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Rapid communication

Feasibility of macrophage mediated on-demand drug release from surface eroding poly(ethylene carbonate)

Dafeng Chu[a,*], Moritz Beck-Broichsitter[a], Catherine Curdy[b], Bernd Riebesehl[b], Thomas Kissel[a]

[a] Department of Pharmaceutics and Biopharmacy, Philipps-Universität, Ketzerbach 63, Marburg D-35032, Germany
[b] Novartis Pharma AG, Novartis Campus, Basel CH-4056, Switzerland

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ABSTRACT

Macrophage induced surface degradation of poly(ethylene carbonate) (PEC) was investigated under in vitro conditions. Degradation of PEC with the MW of 41 kDa (PEC41) was slower than that of PEC with the MW of 200 kDa (PEC200). In terms of macrophage mediated drug release from PEC matrix, in cell-free medium, less than 1% of levofloxacin was released from both PEC samples in 10 days, while more than 60 and 20% of the drug, levofloxacin, can be detected in medium with macrophages from PEC200 and PEC41 films, respectively. This work indicated that on-demand drug delivery induced by macrophages can be achieved with PEC polymer.

Mononuclear phagocytes (MPs) such as macrophages are widely involved various diseases such as rheumatoid arthritis, asthma, atherosclerosis, inflammatory bowel disease, cancer and would infection (Duffield, 2003; Boateng et al., 2008). Therefore, MPs mediated drug delivery is highly attractive and studies on targeted drug release to MPs have been conducted (Batrakova et al., 2011). However, on-demand drug release at the site of disease upon contact with MPs was rarely reported.

In the treatment of disease, to maximize efficiency of therapy and minimize side effects, it is critical to ensure that drug is released only when needed. Biocompatible and biodegradable poly(ethylene carbonate) (PEC) is an unique polymer and would provide such an opportunity (Stoll et al., 2001; Unger et al., 2007; Acemoglu, 2004). Different from bulk eroding polymers, such as PLGA and PCL, PEC does not degrade in the pH range of 1–12. Most importantly, the in vivo biodegradation of PEC films in membrane cages was extremely suppressed in comparison to free samples after subcutaneous implantation (Stoll et al., 2001). Thus, it was postulated that PEC was specifically degraded by immune competent cells (Unger et al., 2007), although no direct evidence was obtained from in vitro cell culture study.

In this study, by taking all this knowledge into account, the feasibility of macrophage-mediated degradation of PEC and drug release by this mechanism was investigated under in vitro conditions.

Poly(ethylene carbonate) of 200 kDa (PEC200) was a gift from Novartis Pharma AG (Basel, Switzerland). Poly(ethylene carbonate) of 41 kDa (PEC41) was produced by a thermal hydrolysis method (Inoue et al., 1975).

J774A.1 macrophages were maintained in DMEM containing 4.5 g/L of D-glucose, sodium pyruvate, L-glutamine, 10% of fetal bovine serum and 1% of antibiotic-antimycotics solution (v/v) (PAA, Cölbe, Germany). Cells were passaged every 3–4 days via scraping. The seeding density was approximately 3.6×10^4 cells/cm^2 and the volume of medium used was 1 mL.

PEC films were prepared at 20 bars and 140 °C using the Schwabenthan Polystat 200 T laboratory press (Berlin, Germany) with a 1 mm thick stainless steel mold for 10 min. PEC200 and PEC41 films were divided into three groups ($n = 3$ for each group), respectively. In groups one and two, macrophages were directly cultured on the surface of films. Group three was performed as control experiment by conditioning the films in the cell culture medium only. After 10 days of culture, group one was placed in 1% aqueous Triton X-100 detergent for 60 min to lyse the cells, and then thoroughly rinsed in distilled water. Group two was fixed with 2.5% para-formaldehyde for 8 h, and later dried with

* Corresponding author. Present address: Department of Chemical Engineering, University of Waterloo, 200 University Avenue West, N2L 3G1, Waterloo, Ontario, Canada. Tel.: +1 519 8884567x36864; fax: +1 519 884 8009.
E-mail addresses: d5chu@uwaterloo.ca, chudafeng@gmail.com (D. Chu).

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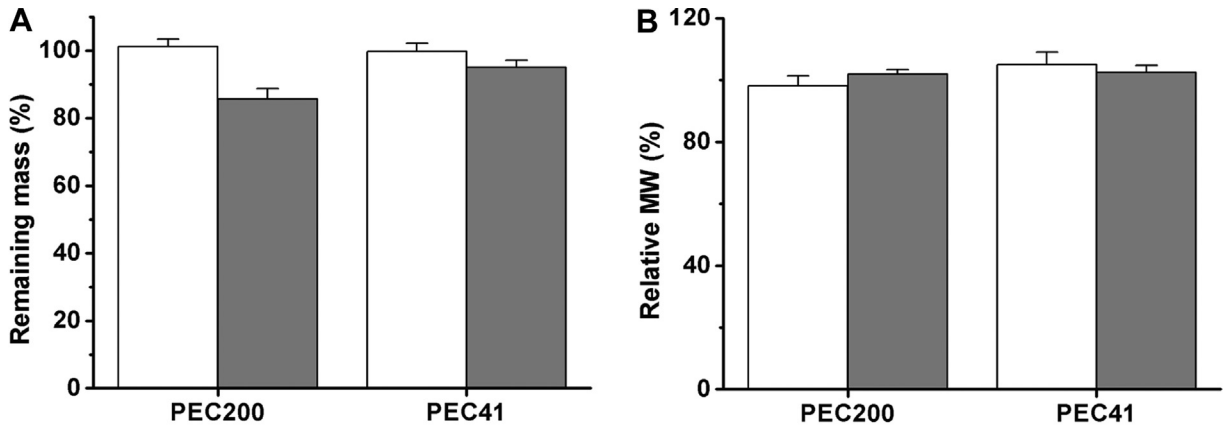

Fig. 1. Remaining mass (A) and change in MW (B) of PEC200 and PEC41 after 10 days with (gray) and without macrophages (white). Values are presented as mean ± SD ($n = 3$).

alcohol. Group three was only rinsed with distilled water. Surfaces and cross-sections of polymer films were imaged on a JEOL JSM-7500F Cryo-SEM (Tokyo, Japan).

Levofloxacin (Sigma–Aldrich, St. Louis, USA) and PEC200 or PEC41 (1: 99, w/w) was dissolved in dichloromethane (10%, w/v) and cast over the bottom of a glass Petri dish, which was placed for 24 h to evaporate the solvent slowly and then dried in vacuum. Macrophages were directly cultured on the surface of films ($n = 3$). The films of control group were only incubated in the cell culture medium. 0.8 mL of the medium was replaced with the fresh one every 2 days. Afterwards, 0.2 mL of acetonitrile was added to 0.2 mL of the medium to precipitate proteins. The supernatant was subject to HPLC analysis (Gao et al., 2007).

As shown in Fig. 1A, after 10 days of macrophage culturing, the degradation of PEC was evident with mass loss of 14 and 5% for PEC200 and PEC41, respectively. The faster degradation of PEC with high MW was similar with the results of in vivo degradation study (Acemoglu et al., 1997). No degradation occurred in cell culture medium without macrophages. The MW remained unchanged for all PEC samples in presence of macrophages and cell-free medium (Fig. 1B). Surface and cross-section analysis of PEC200 films after macrophage detachment from the sample surfaces revealed that

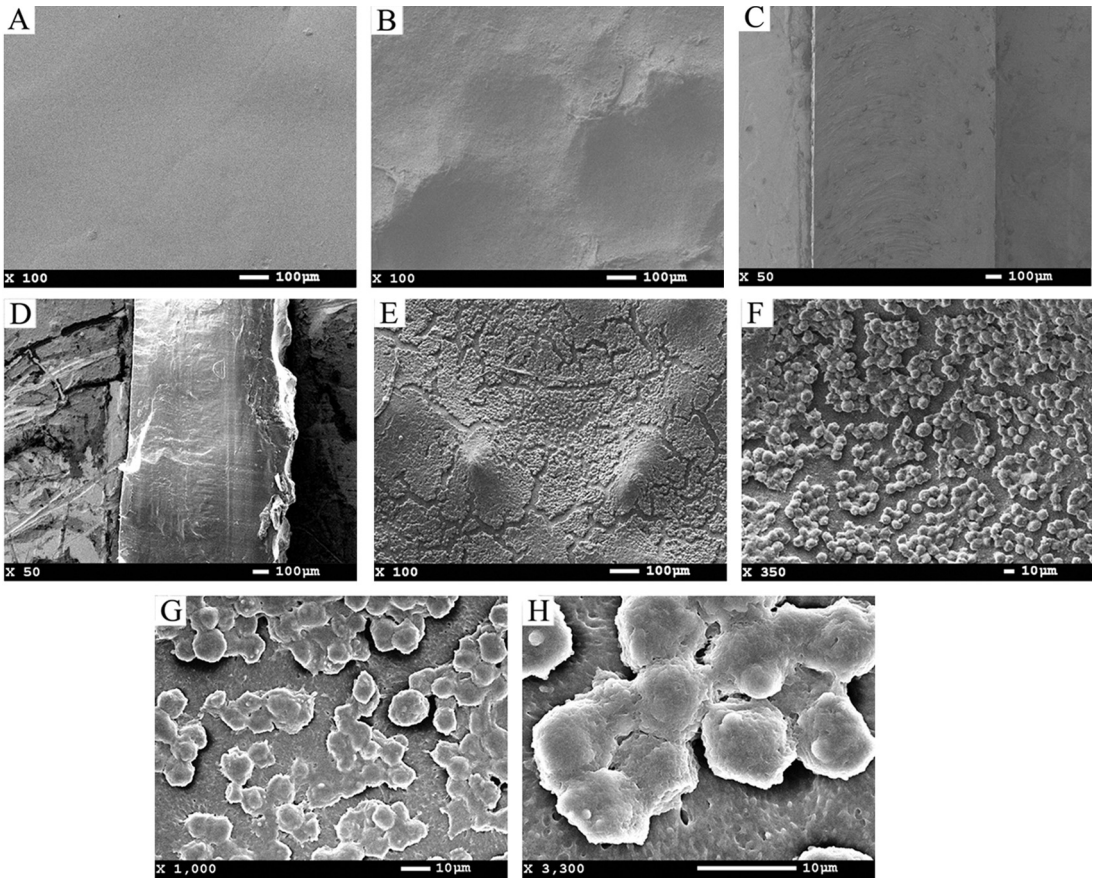

Fig. 2. SEM micrographs of 200 kDa PEC films after 10 days of macrophage culturing: surface (A) and cross-section (C) at day 0; surface (B) and cross-section (D) after the removal of macrophages and surface with macrophages (E)–(H).