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Colloid, adhesive and release properties of nanoparticular ternary complexes between cationic and anionic polysaccharides and basic proteins like bone morphogenetic protein BMP-2

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ABSTRACT

Herein we describe an interfacial local drug delivery system for bone morphogenetic protein 2 (BMP-2) based on coatings of polyelectrolyte complex (PEC) nanoparticles (NP). The application horizon is the functionalization of bone substituting materials (BSM) used for the therapy of systemic bone diseases. Nanoparticular ternary complexes of cationic and anionic polysaccharides and BMP-2 or two further model proteins, respectively, were prepared in dependence of the molar mixing ratio, pH value and of the cationic polysaccharide. As further proteins chymotrypsin (CHY) and papain (PAP) were selected, which served as model proteins for BMP-2 due to similar isoelectric points and molecular weights. As charged polysaccharides ethylenediamine modified cellulose (EDAC) and trimethylammonium modified cellulose (PQ10) were combined with cellulose sulphatesulfate (CS). Mixing diluted cationic and anionic polysaccharide and protein solutions according to a slight either anionic or cationic excess charge colloidal ternary dispersions formed, which were cast onto germanium model substrates by water evaporation. Dynamic light scattering (DLS) demonstrated, that these dispersions were colloidally stable for at least one week. Fourier Transform Infrared (FTIR) showed, that the cast protein loaded PEC NP coatings were irreversibly adhesive at the model substrate in contact to HEPES buffer and solely CHY, PAP and BMP-2 were released within long-term time scale. Advantageously, out of the three proteins BMP-2 showed the smallest initial burst and the slowest release kinetics and around 25% of the initial BMP-2 content were released within 14 days. Released BMP-2 showed significant activity in the myoblast cells indicating the ability to regulate the formation of new bone. Therefore, BMP-2 loaded PEC NP are suggested as novel promising tool for the functionalization of BSM used for the therapy of systemic bone diseases.

1. Introduction

Systemic hard tissue diseases like osteoporosis [1,2] and multiple myeloma [3,4] can lead to fractures, defects or lesions of bone, which must be cured by bone substitute materials (BSM). Among them, bone cements [5] and xerogels [6] are commonly used to fill up larger scale defects, whereas biomechanically adapted osteosynthetic Ti/Al alloy materials [7] are used to stabilize fractures. In the sense of a local therapy, these BSM can be further functionalized by pure drugs or drug delivery systems (DDS) exposing specific therapeutics for such bone diseases. In the past decade highly specific proteinogenic growth factors have been integrated directly or via DDS in BSM aiming at hard tissue regeneration. However, finding an appropriate DDS for growth factors in bone regeneration remains a challenge, because of uncontrolled burst release and high doses needed to elicit biological responses [8]. Recently, bone morphogenetic protein 2 (BMP-2), a growth factor, which belongs to the tissue growth factor beta (TGF-β) superfamily and acts as potent inducer of bone formation, has been encapsu-

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lated into polymeric particles, such as PLGA, or into heparin-based microparticles, to achieve its sustained release and stimulate bone forming cells [9,10].

Following this concept of polymer particle assisted BMP-2 delivery, we report an approach, which is based on the use of dispersed polyelectrolyte complex (PEC) nanoparticles (NP) prepared by mixing polycation and polyanion solutions [11,12]. These PEC NP can be further loaded with drugs and deposited onto substrates of choice. This novel PEC NP approach is very appealing, since the preparation protocol is relatively simple, based on biorelated components in aqueous media and mild conditions and the generated particles could be controlled with respect to size, shape, density, surface charge and chemical composition, as it was recently reviewed [13]. In recent studies, we integrated charged low molecular osteotherapeutic drugs like antibiotics, bisphosphonates, statins and proteasome inhibitors into PEC NP and attached them onto model or bone substitute material (BSM) surfaces and determined their release behavior [14–17]. Moreover cytocompatibility was studied *in vitro* based on human mesenchymal stromal cells (hMSC) and human peripheral blood monocytes (hPBMC) [18,17]. Based on this work on PEC NP loaded with low molecular drugs, in the present work we introduce now PEC NP loaded with the higher molecular osteotherapeutic BMP-2 or the two model proteins chymotrypsin (CHY) and papain (PAP), respectively. These two proteins were chosen for their lower costs and similar properties with respect to molecular weight and isoelectric point allowing to perform extensive initial experiments on the variation of parameters like pH and composition before switching to the less available BMP-2. In this work the colloid, interfacial and release properties of protein loaded PEC NP is characterized by colloid titration, dynamic light scattering (DLS) and Fourier Transform Infrared (FTIR) spectroscopy.

From a fundamental polymer science perspective our protein loaded PEC NP systems are related to the ternary polycation/polyanion/protein complexes reported by Lindhoud and Cohen Stuart [19,20] and to the binary polyelectrolyte/protein complexes reported earlier by Dubin [21]. In these works, the dynamic diffusive and flexible behavior of proteins within dispersed polyelectrolyte complex phases plays a major role, which is influenced by pH, salt and composition. Hence, we see a link to our experimental work on the release of proteins from adhesive PEC NP phases expected to range from internal diffusion up to release in the external aqueous medium.

2. Experimental

2.1. Materials

2.1.1. Chemicals

As polyelectrolytes the cationic polysaccharides ethylenediamine cellulose (EDAC, degree of substitution $d_S = 0.7$, $M_W \approx 100.000$ g/mol, EDA-CELL-T17, TITK, Rudolstadt, Germany) and polyquaternium-10 (PQ10, $d_S = 0.5$, BOC Sciences, New York, USA) and the anionic polysaccharide cellulose sulfate (CS, $d_S = 0.5$, Euroferm, Erlangen, Germany) were used and diluted to 0.002 M solutions related to their repeating units (formulae in Fig. 1). Values of pH = 4, 7, 10 were adjusted by the addition of small portions of either 0.1 M HCl or 0.1 M NaOH [22].

As proteins chymotrypsin (CHY, Sigma, Germany) with molecular weight $M_W \approx 25.000$ g/Mol and isoelectric point IEP = 8.75 [22], papain (PAP, Sigma, Germany) with $M_W \approx 24.000$ g/mol and IEP = 8.75 [22] and bone morphogenetic protein-2 (BMP-2, ProSpec GmBH, Heidelberg, Germany) with $M_W \approx 30.000$ g/mol and isoelectric point IEP ≈ 8.5 [23] were used.

2.1.2. Polyelectrolyte complex (PEC) preparation

Binary complexes between cationic and anionic polysaccharides were prepared for molar mixing ratios varying from n−/n+ = 0.5–1.5. For binary PEC with n−/n+ = 0.5–0.9 (cationic excess) the 0.002 M polycation solution was presented and the 0.002 M polyanion solution was added with respect to the true charge concentration (see PCD), while for binary PEC with n−/n+ = 1.1 − 1.5 the 0.002 M polyanion solution was presented and the 0.002 M polycation solution was added. For PEC with n−/n+ = 1.0 both mixing orders were applied and studied.

Ternary complexes of polycation/polyanion/protein were prepared by mixing 0.002 M polycation (EDAC or PQ10) with 0.002 M protein solution and adding this polycation/protein mixture to the 0.002 M polyanion (CS) solution in certain amounts. Thereby in the final polycation/polyanion/protein mixture a molar mixing ratio n−/n+ = 1.1 related to polycation and polyanion was valid and the molar amount of protein (n_{PROT}) was half of the sum of the molar polycation and polyanion amount according to $n_{PROT} = (n- + n+)/2$. Consequently, the molar fraction of protein P in the ternary polycation/polyanion/protein mixture was expressed as $P = n_{PROT}/(n_{PROT} + n- + n+)$. Applying this procedure, the molar fraction of protein P and thus the protein content was always equivalent in various samples regardless of different compositions like EDAC/CS/protein and PQ10/CS/protein and of different n−/n+ values. As standard value P = 0.33 was applied. Typical samples of ternary complexes for CHY and PAP to be used for DLS measurements (see below) had total volumes of 2.500 ml consisting of 1.666 ml PEC dispersion and 0.833 ml protein solution. Typical samples of BMP-2 containing ternary complexes were lower in volume due to its availability and had total volumes of 0.150 ml consisting of 0.100 ml PEC dispersion and 0.050 ml protein solution.

2.1.3. PEC coating preparation

Binary polycation/polyanion or ternary polycation/polyanion/protein coatings were prepared by casting typically 100 microliters of the polycation/polyanion or polycation/polyanion/protein dispersion onto Ge substrates and drying by 50 °C (binary systems) and 40 °C (ternary systems) in an oven for 15 min. In typical polycation/polyanion/protein coatings casted from 100 μl dispersions (67 μl 0.002 M PEC dispersion, 33 μl 0.002 M protein solution) the protein content was 6.7 μg.

2.2. Analytical methods

2.2.1. Colloid titration

Colloid titration is a useful technique to determine the molar concentration of charges in polyelectrolyte solutions, which can be routinely realized by the particle charge detector (PCD, Mutek, Herrsching, Germany). PCD is based on titrating a sample PEL solution (titrand) by an oppositely charged low molecular polyelectrolyte (PDADMAC or PES) solution (titrator) until a zeta-potential value of zero is reached. The zeta-potential is defined as the voltage [mV], which is needed to compensate the sheared ion cloud, when a PTFE piston is periodically moved within a PTFE tube filled with the sample PEL solution. Herein a volume of 0.5 ml of a 0.002 M sample PEL solution was further diluted to 10 ml (0.0001 M) and the titrator solution (0.001 M) was added. From the ratio between the volume of the consumed titrator solution (V_{TIT}) and the volume of the sample PEL solution (V_{PEL}) the charge factor $F = V_{TIT}/V_{PEL}$ was determined. Monomer units bearing one potential charged group (e.g. monobasic acids) can ideally have a factor of F = 1, those bearing 2–3 charged groups (e.g. di- and tribasic acids) can ideally have values of F = 2–3. In order to define an exact molar mixing ratio (n−/n+) of a given PEC dispersion, it has to be directly related to the charge factors of its polycation and polyanion components. In this report the abbreviation e.g. “PEC-0.50” and e.g. “PEC-1.50” are used