *in vivo* studies (s.c. implantation of discoids in rats) indicated that surface erosion controlled drug release from the discoids (drug loading 40% w/w). Oligomers did not decrease cell viability *in vitro* and the implanted discoids (s.c., rats) did not evoke any cytokine activity *in vivo*. In summary, surface erosion controlled drug release and the safety of photocrosslinked poly(ester anhydride) were demonstrated in this study.

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1. Introduction

Poly(ester anhydride)s have been developed in order to combine advantageous properties of the polyesters and polyanhydrides [1–6]. Polyesters and polyanhydrides are well-characterized biocompatible materials for drug delivery and other medical applications. Aliphatic polyesters, such as poly(ϵ -caprolactone)s and polylactides, possess usually good mechanical strength but they often show bulk erosion, and thus, they may not be ideal candidates for achieving controlled, linear drug release [7,8]. In contrast to the polyesters, polyanhydrides are surface eroding polymers which means that they enable controlled, linear drug release, but in turn, they often are fragile which may hinder the development of controlled drug delivery systems [9,10]. In recent years, thermoplastic poly(ester anhydride) delivery systems have been developed for example for intratumoral injection of paclitaxel [11–13], and for DNA encapsulation and cellular transfection [14].

Recently, Seppälä et al. have developed thermoplastic and cross-linked poly(ester anhydride)s which are prepared from different polylactone oligomers [15,16]. Molecular architecture (linear or star-shaped), molecular size, and hydrophobicity of the oligomers can be readily modified during the synthesis of the oligomers [15,17]. For example, by changing the molecular architecture or adjusting the molecular size, the physical state of the oligomer at room temperature can be changed from a solid to a viscous liquid [17]. In addition, by increasing the hydrophobicity of the polyester prepolymers by functionalizing the oligomers with alkenylsuccinic anhydrides containing alkenyl chains of various lengths, the erosion rate of the poly(ester anhydride)s can be modified [16,17]. Furthermore, some of the crosslinkable versions can be photocrosslinked under mild conditions with visible light to form network-structured poly(ester anhydride)s.

The advantages of photocrosslinking are that a wide variety of drugs and heat-sensitive macromolecules can be entrapped as solid powders into the photocrosslinkable polymer network under mild reaction conditions without the need for heat or solvents [18–20]. In addition, photocrosslinking is a suitable method for *in situ* administration as injectable oligomers can be polymerized in a rapid and controlled manner at physiological temperatures [20,21]. Previously,

* Corresponding author. Tel.: +358 403553878; fax: +358 17162252.

E-mail address: juha.monkare@uef.fi (J. Mönkäre).

¹ These authors share equal contribution.

many photocrosslinkable polymers have been studied as drug delivery systems in which the drug release has been typically diffusion-controlled [22–27].

Earlier *in vitro* studies have indicated that crosslinked poly(ester anhydride)s based on functionalized polyester oligomers could be used for controlled drug delivery or tissue engineering applications [15,28,29]. However, no study has reported the functionality and biocompatibility of these polymers as drug delivery systems *in vivo*. Therefore, the aim of these *in vitro* and *in vivo* experiments was to evaluate the erosion, drug release and preliminary safety of photocrosslinked poly(ester anhydride) based on the star-shaped poly(ϵ -caprolactone) prepolymer functionalized with succinic anhydride. A small water-soluble drug, propranolol HCl (M_w 296 g/mol, solubility 50 mg/ml), was used as a model drug to confirm erosion controlled release *in vitro* and *in vivo*. In general, we are developing photocrosslinked poly(ester anhydride)s for parenteral and oral drug delivery systems, including *in situ* forming drug delivery systems. A wide spectrum of therapeutic agents, ranging from small-molecular drugs to proteins, can be entrapped as solid powders into the polymer network under mild reaction conditions with the drug release being controlled by surface erosion of the polymer.

2. Materials and methods

2.1. Materials

Oligomers were polymerized from ϵ -caprolactone (Solvay Interlox Ltd., Warrington, England) in the presence of stannous octoate (SnOct_2 , Sigma-Aldrich Chemie, Germany) and pentaerythritol (Sigma-Aldrich Chemie, Germany). The ϵ -caprolactone was dried over molecular sieves. Stannous octoate as an initiator and pentaerythritol as a co-initiator were used without further treatment in the ring-opening polymerization of the oligomers. Succinic anhydride (Sigma-Aldrich Chemie, Germany) and methacrylic anhydride (Sigma-Aldrich Chemie, Germany) were used in the functionalizations, and camphorquinone (Sigma-Aldrich Chemie, Germany) was used as an initiator for photo-curing. They were used as received.

Propranolol HCl (M_w 296 g/mol, solubility 50 mg/ml, Societa Italiana Medicinali Scandicci, Firenze, Italy) was used as the model drug in the study. Acetonitrile (JT Barker, Netherlands), trifluoroacetic acid (Sigma-Aldrich, USA) and triethylamine (Sigma-Aldrich, Belgium) were HPLC grade. pH 7.4 (0.2 M NaOH- KH_2PO_4) buffer was used in the erosion (i.e. polymer weight loss) and drug release studies. NaOH was received from FF-Chemicals (Yli-Ii, Finland) and KH_2PO_4 from Merck (Darmstadt, Germany).

2.2. Animals

Male Wistar rats (10–12 weeks, 200–300 g) (Kuopio, Finland) were housed in an environment-controlled room temperature 22 ± 1 °C, relative air humidity $55 \pm 15\%$ and 12/12 h light/dark cycle with lights on at 7 am with food (Lactamin R36, Sweden) and tap water available *ad libitum*. The Institutional Animal Care and Use Committee of the Provincial Government approved the experiments. Procedures were conducted in accordance with the guidelines set by the European Community Council Directives 86/609/EEC.

2.3. Preparation of poly(ester anhydride) oligomers

The synthesis and properties of the poly(ϵ -caprolactone) based poly(ester anhydride) oligomers have been described earlier [28,29]. Briefly, ϵ -caprolactone monomers were polymerized to star-shaped hydroxyl telechelic oligomers by ring-opening polymerization. In the next step, the hydroxyl termination was changed to acid termination with succinic anhydride. Finally, in order to obtain crosslinkable poly(ester anhydride) oligomers with labile anhydride bonds, acid-

terminated oligomers were allowed to react with methacrylic anhydride.

2.4. Preparation of poly(ester anhydride) discoids

Poly(ester anhydride) discoids were prepared as follows. The liquid methacrylated prepolymers, camphorquinone (1% w/w) and propranolol HCl powder (10, 20, 40 and 60% w/w) were stirred until homogeneity was achieved and the mixture was then applied to a mold in order to produce discoids (2 mm in thickness, 5 mm in diameter and 50 mg in weight). The discoids were photocured in a Teflon mold in a Triad 2000 light curing oven with visible light (350–550 nm, DeguDent) to produce crosslinked polymers. The exposure time in the irradiation chamber was 10 min. The light intensity at the surface level of the cured discoids was measured with an HD2102.1 photo-radiometer (Delta Ohm, Italy) to be on average 220 mW/cm².

The gel contents of the discoids were measured by extracting the soluble phase in dichloromethane at room temperature for 24 h. Attenuated total reflectance infrared spectroscopy (ATR-FTIR, Nicolet Magna-FTIR spectrometer 750 equipped with PIKE Technologies GladiATR with diamond crystal plate) was used to monitor the double bond conversion of the methacrylated oligomers. The double bond conversion in the discoids was calculated based on the decrease in absorbance near 1637 cm⁻¹, a characteristic absorbance of the methacrylate double bond [28,30].

2.5. Preparation of polyester discoids

In order to evaluate the effect of anhydride bonds on the erosion of and drug release from the poly(ester anhydride) discoids, the corresponding polyester discoids without anhydride bonds were prepared. In the preparation of polyester discoids, the hydroxyl termination was not changed to acid termination. Otherwise the synthesis of the polyester discoids was identical to preparation of poly(ester anhydride) discoids.

2.6. *In vitro* erosion and drug release

The *in vitro* erosion ($n=3$) and drug release ($n=4$) from the poly(ester anhydride) discoids were studied in 40 ml of pH 7.4 buffer in the water bath shaker with orbital shaking at a frequency of 120 strokes/min at +37 °C (Grant OLS200, Cambridge, UK). Fresh buffer was changed every 12 h and the pH of the buffer remained constant at $\text{pH } 7.4 \pm 0.2$.

The erosion of the samples was studied by removing discoids from the pH 7.4 buffer at pre-determined time intervals, and drying them in a vacuum for 48 h prior to weighing. The erosion (%) was calculated by using the Eq. (1).

$$\text{Erosion\%} = 100 - 100 \times \frac{m_d(t) - m_{\text{pro}}(t)}{m_d(0) - m_{\text{pro}}(0)}, \quad (1)$$

where $m_d(0)$ and $m_{\text{pro}}(0)$ are the masses of the discoid and propranolol HCl in the discoid, respectively, at the beginning of the experiment, and, $m_d(t)$ and $m_{\text{pro}}(t)$ are the masses of the discoid and propranolol HCl in the discoid, respectively, at the sampling time point.

The drug release from the discoids was studied by taking samples (5 ml) at predetermined time intervals and the sampling volume was replaced by fresh pre-warmed buffer. The released propranolol HCl in the buffer was analyzed with the HPLC-UV method described below.

The erosion and drug release from the control polyester discoids without anhydride bonds were studied in a similar manner except that the fresh pH 7.4 buffer was changed only every 3–4 days. The pH of the buffer remained at a constant level at $\text{pH } 7.4 \pm 0.2$.

2.7. In vivo erosion and drug release

The erosion of drug-free poly(ester anhydride) discs ($n=3$) and the drug release from 40% w/w propranolol HCl loaded poly(ester anhydride) discs ($n=3-4$) were evaluated in 12 weeks old Wistar male rats weighing 300 g. The discs were purified with ethanol: water (70:30) solution before implantation. The discs were implanted subcutaneously (one disc in three different dorsal locations for each animal) and removed either 12, 24, 36 or 48 h after implantation. Before implantation or the removal of the disc, the rats were anesthetized with a fentanyl citrate/fluanizone/midazolam combination (0.158 mg/5 mg/2.5 mg/kg, s.c.).

To determine the erosion of the drug-free poly(ester anhydride) discs, they were dried in a vacuum for 48 h before weighing. The erosion (%) was calculated by dividing the weight of the removed disc by its original weight.

Drug release was analyzed by dissolving in pH 7.4 buffer (+37 °C) the propranolol HCl loaded discs, which had been removed from the rats, to determine the amount of the remaining propranolol HCl in the discs. Released drug fraction at the sampling point was calculated by Eq. (2).

$$\text{Released drug\%} = 100 \times \frac{m_{\text{pro}}(0) - m_{\text{pro}}(t)}{m_{\text{pro}}(0)}, \quad (2)$$

where $m_{\text{pro}}(0)$ is the theoretical amount of propranolol HCl of the disc, calculated based on the weight of the disc, and $m_{\text{pro}}(t)$ is the amount of propranolol HCl in the disc at the time of its removal. The amount of the propranolol HCl in pH 7.4 buffer was analyzed with HPLC-UV method as described below.

2.8. HPLC analysis

The concentration of propranolol HCl was analyzed with Gilson High Performance Liquid Chromatograph (HPLC). The system consisted of an UV detector (UV/VIS-151), pump (321), autoinjector (234), interface (506C) and integrator (Unipoint 3.0). The mobile phase was a mixture of acetonitrile (40% v/v), water (60% v/v), trifluoroacetic acid (0.1% v/v) and triethylamine (0.15% v/v). The pre-column was a reverse-phase Pelliguard® LC-18 column (20 × 4.6 mm id, particle size 40 μm, Supelco, Bellefonte, PA, USA) and the analytical column was a reverse-phase Supelcosil® C-18 column (150 × 4.6 mm id, particle size 5 μm, Supelco, Bellefonte, PA, USA). The injection volume was 20 μl, flow rate 1 ml/min, and propranolol HCl was detected at 289 nm. The calibration curve of the propranolol HCl was linear over the range of 0.1–25 μg/ml with a correlation coefficient $R^2 > 0.998$. The area under the curve was used for the quantification of the drug concentration.

2.9. In vitro cytotoxicity of oligomers

Human gingival fibroblasts were plated at a density of 25,000 cells/cm² in 96-wells and allowed to adhere for 24 h. A dilution series of the oligomers with different functionalities (either hydroxyl terminated, carboxyl acid terminated or methacrylated) were prepared in acetone, and pipetted onto the top of the cells in 6 replicates. The final concentrations of acetone and oligomers were 2.5% and 50–400 μg/ml, respectively. The cells were cultured for an additional 24 h and washed with phosphate buffered saline. The cell activities were determined using AlamarBlue™ assay (BioSource International) in colorimetric format. Measured absorbances (Multiskan MS ELISA plate reader, Labsystems, Finland; 560 nm and 595 nm) were used to calculate the reduction of AlamarBlue reagent in accordance with the manufacturer's instructions. The activity of acetone-treated cells was set to 100%.

2.10. Cytokine release

In order to evaluate the preliminary *in vivo* toxicity of poly(ester anhydride) discs, the cytokine release was measured after the implantation of the drug-free disc in rats. A disc purified in 100% ethanol was implanted subcutaneously dorsally (one disc per animal, polymer dose 200 mg/kg) into 10 week old Wistar male rats weighing 200–300 g ($n=5$). Rats were anesthetized under isoflurane anesthesia (5% for induction, 2–3% for maintenance) during the implantation. Control group was sham-operated and received 0.9% NaCl (300 μl). Blood samples were collected from the femoral vein 12, 24, 36, 48 and 120 h after the implantation into heparinized capillaries and the plasma was separated by centrifugation. All the discs had eroded within 120 h as confirmed by the visual inspection of the implantation site. The concentrations of nine cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, GM-CSF, TNF-α and IFN-γ) in plasma were measured using Bio-Plex Rat Cytokine 9-Plex Panel with Bio-Plex instrument, based on Luminex xMAP technology following the manufacturer's instructions (Bio-Plex®, Bio-Rad Laboratories Inc.).

2.11. Statistical analysis

The non-parametric Kruskal–Wallis test (SPSS 14.0 for Windows, SPSS Inc.) was used to test the statistical significance of differences between groups in *in vitro* erosion and drug release studies. The *post hoc* test [31] was employed to test the significance of the differences of the means. The level of significance was taken as $p < 0.05$. Pearson's product moment correlation was calculated by using GraphPadPrism 4.03 for Windows (GraphPad Software Inc) software.

Results from *in vitro* cytotoxicity experiments were analyzed using Kruskal–Wallis test and Dunn's Multiple Comparison post-test (GraphPadPrism 4.03 for Windows, GraphPad Software Inc) and the level of significance was taken as $p < 0.05$. Results from the cytokine release experiments were analyzed using two-way ANOVA and Bonferroni post test (GraphPadPrism 4.03 for Windows, GraphPad Software Inc) and the level of significance was taken as $p < 0.05$.

3. Results and discussion

3.1. Preparation of poly(ester anhydride) discs

The synthesis of the photocrosslinked poly(ester anhydride) networks is illustrated in Fig. 1. The preparation of the drug-loaded discs was performed at room temperature which requires an appropriate viscosity of photocrosslinkable oligomers. By using 10 mol% of pentaerythritol as a co-initiator, transparent viscous oligomer liquid (molecular weight 1160 g/mol) suitable for the photocrosslinking and the mixing of the drug powder (10–60% w/w) at room temperature was obtained. After the photocrosslinking, the samples were elastic and the extraction of the samples in dichloromethane showed gel contents above 95% indicative nearly complete crosslinking of precursors. In addition, final double bond conversions of the photocrosslinked discs were between 92 and 98%.

It is noteworthy that in the present process there was no need to dissolve the drug into any solvent since the drug could be simply mixed with the oligomers as a plain drug powder. In typical preparation methods of biodegradable polymeric drug delivery systems, such as hot melt extrusion and solvent casting methods, one must heat the polymer mass or dissolve the drug in an organic solvent in order to mix the polymer and the drug which may result in loss of compound activity, particularly, in the cases of peptides and proteins [32]. Organic solvents can be also problematic due to toxic solvent residues [32]. In addition to the mentioned advantages, the present polymer enabled a high drug loading degree, up to 60% w/w.

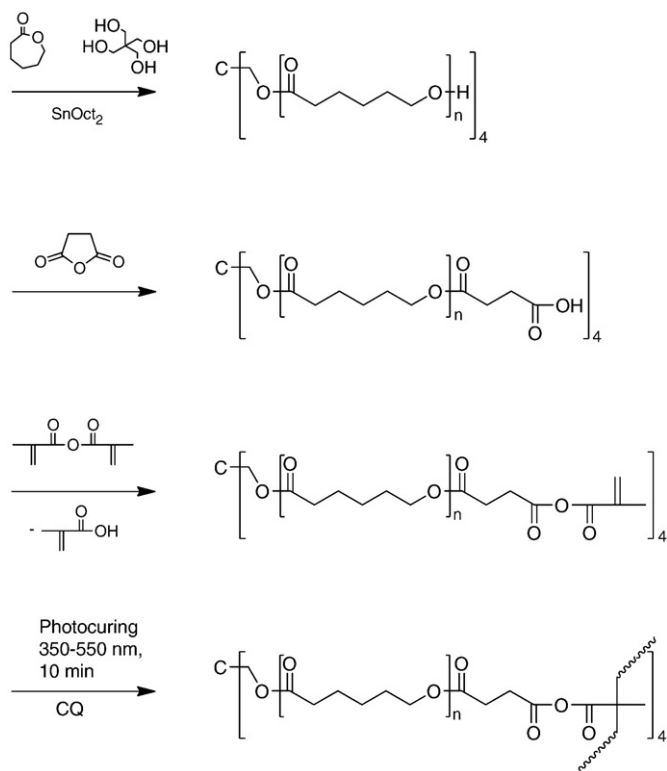


Fig. 1. Preparation of poly(ester anhydride) networks.

3.2. Poly(ester anhydride) erosion and drug release *in vitro* and *in vivo*

Surface erosion of photocrosslinked poly(ester anhydride)s occurred both *in vitro* and *in vivo*. Fig. 2 clearly shows that the dimensions of drug-free poly(ester anhydride) discs shrunk steadily and the discoid core remained intact during the *in vitro* and *in vivo* studies.

In vitro (pH 7.4, 37 °C), after a lag-time of 4–8 h, the studied poly(ester anhydride) discoids eroded linearly (R^2 0.961–0.990) within 24 to 48 h (Fig. 3, Table 1). Poly(ester anhydride) discoids with the propranolol HCl loading from 10 to 40% w/w tended to have a slightly higher erosion rate than drug-free and 60% w/w drug loaded discoids (Fig. 3). The steady-state erosion rate of propranolol HCl loaded discoids varied from 2.61%/h (60% w/w) to 4.05%/h (20% w/w) (Table 1). However, one can conclude that the drug loading degree did not influence greatly the erosion of the poly(ester anhydride) discoids as the statistically significant ($p < 0.05$) differences were detected only between 20 and 60% w/w loaded discoids at 4 h, between 10 and 40%

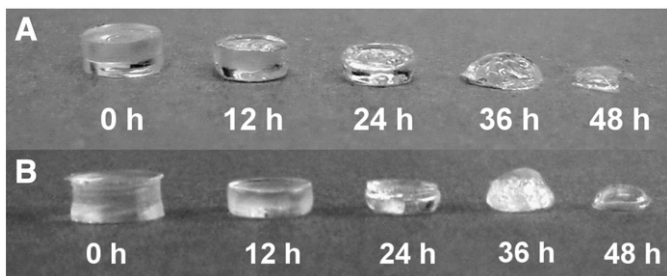


Fig. 2. Core of drug-free poly(ester anhydride) discoids remained intact whereas the dimensions decreased steadily during the *in vitro* (A) and *in vivo* (rats, s.c.) (B) studies. Discoids at 0, 12, 24, 36 and 48 h after incubation in 7.4 buffer and implantation in rats. Initial diameter 5 mm, height 2 mm and weight 50 mg.

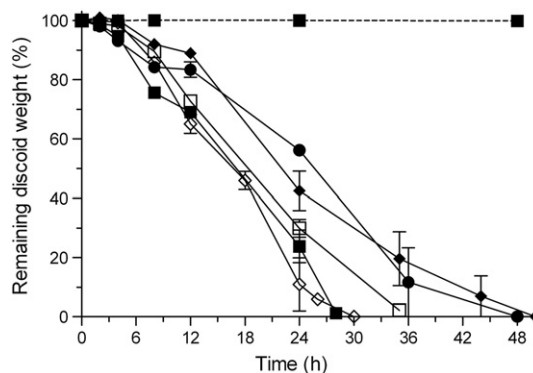


Fig. 3. Erosion of drug-free and drug-loaded poly(ester anhydride) (solid line) was linear *in vitro* (pH 7.4, +37 °C), while corresponding polyester without anhydride bonds (dashed line) did not erode. Drug loading degrees: ● 0% ■ 10%, ◇ 20%, □ 40% and ◆ 60% w/w propranolol HCl. Mean \pm SEM, $n = 3$. Statistically significant ($p < 0.05$) differences were found between 20 and 60% w/w at 4 h, between 10 and 40% (w/w) at 8 h, between 0 and 20% (w/w) at 12 h and between 0 and 10% w/w at 24 h.

w/w loaded discoids at 8 h, between 0 and 20% w/w loaded discoids at 12 h and between 0 and 10% w/w loaded discoids at 24 h. For comparison, propranolol HCl loaded (10% w/w) discoids from corresponding polyester discoids without anhydride bonds hardly eroded at all in 48 h (Fig. 3). The difference between the *in vitro* erosion of photocrosslinked poly(ester anhydride) and polyester discoids (Fig. 3) clearly shows that the erosion of the poly(ester anhydride) is due to the rapid hydrolysis of the anhydride bonds. During the erosion process, anhydride bonds are remaining intact inside the poly(ester anhydride) matrix until they are exposed to the water as the erosion front proceeds [28].

In vivo (s.c., rats), erosion of the drug-free poly(ester anhydride) discoid showed linear (R^2 0.925) erosion between 12 and 48 h (Fig. 4). The *in vivo* erosion profile of the drug-free discoid closely followed the *in vitro* erosion profile of the corresponding discoid although statistically significant differences ($p < 0.05$) were detected between the *in vitro* and *in vivo* profiles at 12 h and 24 h (Fig. 4). The similarity between the *in vitro* and *in vivo* erosion of the poly(ester anhydride) suggests that the *in vivo* erosion is based on the hydrolytic degradation of the anhydride bonds. If enzymes or other biological factors were playing a significant role in the erosion process, then the erosion would be quicker *in vivo* than *in vitro* [33].

Surface erosion is defined as the erosion of the polymer from the external surfaces of the polymer matrix [8]. Therefore, as long as the external area of the polymer matrix remains constant, a linear drug release can be achieved if the drug release is erosion controlled. Göpferich and Tessmar [9] stated that a hydrophilic model compound should be used if one wishes to examine the erosion controlled release from polyanhydrides. Therefore, in this study, a small water-soluble drug, propranolol HCl (M_w 296 g/mol, solubility 50 mg/ml), was used as the model drug to evaluate whether release from photocrosslinked poly(ester anhydride)s was erosion controlled.

In vitro (pH 7.4, 37 °C), propranolol HCl (10–40% w/w) release from poly(ester anhydride) discoid showed a linear (R^2 0.980–0.990) drug release with the lag time of 8 h (Fig. 5, Table 1). It was noticed already in the erosion studies that there is a lag time before the beginning of the linear erosion phase (Table 1). Thus, it is suggested that the lag time of the drug release is related to the similar delay in the beginning of the polymer erosion. When the drug loading degree was 60% w/w, there was no lag time in the release profile, instead an initial burst release of 10% within the first hour was observed (Fig. 5). The burst release can be explained for example by manufacturing conditions and the heterogeneity of polymer matrices as discussed by Huang and Brazel [34]. The steady-state propranolol HCl release rate from poly(ester anhydride) discoids varied from 2.10%/h (60% w/w)

Table 1
In vitro erosion of and propranolol HCl release from poly(ester anhydride) discoids.

Drug loading degree (% w/w)	Linear release period (h)	R ² -value of release	Release rate ^a %/h (mean ± SEM)	Linear erosion period (h)	R ² -value of erosion	Erosion rate ^a %/h (mean ± SEM)	R ² correlation ^b
0	N/A	N/A	N/A	0–48	0.987	2.04 ± 0.04	N/A
10	8–28	0.990	3.32 ± 0.13	8–28	0.970	3.77 ± 0.12	0.973
20	8–32	0.990	3.16 ± 0.05	8–30	0.961	4.05 ± 0.13	0.982
40	8–36	0.980	2.68 ± 0.10	8–35	0.990	3.23 ± 0.06	0.995
60	1–36	0.987	2.10 ± 0.18	4–35	0.965	2.61 ± 0.17	0.988

^a The release and erosion rates are calculated from the reported linear release ($n=4$) and erosion ($n=3$) periods.

^b Correlation between the drug release rate and the erosion rate (Pearson's correlation test).

to 3.32%/h (10% w/w) (Table 1). The drug release profiles were found to be statistically ($p>0.05$) similar when the drug loading degrees were 10–40% w/w. Statistically significant differences in propranolol HCl release were detected between the 10 and 60% w/w loaded discoids at 1 and 8 h and between 20 and 60% w/w loaded discoids at 1, 6 and 8 h. Thus, the drug loading degree (10–40% w/w) could be varied extensively without affecting the drug release rate.

In order to study if poly(ester anhydride) *in vitro* erosion and the resulting drug release kinetics are similar, Pearson's product moment correlation was determined (Table 1, Fig. 6). High R²-values (>0.973) were found for all the studied drug loaded (10–60% w/w) poly(ester anhydride) discoids confirming erosion controlled drug release from the poly(ester anhydride).

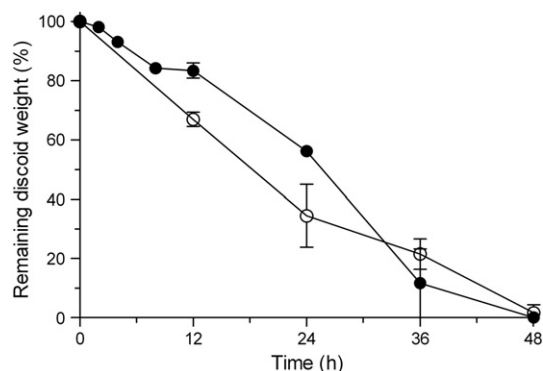


Fig. 4. Erosion of drug-free poly(ester anhydride) discoids *in vitro* (●) and *in vivo* (rat, s. c.) (○). Mean ± SEM, $n=3$. Statistically significant ($p<0.05$) differences were found at 12 h and 24 h.

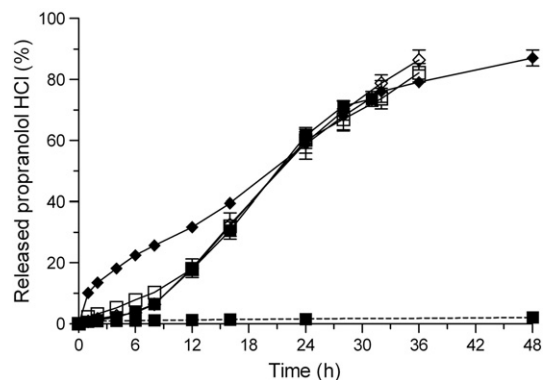


Fig. 5. Drug release from poly(ester anhydride) discoids (solid line) and corresponding polyester discoids without anhydride bonds (dashed line) *in vitro* (pH 7.4, +37 °C). Drug loading degrees: ■ 10%, ◇ 20%, □ 40% and ◆ 60% propranolol HCl. Mean ± SEM, $n=4$. Statistically significant ($p<0.05$) differences were found between the drug loading degrees of 10 and 60% w/w (from 1 to 8 h), and 20 and 60% w/w (at 1, 6 and 8 h).

In order to clarify the effect of the anhydride bonds on the release, the drug release from corresponding polyester discoids without anhydride bonds was studied. Fig. 5 clearly indicates the importance of anhydride bonds in the drug release as very little propranolol HCl was released from 10% w/w loaded polyester discoids within 48 h. In contrast to the poly(ester anhydride) discoids, the drug release rates from the polyester discoids increased with increasing drug loading degrees. Drug release rates were 0.04, 0.10 and 0.38%/h from 10, 40 and 60% w/w propranolol HCl loaded discoids in the first 48 h of the drug release.

In vivo (s.c., rats), propranolol HCl release from poly(ester anhydride) discoids (40% w/w) exhibited a linear drug release (R²0.953) between 12 and 48 h (Fig. 7). However, the drug release rate of the linear release phase was lower *in vivo* (1.66%/h) than *in vitro* (2.68%/h). The *in vivo* drug release (Fig. 7) is consistent with the *in vivo* erosion (Fig. 4) both lasting approximately 48 h, indicating surface-erosion controlled drug release.

3.3. Safety evaluation of poly(ester anhydride) *in vitro* and *in vivo*

In vitro cytotoxicity of oligomers with different functionalities (hydroxyl terminated, carboxyl acid terminated and methacrylated) were evaluated by the AlamarBlue test which is based on the reduction of resazurin to resofurin in the cytosol of the living cells [35]. Unreacted methacrylated poly(ester anhydride) oligomers are the starting material of the photocrosslinking and during *in situ* polymerization their leaching could possibly cause toxicity. Carboxyl acid terminated oligomers are degradation products of crosslinked poly(ester anhydride) networks, and thus also their toxicity needs to be assessed. These oligomers were compared with the hydroxyl terminated oligomer since it is approved by the US Food and Drug

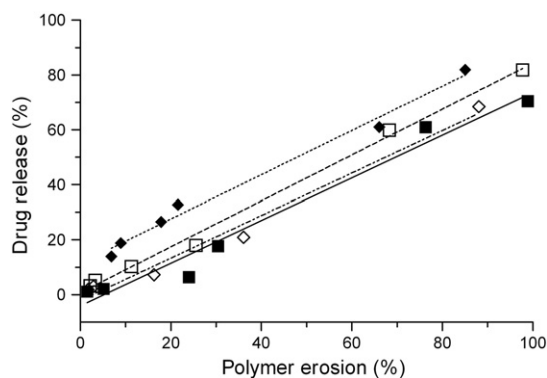


Fig. 6. The clear correlation between the erosion of and the drug release from poly(ester anhydride) discoids *in vitro* (pH 7.4, +37 °C) was observed. Only mean values are shown for the sake of clarity. Drug loading degrees: ■ 10% (solid line), ◇ 20% (dotted-dashed line), □ 40% (dashed line), and ◆ 60% (dotted line) propranolol HCl.

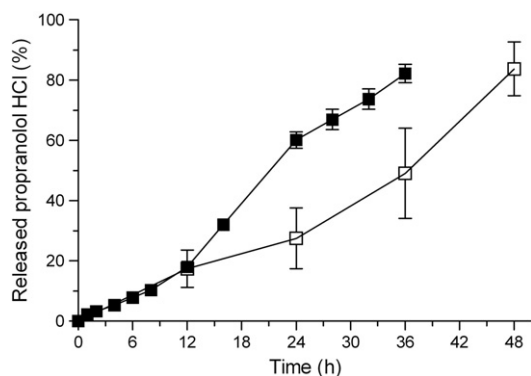


Fig. 7. Both *in vitro* (pH 7.4, +37 °C) (■) and *in vivo* (rat, s.c.) (□) drug release from poly(ester anhydride) discs loaded with 40% propranolol HCl followed closely linear kinetics. Mean ± SEM, $n = 3-4$.

Administration for clinical use in medical and drug delivery devices [36]. Human gingival fibroblasts were chosen as the model cell line since, in addition to the controlled drug release applications, poly(ester anhydride) networks could be also used in tissue engineering applications [29].

All of the studied oligomers were non-cytotoxic at the level of their solubility (Fig. 8). Cell activities with oligomer concentrations below 50 µg/ml were in the range of 85%–100% (Fig. 8), and no statistically significant differences were found between the acetone-treated control cells and oligomer-treated cells. At concentrations above 100 µg/ml, the oligomers precipitated on the cells in aqueous solutions. These precipitates dose-dependently decreased cell activities (data not shown), as they hindered the cellular transport of gases and nutrients affecting viability. These results are in the agreement with earlier publications showing that the crosslinked networks formed from acrylated and methacrylated oligomers are generally well tolerated [37,38] even though there have been some reports of *in vitro* cytotoxicity of the methacrylated and dimethacrylated oligomers [39].

The *in vivo* safety of poly(ester anhydride) networks was evaluated in rats by measuring cytokine secretion after the subcutaneous implantation of poly(ester anhydride) discs. Cytokines are cell signaling proteins released in many pathological conditions e.g. infection and inflammation and produced in response to microbes and other antigens. The introduction of foreign materials, such as polymer implants, into the body evokes tissue injury and subsequently inflammation and wound healing [40,41]. After the implantation of biomaterials, cytokines are secreted by many cells, such as macrophages or neutrophils, promoting inflammation and wound healing processes [42]. Thus, plasma concentrations of cytokines can be

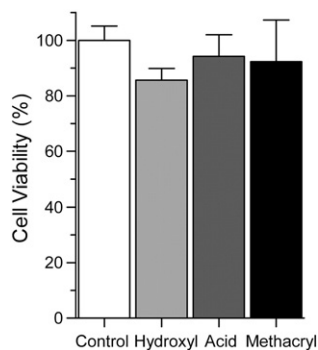


Fig. 8. Cell viability after incubating 50 µg/ml either hydroxyl terminated, carboxyl acid terminated, or methacrylated oligomers with human gingival fibroblasts for 24 h (mean ± SD, $n = 6$). No statistical differences ($p < 0.05$) were found between groups. Acetone-treated cells were used as a control group.

considered as markers of immunogenic responses to the polymer materials [41–45].

In this study, the rat plasma concentrations of nine cytokines were measured 12, 24, 36, 48 and 120 h after the subcutaneous implantation of drug-free poly(ester anhydride) discs (Table 2). TNF-α levels were not included in the Table 2 since the majority of the measurements were below the detection limit (1.95 pg/ml). The cytokine levels of the control group (NaCl 0.9%, $n = 5$) were comparable to the poly(ester anhydride) group ($n = 5$). The only exceptions were the unexpectedly lower IL-1α levels at 120 h and the lower IFN-γ levels at 24 h after the implantation of poly(ester anhydride) discs. Based on the present results, the studied photocrosslinked poly(ester anhydride) network did not provoke any major immunogenic responses. These results are in concordance with previously published safety studies of thermoplastic poly(ester anhydride) implants and injectable pastes that have indicated no or only minor inflammatory reactions in mice and rats [13,46,47].

Table 2

Poly(ester anhydride) discs (s.c., 200 mg/kg) did not induce plasma cytokine levels in rats when compared with 0.9% NaCl administration.

Hours after administration	Cytokine	Concentration in plasma (pg/ml) (mean ± SEM, $n = 5$)	
		NaCl 0.9%	Polymer
12	IL-1α	190 ± 60	117 ± 41
		132 ± 21	130 ± 47
		311 ± 109	306 ± 69
		212 ± 33	155 ± 43
		471 ± 78***	74 ± 30***
12	IL-1β	137 ± 53	186 ± 29
		287 ± 100	247 ± 64
		478 ± 330	198 ± 51
		1469 ± 483	869 ± 420
		225 ± 58	102 ± 27
12	IL-2	256 ± 130	74 ± 27
		166 ± 64	96 ± 33
		174 ± 56	153 ± 73
		358 ± 251	185 ± 82
		807 ± 551	116 ± 23
12	IL-4	58 ± 23	23 ± 10
		46 ± 14	22 ± 10
		49 ± 14	38 ± 17
		81 ± 49	46 ± 21
		152 ± 88	33 ± 10
12	IL-6	398 ± 132	143 ± 41
		206 ± 69	188 ± 54
		221 ± 61	203 ± 90
		411 ± 264	291 ± 93
		709 ± 483	158 ± 17
12	IL-10	113 ± 38	50 ± 10
		71 ± 26	53 ± 16
		79 ± 23	84 ± 26
		103 ± 35	101 ± 22
		138 ± 48	59 ± 13
12	GM-CSF	52 ± 21	21 ± 8
		44 ± 14	18 ± 6
		43 ± 14	33 ± 13
		65 ± 38	37 ± 15
		123 ± 68	27 ± 7
12	IFN-γ	381 ± 131	116 ± 42
		678 ± 246**	76 ± 28**
		490 ± 270	81 ± 8
		279 ± 137	88 ± 34
		573 ± 190	142 ± 68

*** $p < 0.001$, ** $p < 0.01$, Polymer vs. NaCl 0.9%.

4. Conclusions

In vivo functionality and biocompatibility of the photocrosslinked poly(ester anhydride) network were demonstrated. The present results indicate that poly(ester anhydride)s enable a high drug loading degree (up to 60% w/w), and are capable of surface erosion controlled drug release both *in vitro* and *in vivo*. *In vitro* cytotoxicity and *in vivo* cytokine secretion experiments indicated that the polymer is safe for use in drug delivery systems.

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