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System with embedded drug release and nanoparticle degradation sensor showing efficient rifampicin delivery into macrophages

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Abstract

We have developed a biodegradable, biocompatible system for the delivery of the antituberculotic antibiotic rifampicin with a built-in drug release and nanoparticle degradation fluorescence sensor. Polymer nanoparticles based on poly(ethylene oxide) monomethyl ether-*block*-poly(ε-caprolactone) were noncovalently loaded with rifampicin, a combination that, to best of our knowledge, was not previously described in the literature, which showed significant benefits. The nanoparticles contain a Förster resonance energy transfer (FRET) system that allows real-time assessment of drug release not only *in vitro*, but also in living macrophages where the mycobacteria typically reside as hard-to-kill intracellular parasites. The fluorophore also enables *in situ* monitoring of the enzymatic nanoparticle degradation in the macrophages. We show that the nanoparticles are efficiently taken up by macrophages, where they are very quickly associated with the lysosomal compartment. After drug release, the nanoparticles in the cmacrophages are enzymatically degraded, with half-life 88 ± 11 min.

Key words: Tuberculosis; Rifampicin; Nanoparticle; MPEO-*b*-PCL; FRET

Tuberculosis (TB) is a hard to cure infectious disease that affects millions of people around the world. It is caused by various strains of mycobacteria, typically *Mycobacterium tuberculosis*.[1–3] *M. tuberculosis* is a slender, non-motile and acid-fast Gram-positive bacillus, measuring approximately 0.5-3 μm.[4,5] Mycobacteria possess waxy coating on the surface of their cell wall, which is mainly composed of mycolic acids (MAs). MAs are long 2-alkyl and 3-hydroxyl fatty acids that are typically 70-90 carbon atoms long.[6,7] Because of the MAs, *M. tuberculosis* is resistant to dehydration, has low permeability to hydrophobic antibiotics and has high ability to survive.[8] It is also able to survive and persist in alveolar macrophages at the typical entry point of the infection.[9] Thus, treatment of TB is difficult, and conventional treatment with first-line antituberculotics e.g., isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol must be complex and administered continuously for approximately 6 months. Second-line pharmacotherapy, including aminoglycoside antibiotics, cycloserine, ethionamide, and fluoroquinolones, is often required in the case of multidrug-resistant TB.[2,4,10,11]

Recently, encapsulation of antimicrobial drugs in nano-formulation systems has emerged as an innovative and promising alternative. These systems enhance the therapeutic effectiveness and minimize

Abbreviations: C120, coumarin 120; DACCA, 7-(diethylamino)-coumarin-3-carboxylic acid; DL, drug loading; DMEM, Dulbecco's modified Eagle's medium; EE, entrapment efficiency; FDA, fluorescein diacetate; FRET, Förster resonance energy transfer; RFP, rifampicin; HMDSO, hexamethyldisiloxane; MIC, minimal inhibitory concentration; MTT, methylthiazolyldiphenyl-tetrazolium bromide; NPs, nanoparticles; PD, polydispersity; PI, propidium iodide.

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Table 1
Macromolecular characteristic of chosen MPEO-*b*-PCL copolymers.

No.	Sample	M_n (NMR)[a]	M_n (GPC)[b]	M_w (GPC)[b]	*PD* (GPC)[c]	Monodisperse NPs
002	$MPEO_{44}$-*b*-PCL_{15}	3677	3328	5013	1.51	yes
004A	$MPEO_{44}$-*b*-PCL_4	2379	3440	3813	1.11	no
004B	$MPEO_{44}$-*b*-PCL_{27}	5001	5416	6681	1.25	yes
006A	$MPEO_{98}$-*b*-PCL_8	5255	5038	5858	1.16	no

[a] M_n values were calculated by ^{1}H NMR spectroscopy.
[b] M_n was determined by GPC calibrated with PS standards.
[c] *PD* (polydispersity, M_w/M_n) was determined from GPC calibrated with PS standards.

undesirable side effects of numerous antibacterial drugs.[5] Most studies on antibiotics have been performed with polymeric nanoparticles[12–14], solid lipid nanoparticles[15–17], liposomes[18] and micelles[19].

Polyester-based nanoparticles are highly studied drug delivery systems due to nanoscale-related effects, biocompatilility and biodegradability.[20–24] Although drug release takes days and nanoparticle degradation weeks to months in many cases, in living cells, the time period may differ. In this paper, we prepared and studied the biological properties of a biodegradable, biocompatible system for the delivery of the antituberculotic antibiotic rifampicin. The nanoparticles are based on poly(ethylene oxide) monomethyl ether-*block*-poly(ε-caprolactone) (MPEO-*b*-PCL) loaded with rifampicin (RFP), a combination that, to the best of our knowledge, has not been previously described in literature, which showed significant benefits. The hydrodynamic radius of the nanoparticles was kept below 35 nm in all cases to enable efficient distribution within the organism for application either intravenously or as an aerosol to the airways, which may be a problem for larger nanoparticles and microparticles. Rifampicin release is enhanced after internalization by enzymatic degradation of the nanoparticle-forming polymer by endosomal and/or bacterial lipases. Our nanoparticles also contain a Förster resonance energy transfer (FRET) sensor. The novel system allows comparison of the drug release rate and nanoparticle degradation *in vitro* and in living cells. We found that the nanoparticles are efficiently taken up by macrophages, where they become very quickly associated with the lysosomal compartment. The nanoparticles thus become concentrated in the same cells and the same cell compartment as the mycobacteria. Lysosomal localization of the nanoparticles is then followed by drug release and nanoparticle degradation. We also show that released rifampicin is highly biologically active.

Methods

See *Supplementary information* for detailed description of materials and methods employed.

Results

Synthesis of the MPEO-b-PCL matrix

MPEO-*b*-PCL block copolymers were characterized by ^{1}H NMR spectroscopy. Its structure was confirmed by the ^{1}H NMR spectrum, that can be seen in *Supplementary information* Figure S4. GPC investigations of the prepared copolymers showed monomodal distributions, as evidenced by the overlap of the GPC curves (*Supplementary information* Figure S5). Macromolecular characteristics of the copolymers are summarized in Table 1.

The physicochemical characterization of MPEO-*b*-PCL nanoparticles was performed by transmission electron microscopy (TEM), dynamic (DLS) and static (SLS) light scattering and asymmetric-flow field-flow (AFFFF) experiments (Figure 1, *Supplementary information* Figure S6, Tables 1 and 2). For some of the copolymers, the distribution function is portrayed as a narrow peak for all concentrations, and sizes (R_h) in the range of 20-30 nm are obtained (copolymers $MPEO_{44}$-*b*-PCL_{15} and $MPEO_{44}$-*b*-PCL_{27}). For $MPEO_{44}$-*b*-PCL_{15}, the value of the hydrodynamic radius is 20 nm (Figure 2). The rifampicin-loaded particles from this copolymer are somewhat smaller, with a hydrodynamic radius of approximately 10 nm. The nanoparticles from the copolymer with the longer PCL block $MPEO_{44}$-*b*-PCL_{27}, form nanoparticles with a hydrodynamic radius of approximately 35 nm both without and with rifampicin. Further, we focused only on those copolymers that have a monodisperse distribution (Table 2).

In terms of the drug incorporation efficiency, for the $MPEO_{44}$-*b*-PCL_{15} micelles, the drug loading (*DL*) and entrapment efficiency (*EE*) were 12.8% and 27.0% respectively. The results from the DLS/SLS and AFFFF experiments are summarized in Table 2.

Enzymatic degradation of the MPEO-b-PCL nanoparticles

For the lipase from *Pseudomonas* sp. (i.e., the bacterial model of enzymatic apparatus), Figure 3 shows the degradation results conducted inside a DLS cuvette at 37 °C with different copolymer micelles. The changes in the light scattering intensity (derived count rate) and micelle size (hydrodynamic diameter) were recorded simultaneously. Figure 3, *A* shows that the light scattering intensity of the samples drops quickly in most of the enzymatically degraded samples, but the decrease of the light scattering intensity is faster for the copolymers with shorter poly(ε-caprolactone) block. Figure 3, *B* shows that the micelle size (defined by hydrodynamic diameter) is virtually constant until the decrease of light scattering intensity is stopped at a constant limit value (up to 10 min of degradation). From this point, the micelle size increases sharply due to formation of less stable polymeric chains, which have a tendency to aggregate, that is observed as the size of the particles increase.

The enzymatic degradability was evaluated concurrently in the presence of pancreatic lipase (lipase from porcine pancreas)