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Cast adhesive polyelectrolyte complex particle films of unmodified or maltose-modified poly (ethyleneimine) and cellulose sulphate: fabrication, film stability and retarded release of zoledronate

Bernhard Torger, David Vehlow, Birgit Urban, Samaa Salem, Dietmar Appelhans and Martin Müller*

Abstract

The bone therapeutic drug zoledronate (ZOL) was loaded at and released by polyelectrolyte complex (PEC) particle films composed of either pure poly(ethyleneimine) (PEI) or maltose-modified poly(ethyleneimine) (PEI-M) and oppositely charged cellulose sulfate attached to model germanium (Ge) substrates by solution casting. Dispersions of colloidally stable polyelectrolyte complex (PEC) particles in the size range 11–141 nm were obtained by mixing PEI or PEI-M, CS and ZOL in defined stoichiometric ratios. TRANS-FTIR spectroscopy was used to determine the stability of the PEC films against detachment, in-situ-ATR-FTIR spectroscopy for the ZOL loss in the PEC film and UV–VIS spectroscopy for the ZOL enrichment of the release medium. Films of casted ZOL/CS/PEI-M or ZOL/CS/PEI particles were stable in contact to water, while films of the pure drug (ZOL) and of the binary systems ZOL/PEI-M or ZOL/PEI were not stable against detachment. Retarded releases of ZOL from various PEC films compared to the pure drug film were observed. The molecular weight of PEI showed a considerable effect on the initial burst (IB) of ZOL. No significant effect of the maltose modification of PEI-25 K on IB could be found. Generally, after one day the ZOL release process was finished for all measured ZOL/PEC samples and residual amounts of 0-30% were obtained. Surface adhesive drug loaded PEC particles are promising drug delivery systems to supply and release a defined amount of bone therapeutics and to functionalize bone substitution materials.

Keywords: Bone healing; Interfacial drug delivery system; Polyelectrolyte complex particle; Poly(ethyleneimine); Zoledronate; In-situ ATR-FTIR spectroscopy

Background

Functionalization of bone substitution materials (BSM) by local interfacial delivery systems for bone therapeutic drugs is a highly relevant strategy to improve bone healing after fractions associated with systemic bone diseases like osteoporosis or multiple myeloma [1]. Bisphosphonates (BP), especially nitrogen containing ones, are current drugs of choice for the therapy of osteoporosis [2]. Known examples of drug delivery systems (DDS) for BPs are based on calcium phosphate phases [3], sol–gel derived titania systems [4], acrylic bone cements [5], bisphosphonate/fatty acid salt mixtures [6] and poly(D, L-lactide) coatings [7]. Target of this study is to develop an adhesive nanoparticular bisphosphonate delivery system based on biocompatible polyelectrolyte complexes. Typical release parameters like initial burst, residual amount and release rate of the drug are intended to be influenced by macromolecular structure parameters, in a defined way for future use by cell biologists and clinicians.

Recently, in this frame we reported on our approach making use of polyelectrolyte (PEL) complex (PEC) nanoparticles, that were loaded by the BP pamidronate (PAM) and deposited as adhesive films onto planar Ge model substrates [8]. BPs are known to inhibit

* Correspondence: mamuller@ipfdd.de
Leibniz Institute of Polymer Research (IPF) Dresden, Hohe Straße 6, D-01069 Dresden, Germany

osteoclastic activity (bone resorption) via the farnesyl pathway favoring osteoblastic bone formation (remodeling) and are widely used as therapeutics for systemic bone diseases like osteoporosis [2]. A retarded release of PAM under conservation and adhesive stability of the bare PEC particle film was shown by in situ ATR-FTIR spectroscopy, monitoring the depletion of PAM in the cast PEC film matrix. A PEC system based on high molecular poly(ethyleneimine) (PEI-750 K) and cellulose sulfate (CS) was used and various factors of PAM release were studied. Generally, with this system drug releases were obtained, which were much more retarded compared to releases from or dissolution of the pure drug film.

Herein we like to follow the former work and apply both the introduced preparative and analytical concept on PEC systems involving cationic PEI of different molecular weights (1.3 kg/mol), 25 kg/mol, 750 kg/mol), cationic maltose modified PEI (PEI-M) and anionic CS. The PEI/CS system was chosen because of the high functional group number and density of both ammonium groups (PEI) and sulfate groups (CS) and the availability of both PELs. Furthermore, especially for branched/linear PEL combinations like PEI/CS high structural densities enabling better drug entrapment are expected. Moreover recent studies revealed, that among several compositions the combination PEI/CS resulted in a very benign interaction to human mesenchyme stromal cells (hMSC), which was evidenced by metabolic activity and fluorescence imaging studies recently [9]. This is conflicting to studies revealing, significant cell toxicity of uncomplexed PEI [10]. Therefore, PEC systems composed of PEI-M are expected to provide an enhanced biocompatibility compared to unmodified PEI. PEI-M systems have been established in the last years as potent carrier systems for model drugs ATP [11,12], si-RNA and DNA [9,13] and show a benign interaction to different relevant cell types [9,10]. Herein, zoledronate (ZOL) was chosen, since it is to date an established BP of choice for the systemic therapy of osteoporosis [14] and multiple myeloma [15], which is more potent compared to PAM. However, BPs like PAM and ZOL are suspected for certain side effects like accumulation in the bone matrix due to the strong BP/calcium interaction leading to osteonecrosis [16] and like insufficient renal clearance [17]. Therefore, the validation of a local, sustainable, retarded release of a limited small amount of ZOL from PEC films at model interfaces or BSM is a highly relevant task. In this report we address and point out colloidal properties, adhesiveness and retarded release of ZOL loaded PEC particle systems based on PEI or PEI-M and CS under variation of PEI molecular weight and the PEC particle net charge sign.

Methods

Chemicals

Zoledronic acid was obtained from Chemos GmbH (Regenstauf, Germany) and zoledronate (ZOL) solutions were prepared by dissolving in 0.0002 M PBS buffer and adjusting pH = 7.3 by addition of 0.1 M NaOH. Branched poly(ethyleneimine) with molecular weights of 1.3 kg/Mol (PEI-1.3 K), 25 kg/Mol (PEI-25 K) and of 750 kg/Mol (PEI-750 K) (gift of BASF, Ludwigshafen) and cellulose sulphate (CS, 2.900 kg/Mol, degree of substitution DS = 2.9-2.8, ACROS) were dissolved to 0.002 M solutions related to their monomer units. PEI-M-25 K was synthesized according to a protocol published therein [11]. Macromolecular structures are given in the Figure 1.

Since for PEI-M-25 K, unlike PEI-1.3 K, PEI-25 K, PEI-750 K and CS, no defined molecular weight of the repeating unit is available, 0.5 mg/ml PEI-M-25 K solutions were prepared. To get the concentration of truly charged monomer units of these polyelectrolytes (PEL), colloid titration (PCD) was applied (see below). Respective factors for PEI-1.3 K, PEI-25 K, PEI-750 K and CS were F = 0.67, 0.54, 0.70 and 2.9 L5, respectively, related to the volume ratios of 0.001 M PEL and 0.001 M titrator solution (see PCD measurements). For 0.5 mg/ml PEI-M a respective factor F = 0.90 was obtained. PBS buffer was prepared from respective buffer tablets. Ge internal reflection elements (IRE) were obtained from Komlas GmbH, Berlin.

Preparation of drug loaded polyelectrolyte complex (PEC) particles

For PEI and CS solutions of 0.002 M and for PEI-M solutions of 0.5 mg/ml were prepared in 0.0002 M PBS buffer, respectively. This unusually low PBS concentration was applied for analytical reasons concerning bisphosphonate detection by ATR-FTR spectroscopy (see below). For the preparation of binary ZOL/polycation samples defined volumes (1–5 ml) of polycation (PC: PEI or PEI-M) solutions were incubated with varying volumes of a 0.01 M ZOL in 0.0002 M PBS buffer solution under stirring to get stoichiometric ratios ZOL/PC of 1:5 related to the molar concentration of ZOL molecules and charged monomer units of PC. For the preparation of ternary ZOL/CS/PC samples defined volumes of 0.002 M CS solutions were mixed slowly to the binary ZOL/PC solutions under stirring to get stoichiometric ratios CS/PC of either n-/n + = 0.9 or n-/n + = 1.1 related to the molar concentration of charged monomer units of CS and PC. For the CS/PEI combination such prepared ternary PEC samples are denoted as ZOL/CS/PEI-0.9-1:5 or ZOL/CS/PEI-1.1-1:5. For CS/PEI-0.9 dispersions CS (minority) was dosed into PEI (majority), while for CS/PEI-1.1 dispersions PEI (minority) was dosed into CS (majority) solutions, whereby in both cases at first ZOL solutions were given

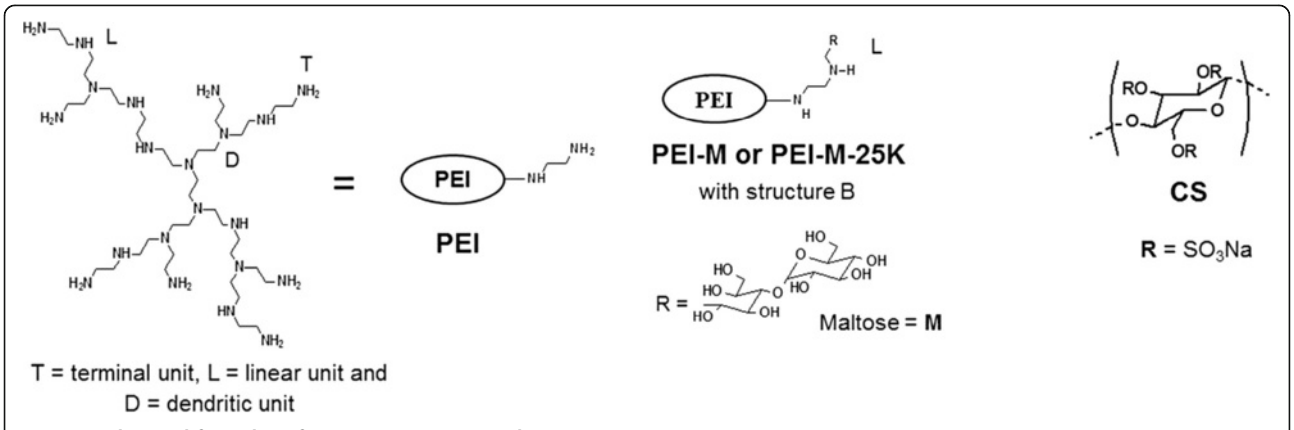

Figure 1 Chemical formulae of PEI-XK, PEI-M-25 K and CS.

to PEI solutions. Finally, 10 microliter of 0.01 M $CaCl_2$ solution were added to 200 microliter of the ZOL/PEI/CS dispersions.

Deposition of drug loaded PEC particles and drug release

3 × 16.6 microliter (totally ≈50 microliter) of the respective ZOL/CS/PEI/$CaCl_2$ mixture in 0.2 mM PBS described above were consecutively solution casted onto the Ge IRE to homogeneous films and dried at 50°C in the vacuum oven for 30 min on top of each other, respectively. For the ternary ZOL/CS/PEI-750 K-0.9-1:5 system such a film contains a ZOL mass of ≈ 4.6 μg. ZOL release was initiated by the direct contact of the ZOL/PEC films to 0.1 mg/ml PBS buffer (release medium). ZOL release was measured by in-situ ATR-FTIR spectroscopy (see below). Each sample was measured in the dry state, after 5, 30, 60, 120 and 1200 min in triplicate, respectively.

Colloid titration

Concentrations of charged monomer units of PELs used in this study were determined by the Particle Charge Detector (PCD, Mutek, Herrsching, Germany) according to standard protocols using low molecular 0.001 M PDADMAC or 0.001 M PVS solutions as titrating solutions. In principle sample solutions or dispersions bearing charged species (polymers, particles) are titrated by either PDADMAC or PVS until the zeta-potential measured upon moving a PTFE piston within a PTFE vessel is zero. Herein two titration modes i.e., a slow (titration steps of 10 μl) and a rapid titration mode (titration steps of 50 μl) were used (see titration curves in Additional file 1). The true concentration of charged monomer units in the PEL solutions, whose initial concentration was 0.002 M, related to their both charged and uncharged monomer units, was calculated from the consumed amount of the titrator solution. The true concentration of charged monomer units was always smaller compared to the monomer unit concentration of 0.002 M. The ratio between true charged monomer and monomer concentration can be expressed by the factor F (see above).

Dynamic light scattering (DLS)

ZOL/PEC and PEC particle sizes were determined by dynamic light scattering (DLS) at the Jianke Portable Particle Sizer (Jianke Instruments Co. Ltd, Wuhu, P.R. China) using a scattering angle of 90°. The samples were hold in 10 mm cuvettes with circular bottom. The hydrodynamic radius R_H was estimated using the Stokes-Einstein equation. Intensity weighted DLS data were considered, as it was applied earlier therein [18]. Errors of R_H are related to the standard deviation of at least three different measurements. The ALV-5000/E/EPP-Software of ALV GmbH, Langen, Germany was used for calculations of DLS parameters.

Ex-situ TRANS-FTIR spectroscopy

TRANS-FTIR was applied using the Ge IRE taken for the in-situ-ATR-FTIR spectra. These Ge IREs were mounted at standard sample holders and the IR beam transmitted the Ge-IRE at its focus. FTIR spectra were recorded taking uncoated Ge-IREs as reference and coated ones as sample. The coated ones were measured in the initial dry state (t = 0 min) and after contact to water for defined release times (t = 1, 60, 1200 min) and subsequent drying. After actual measurements the dry coated Ge-IREs were again contacted to the release medium for a defined period and again taken out and dried for the next measurements. Film stability was checked by the constancy or decrease of diagnostic IR bands of the polymer film matrix.

In-situ ATR-FTIR spectroscopy

ZOL/PEC films deposited onto Ge internal reflection elements (IRE) were characterized by in-situ-ATR-FTIR spectroscopy using the SBSR concept [19] resulting in well compensated ATR-FTIR spectra. A dedicated ATR-FTIR

attachment (OPTISPEC, Zurich, Switzerland) was operated on the IFS 55 Equinox spectrometer (BRUKER Optics GmbH, Ettlingen, Germany) equipped with globar source and MCT detector. 100 scans were collected at spectral resolution of 2 cm^{-1} using Happ-Gänzel apodisation for each recorded spectrum. Quantification of concentrations c at polymer films by ATR-FTIR spectroscopy is based on the absorbance or integral A of a given IR band using a modified Lambert-Beer law according to Fringeli [19] and Harrick [20], which is given in equation (1):

$$A = N\varepsilon c d_E \quad (1)$$

Equation (1) includes the number of reflections N = 11, absorption coefficient ε of the given band and the effective thickness d_E instead of the true sample thickness d in TRANS-FTIR. The value of d_E can be calculated knowing the refractive indices n_1, n_2, n_3 of the IRE, polymer and bulk medium, respectively, depth of penetration d_P and incident angle θ of the IRE. Earlier it has been shown, that band integrals from ATR-FTIR spectra are directly proportional to the molar amount of IR absorbing monomer units or components in polymer films, if the thickness of the polymer film does not exceed around 300 nm (thin film case) [21]. For casted PEC films used in this report this assumption was valid. ZOL release from the PEC films was measured in the dry state (t = 0 min) and after defined times (t = 5, 30, 60, 120, 1200 min) of contact to the release medium in the wet state.

Spectral curve fitting analysis and quantification of actual ZOL content

The diagnostic but highly overlapped ATR-FTIR spectral region between 1320 and 900 cm^{-1} on ZOL/PEC films was analysed by the curve fitting routine of the OPUS software (BRUKER Optics GmbH, Ettlingen, Germany) following a procedure, which was reported recently and therein applied on the pamidronate/PEC system [8].

UV/VIS spectroscopy

UV–VIS spectra were recorded at the spectropolarimeter J810 (JASCO, Großumstadt, Germany), equipped with an air cooled powerful Xenon source (150 W), piezoelastic modulator and head-on photomultiplier tube as detector in the range 163–900 nm, which enabled the detection of very low ZOL concentrations in the release medium.

Scanning force microscopy (SFM)

SFM images were recorded from casted films of the ZOL/PEC and ZOL free PEC particles by the device Nanostation II of Bruker Nano GmbH (Karlsruhe, Germany) using silicon probe tips from Nanosensors (Darmstadt, Germany) having radii of around 10 nm. Dry film samples on the Ge IRE used for ATR-FTIR were measured in "non-contact mode" under room conditions in topography, error and phase mode and scanning parameters were optimized by minimizing the signal in the error mode. The thickness of PEC films was determined at scalpel cutted film regions based on the height difference between undamaged film and naked Ge surface. Surface profiles were generated from SFM raw data by the SISCANPro software (BRUKER Nano GmbH, Karlsruhe, Germany) using SFM images in topography mode.

Results and discussion

Size of PEC particles

Particle sizes of unloaded and ZOL loaded (ZOL/PEC-1:5) complexes of PEI-1.3 K/CS, PEI-25 K/CS, PEI-750 K/CS and PEI-M-25 K-1:5/CS were determined by dynamic light scattering (DLS). Both PEC-0.9 and PEC-1.1 samples were prepared and analysed, respectively. In the Table 1 hydrodynmic radii (R_H), count rates and assumed net charges for these PEC samples are shown. Particle sizes in the range R_H = 11–141 nm were obtained and PEC dispersions showed colloidal stability (only ZOL/CS/PEI-1.3 K-0.9-1:5 tended to colloidal instability) in the sense, that PEC particles do not aggregate at a significant rate.

Obviously, both sizes and count rates for PEC-1.1 particles were in nearly all cases smaller compared to PEC-0.9 particles. Since it is claimed, that PEC particles consist of a neutral charge compensated core and a shell of the respective excess PEL component [22], the branched polycations PEI-1.3 K, PEI-25 K, PEI-M-25 K and especially PEI-750 K are assumed to form a larger shell compared to the linear CS. Loading of ZOL into PEC particles generally enlarged particle sizes with exception of the CS/PEI-25 K-0.9 system.

No distinct molecular weight effect of PEI on the particle size was found for bare (unloaded) PEC-0.9 and PEC-1.1 particles containing unmodified PEI. CS/PEI-1.3 K particles seem to be smallest, but these R_H values are based on low count rate values. This finding is based on the fact, that herein prepared secondary PEC particles are composed of several aggregated primary PEC particles and primary PEC particles are composed of few oppositely charged PEL [22,23]. Hence in PEC particles with low molecular PEI more macromolecules are included but the particle size is around constant. However ZOL loaded PEC-0.9 and PEC-1.1 particles show a molecular weight dependence of particle size. Presumably, high molecular PEI-750 K can uptake or store more ZOL molecules compared to lower molecular PEI-25 K or PEI-1.3 K in their respective complexes with CS.

Stability of PEC particle films

The stability against detachment of the pure ZOL, the binary mixture ZOL/PEI-M and the ternary PEC dispersion

Table 1 Sizes (R_H), count rates and net charge signs for various PEC samples

PEC sample	R_H / [nm]	Count rate /[kHZ]	Net charge sign
CS/PEI-1.3 K-0.9	42 ± 5	19	(+)
CS/PEI-1.3 K-1.1	11 ± 5	4*	(–)
ZOL/CS/PEI-1.3 K-0.9-1:5	416 ± 10**	5*	(+)
ZOL/CS/PEI-1.3 K-1.1-1:5	45 ± 5	72	(–)
CS/PEI-25 K-0.9	73 ± 5	87	(+)
CS/PEI-25 K-1.1	28 ± 5	6	(–)
ZOL/CS/PEI-25 K-0.9-1:5	70 ± 5	119	(+)
ZOL/CS/PEI-25 K-1.1-1:5	62 ± 5	92	(–)
CS/PEI-750 K-0.9	60 ± 10	110	(+)
CS/PEI-750 K-1.1	35 ± 6	20	(–)
ZOL/CS/PEI-750 K-0.9-1:5	105 ± 10	215	(+)
ZOL/CS/PEI-750 K-1.1-1:5	73 ± 5	138	(–)
CS/PEI-M-25 K-0.9	71 ± 5	6	(+)
CS/PEI-M-25 K-1.1	53 ± 5	4*	(–)
ZOL/PEI-M-25 K-0.9-1:5	95 ± 10	25	(+)
ZOL/PEI-M-25 K-1.1-1:5	141 ± 5	11	(–)

*very low countrate **unstable sample (aggregation).

ZOL/CS/PEIM casted onto the Ge model substrate was measured by TRANS-FTIR spectroscopy. In the Figure 2 TRANS-FTIR spectra of solution casted films of pure ZOL (a), ZOL/PEI-M-1:1 (b) and of ZOL/CS/PEI-M-25 K-1.1-1:5 (c) are shown for the initial freshly casted dry state (black) and the end dry state after contact to water for one day and subsequent drying (blue).

The TRANS-FTIR mode was chosen, since it has sensitivity for loss processes through the whole thickness of the ZOL/PEC films regardless if thin or thick. Whereas, the ATR-FTIR mode is more sensitive for loss processes in zones of PEC films close to the substrate, since the evanescent field decays exponentially and losses in outer film regions of thick films are detected with less sensitivity compared to those of thin films (see Experimental). Poor film stability at the Ge substrate was found for the casted pure ZOL solution (a) and the binary mixture ZOL/PEI-M-25 K-1:1 (b), which can be rationalized by the nearly complete vanishing of the initial dry spectrum intensity (black), so that only traces of ZOL or ZOL/PEI-M-25 K-1:1 (blue) were present. Whereas, good film stability was found for the casted ternary PEC dispersion of ZOL/CS/PEI-M-25 K-1.1-1:5 (c), whose film thickness ranged around 200 nm determined by AFM cuts. Obviously, only the ternary ZOL/CS/PEI-M-25 K-1.1-1:5 system was stable against dissolution and detachment by the release medium. We argue, that the ZOL/CS/PEI-M-25 K-1.1-1:5 particles in their casted films undergo a process of partly shrinking, flattening and merging upon drying and water loss, leading to films comparably stable against detachment like those of latex particles used e.g. as paints [24,25]. Furthermore, in the Figure 3 the long-term adhesive stability of this PEC film rationalized by the intensity of the PEC diagnostic ν(O = S = O) band versus time for ZOL/CS/PEI-M-0.9-1:5 (a) and ZOL/CS/PEI-M-1.1-1:5 (b) is shown.

The value for the initial dry state was taken to normalize all later states. Generally, no significant decrease from the initial 100% value could be obtained, which is a direct proof of the stability of all PEC films against detachment. Moreover, there was a slight percentage increase for the PEC-1.1 samples, which might be caused on the one hand by band shifts of the ν(O = S = O) band between the initial dry and the successive dry states after rinsing. On the other hand we attribute this effect to the compaction of the PEC phase, when the initially casted PEC films undergo structural rearrangements in contact to water, so that the subsequent drying steps result in denser structures compared to

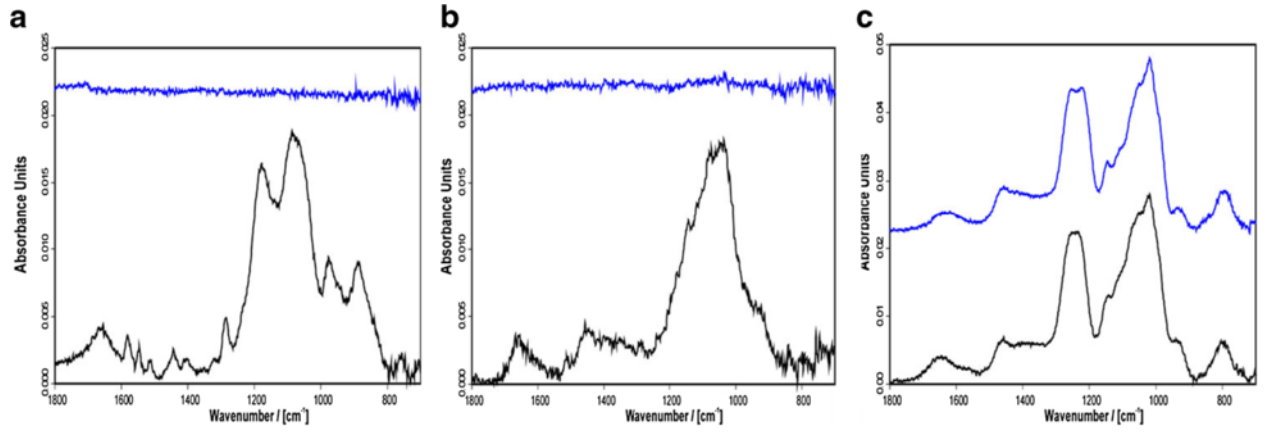

Figure 2 TRANS-FTIR spectra of solution casted films at Ge-IRE substrates. FTIR spectra on pure ZOL **(a)**, ZOL/PEI-25 K-M-1:1 **(b)** and ZOL/CS/PEI-25 K-M-1.1-1:5 **(c)** for the initial dry state (0 min, black, bottom) and the end dry state after 1 day contact to release medium (blue, top) are given.

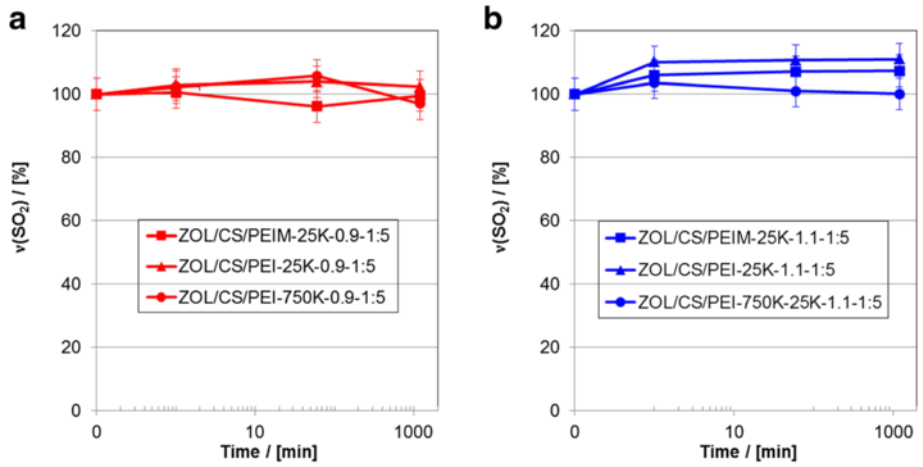

Figure 3 Stability of ZOL loaded PEC films at Ge-IRE substrates against detachment. The $v(SO_2)$ band integral from TRANS-FTIR spectra of thin PEC-0.9 **(a)** and PEC-1.1 **(b)** films of ZOL/CS/PEI-M-25 K-1:5 (cubes), ZOL/CS/PEI-25 K-1:5 (triangles) and ZOL/CS/PEI-750 K-1:5 (circles) is plotted versus time (0, 1, 60, 1200 min) of contact to release medium (dry state).

the initial one. This supports the shrinking/merging scenario raised before as an argument for the good PEC film stability against detachment. Especially, drug loaded PEC films are assumed to be prone for this effect, since the space formerly filled by the drug (ZOL) is now filled with pure PEC material. Finally, it has to be pointed out, that the film stability proven herein is a prerequisite for the functionalization of BSM like bone cements or implants by interfacial drug delivery systems (DDS) in future clinical applications. Obviously, of the drug delivery systems assessed in this work, the ternary drug/polycation/polyanion systems fulfill this requirement in contrast to the binary drug/polycation systems.

Morphology of PEC films

Additionally, AFM measurements on the freshly prepared dry films and on those after the contact with water were performed. In the Figure 4 AFM images of ZOL/CS/PEI-XK-0.9-1:5 and ZOL/CS/PEI-M-25 K-0.9-1:5

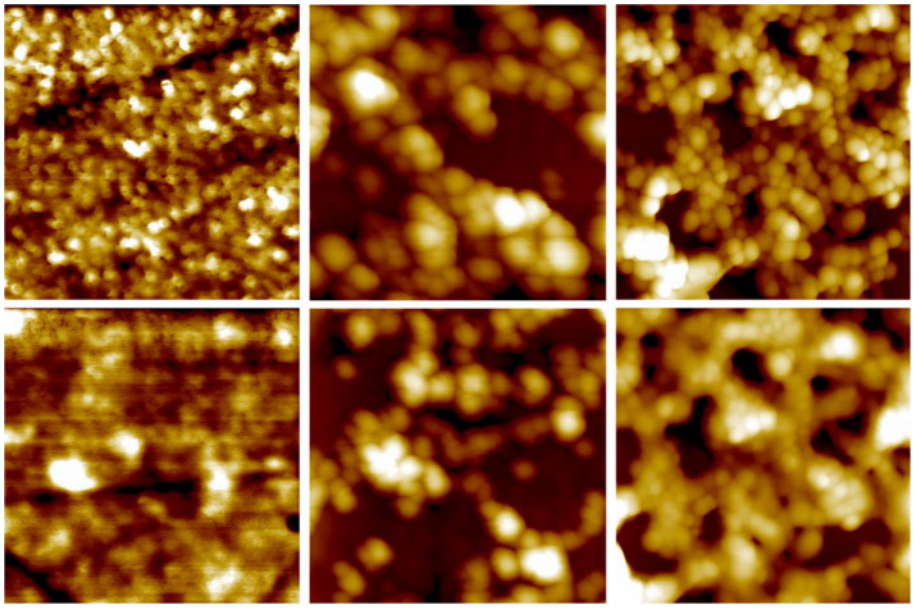

Figure 4 AFM images of solution casted ZOL loaded PEC films on Ge-IRE substrates. AFM images (topography, 4 × 4 um) are shown in the dry state before (top row) and after release (bottom row). In the left column films of ZOL/CS/PEI-M-25 K-0.9-1:5, the middle column those of ZOL/CS/PEI-25 K-0.9-1:5 and right column of ZOL/CS/PEI-750 K-0.9-1:5 are given.

films after water contact for one day and subsequent drying are shown.

At first, no significant difference between respective AFM images of the freshly casted film (top) and the rinsed film (bottom), after drying respectively, could be observed. This finding supports the stability of ZOL/PEC films after contact to the release medium. Secondly, small PEC particles, whose diameters were sizing around 100 nm could be found. This value can not be directly compared to diameters found by DLS measurements (Table 1), since on the one side casted PEC particles are flattened and are assumed to have a hemispheric profile [26] leading to larger values. On the other side, dried casted PEC particles lost water to a considerable amount leading to smaller values compared to diameters obtained from DLS. Which effect, flattening or shrinking, is dominating or whether both approximately compensate, cannot be figured out exactly. Nevertheless we speculate that drying of casted PEC films is associated with a process of shrinking, flattening and partly merging of individual PEC particles. This results in the remarkable stability of PEC particle films against detachment (see above).

Zoledronate release from PEC films

ZOL release was determined as the drug loss in the polymer film by *in-situ* ATR-FTIR spectroscopy, which is linearly sensitive to concentration changes of a variety of surface active compounds (e.g. drugs, polyelectrolytes, proteins) at and in thin films, whose thickness is not exceeding around 300 nm [21,27-30]. In the Figure 5 typical ATR-FTIR spectra recorded during ZOL release from thin PEC films of ZOL/CS/PEI-M-25 K-0.9-1:5 (a) and ZOL/CS/PEI-750 K-0.9-1:5 (b) for the initial (black) and end dry state (red) and for the contact to release medium (blue) after 5–1200 min (≈1 day) are shown.

Significant changes in the range between 1200 and 1000 cm^{-1}, in which diagnostic IR bands of ZOL appear, were obtained. An assignment of these IR bands is given in the Table 2.

Since this spectral region is highly overlapped, the exact intensities (absorbances, integrated areas) of these bands have to be obtained by line shape analysis of the spectral region between 1300 and 900 cm^{-1}, which was introduced for another drug/PEC system therein [8]. Generally, from the ratio of band intensities assigned to ZOL and those assigned to the PEC (CS) the actual relative amount of ZOL in the PEC film can be obtained (see Methods). In the Figure 6a kinetic curves of the percentage content values of ZOL normalized by the initial content are shown for the ZOL loaded PEC-0.9 systems of CS/PEI-M-25 K, CS/PEI-25 K and CS/PEI-750 K, respectively. Additionally, in the Figure 6b kinetic curves of the ZOL loaded PEC-1.1 systems of CS/PEI-M-25 K, CS/PEI-25 K and CS/PEI-750 K are given.

A significant retardation of drug release with respect to the pure ZOL film can be rationalized for all ZOL/PEC samples. Obviously, there is a retention of the drug in the PEC matrix, whose initial amount is around 4.6 μg per PEC film sample (see Experimental). This amount could be easily increased to compare e.g. with that of ZOL loaded poly(D,L-lactide) (PDLLA) coatings used to modify intramedullary implants healing fractures of rat tibia containing 20 μg ZOL as reported by Greiner [7].

Two parameters could be read out further from these curves. At first the initial burst (IB) can be rationalized from the difference between 100% at time zero and a given percentage after 5 min. Secondly, from the percentage value at the end of the observed release process (typically after around one day) the residual amount (RA) of the drug can be obtained. In Table 3 values of IB and RA of the various PEC samples are summarized.

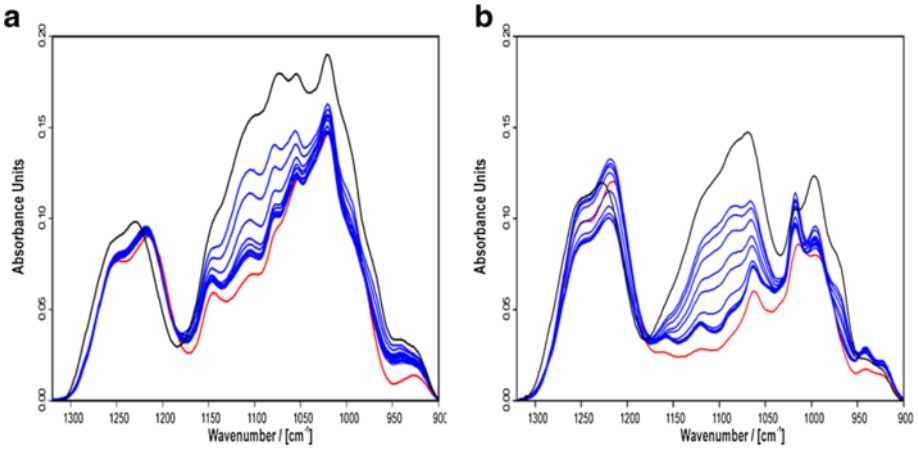

Figure 5 ATR-FTIR spectra of solution casted ZOL loaded PEC films. Kinetic FTIR spectra series for ZOL/CS/PEI-M-25 K-0.9-1:5 **(a)** and ZOL/CS/PEI-750 K-0.9-1:5 **(b)** are provided for the initial (black) and end dry state (red) and the contact to the release medium (5–1200 min, blue).

Table 2 Assignment of diagnostic IR bands between 1300 and 800 cm^{-1} in ZOL/CS/PEI(M) samples

Wavenumber / [cm^{-1}]	Vibration mode	Molecular species
1255	ν_a(O-S-O)	CS
1214	ν_a(O-S-O)	CS
1120	ν_a(O-P-O)	ZOL
1070	ν_a(O-P-O	ZOL
1000	ν_s(O-S-O)	CS
950	ν_s(O-S-O)	CS

At first, for all three unmodified PEC samples the IB values decreased significantly with the molecular weight, resulting in lower IB values (37-39%) for the PEI-750 K/CS systems and higher IB values (72-75%) for the PEI-1.3 K/CS system. Presumably, the hyperbranched PEI structure serves as a sponge for oppositely charged compounds like the drug bisphosphonate, for which counter ion condensation might be seen as a major driving force. Hence, the higher molecular weight PEI might provide a larger diffusion region compared to lower molecular weight PEI, when the PEC film gets hydrated and dissolution and release of the drug sets in. This result is supported by data provided in the additional information, where high molecular PEI-750 K showed also an insufficient titration behavior even in the slow titration mode due to longer diffusion times. However, no significant molecular weight effect on the RA values could be found for the unmodified PEC samples. Obviously, the drug release process for all studied PEC systems stopped or highly delayed after around one day.

Secondly, no large differences in both IB and RA values could be identified between the maltose modified PEC samples of ZOL/CS/PEI-M-25 K (IB: 21-57%, RA: 6-12%) and the unmodified samples of ZOL/CS/PEI-25 K (IB: 27-54%, RA: 0-8%), respectively. Both PEC samples are based on the same PEI molecular weight containing equal amounts of charges. Thus, we assume, that ZOL interaction to these PEC samples is governed by electrostatic attraction between negatively charged ZOL and oppositely charged PEI-25 K or PEI-M-25 K.

Thirdly, the net charge of all ZOL/PEC particle system shows no clear effect on IB and RA for PEC systems containing low molecular PEI-1.3 K and PEI-25 K. The RA values for both PEI molecular weights cases and for PEC-0.9 and PEC-1.1 particles are low. Whereas, for the PEI-750 K containing system PEC-1.1 particles show a moderate (27%) and PEC-0.9 particles a low RA value (10%) and the IB values for PEC-0.9 and PEC-1.1 particles are similar and moderate. Hence, we conclude, that a high PEI molecular weight and the PEC-1.1 particle system show actually the best release performance.

Zoledronate enrichment of the release medium

Parallel to the ATR-FTIR data on ZOL loss of the polymer films, ZOL enrichment of the release medium was measured by UV–VIS-spectroscopy. A concentration series of UV–VIS spectra on ZOL solutions is given in the Figure 7a and the related calibration plot in Figure 7b.

According to these spectra and the calibration plot a concentration of around 0.00001 M (10^{-5} M) of ZOL can be detected conveniently. A respective kinetics of ZOL enrichment is plotted versus time for ZOL/CS/PEI-750 K-1.1-1:2 in the Figure 7c. The known ZOL content in the PEC film was taken as 100%. The concentration enrichment shows an initial burst after 5 min of around 40%. Afterwards the released ZOL concentration increases

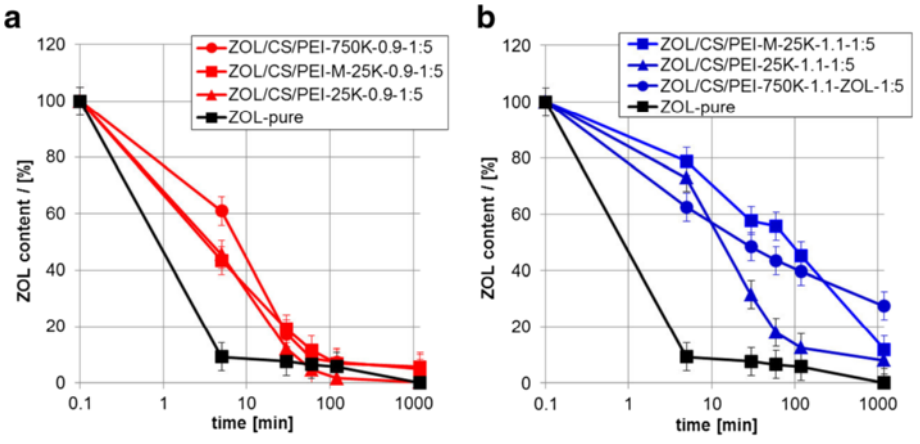

Figure 6 Release kinetics of ZOL from PEC films. The relative ZOL content for PEC-0.9 **(a)** and PEC-1.1 **(b)** systems of ZOL/CS/PEI-M-25 K-1:5 (cubes), ZOL/CS/PEI-25 K-1:5 (triangles) and ZOL/CS/PEI-750 K-1:5 (circles) samples is plotted versus exposure time (0, 5, 30, 60, 120, 1200 min) to the release medium (0.1 mg/ml PBS). For comparison the kinetic curve for a pure ZOL film (black) is shown.

Table 3 Initial burst (IB, after 5 min) and residual amount (RA, after 1200 min) values for various PEC samples

PEC sample	IB / [%]	RA / [%]
ZOL/CS/PEI-1.3 K-0.9-1:5	72 ± 5	9 ± 5
ZOL/CS/PEI-1.3 K-1.1-1:5	75 ± 5	0 ± 5
ZOL/CS/PEI-25 K-0.9-1:5	54 ± 5	0 ± 5
ZOL/CS/PEI-25 K-1.1-1:5	27 ± 5	8 ± 5
ZOL/CS/PEI-750 K-0.9-1:5	39 ± 5	10 ± 5
ZOL/CS/PEI-750 K-1.1-1:5	37 ± 5	27 ± 5
ZOL/CS/PEI-M-25 K-0.9-1:5	57 + 5	6 + 5
ZOL/CS/PEI-M-25 K-1.1-1:5	21 + 5	12 + 5

further and levels off at a concentration c_{ZOL} = 0.00006 M, which corresponds to a release of ~41.3 μg ZOL from the polymer film within 2 hours. Comparable ZOL amounts were used by Greiner [7] for the release from PDLLA films on intramedullary implants for rat tibia fixation. Concerning a physicochemical description of the release kinetics several models are known. Herein, we applied the kinetic analysis according to the classical Ritger/Peppas model [31] to represent the UV–VIS data on ZOL release given in Figure 7c see (Additional file 2). Generally, from this analysis dissolution rather than diffusion of ZOL at PEC films was concluded.

Conclusions

-Colloidally stable dispersions of polyelectrolyte complex (PEC) particles loaded by osteotherapeutic zoledronate (ZOL) in the size range 11–141 nm could be prepared by mixing pure (PEI) or maltose-modified poly (ethyleneimine) (PEI-M) with cellulose sulfate (CS) in the presence of ZOL.

-Casted films of ternary ZOL/CS/PEI-M or ZOL/CS/PEI PEC particles were stable in contact to water, while films of the pure drug (ZOL) and of the binary system ZOL/PEI-M or ZOL/PEI were not stable against detachment.

-ZOL release from PEC films could be detected by in-situ ATR-FTIR (drug loss in the PEC film) and by UV–VIS spectroscopy (drug enrichment in the release medium). As release parameters the initial burst (IB) after 5 minutes and the residual amount (RA) after one day were read out from these data.

-Retarded releases of ZOL from various PEC films compared to the pure drug film were observed. Generally, after one day the ZOL release process was finished for all measured ZOL/PEC samples and RA values between 0-27% were obtained. With increasing molecular weight, IB values decreased due to slower diffusion times of ZOL out of the PEC samples, so that actually, PEC-1.1 particles of CS/PEI-750 K have the best release performance (IB = 37%, RA = 27%). No significant effect of the maltose modification of PEI-25 K neither on IB nor on RA could be found.

-Generally, we conclude that IB values are acceptable, but RA values after one day are actually too low for long-term clinical applications. Assuming molecular ZOL dimensions < 1 nm, we consider compactness as the main molecular parameter influencing ZOL retention in the PEC based delivery systems. Therefore future achievements will be addressed to densify the PEC matrix further by using additional biocrosslinkers and

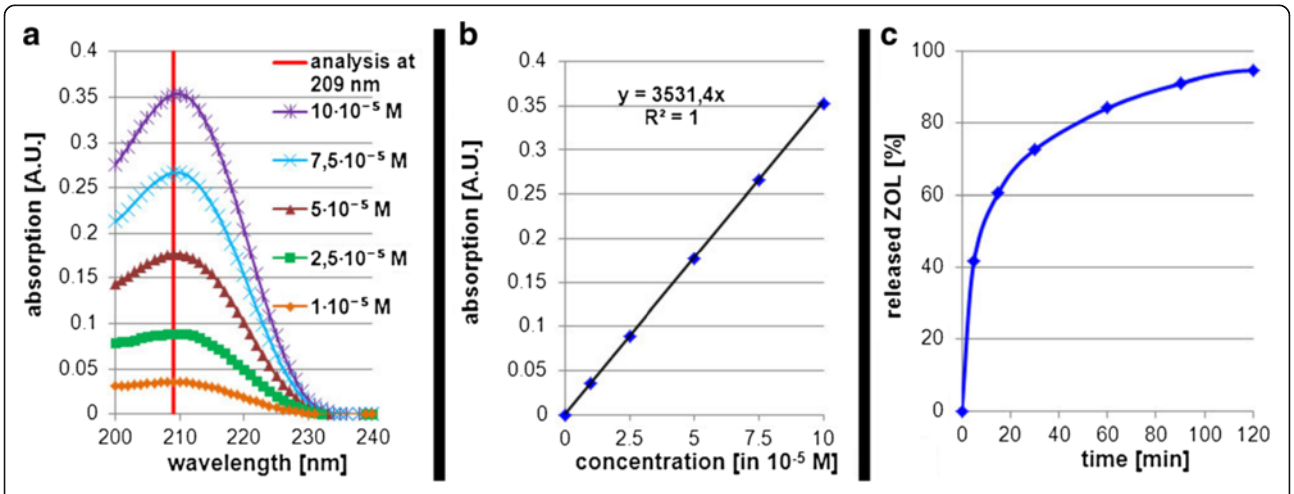

Figure 7 UV–VIS studies on the enrichment of the release medium (0.1 mg/ml PBS) by ZOL. UV–VIS spectra of ZOL solutions in the concentration range c_{ZOL} = 0.00001-0.0001 M **(a)**, the calibration curve for ZOL solutions in the concentration range c_{ZOL} = 0.00001-0.0001 M **(b)** and the kinetics of ZOL enrichment released from ZOL/CS/PEI-750 K-1.1-1:2 particle films **(c)** are given.

further PEL combinations enabling smaller mesh and pore sizes and involving additional molecular interaction forces. Nevertheless, the PEL complexation concept for controlled delivery of ZOL offers an additional advantage: While for systemic medication large amounts in the range of milligrams of ZOL are used, ZOL/PEC films contain ZOL in the range of micrograms. Herein 4.6 μg ZOL are provided per film sample, which can be easily upscaled to be large enough for local therapy (see [7]) and preventing systemic side effects.

-Finally PEC particles have already started to be applied at relevant bone substituting materials like bone cements or implants and tested for their biocompatibility and interaction to bone cells [9]. In final conclusion, surface adhesive drug loaded PEC particles are promising drug delivery systems to supply and release a defined amount of bone therapeutics from BSM.

Additional files

Additional file 1: Molar amount of PEL charges. Colloid titration results on PEI-25 K, PEI-750 K, PEI-M-25 K, CS.

Additional file 2: Kinetic analysis of ZOL release from PEC films. Results based on the model of Ritger/Peppas.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
BT, DV and BU prepared ZOL loaded PEC dispersions and carried out the in-situ-ATR-FTIR measurements. DV carried out the UV–VIS measurements. SS and DA synthesized and supplied the PEI-M-K25 sample. MM, BT and DV designed the in-situ-ATR-FTIR experiments. BT, DA and MM drafted and iteratively refined the manuscript. All authors approved the final manuscript.

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