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Research paper

Molecular weight-dependent degradation and drug release of surface-eroding poly(ethylene carbonate)

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ABSTRACT

Poly(ethylene carbonate) (PEC) is a unique biomaterial showing significant potential for controlled drug delivery applications. The current study investigated the impact of the molecular weight on the biological performance of drug-loaded PEC films.

Following the preparation and thorough physicochemical characterization of diverse PEC (molecular weights: 85, 110, 133, 174 and 196 kDa), the degradation and drug release behavior of rifampicin- and bovine serum albumin-loaded PEC films was investigated *in vitro* (in the presence and absence of cholesterol esterase), in cell culture (RAW264.7 macrophages) and *in vivo* (subcutaneous implantation in rats). All investigated samples degraded by means of surface erosion (mass loss, but constant molecular weight), which was accompanied by a predictable, erosion-controlled drug release pattern. Accordingly, the obtained *in vitro* degradation half-lives correlated well with the observed *in vitro* half-times of drug delivery (R^2 = 0.96). Here, the PEC of the highest molecular weight resulted in the fastest degradation/drug release. When incubated with macrophages or implanted in animals, the degradation rate of PEC films superimposed the results of *in vitro* incubations with cholesterol esterase. Interestingly, SEM analysis indicated a distinct surface erosion process for enzyme-, macrophage- and *in vivo*-treated polymer films in a molecular weight-dependent manner.

Overall, the molecular weight of surface-eroding PEC was identified as an essential parameter to control the spatial and temporal on-demand degradation and drug release from the employed delivery system.

1. Introduction

Advances in the field of chemical engineering and the biomedical sciences have made polymer drug carriers available, which allow for modification of the delivery rate of the embedded therapeutic agent [1]. The drug release may occur constantly over an extended period of time, typically by means of polymer degradation and/or diffusion through the polymer matrix, with the aim of obtaining constant plasma levels, reducing side effects and prolonging dosing intervals [2]. Biodegradable polyesters such as poly (lactide-*co*-glycolide) have been most-frequently utilized as matrix materials for controlled drug delivery devices [3,4]. However, the hydrolytic degradation behavior (i.e., bulk erosion [5], formation of acidic microclimate within the drug carrier [6]) often results in a rather uncontrolled drug release profile [7] and chemical instability for numerous therapeutic agents [8,9].

In contrast, polymers that degrade solely by surface erosion have demonstrated more predictable drug release kinetics [10]. Biodegradable poly(ethylene carbonate) (PEC) is a promising example of such a polymer [11]. Diverse PEC-based drug delivery vehicles have been shown to degrade from the surface *in vitro* and *in vivo* [11–13], resulting in a favorable drug release profile [14,15]. Furthermore, the degradation of PEC is triggered by specific enzymes and cells [16,17] making this type of polymer especially attractive for on-demand drug release to desired sites within the body [18]. Despite the significant potential of PEC for controlled drug delivery applications, only scant information is available describing the biomedical performance (i.e., biodegradation and drug release) of drug delivery vehicles composed of PEC of different molecular weight [19].

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Nomenclature

Abbreviations

BSA	bovine serum albumin
$Đ$	dispersity
DSC	differential scanning calorimetry
$[\eta]$	intrinsic viscosity
η_{sp}	specific viscosity
FITC	fluoresceine isothiocyanate
GPC	gel permeation chromatography
M_w	weight-average molecular weight
PBS	phosphate-buffered saline
PEC	poly(ethylene carbonate)
SD	standard deviation
SEM	scanning electron microscopy
$t_{1/2}$	half-time/half-life
T_g	glass transition temperature
wt.%	weight percent
XRPD	X-ray powder diffraction

In this respect, the current study aimed to close this gap by investigating the impact of the polymer molecular weight on the degradation of and drug release rate from surface-eroding PEC films in detail. Polymers and polymer films either loaded with rifampicin or fluorescently-labelled bovine serum albumin (BSA) were thoroughly characterized before studying their *in vitro* degradation and drug release behavior in the absence and presence of the enzyme cholesterol esterase. Degradation tests in cell culture (i.e., RAW264.7 macrophages) and *in vivo* (i.e., subcutaneous implantation in rats) underlined the unique potential of PEC for prolonged drug delivery applications.

2. Materials and methods

2.1. Materials

PEC, QPAC®25 was purchased from Empower Materials (USA). PEC polymers of lower molecular weight were obtained by thermal hydrolysis in boiling water (Fig. S1, Supplementary materials) [16,17,19]. Rifampicin (≥97%), fluorescein isothiocyanate (FITC)-labeled BSA (≥7 mol FITC/mol BSA) and cholesterol esterase (from porcine pancreas) were acquired from Sigma-Aldrich (UK). All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Gel permeation chromatography (GPC)

Samples were dissolved in chloroform (3–4 mg/ml). After filtration (0.2 μm; Acrodisc®, Pall, Germany), 100 μl of the sample solution was injected into the system, consisting of two columns from Polymer Laboratories (PL-gel MIXED-D; 300 × 7.5 mm; bead diameter 5 μm) and a differential refractive index detector (SpectraSystem RI-150, Thermo Electron Corp., USA). The elution was performed with chloroform at a flow rate of 1 ml/min and toluene as flow-rate marker. Poly(methyl methacrylate) standards of known molar masses were used for calibration.

2.3. Viscosity measurements

The viscosity of PEC samples dissolved in chloroform was measured using a capillary viscometer of the Ubbelohde Semi-Micro dilution type (No. 50, N213, CANNON Instrument Company, USA) at 25.0 ± 0.2 °C. Values for the specific (η_{sp}) and intrinsic viscosity ($[\eta]$) were calculated using standard equations.

2.4. Differential scanning calorimetry (DSC)

The glass transition temperature (T_g) of polymers and polymer films was determined using a differential scanning calorimeter (Discovery DSC, TA Instruments, Denmark). Samples (~3–5 mg) were scanned at a rate of 10 °C/min from −40 °C to 190 °C under a nitrogen atmosphere.

2.5. X-ray powder diffraction (XRPD)

Wide-angle XRPD patterns were recorded on a X'Pert PROMPD X-ray diffractometer (PANalytical, The Netherlands). Samples were measured in Bragg Brentano reflection mode in the 2θ range of 5-37° using a PIXel detector (step size of 0.039°). The X-ray source was Ni-filtered $CuK_{\alpha 1}$ radiation (λ = 1.541 Å). The operating current and voltage were 40 mA and 45 kV, respectively. The aluminum sample holder was spun throughout data collection to avoid orientation artifacts.

2.6. Scanning electron microscopy (SEM)

Samples were placed on double-sided carbon tape, mounted on stubs and sputter coated with a 5 nm layer of gold using a Leica EM ACE200 (Germany) prior to sample imaging. The images were acquired with an FEI/Philips XL30 FEG (USA) at an acceleration voltage of 2 kV using the secondary electron detector.

2.7. Preparation of polymer films

PEC films were fabricated by a solvent casting technique [20,21]. Therefore, PEC without or with added rifampicin (10 wt.% per polymer mass) were accurately weighed and then dissolved in chloroform at a concentration of 100 mg/ml. For films loaded with BSA, PEC was first dissolved in chloroform (100 mg/ml) containing Span® 80 (1 mg/ml). BSA was dissolved in water (10 mg/ml) in parallel before mixing the two solutions (ratio of 9/1 (v/v)) using a homogenizer (T25 Ultra-Turrax®, IKA, Germany). Film solutions (500 μl) were then transferred to custom-made Teflon® dishes (diameter: 10 mm, height: 3 mm) and the organic solvent was allowed to evaporate under a fume hood overnight. The films were then cut into discs, and dried under reduced pressure (~0.1 mbar).

2.8. Enzymatic polymer degradation

Polymer film samples (~25 mg) were transferred to 2 ml of phosphate-buffered saline (PBS, pH 7.4) without or with added cholesterol esterase (0.1 mg/ml). The films were incubated at 37 °C with shaking (Rotatherm®, Gebr. Liebisch, Germany). The medium was exchanged every 3–4 d. At predetermined time points the incubation was terminated by removal of the supernatant. Polymer films were washed several times with distilled water and subsequently freeze-dried (Beta I, Christ, Germany) overnight. The remaining film mass and molecular weight was determined gravimetrically (BP 211 D, Sartorius, Germany) and by GPC as described above, respectively.