Long-Term Retarded Release for the Proteasome Inhibitor Bortezomib through Temperature-Sensitive Dendritic Glycopolymers as Drug Delivery System from Calcium Phosphate Bone Cement

Thu Hang Lai, Bettina Keperscha, Xianping Qiu, Brigitte Voit,* and Dietmar Appelhans*

For the local treatment of bone defects, highly adaptable macromolecular architectures are still required as drug delivery system (DDS) in solid bone substitute materials. Novel DDS fabricated by host–guest interactions between β-cyclodextrin-modified dendritic glycopolymers and adamantane-modified temperature-sensitive polymers for the proteasome inhibitor bortezomib (BZM) is presented. These DDS induce a short- and long-term (up to two weeks) retarded release of BZM from calcium phosphate bone cement (CPC) in comparison to a burst release of the drug alone.

Different release parameters of BZM/DDS/CPC are evaluated in phosphate buffer at 37 °C to further improve the long-term retarded release of BZM. This is achieved by increasing the amount of drug (50–100 µg) and/or DDS (100–400 µg) versus CPC (1 g), by adapting the complexes better to the porous bone cement environment, and by applying molar ratios of excess BZM toward DDS with 1:10, 1:25, and 1:100. The temperature-sensitive polymer shells of BZM/DDS complexes in CPC, which allow drug loading at room temperature but are collapsed at body temperature, support the retarding long-term release of BZM from DDS/CPC. Thus, the concept of temperature-sensitive DDS for BZM/DDS complexes in CPC works and matches key points for a local therapy of osteolytic bone lesions.

1. Introduction

Dendritic glycopolymers represent a steadily growing research field in the ongoing evolution of drug delivery systems (DDS), therapeutics, diagnostics, and nanomedicine among others. [1–8] This is triggered by the promising macromolecular architecture combined with multifunctional properties. Especially, their surface compositions combined with the branched molecular architecture control multiple key features such as high water and physiological environment solubility, complexing and conjugation properties, various biological interactions (e.g., inhibition, aggregation,[9] biocompatibility or tubulating properties[10]), and self-assembly processes.[11] These features are the base for the design and formation of biologically active biohybrid structures.[11–13] Overall, dendritic glycopolymers are one of the highly sophisticated and tunable nanomaterials for various biomedical applications[1–8] due to the ability to undergo H-bond-driven interactions[2,14] to cross biological barriers[1,3,15,16] and to enhance the transport of gene material to the right biological compartment[4,5,15] as well to adapt to soft and solid biological environments as inhibitor and DDS as well as bio-adhesive.[1–3,8,16–20]

The intelligent use of DDS in bone substitute materials for the local treatment of bone defects still needs ambitiously adaptable macromolecular architectures to induce long-term retarding drug release from a complex environment such as calcium phosphate bone cement (CPC).[19] CPC is considered as DDS itself for many drugs leading in most cases to a burst drug release, followed by a plateau-like release over a defined period.[19] This process is also accompanied by a slow degradation of CPC over days and weeks. For overcoming these drawbacks, first steps toward a local therapy of osteolytic bone lesions (e.g., multiple myeloma)[21] were attempted by using H-bond-active and positively charged dendritic glycopolymers as DDS (PEI-Mal B[22] in Figure 1) in CPC. Retarded short-term release (up to 96 h) of the proteasome inhibitor bortezomib[23] (BZM) from drug/DDS/CPC composites could be shown. These results motivated us to improve the molecular architecture and surface composition of dendritic glycopolymers as DDS for inducing better...
A) Schematic approach of the synthesis of temperature-sensitive dendritic glycopolymers $3$ by host–guest interactions. B) Investigations of BZM complexation by dendritic glycopolymers and drug delivery system (DDS) $3$ and their integration in calcium phosphate cements (CPC). The presentation of drug/DDS complexes in CPC is simplified in analogy to ref. $^{[19]}$, but also some amounts of interdendritic bridges between drug/DDS as smaller aggregates are imaginable. C) Retarding release of drug bortezomib (BZM) release from drug/DDS/CPC composites and comparing two DDS, $3$ and PEI-Mal B, for validating short- ($\leq 24$ h) and long-term retarding BZM release. Release of BZM from composites with i) $50 \mu$g BZM, $100 \mu$g DDS, and $1$ g CPC and ii) $100 \mu$g BZM, $200 \mu$g DDS, and $1$ g CPC, observed over $96$ h (C) and $336$ h (Figure S21, Supporting Information).

Figure 1. A) Schematic approach of the synthesis of temperature ($T$)-sensitive dendritic glycopolymers $3$ by host–guest interactions. B) Investigations of BZM complexation by dendritic glycopolymers and drug delivery system (DDS) $3$ and their integration in calcium phosphate cements (CPC). The presentation of drug/DDS complexes in CPC is simplified in analogy to ref. $^{[19]}$, but also some amounts of interdendritic bridges between drug/DDS as smaller aggregates are imaginable. C) Retarding release of drug bortezomib (BZM) release from drug/DDS/CPC composites and comparing two DDS, $3$ and PEI-Mal B, for validating short- ($\leq 24$ h) and long-term retarding BZM release. Release of BZM from composites with i) $50 \mu$g BZM, $100 \mu$g DDS, and $1$ g CPC and ii) $100 \mu$g BZM, $200 \mu$g DDS, and $1$ g CPC, observed over $96$ h (C) and $336$ h (Figure S21, Supporting Information).

2. Results and Discussion

This study reports the controlled and retarded release of the proteasome inhibitor BZM from drug/DDS/CPC composites (Figure 1B). For that, temperature-sensitive dendritic glycopolymers $3$ (Figure 1A) were introduced as possibly better adaptable DDS into CPC compared to previously used dendritic glycopolymer PEI-Mal B (Figure 1B).$^{[19]}

2.1. Synthesis and Characterization of Temperature-Sensitive Core–Shell Architectures $3$

The synthetic approach of the core macromolecule PEI-Mal B-[$CD\]$_{n}$ (1) is presented in Figure 2A. The first step was the
substitution reaction of 6-monotosyl-β-cyclodextrin (4) with propargylamine to obtain mono-6-deoxy-6-propargylamine-β-CD (5). Then, βCD-modified PEG2 acid (6) was synthesized by the copper-catalyzed azide-alkyne cycloaddition (CuAAC) between 5 and azido-modified PEG2 acid. After the amidation reaction between hyperbranched PEI (M_w = 25 kDa) and ester-activated 6 to form desired β-CD-modified PEI macromolecule (7), the carbohydrate shell of 1 was introduced by a reductive amination \(^{22}\) of 7 with maltose (Figure 2A). Further details for the NMR characterization of 1, 5, 6, and 7 are presented in the Supporting Information. In addition, the amount of coupled β-CD and maltose units in 1 was determined by elemental analysis (Table S6, Supporting Information). Moreover, spectroscopic experiments were carried out to determine the amount of β-CD in 1 available for host–guest interaction using an adamantane-modified fluorescein derivative (Table S6A, Supporting Information). From this we can conclude that the desired dendritic glycopolymer 1 (Figure 1A) with about 5 (1a) and 10 β-CD units (1b), respectively, had been prepared.

Thermoresponsive PNIPam and PNVCL (2 in Figure 1) were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization with adamantane modified chain transfer reagent (9) \(^{28}\) presented in Figure 2B. 9 (Figure S5, Supporting Information) was prepared by a Steglich esterification of 2-(dodecylthio-carbonothioylthio)-2-methylpropionic acid (8) and 1-(hydroxymethyl)adamantane. \(^{28}\) Subsequently, Ada-PNIPam and Ada-PNVCL (2) with molecular weights of around 3000, 5000–6000, and 10 000 g mol\(^{-1}\) (Tables S1 and S6, Supporting Information), were synthesized by RAFT polymerization followed by the removal of thiocarbonylthio end group via radical-induced reduction with tris(trimethylsilyl)silane (TTMS).\(^{29}\)

The molecular weights of the polymers were characterized by \(^1\)H-NMR and gel permeation chromatography (GPC) (Table S6B and Figures S5–S9, Supporting Information), showing a dispersity (Table S6, Supporting Information) of ≤1.25 for Ada-PNIPam (2a–b) and <1.9 for Ada-PNVCL (2e–f), respectively, while their corresponding temperature-sensitive behavior was proven by dynamic light scattering (DLS) and UV–vis experiments (Table S6C, Supporting Information; Figure 3A, C, and Figure S10, Supporting Information). As seen from the broad dispersity, RAFT agent 9 was not able to produce PNVCL in a highly controlled fashion, probably due to the attachment of the adamantly substituent. Nevertheless, end-functionalized Ada-PNVCL (2e–f) were prepared as proven by NMR and used for the formation of 3.

Novel temperature-sensitive core–shell macromolecules (3) (Figure 1) were realized as white solids by host–guest interactions of PEI-MalB-[CD]_n (1) with either Ada-PNIPam or Ada-PNVCL (2) followed up by a dialysis and freeze-drying (composition details see Table S2, Supporting Information). In the next step, their temperature-sensitive characteristics and complexing properties toward BZM were evaluated. LCST values are requested to be around body temperature. Comparing LCST
values of 2 and 3 (Table S6C, Supporting Information; Figure 3A,C) show that the LCST values of 3 are with 34–38 °C (PNIPAm shell) and ≈46 °C (PNVCL shell) higher as found for Ada-PNIPAm (below 37 °C, 2a + d) and for Ada-PNVCL (≈38 °C, 2e) due to the steric inhibition based on the formation of mesoglobules and the increased hydrophilicity imposed on the temperature-sensitive polymer chains by the glycopolymer core. However, determination of the aggregation temperatures (T_{ag}) of 2 and 3 by DLS experiments (Table S6B,C, Supporting Information; Figure 3; Figures S10–S12, Supporting Information) clarified, that 3 have similar T_{ag} as isolated Ada-polymers 2. Intermolecular aggregation of 3 starts at temperature significantly below the determined LCST (Table S6C, Supporting Information). Thus, PEI-Mal B-[CD/Ada-PNIPAm]_n (3) start to aggregate already above 28 °C and form especially above 34 °C large aggregates (>1 µm) due to a high degree on interchain interactions. In contrast, PEI-Mal B-[CD/Ada-PNVCL]_n (3) are more stable and show the formation of smaller aggregates (50–150 nm) starting at about 32 °C and large aggregates appear only after 38 °C (Figure 3B,D). Thus, all prepared DDS show the desired temperature sensitivity for loading the drug at room temperature and achieving a polymer chain aggregation hopefully leading to a better drug retaining within the DDS at body temperature.

2.2. Application of 3 as Drug Delivery System

In summary, five new temperature-sensitive DDS 3 based on host–guest interaction of β-CD-modified dendritic glycopolymer 1 and adamantane-modified PNIPAm or PNVCL (2) were synthesized (Table S6C, Supporting Information) suited to be tested by uptake and release experiments with BZM.

2.2.1. Uptake of BZM

The complexation capacity of PEI-Mal B-[CD/Ada-PNIPAm]_n and PEI-Mal B-[CD/Ada-PNVCL]_n (3) toward BZM was determined at room temperature by ultrafiltration experiments combined with UV–vis measurements as presented in a previous study.\(^\text{[30]}\) The results of the complexation study with molar ratios of 1:10, 1:25, and 1:50 (DDS:BZM) over 20 h in phosphate buffered saline (PBS)/NaCl buffer (pH 7.4) are summarized in Figure 1B and Figure S14 (Supporting Information). For example, Figure 1B shows that the complexation behavior of temperature-sensitive 3 as DDS is comparable with previously published results of dendritic glycopolymer with pure maltose shell (Figure 1B—PEI-Mal B).\(^\text{[19]}\) High complexation values between 60% and 76% were obtained for all DDS after 20 h.

2.2.2. Release of DDS from CPC

The successful use of temperature-sensitive dendritic glycopolymer 3 as DDS in CPC requires a very retarded release of DDS itself from CPC composites. Therefore, the release profile of 3 from DDS/CPC composite was studied. After labeling
of DDS with rhodamine B isothiocyanate (Rh-DDS). Rh-DDS (400 µg) was incorporated in thin CPC disks prepared by a published process (Figure S15, Supporting Information).[19]

Figure 4A presents the release profile of different dye-labeled DDS from Rh-DDS/CPC composites, including the reference of the pure dendritic glycopolymer, Rh-PEI-Mal B. With the exception of Rh-DDS Rh-PEI-Mal B-[CD/Ada-PNIPAm(3k)]n, the temperature-sensitive Rh-DDS has a higher accumulated release than Rh-PEI-Mal B defined as reference system from previous study.[19] About 42% of Rh-PEI-Mal B-[CD/Ada-PNIPAm] was released after 24 h, while about 30% of Rh-PEI-Mal B was released in 96 h. The retarded release of Rh-PEI-Mal B can be explained by the fact that smaller macromolecules as Rh-PEI-Mal B may be more compactly incorporated in CPC than those of larger dimensions as found in the case of temperature-sensitive DDS 3 (Figure 1). From this, one would expect a faster release of BZM from drug/DDS 3/CPC composites compared to previous release studies based on BZM/PEI-Mal B/CPC composites.[19]

2.2.3. Release of BZM from BZM/DDS/CPC

After the establishment of complexing DDS for the drug BZM, the release of BZM from drug/DDS/CPC composites (Table S5, Supporting Information) was studied. The following CPC composites with a concentration of 50 µg BZM and 100 µg DDS were tested: PEI-Mal B-[CD/Ada-PNIPAm(3k)]n/CPC and PEI-Mal B-[CD/Ada-PNIPAm(5k)]n/CPC (n = 5 or 10). The results of BZM release from different CPC compositions over 96 h are presented in Figure 4B. During the first 24 h, the release profile of BZM from PEI-Mal B-[CD/Ada-Polymer]n/CPC shows a high initial burst. After 96 h, about 82% BZM from CPC composite, 72–76% BZM from PEI-Mal B-[CD/Ada-PNIPAm(3k)]n/CPC and 69–73% BZM from PEI-Mal B-[CD/Ada-PNIPAm(5k)]n/CPC are released. PEI-Mal B-[CD/Ada-PNVCL(5k)]n/CPC of all DDS 3 shows the lowest release percentage of BZM. In general, the release experiments were carried out at 37 °C near or above the LCST of DDS 3. At this temperature the shell polymers of DDS start to form mesoglobules simultaneously inducing a better complexation of BZM within DDS. Nevertheless, the collapse of DDS during the hardening of drug/PEI-Mal B-[CD/Ada-PNVCL(5k)]n/CPC composites leads to an improvement of up to 13% of retarded release of BZM compared to BZM/CPC composites. The additional attachment of Ada-Polymer 2 with low molecular weight shows a positive effect on the working hypothesis “Retarding release of BZM.”

As the release experiments of PEI-Mal B-[CD/Ada-PNVCL(5k)]n have one of the best BZM release profile (Figure 4B), the concentration of BZM to DDS in CPC composites was changed from 50 µg/100 µg to 50 µg/200 µg to further analyze the BZM release behavior (Figure 4C). Compared with the release profile of BZM from CPC and PEI-Mal B-[CD]10/CPC
composites (Figure 4C), PEI-Mal B-[CD/Ada-PNIPAm(10k)]_{10}/CPC composites have a significantly decreased initial burst of only 55–63% BZM after 24 h (24 h: short-term release) as well as a decreased plateau-like release leading to 63–73% BZM after 336 h (Figure S19, Supporting Information, long-term release). The direct comparison of the release profile of BZM from PEI-Mal B-[CD/Ada-PNIPAm(10k)]_{10} with different concentration clarifies that after 96 h about 69% BZM from the composition 50 µg/100 µg and 60% BZM from the composition 50 µg/200 µg, respectively, are released (Figure 4C). The higher the DDS:BZM ratio in the CPC composite, the higher is the drug complexation ability and a better integration of drug/DDS complexes in CPC composites resulting in a slower release of BZM.

In addition, different combinations of BZM and PEI-Mal B-[CD/Ada-PNIPAm(10k)]_{10}, having long Ada-PNIPAm chains with Mₙ of 10000 g mol⁻¹ (Table S6C, Supporting Information), in CPC composites were prepared and compared with the release profile of BZM from CPC (Figure 4D). The following concentration pairs for BZM to PEI-Mal B-[CD/Ada-PNIPAm(10k)]_{10} in CPC composites were investigated: 50 µg/100 µg, 50 µg/200 µg, and 100 µg/200 µg. After 96 h, the DDS/CPC composites show a significantly decreased release of BZM for all concentrations of BZM to DDS. Furthermore, the ability of a higher complexation of BZM combined with a decreased release profile by a higher amount of DDS than BZM alone is evidenced with these release experiments for complexation ratios of 50 µg/100 µg and 50 µg/200 µg. The same short- and long-term retarding release profile of BZM is given compared to DDS 3, PEI-Mal B-[CD/Ada-PNIPAm(10k)]_{10} (Figure 4C). Moreover, the increase of both amounts of BZM and DDS to 100 and 200 µg promotes a delayed drug release in contrast to DDS decorated with shorter Ada-PNIPAm chains (Figure 4B), but also to the other complexation ratios in the experimental series (Figure 4D). In the best case, BZM to PEI-Mal B-[CD/Ada-PNIPAm(10k)]_{10} with 100 µg:200 µg, only 50% drug release after 24 h was observed, with a strong long-term sustained release resulting in only about 55% BZM release after 96 h compared to 82% release for the pure BZM from CPC.

Surprisingly, the BZM release behavior of temperature-sensitive DDS 3 (Figure 1) provides similar characteristics compared to the previously studied DDS PEI-Mal B (Figure 1C) reported by Striegler et al.[19] In the recent and previous[19] study, the same concentration pairs of BMZ/DDS were used: 50 µg/200 µg as low dosage and 100 µg/200 µg as high dosage incorporated in 1 g CPC, respectively. For low dosage experiment, the novel temperature-sensitive DDS were successfully synthesized by host-guest interactions of β-CD-modified dendritic glycopolymer with adamantane-modified PNIPam and PNVCL for enhancing retarded release of encapsulated drugs by polymer chain collapse. All DDS are suited materials for complexing proteasome inhibitor BZM in solution and the resulting drug complexes are smoothly transferable into CPC composite. With these highly adaptable drug/DDS complexes in the solid CPC, these highly adaptable drug/DDS complexes in the solid CPC promising retarded release characteristics of BZM are available. These desired release characteristics of proteasome inhibitor drug is still addressable when increasing the amount of drug and/or DDS in CPC. The evaluated release characteristics from DDS/CPC composites match key points to establish DDS for a local therapy of osteolytic bone lesions.[19,23,31] Further efforts will be undertaken to better understand the retarding properties of (temperature-sensitive) dendritic glycopolymers as DDS in CPC and their biological influence on osteolytic bone lesions.

3. Conclusion

Temperature-sensitive DDS were successfully synthesized by host-guest interactions of β-CD-modified dendritic glycopolymer with adamantane-modified PNIPam and PNVCL for enhancing retarded release of encapsulated drugs by polymer chain collapse. All DDS are suited materials for complexing proteasome inhibitor BZM in solution and the resulting drug complexes are smoothly transferable into CPC composite. With these highly adaptable drug/DDS complexes in the solid CPC, the higher is the drug complexation ability and a better integration of drug/DDS complexes in CPC composites resulting in a slower release of BZM.

4. Experimental Section

Materials and Characterization: All reagents were used as obtained commercially without further purification except N-isopropylacrylamide (NIPAm), N-vinylcaprolactam (NVCL), and 2,2′-azobis-(isobutyronitrile) (AIBN). NIPAm and NVCL were purified by recrystallization from n-hexane at room temperature and AIBN initiator was purified by recrystallization from methanol at 50 °C. Reactions conditions, purification methods, and characterization of all prepared materials not reported here are described in the Supporting Information.

Uptake Experiments: The determination of the BZM uptake by dendritic glycopolymers was carried out by ultrafiltration procedures with solvent resistant stirred cells (47 mm, Merck) and polyethersulfone (PES) membranes (MWCO 1 kD, Pall Life Science) at a rotation speed of 200 rpm under 5 atm pressure of nitrogen at 25 °C. Following a previously published approach[19] DDS: BZM complexation ratios of 1:10, 1:25, and 1:50, respectively, were used. These complexes (Table S4, Supporting Information) fabricated in water or PBS buffer were separated after 20 h of stirring by ultrafiltration in three stirred cells per 40 mL at 0, 3, and 20 h. Samples with a volume of 2 mL were taken at 18, 23, and 28 mL and the complexed amount of BZM was calculated by UV–vis measurements at λ(BZM) = 271 nm.
Release Experiments: The release of BZM from drug/DDS/CPC composite was studied in an aqueous 4 wt% disodium hydrogen phosphate (Na2HPO4) solution at 37 °C. A total volume of 400 µL of 4 wt% Na2HPO4 solutions, containing the corresponding concentration of BZM and polymer, was used to mix with 1 g calcium phosphate bone cement powder (Table S5, Supporting Information). Subsequently, the cement paste was molded into disks using a silicone mold followed by a drying process at 37 °C (Figure S15, Supporting Information). After 4 d, all cement disks (eight per batch) were placed in UV cuvettes and incubated in 2 mL PBS solution (Figure S15, Supporting Information). In this closed system, the release of BZM from different DDS/CPC composites was observed over two weeks at 37 °C by UV–vis measurements at λ(BZM) = 271 nm. Each release experiment was repeated twice with eight disks so that the accumulated release values at 0, 3, 8, 48, 96, 168, and 336 h were determined. This approach was also used for the determination of release of pure BZM and dye-labeled DDS.

Synthesis of Polymer 10 by RAFT Polymerization: NIPAm or NVCL (n eq.), AIBN (0.5 eq.), and a solution of the RAFT reagent (9 (1 eq.) in 1,4-dioxane (c = 0.25 g (NIPAm) L⁻¹ or 0.6 g (NVCL) L⁻¹) were added to a dry Schlenk tube containing a stirrer bar. Then the solution was degassed using at least a four freeze–pump–thaw cycles, back filled with argon and stirred at 70 °C. The reaction was quenched by liquid nitrogen and solvent was removed in vacuo. The crude product was purified twice by dissolving in tetrahydrofuran (THF) (3 mL) and precipitation in n-hexane (400 mL). The product was obtained as a white-yellow solid (Table S1, Supporting Information).

End Group Removal of 10 by Radical-Induced Reduction: Ada-PNIPAm or Ada-PNVCL (10, 1 eq.), AIBN (0.6 eq.), TTMS (2 eq.), and 1,4-dioxane (c = 0.25 g (Ada-Polymer) L⁻¹) were added to a dry Schlenk tube containing a stirrer bar. The solution was degassed using at least a four freeze–pump–thaw cycles, back filled with argon and stirred at 80 °C for 5 h for 10a–d and for 18 h for 10e–g, respectively. The reaction was quenched by liquid nitrogen and the solvent is removed in vacuo. The crude product was purified twice by dissolving in THF (3 mL) and precipitation in n-hexane (400 mL) followed by dialysis in a mixture of THF and water (4:1). After removing THF in vacuo and freeze drying, the product 2 was quantitatively obtained as a white solid.

PEI-Mal B-[CD]/Ada-Polymer)ₙ: PEI-Mal B-[CD]ₙ (1, n = 5 or 10, 1 eq.) and Ada-PNIPAm or Ada-PNVCL (2, 2n eq.) were dissolved in water (c = 2 g (PEI-Mal B-[CD]ₙ) L⁻¹) and stirred in an ice bath for 1 d. The crude product was purified by dialysis (regenerated cellulose tubing membranes, MWCO 25 kDa) in deionized water for 1 d. The dialysis was carried out in a 3 L beaker which was cooled outside by an ice bath in a 5 L beaker and the solvent was changed every 30 min. After freeze drying the desired product was obtained as white solid (Table S2, Supporting Information).

Keywords
calcium phosphate cements, dendritic glycopolymers, drug delivery systems, proteasome inhibitors, temperature-sensitive polymers

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
The authors thank the German Research Foundation (DFG) for funding this study within the framework of the SFB/TR79 “Materials for tissue regeneration within systemically altered bone.” The authors want to thank Mrs. C. Harnisch for carrying out GPC measurements and Mr. R. Schulze for carrying out elemental analysis.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest
The authors declare no conflict of interest.

[1] K. Hatano, K. Matsuoka, D. Terunuma, Chem. Soc. Rev. 2013, 42, 4574.
[2] Y. M. Chabre, R. Roy, Chem. Soc. Rev. 2013, 42, 4657.
[3] D. Appelhans, B. Klijnert-Maculiewicz, A. Janaszewska, J. Lazniewska, B. Voit, Chem. Soc. Rev. 2015, 44, 3968.
[4] K. C. Wood, S. R. Little, R. Langer, P. T. Hammond, Angew. Chem., Int. Ed. 2005, 44, 6704.
[5] H. Arima, Y. Chihara, M. Arizono, S. Yamashita, K. Wada, F. Hirayama, K. Uekama, J. Controlled Release 2006, 116, 64.
[6] S.-K. Wang, P.-H. Liang, R. D. Astronomo, T.-L. Hsu, S.-L. Hsieh, D. R. Burton, C.-H. Wong, Proc. Natl. Acad. Sci. USA 2008, 105, 3690.
[7] A. Papadopoulos, T. C. Shiao, R. Roy, Mol. Pharm. 2012, 9, 394.
[8] Y. Miura, Y. Hoshino, H. Seto, Chem. Rev. 2016, 116, 16713.
[9] S. P. Bernhard, M. S. Fricke, R. Haag, M. J. Cloninger, Polym. Chem. 2020, 11, 3849.
[10] A. Köth, D. Appelhans, D. Robertson, B. Tiersch, J. Koetz, Soft Matter 2011, 7, 10581.
[11] V. Perce, P. Leowanawat, H.-J. Sun, O. Kulikov, C. D. Nusbaum, T. M. Tran, A. Bertin, D. A. Wilson, M. Peterca, S. Zhang, N. P. Kamat, K. Vargo, D. Mook, E. D. Johnston, D. J. Pochan, Y. Chen, Y. M. Chabre, T. C. Shiao, M. Bergeron-Blek, S. André, R. Roy, H.-J. Gabius, P. A. Heiney, J. Am. Chem. Soc. 2013, 135, 9055.
[12] J. Daeg, X. Xu, L. Zhao, S. Boye, A. Janke, A. Temme, J. Zhao, A. Ledrer, B. Voit, X. Shi, D. Appelhans, Biomacromolecules 2020, 21, 199.
[13] F. Ennen, P. Fener, G. Stoychev, S. Boye, A. Ledrer, B. Voit, D. Appelhans, ACS Appl. Mater. Interfaces 2016, 8, 6261.
[14] M. Schmitz, N. Candelise, E. Kanata, F. Llorens, K. Thüne, A. Villar-Piqué, S. M. Da Silva Correia, D. Dafou, T. Sklaviadis, D. Appelhans, I. Zeir, Mol. Neurobiol. 2020, 57, 1863.
[15] S. Tietze, I. Schau, S. Michen, F. Ennen, A. Janke, G. Schackert, A. Aigner, D. Appelhans, A. Temme, Small 2017, 13, 1700072.
[16] E. Aso, I. Martinsson, D. Appelhans, C. Effenberg, N. Bensery-Cases, J. Cladera, G. Gouras, I. Ferrer, O. Klementieva, Nanomed.: Nanotechnol., Med. Biol. 2019, 17, 198.
[17] S. Bhatia, D. Lauster, M. Bardua, K. Ludwig, S. Angiotta-Uberti, N. Popp, U. Hoffmann, F. Paulus, M. Budt, M. Stadtmüller, T. Wolff, A. Hamann, C. Böttcher, A. Herrmann, R. Haag, Biomaterials 2017, 138, 22.
[18] J. H. Ennist, H. R. Termuhlen, S. P. Bernhard, M. S. Fricke, M. J. Cloninger, Biocconjuate Chem. 2018, 29, 4030.
[19] C. Striegler, M. Schumacher, C. Effenberg, M. Müller, A. Seckinger, R. Schnettler, B. Voit, D. Hose, M. Gelinsky, D. Appelhans, Macromol. Biosci. 2015, 15, 1283.
[20] J. Majoijen, J. S. Haataja, D. Appelhans, A. Lederer, A. Olszewska, J. Seitsonen, V. Aseyev, E. Kontturi, H. Rosilo, M. Österberg, N. Houbenov, O. Ikalka, J. Am. Chem. Soc. 2014, 136, 866.
[21] R. A. Kyle, S. V. Rajkumar, N. Engl. J. Med. 2004, 351, 1860.
[22] D. Appelhans, H. Kömer, M. A. Quadir, S. Richter, S. Schwarz, J. Van Der Vlist, A. Aigner, M. Müller, K. Loos, J. Seidel, K.-F. Arndt, R. Haag, B. Voit, Biomacromolecules 2009, 10, 1114.
[23] B. Klein, A. Seckinger, T. Moehler, D. Hose, Recent Results Cancer Res. 2011, 183, 39.
[24] M. Heskins, J. E. Guillet, J. Macromol. Sci., Part A 1968, 2, 1441.
[25] F. Meeussen, E. Nies, H. Berghmans, S. Verbrugghe, E. Goethals, F. Du Prez, Polymer 2000, 41, 8597.
[26] G. Huang, J. Gao, Z. Hu, J. V. St. John, B. C. Ponder, D. Moro, J. Controlled Release 2004, 94, 303.
[27] H. Vihola, A. Laukkanen, H. Tenhu, J. Hirvonen, J. Pharm. Sci. 2008, 97, 4783.
[28] S. Tang, B. D. Olsen, Front. Chem. 2014, 8, 23.
[29] Y. K. Chong, G. Moad, E. Rizzardo, S. H. Thang, Macromolecules 2007, 40, 4446.
[30] N. Polikarpov, D. Appelhans, P. Welzel, A. Kaufmann, P. Dhanapal, C. Bellmann, B. Voit, New J. Chem. 2012, 36, 438.
[31] F. Schulze, B. Keperscha, D. Appelhans, A. Rösen-Wollf, Biomacromolecules 2019, 20, 2713.