Journal of Controlled Release 244 (2016) 314–325

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Tailoring the physicochemical properties of core-crosslinked polymeric micelles for pharmaceutical applications

Qizhi Hu [a,b], Cristianne J.F. Rijcken [b], Ethlinn van Gaal [b], Paul Brundel [b], Hana Kostkova [c], Tomas Etrych [c], Benjamin Weber [d], Matthias Barz [d], Fabian Kiessling [e], Jai Prakash [a], Gert Storm [a,f], Wim E. Hennink [f], Twan Lammers [a,e,f,*]

[a] Department of Biomaterials Science and Technology, Section: Targeted Therapeutics, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands
[b] Cristal Therapeutics, Oxfordlaan 55, Maastricht 6229EV, The Netherlands
[c] Institute of Macromolecular Chemistry, the Czech Academy of Sciences, Heyrovský Sq. 2, 162 06 Prague 6, Czech Republic
[d] Institute of Organic Chemistry, Johannes Gutenberg-University Mainz, Duesbergweg 10-14, 55128 Mainz, Germany
[e] Department of Nanomedicine and Theranostics, Institute for Experimental Molecular Imaging, University Clinic and Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen 52074, Germany
[f] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht 3584CG, The Netherlands

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form 4 July 2016
Accepted 7 July 2016
Available online 9 July 2016

Keywords:
Nanomedicine
Drug targeting
Polymeric micelles
Core-crosslinking
Drug release

ABSTRACT

To optimally exploit the potential of (tumor-) targeted nanomedicines, platform technologies are needed in which physicochemical and pharmaceutical properties can be tailored according to specific medical needs and applications. We here systematically customized the properties of core-crosslinked polymeric micelles (CCPM). The micelles were based on mPEG-*b*-pHPMAmLac$_n$ (i.e. methoxy poly(ethylene glycol)-*b*-poly[*N*-(2-hydroxypropyl) methacrylamide-lactate]), similar to the block copolymer composition employed in CriPec® docetaxel, which is currently in phase I clinical trials. The CCPM platform was tailored with regard to size (30 to 100 nm), nanocarrier degradation (1 month to 1 year) and drug release kinetics (10 to 90% in 1 week). This was achieved by modulating the molecular weight of the block copolymer, the type and density of the crosslinking agent, and the hydrolytic sensitivity of the drug linkage, respectively. The high flexibility of CCPM facilitates the development of nanomedicinal products for specific therapeutic applications.

1. Introduction

Over the past three decades, nanoparticle technologies have shown great promise for disease treatment. The incorporation of drugs in nanoparticles, together referred to as nanomedicines, can improve the pharmacokinetic profile and disposition of drugs in the body, leading to enhanced efficacy and/or reduced toxicity [1–9]. To date, approximately a dozen nanomedicinal products have been approved (e.g. Doxil®, Abraxane® and Genexol-PM®) [10–12] and more than a hundred are under clinical investigation [2,13–19]. However, in spite of significant progress at the preclinical level, their clinical benefits have remained relatively modest [20–23]. To improve the clinical performance of nanomedicinal products, their pharmaceutical properties should be customized for a specific disease and application, and ideally also to the characteristics and needs of a specific patient [24,25].

The physicochemical and pharmaceutical properties of nanomedicines strongly influence their in vivo therapeutic performance. Among these, particle size is a critical characteristic that governs the circulation kinetics and biodistribution profile of nanomedicines after systemic administration [26,27]. There is general consensus that nanoparticle size should be large enough to avoid renal excretion and at the same time small enough to evade rapid uptake by the mononuclear phagocyte system (MPS), leaving a size window between ~5–200 nm [28,29]. For tumor targeting, a relatively small particle size of 5–50 nm in diameter is generally desired to enable besides efficient tumor accumulation via the "enhanced permeability and retention (EPR) effect" [30,31] also deep penetration into the tumor interstitium [32,33]. The release profile of incorporated drug(s) is also vital for the therapeutic performance of nanomedicines [34]. While excessive drug release as a burst in the circulation could give rise to toxic systemic drug levels, delayed drug release could result in sub-therapeutic drug levels at the target site, both leading to poor clinical performance [35]. Furthermore,

* Corresponding author at: Department of Biomaterials Science and Technology, Section: Targeted Therapeutics, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands.
E-mail address: tlammers@ukaachen.de (T. Lammers).

http://dx.doi.org/10.1016/j.jconrel.2016.07.012
0168-3659/

upon complete drug release, the nanoparticles should disintegrate and the (generated) degradation products should be eliminated. This controllable biodegradation is essential to avoid the excessive accumulation of nanoparticle constituents within the body.

To optimally exploit the potential of nanomedicine formulations, the above-mentioned attributes should be tailored towards specific therapeutic needs and uses. However, physicochemical and pharmaceutical properties that render optimal therapeutic outcome are often unknown, and can only be identified through systematic in vivo evaluation. Thus, a tunable platform is needed, via which nanomedicines with diverse physicochemical and pharmaceutical properties can be generated in a simple and straightforward manner.

In the last decades, polymeric micelles (PM) based on amphiphilic AB block copolymer methoxy poly(ethylene glycol)-*b*-poly[*N*-(2-hydroxypropyl) methacrylamide-lactate] (mPEG-*b*-pHPMAmLac$_n$) have emerged as promising nanocarriers [17,36–40]. This block copolymer consists of a permanently hydrophilic PEG block and a thermosensitive pHPMAmLac$_n$ block. At temperatures above the so-called critical micelle temperature (CMT), the thermosensitive block becomes hydrophobic, allowing the block copolymers to self-assemble into micellar structures in aqueous media. However, in spite of relatively low critical micelle concentrations (CMC), PM generally suffer from poor in vivo stability following intravenous administration because of adsorption of block copolymers to plasma proteins [41,42].

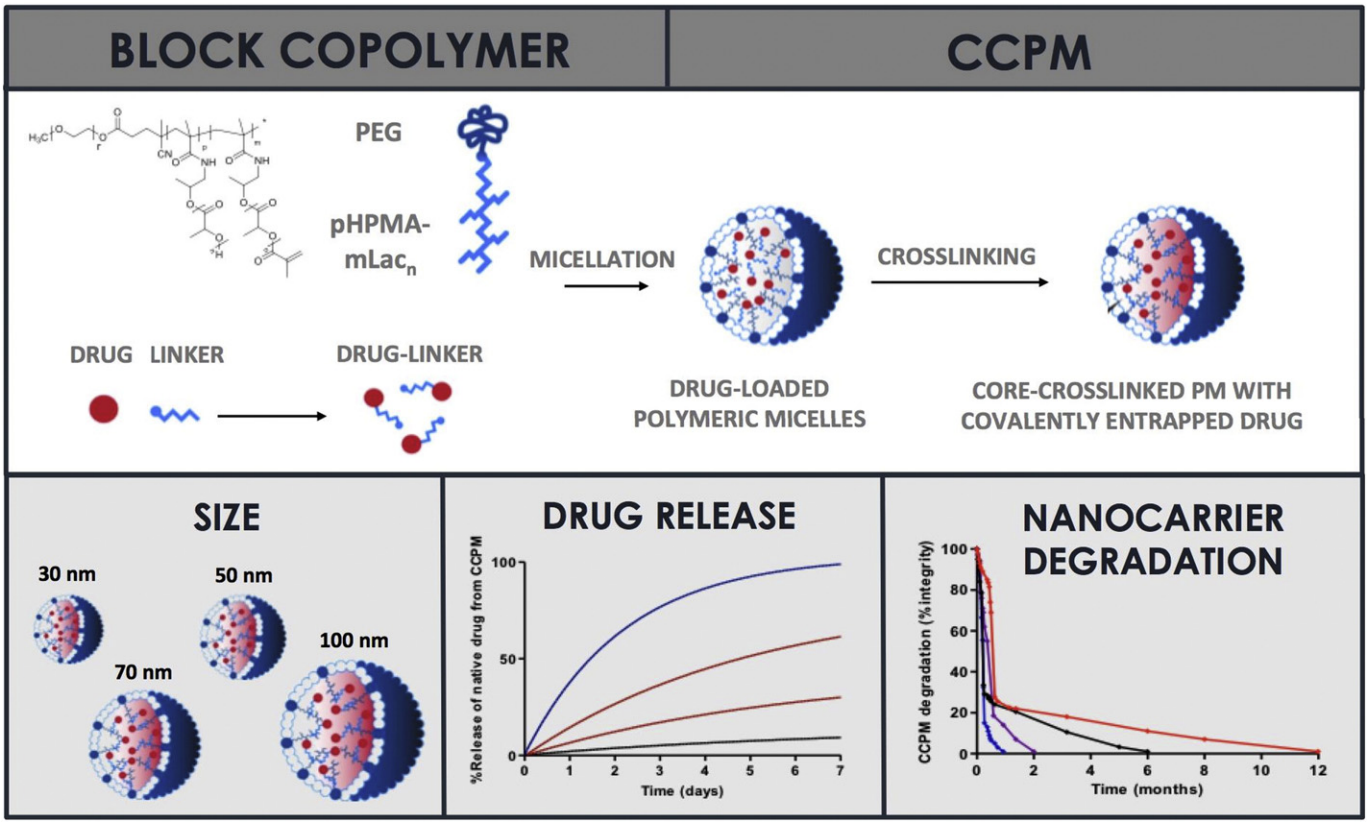
To stabilize PM for in vivo applications, they can be crosslinked by polymerizing the methacrylate groups on the block copolymer side chains, yielding core-crosslinked polymeric micelles (CCPM) [43–45]. Furthermore, through the attachment of a polymerizable linker, drugs can be covalently conjugated to the block copolymeric network and thereby transiently entrapped within the micellar core (Scheme 1) [37,43,46]. Thus far, various drugs (e.g. doxorubicin (DOX), dexamethasone (DEX) and docetaxel (DTX)) have been covalently entrapped within 60–70 nm-sized CCPM, which demonstrated excellent therapeutic efficacy and tolerability after systemic administration as a result of prolonged circulation kinetics and improved accumulation at pathological sites [36–38,40]. Based on this promising proof-of-principle, a GMP-based manufacturing and purification process has been established for the production and scale-up of these CCPM, and a DTX-containing CCPM formulation, termed CriPec® docetaxel, has recently entered phase I clinical trials [47].

In the present manuscript, we demonstrate the broad physicochemical and pharmaceutical tunability of CCPM composed of mPEG-*b*-pHPMAmLac$_n$ block copolymers, by showing that their particle size, degradation profile and drug release kinetics can be precisely controlled. Importantly, we illustrate that these key properties can be tailored not only independently but also simultaneously, thereby enabling the generation of nanomedicinal products with tunable characteristics and optimal therapeutic performance.

2. Materials and methods

2.1. Materials

N,*N*′-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), 4-methoxyphenol, methacrylic anhydride, ammonium acetate, formic acid, Mukaiyama's reagent (2-chloro-1-methyl-pyridinium iodide), potassium peroxymonosulfate (oxone), potassium persulfate (KPS), tetramethylethylenediamine (TEMED) and trifluoroacetic acid (TFA) were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands). Acetonitrile (ACN), dichloromethane (DCM), diethyl ether (DEE), dimethylformamide (DMF) and tetrahydrofuran (THF) were purchased from Biosolve (Valkenswaard, The Netherlands). Absolute ethanol and triethylamine (TEA) were purchased from Merck (Darmstadt, Germany). Docetaxel (DTX) was obtained from Phyton Biotech (Ahrensburg, Germany). 6-(Methacryloylamino) hexanoylhydrazide-doxorubicin (DOX-MA) was produced at the Institute of



Scheme 1. Schematic synthesis of core-crosslinked polymeric micelles (CCPM) with covalently entrapped drugs and overview of the refinements performed as part of this study.