

# Glucocorticoid-Loaded Core-Cross-Linked Polymeric Micelles with Tailorable Release Kinetics for Targeted Therapy of Rheumatoid Arthritis\*\*

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Glucocorticoids (GCs) play a prominent role in the therapeutic management of rheumatoid arthritis (RA) and other chronic inflammatory diseases.<sup>[1]</sup> However, the occurrence of significant adverse effects, especially when high doses are required, forms an important limitation with respect to the clinical application of GCs.<sup>[1]</sup> Targeted delivery strategies for improving the accumulation of GCs in the target tissue have therefore attracted considerable attention in recent years, especially for RA therapy.<sup>[2]</sup> In RA, such strategies commonly

exploit the up to 40-fold increase in the permeability of the blood–joint barrier of the inflamed synovium for macromolecules and nanoparticulates.<sup>[2b,3]</sup> This preferred distribution of systemically administered nanomedicines to arthritic joints enables the inflammation-directed targeting of GCs, thereby improving efficacy and reducing systemic GC toxicity.<sup>[2a,4]</sup>

In the last decades, many types of nanomedicines, including liposomes,<sup>[5]</sup> polymeric drug derivatives,<sup>[6]</sup> protein–drug conjugates,<sup>[7]</sup> polymeric micelles,<sup>[8]</sup> and other types of nanoparticles,<sup>[9]</sup> have been developed for drug targeting. Specific properties, such as size, circulation time, (bio)degradability, cellular distribution and internalization, and drug release kinetics, are important factors that determine the efficacy of a system for a given therapeutic application. Core-cross-linked polymeric micelles (PMs) based on poly(ethylene glycol)-*b*-poly[*N*-(2-hydroxypropyl)methacrylamide-lactate] (mPEG-*b*-pHPMAMlac<sub>n</sub>) have attracted considerable attention because of their favorable characteristics in this regard. Their 1) degradability under physiological conditions, 2) 50–100 nm size range (which is very suitable for drug targeting purposes), 3) prolonged circulation kinetics, and 4) flexibility with regard to their physicochemical properties make core-cross-linked PMs highly suitable for systemic drug targeting.<sup>[10]</sup>

After obtaining promising results with PMs for tumor-targeting of doxorubicin,<sup>[11]</sup> we herein for the first time demonstrate the potential of PMs for GC targeting to joints affected by RA. Since the favorable circulation kinetics of core-cross-linked polymeric micelles in mice have been demonstrated previously,<sup>[12]</sup> this study focuses on a novel chemical concept of using thioether esters to tailor the release of the targeted drug. Dexamethasone (DEX), a highly potent GC, was derivatized with three different methacrylated linkers through ester bonds, thereby creating polymerizable DEX derivatives. By employing linkers that additionally contain thioethers with different degrees of oxidation, the hydrolysis rate of the ester bond, and therefore the drug release rate upon entrapment into the micellar core, is potentially controllable. The tailorable drug release kinetics of the different DEX-loaded PMs were confirmed in vitro, and the anti-arthritic efficacy of the micellar nanomedicine with the most rapid release kinetics was evaluated in two different animal models of inflammatory arthritis.

PMs were prepared from block copolymers consisting of a hydrophilic PEG block and a thermosensitive

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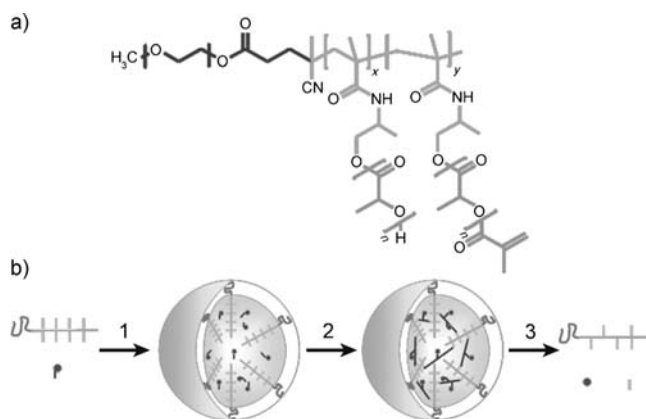
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pHPMAmLac<sub>n</sub> block, which is either hydrophilic or hydrophobic depending on the temperature (Figure 1a).<sup>[13]</sup> At a temperature below its lower critical solution temperature (LCST), the pHPMAmLac<sub>n</sub> block is hydrophilic, and there-



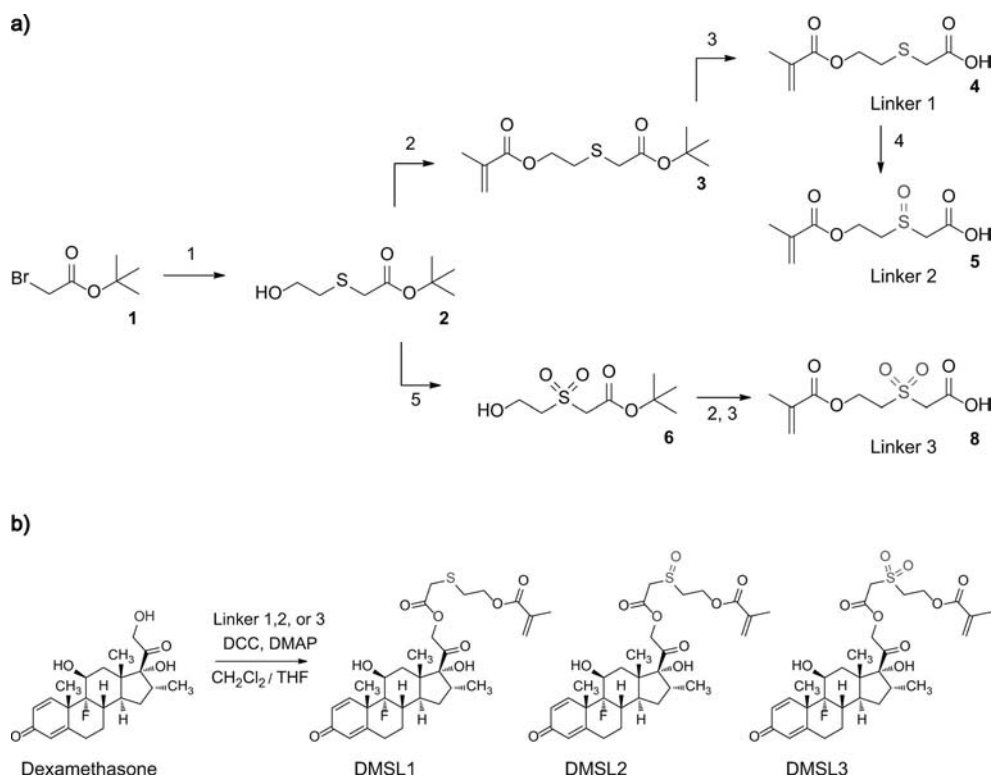
**Figure 1.** Schematic representation of dexamethasone-loaded core-cross-linked polymeric micelles (DEX-PMs). a) Chemical structure of thermosensitive mPEG-*b*-pHPMAmLac<sub>n</sub> block copolymers. b) Preparation, degradation, and drug release from DEX-PMs. 1) Micelle formation and encapsulation of hydrophobic DEX derivatives by rapid heating; 2) copolymerization of methacrylated lactate side chains and DEX derivatives in the micelle core; 3) hydrolytic liberation of lactate moieties, drugs, and drug linkers, leading to micellar destabilization and drug release.

fore, in aqueous conditions, is fully soluble. However, when the temperature is raised above its LCST, the pHPMAmLac<sub>n</sub> block will become hydrophobic, thereby rendering the block copolymer amphiphilic, which in aqueous media results in the formation of micellar nanostructures that are composed of a hydrophobic pHPMAmLac<sub>n</sub> core and a hydrophilic PEG shell. Subsequent cross-linking of the micellar core by polymerization of the partially methacrylated lactate side chains of the pHPMA backbone ensures particle stability below the critical micelle concentration (CMC), thereby preventing rapid dissociation upon systemic administration, and assuring prolonged circulation times.<sup>[14]</sup>

Over time, the ester bonds that link the lactate groups and cross-links to the

polymeric backbone will undergo hydrolysis in aqueous conditions, which eventually results in destabilization of the cross-linked core. The outer shell of the PM is composed of hydrophilic PEG blocks, which is a key component for providing the PM with stealth-like properties, resulting in prolonged circulation times and efficient drug targeting to tumors and to sites of inflammation.<sup>[12,15]</sup>

To allow transiently stable entrapment of DEX in the PM, three different methacrylate-functionalized linkers were synthesized (Scheme 1). In a final single reaction step, these polymerizable linkers were coupled with high efficiency to dexamethasone, thereby generating polymerizable DEX derivatives. To prepare DEX-loaded PMs, the hydrophobic DEX derivatives were first loaded into the micellar core during micelle formation and subsequently covalently entrapped in the particle core upon free radical polymerization of the methacrylate moieties (Figure 1b). A highly efficient encapsulation of the DEX derivatives (> 80%) was obtained at a drug/polymer feed ratio of 10% (w/w). Consequently, the ratio of polymer methacrylate units versus the number of DEX derivatives was calculated to be 1.8. Importantly, it was shown that quantitative methacrylate conversion took place, as determined using a previously established HPLC method,<sup>[16]</sup> indicating that all entrapped DEX derivatives were covalently incorporated in the polymeric structure. The resulting DEX-loaded core-cross-linked PMs were relatively



**Scheme 1.** Synthesis of polymerizable sulfide (DMSL1), sulfoxide (DMSL2), and sulfone (DMSL3) ester dexamethasone derivatives. a) Synthesis of methacrylated linkers containing a thioether moiety with different degrees of oxidation: 1) 2-mercaptoethanol, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 2) methacryloylchloride, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 3) trifluoroacetic acid, 4) NaIO<sub>4</sub>, MeCN/H<sub>2</sub>O, 5) NaIO<sub>4</sub>, RuCl<sub>3</sub>, MeCN/CCl<sub>4</sub>/H<sub>2</sub>O. b) Conjugation of the linkers to dexamethasone in a single reaction step to create polymerizable, hydrolytically cleavable dexamethasone derivatives. DCC = *N,N*-dicyclohexylcarbodiimide, DMAP = 4-(dimethylamino)pyridine.

monodisperse with a diameter of around 70 nm and a polydispersity index of 0.1.

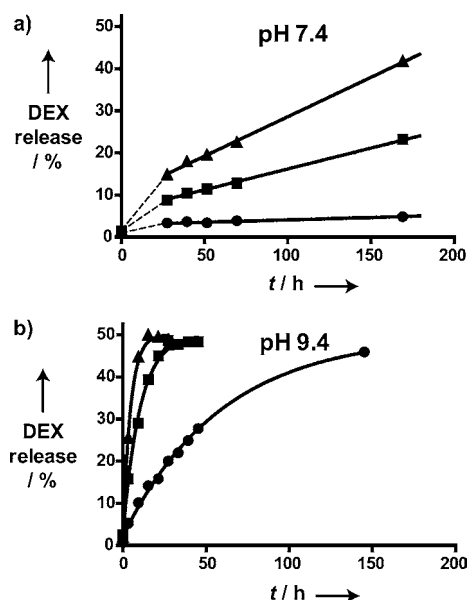
To allow for tailorable release kinetics, hydroxyethyl-methacrylate was conjugated to dexamethasone through a sulfide (DMSL1), a sulfoxide (DMSL2), and a sulfone (DMSL3) ester. The presence of an electron-withdrawing moiety in close proximity of the ester bond reduces the electron density of the ester bond; this accelerates the hydrolysis of this bond by hydroxy ions, thereby releasing the conjugated drug more rapidly.<sup>[17]</sup> The linkers presented herein contain a thioether, which is a stronger electron-withdrawing group than an ether moiety.<sup>[18]</sup> Moreover, the degree of oxidation of the sulfur in the thioether can be modified, which is expected to change its electron-withdrawing properties, thus allowing the adjustment of the hydrolysis rate of the neighboring ester bond. These linkers with different hydrolysis kinetics ensure proper release of DEX over time, and allow the rate of release from the core-cross-linked PMs to be tailored. Indeed, under physiological conditions (37 °C, pH 7.4), when compared to the relatively slow DEX release from PMs containing sulfide ester linked DEX (DMSL1, < 5% in 7 days), the DEX release from PMs containing the sulfoxide ester linked DEX (DMSL2,  $t_{1/2}$  (18.4 ± 0.4) days) or the sulfone ester linked DEX (DMSL3,  $t_{1/2}$  (8.9 ± 0.1) days) was much faster (Figure 2a). When the ester hydrolysis was accelerated by incubating the PMs at pH 9.4, the DEX release, as expected, followed first-order kinetics where DMSL3 ( $t_{1/2}$  (3.2 ± 0.3) h) was cleaved faster than DMSL2 ( $t_{1/2}$  (7.1 ± 0.4) h), which in turn was cleaved considerably faster than DMSL1 ( $t_{1/2}$  (41.3 ± 1.5) h; Fig-

ure 2b). The release kinetics at pH 9.4 were approximately a factor 100 faster than at pH 7.4, thus demonstrating that the hydrolysis reaction was driven by OH<sup>-</sup> ions.<sup>[19]</sup> These data convincingly demonstrate that the release kinetics of DEX from core-cross-linked PMs can be tailored by employing DEX derivatives that contain thioether esters with different degrees of oxidation, that is, a sulfide, sulfoxide, or sulfone ester.

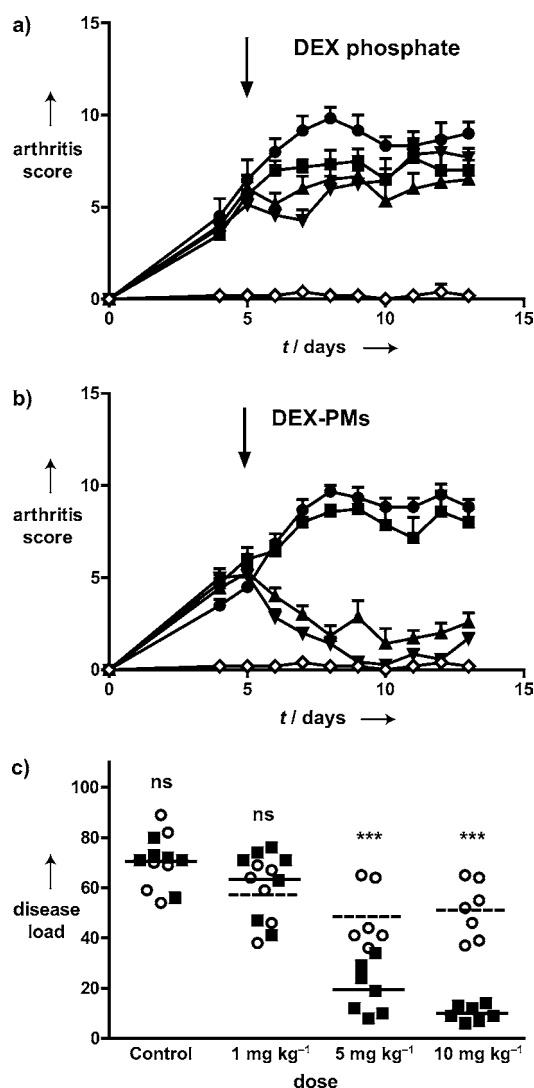
To demonstrate that PMs hold significant potential as nanomedicines for systemic RA treatment, the anti-arthritic efficacy of DEX-PMs that were prepared using DMSL3 (selected because of the highest DEX release rate, taking into account the acute inflammatory character of both animal models used) was evaluated in vivo. Mice with collagen antibody-induced arthritis (CAIA; a model characterized by acute and strong joint inflammation)<sup>[20]</sup> were treated intravenously (i.v.) with a single administration of phosphate-buffered saline (PBS), unloaded PMs, free DEX phosphate or DEX-PMs, five days after disease induction. By using different doses of free DEX phosphate and DEX-PMs, that is, 1, 5, and 10 mg kg<sup>-1</sup>, the dose-dependent efficacy of DEX-PMs in relation to that of the free drug was assessed (Figure 3a).

Treatment with free DEX phosphate merely resulted in a moderate, dose-dependent, and short-term reduction in clinical signs of arthritis, which was only significant on days seven, eight, and ten for 5 mg kg<sup>-1</sup> ( $p < 0.05$ , two-way ANOVA vs. PBS), and on days six to nine ( $p < 0.05$ ) for 10 mg kg<sup>-1</sup> (Figure 3a). In contrast, mice treated with DEX-PMs (5 or 10 mg kg<sup>-1</sup>) showed a strong and long-lasting reduction in arthritic symptoms compared to controls ( $p < 0.01$  from day 6 onwards, in case of 5 mg kg<sup>-1</sup>;  $p < 0.001$  from day 6 onwards, in case of 10 mg kg<sup>-1</sup>; Figure 3b). Moreover, mice that received DEX-PMs dosed at 10 mg kg<sup>-1</sup> showed no or very little signs of arthritis from day nine onwards (i.e. 4 days after treatment), as illustrated by the arthritis scores that were comparable to those of healthy mice. Since PMs have a circulation half-life of around one day,<sup>[12]</sup> these long-lasting anti-inflammatory effects likely result from a preferential accumulation of the nanocarriers in inflamed joints, thereby enabling local release of dexamethasone, and not merely from an extension of the circulation kinetics of the GC. The pronounced improvement in therapeutic efficacy of DEX upon encapsulation in PMs is further illustrated by the total disease load (defined as the area under the arthritis score curve) of mice treated with DEX-PMs compared to those treated with free DEX phosphate (Figure 3c,  $p < 0.001$ , one-way ANOVA). The change in ankle diameter, as a measure of inflammation-related joint swelling, confirmed these findings (Figure S1 in the Supporting Information).

Subsequently, DEX-loaded PMs were also evaluated in rats with adjuvant-induced arthritis (AA; a widely used arthritis model displaying a T-lymphocyte-mediated inflammatory response against cartilage proteoglycan, which is in many respects similar to human RA).<sup>[21]</sup> In this case, rats with established adjuvant-induced polyarthritis were treated with a single i.v. administration of free DEX phosphate (10 mg kg<sup>-1</sup>), DEX-PMs (10 mg kg<sup>-1</sup>), or PBS (Figure 4). Although the administration of free DEX phosphate resulted in a significant alleviation of the clinical signs of arthritis, as

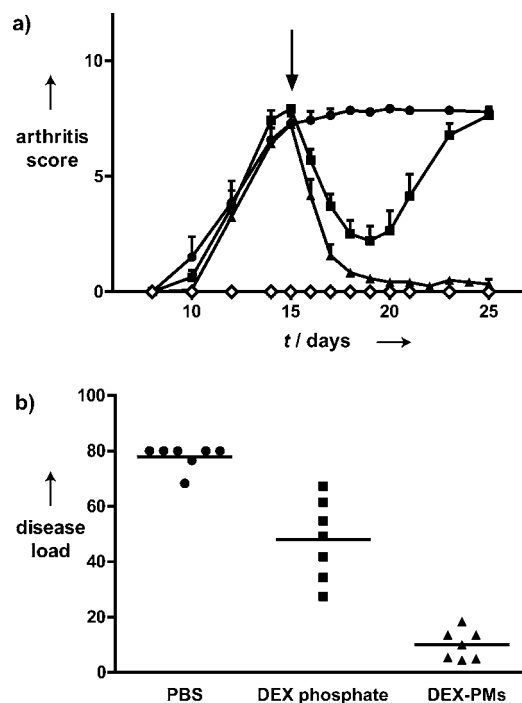


**Figure 2.** The release of DEX from core-cross-linked PMs loaded with different DEX derivatives at 37 °C and a) pH 7.4 or b) pH 9.4. DEX is covalently linked to the hydrophobic core of the micelle through hydrolysable linkers based on a sulfide ester (●, DMSL1), a sulfoxide ester (■, DMSL2), or a sulfone ester (▲, DMSL3; Scheme 1). The release rate from the PMs was dependent on the degree of oxidation of the sulfur atom in the DEX linker, with the order in release rate: DMSL3 > DMSL2 > DMSL1.



**Figure 3.** Therapeutic efficacy of free dexamethasone phosphate (DEX phosphate) and DEX-PMs in mice with CAIA. Polyarthritis was induced by i.v. injection of a collagen antibody cocktail on day 0, followed by an intraperitoneal (i.p.) administration of lipopolysaccharide (LPS) on day 3. a, b) The arthritis score, representing disease activity, was determined daily by using the scoring system presented in Table S1 in the Supporting Information. On day 5 (arrow), when all CAIA-induced mice presented clear signs of inflammation, they received an i.v. injection of DEX phosphate (a) or DEX-PMs (b) dosed at 1 (■), 5 (▲), or 10 mg kg<sup>-1</sup> (▼). Control mice (●) received PBS or unloaded micelles. One group of mice without CAIA served as healthy control (◇). The data is presented as the mean score with standard error of the mean (SEM) of each group. c) The disease load of each individual mouse upon treatment with free DEX phosphate (○) or DEX-PMs (■). The disease load was defined as the area under the arthritis score curve from treatment (day 5) until the end of the study (day 13). The mean of the experimental group is presented as a straight line (dashed for free DEX phosphate; solid for DEX-PMs). Control animals were injected with PBS (free DEX phosphate group) or unloaded micelles (DEX-PM group). At a dose of 5 or 10 mg kg<sup>-1</sup>, but not at 1 mg kg<sup>-1</sup>, there was a significant reduction of mean disease load after treatment with DEX-PMs when compared to free DEX phosphate (one-way ANOVA followed by Bonferroni's multiple comparison test). \*\*\*,  $p < 0.001$ ; ns, not significant.

indicated by the arthritis score (Figure 4a;  $p < 0.001$  as compared to PBS; from day 17 till 21; two-way ANOVA) and ankle swelling (Figure S2 in the Supporting Information;  $p < 0.001$ ; from day 17 until 23;  $p < 0.05$  on day 25), this effect was only transient and started to weaken from day five after treatment. In contrast, upon treatment with DEX-PMs at the same dose, an immediate and prolonged anti-arthritic effect was achieved ( $p < 0.001$  as compared to PBS; from day 16 onwards; for both arthritis score and ankle diameter). DEX-PMs were significantly more effective than free DEX phosphate in terms of reducing the arthritis score ( $p < 0.05$  on day 16 and 20;  $p < 0.001$  from day 21 onwards), as well as minimizing the degree of ankle swelling ( $p < 0.001$  on day 23 and 25). Finally, while free DEX phosphate (10 mg kg<sup>-1</sup>) had a limiting effect on the disease load ( $p < 0.01$  as compared to PBS; one-way ANOVA), the total disease load of arthritic rats treated with DEX-PMs was considerably lower than the disease load of rats treated with either PBS or free DEX phosphate ( $p < 0.001$  for both; Figure 4b).



**Figure 4.** Therapeutic efficacy of free DEX phosphate and DEX-PMs in rats with AA. Arthritis was induced on day 0 by subcutaneous administration of *Mycobacterium tuberculosis* H37Ra in the base of the tail. On day 15 (arrow), rats ( $n = 7$ , all groups) with established AA were treated with a single administration of 10 mg kg<sup>-1</sup> of free DEX phosphate (■) or DEX-PMs (▲). Control rats received PBS (●) and one group of rats without AA served as healthy controls (◇). a) The arthritis score, representing disease activity, of all rats (shown as mean with SEM of each group) was determined daily using the scoring system presented in Table S2 in the Supporting Information. b) The disease load, defined as the area under the arthritis score curve from treatment (day 15) until the end of the study (day 25), of each individual rat, is presented together with the mean of the treatment group as a straight line. The disease load of animals in each treatment group was significantly different from those in the other groups ( $p < 0.001$ , one-way ANOVA followed by Bonferroni's multiple comparison test).

An important advantage of the PM system for glucocorticoid targeting presented herein is the tailorability of the release rate of dexamethasone from the carrier system, which allows further optimization of drug release kinetics for the treatment of RA, as well as other diseases. Future experiments will have to elucidate, for example, the optimal release kinetics for the treatment of different diseases. Furthermore, by incorporating a combination of DEX derivatives with different conversion rates within a single micellar nanomedicine, it will be possible to fine-tune the DEX release profile, for example, by employing a rapid-release and with a slow-release component. Another advantage of the presented system is the entrapment of the therapeutic agent in the hydrophobic core of the PM, which limits any unwanted exposure to its environment. Finally, since the synthesis procedure for preparing polymerizable DEX derivatives only requires the presence of the drug in the final step of the process, the linker technology presented herein is broadly applicable for the preparation of core-cross-linked PMs or other (polymeric) nanocarriers entrapping a wide range of therapeutic agents (that contain a reactive hydroxy moiety), thus making this system very versatile and promising, not only for targeted RA therapy but for the treatment of many other disorders.

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- [1] J. N. Hoes, J. W. G. Jacobs, F. Buttgerit, J. W. J. Bijlsma, *at. Rev. Rheumat.* **2010**, *6*, 693.
- [2] a) J. M. Metselaar, M. H. M. Wauben, J. P. A. Wagenaar-Hilbers, O. C. Boerman, G. Storm, *Arthritis Rheum.* **2003**, *48*, 2059; b) L.-D. Quan, P. E. Purdue, X.-m. Liu, M. Boska, S. Lele, G. Thiele, T. Mikuls, H. Dou, S. Goldring, D. Wang, *Arthritis Res. Ther.* **2010**, *12*, R170.
- [3] I. Kushner, J. A. Somerville, *Arthritis Rheum.* **1971**, *14*, 560.
- [4] D. Wang, S. C. Miller, M. Sima, D. Parker, H. Buswell, K. C. Goodrich, P. Kopečková, J. Kopeček, *Pharm. Res.* **2004**, *21*, 1741.
- [5] a) V. P. Torchilin, *Nat. Rev. Drug Discovery* **2005**, *4*, 145; b) Y. Malam, M. Loizidou, A. M. Seifalian, *Trends Pharmacol. Sci.* **2009**, *30*, 592.
- [6] a) J. Kopeček, P. Kopečková, *Adv. Drug Delivery Rev.* **2010**, *62*, 122; b) D. B. Pike, H. Ghandehari, *Adv. Drug Delivery Rev.* **2010**, *62*, 167.
- [7] a) D. J. FitzGerald, A. S. Wayne, R. J. Kreitman, I. Pastan, *Cancer Res.* **2011**, *71*, 6300; b) E. Neumann, E. Frei, D. Funk, M. D. Becker, H. H. Schrenk, U. Muller-Ladner, C. Fiehn, *Expert Opin. Drug Delivery* **2010**, *7*, 915.
- [8] a) K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Delivery Rev.* **2001**, *47*, 113; b) E. S. Lee, Z. Gao, D. Kim, K. Park, I. C. Kwon, Y. H. Bae, *J. Controlled Release* **2008**, *129*, 228; c) G. Gaucher, R. H. Marchessault, J.-C. Leroux, *J. Controlled Release* **2010**, *143*, 2; d) Y. Bae, S. Fukushima, A. Harada, K. Kataoka, *Angew. Chem.* **2003**, *115*, 4788; *Angew. Chem. Int. Ed.* **2003**, *42*, 4640.
- [9] a) Z. Poon, J. B. Lee, S. W. Morton, P. T. Hammond, *Nano Lett.* **2011**, *11*, 2096; b) F. P. Dong, W. P. Guo, J. H. Bae, S. H. Kim, C. S. Ha, *Chem. Eur. J.* **2011**, *17*, 12802.
- [10] M. Talelli, C. J. F. Rijcken, C. F. van Nostrum, G. Storm, W. E. Hennink, *Adv. Drug Delivery Rev.* **2010**, *62*, 231.
- [11] M. Talelli, M. Iman, A. K. Varkouhi, C. J. F. Rijcken, R. M. Schiffelers, T. Etrych, K. Ulbrich, C. F. van Nostrum, T. Lammers, G. Storm, W. E. Hennink, *Biomaterials* **2010**, *31*, 7797.
- [12] C. J. Rijcken, M. Talelli, C. F. van Nostrum, G. Storm, W. E. Hennink, *Eur. J. Nanomed.* **2010**, *3*, 19.
- [13] C. J. F. Rijcken, T. F. J. Veldhuis, A. Ramzi, J. D. Meeldijk, C. F. van Nostrum, W. E. Hennink, *Biomacromolecules* **2005**, *6*, 2343.
- [14] C. J. F. Rijcken, C. J. Snel, R. M. Schiffelers, C. F. van Nostrum, W. E. Hennink, *Biomaterials* **2007**, *28*, 5581.
- [15] a) A. Vonarbourg, C. Passirani, P. Saulnier, J.-P. Benoit, *Biomaterials* **2006**, *27*, 4356; b) H. S. Choi, B. I. Ipe, P. Misra, J. H. Lee, M. G. Bawendi, J. V. Frangioni, *Nano Lett.* **2009**, *9*, 2354; c) H. Maeda, *Bioconjugate Chem.* **2010**, *21*, 797; d) D. Wang, S. R. Goldring, *Mol. Pharm.* **2011**, *8*, 991.
- [16] R. J. H. Stenekes, W. E. Hennink, *Polymer* **2000**, *41*, 5563.
- [17] a) R. G. Schoenmakers, P. van de Wetering, D. L. Elbert, J. A. Hubbell, *J. Controlled Release* **2004**, *95*, 291; b) B. J. Crielaard, S. van der Wal, T. Lammers, H. T. Le, W. E. Hennink, R. M. Schiffelers, G. Storm, M. H. A. M. Fens, *Eur. J. Pharm. Sci.* **2012**, *45*, 429.
- [18] J. M. Harris, A. Kozłowski, US Patent 5,672,662, **1997**.
- [19] W. N. E. van Dijk-Wolthuis, M. J. van Steenberg, W. J. M. Underberg, W. E. Hennink, *J. Pharm. Sci.* **1997**, *86*, 413.
- [20] L. M. Khachigian, *Nat. Protoc.* **2006**, *1*, 2512.
- [21] W. v. Eden, J. E. R. Tholet, R. v. d. Zee, A. Noordzij, J. D. A. van Embden, E. J. Hensen, I. R. Cohen, *Nature* **1988**, *331*, 171.