Design and synthesis of FRET-trackable HPMA-based biodegradable conjugates for drug/gene delivery

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The introduction of degradable oligopeptide sequences into the backbone of *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-drug conjugates resulted in prolonged circulation time and increased tumor to tissue ratio [1]. In order to independently evaluate the fate of the polymer backbone and the drug (epirubicin) we prepared conjugates double-labeled with a FRET pair — the polymer backbone was labeled with Cy5 (via an enzymatically non-cleavable bond), whereas the oligopeptide side-chains were terminated either in Cy3 (drug model) or epirubicin (EPR). Upon enzymatic cleavage of EPR or Cy3 the FRET signal receded. The emission spectra of the conjugates before and after incubation with enzyme solution were measured with an LS 55 Luminescence Spectrometer (Perkin Elmer) (Fig. 1 insert). The internalization of the conjugate and drug release at the single A2780 cell level were visualized using a 3D super-resolution Vutara SR-200 fluorescence microscope.

Another design of HPMA copolymers is the dynamic conjugates for the delivery of siRNA/miRNA. The copolymer carrier is composed of HPMA, *N*-(2-(2-pyridyldithio)ethyl)methacylamide (PDTEMA), *N*-butylmethacrylamide, and *N*-(3-aminopropyl)methacrylamide (APMA). The amino group of APMA serves for reversible modification with semitelechelic (ST) poly HPMA via a pH-sensitive bond based on 2-propionic-3-methylmaleic anhydride (CDM). A thiol-disulfide exchange reaction was used to bind miRNA to PDTEMA side chains via reducible disulfide bonds. Upon internalization and destabilization of the endosomal membrane the reductive environment cleaves covalently bound RNA from the carrier with ultimate localization in the cytoplasm [2]. Both polymer carriers and ST-polyHPMA were synthesized by RAFT (co)polymerization followed by post-polymerization end-modification, and labeled with Cy5/Cy3, respectively. Conjugation of miRNA and its release were confirmed by agarose gel electrophoresis.

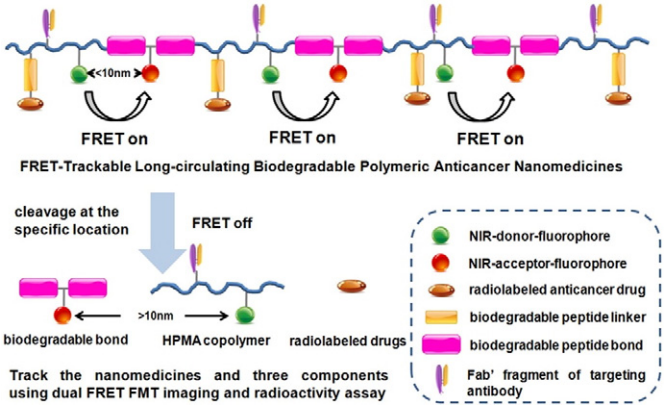

Fig. 1. Rationale of the simultaneous tracking of the fate of the carrier and drug by FRET and nuclear imaging.

Keywords: backbone degradable HPMA copolymers, FRET, RNA, drug delivery

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References

[1] J. Yang, J. Kopeček, Macromolecular therapeutics, J. Control. Release (2014), http://dx.doi.org/10.1016/j/jconrel2014.04013.
[2] B.B. Lundy, A. Convertine, M. Miteva, P.S. Stayton, Neutral polymeric micelles for RNA delivery, Bioconjugate Chem. 24 (2013) 398–407.

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Cell-transfecting multilayered surfaces from poly(amido amine)s

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Polyelectrolyte multilayers (PEMs) fabricated via dip coating layer-by-layer (LbL) assembly has gained a great interest in various fields. In the biomedical fields, these thin PEM coatings have been investigated for their possibility to modify cell-surface interaction and to add additional functionality for therapeutic effects [1]. Multilayers have been fabricated on a wide range of polymer-based biomaterials, metal stents, sacrificial colloidal templates, to even the surface of cells to provide controlled and/or triggered release of (pro)drugs, growth factors, therapeutic proteins and DNA.

Poly(amido amine)s, here represented with the copolymer of cystamine bisacrylamide and 4-aminobutanol (pCABOL) have been used to prepare PEMs with DNA [2]. The PEM is designated pCABOL-(DNA#pCABOL)n with n representing the number of (DNA#pCABOL) bilayers. Build-up profiles followed by increase in UV absorbance at 260 nm were found to progress linearly, indicating the easy tenability of the amount of incorporated materials by the layer numbers. Contact angle measurements indicated complete surface coverage after deposition of 4 bilayers, with static water contact angles showing hydrophobic and hydrophilic topmost layers of pCABOL and DNA, respectively. In vitro studies showed that the PEMs rapidly release pCABOL-DNA polyplexes within 1 h incubation under physiological conditions. This initial release accounts for up to 60% of the initial DNA content and was found to be critical in facilitating cell transfection.

For the studies of cell-transfection properties, PEMs were built on of poly-D-lysine-coated polystyrene well plates using plasmid DNA encoding for GFP protein. COS-7 cells were cultured on PEMs in complete medium with serum. Transfection efficiency (TE) was found to depend on cell seeding density (optimum at 70,000 cells/cm^2) and was found to gradually increase with bilayer number, reaching an optimal value at 10 bilayers. The same PEMs were also fabricated on top of poly(ε-caprolactone) (PCL), heparinized PCL, and poly(lactic acid) (PLA), diminishing the properties of the underlying substrates, significantly improving cell adhesion (in case of PCL and PLA), and adding cell transfection properties. At the optimal culture conditions, up to 50% TE was achieved. Further, as proof of concept the PEM was also built on 3D-printed PCL scaffold. Cell transfection was successfully achieved on cells attaching and proliferating on the surface of the scaffold.

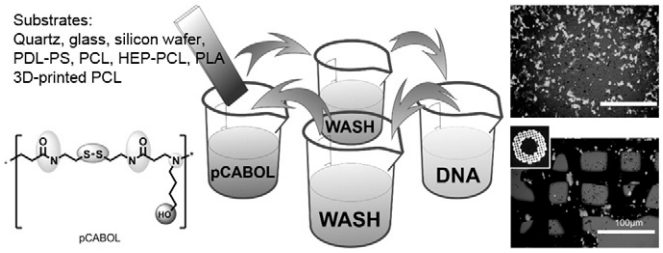

Fig. 1. (DNA#pCABOL) polyelectrolyte multilayers for surface-mediated cell transfection.

Keywords: multilayer, thin film, cell transfection, surface-mediated transfection

References

[1] B.M. Wohl, J.F.J. Engbersen, Responsive layer-by-layer materials for drug delivery, J. Control. Release 158 (2012) 2–14.
[2] C. Lin, Z. Zhong, M.C. Lok, X. Jiang, W.E. Hennink, J. Feijen, J.F.J. Engbersen, Novel bioreducible poly(amido amine)s for highly efficient gene delivery, Bioconjugate Chem. 18 (2006) 138–145.

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Cell specific doxorubicin delivery through the temperature responsive lipopolymer nanocarriers engineered by the combination of RAFT polymerization and click chemistry

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Recently, stimuli-responsive nanocarriers have obtained more attention for the targeted and controlled delivery of therapeutics [1]. Among them, nanocarriers with targeting ligands and temperature sensitivity are highly useful for cell specific drug delivery. Importantly, the amphiphilic behavior of these materials can easily help to form micelles or micelle-like aggregates with a hydrophobic core and hydrophilic surface based on the self-assembly in aqueous media. Our contribution promotes a biocompatible thermoresponsive lipopolymer hybrid system with an active targeting capacity by supramolecular decorations, which offer an additional advantage to enhance the specificity of site directed cell surface for improved endocytosis [2].

Here, phosphatidylethanolamine-poly(*N*-isopropylacrylamide)$_n$ (PE-p(NIPAM)$_n$) ($n = 25, 40, 60$) conjugates have been synthesized by the combination of reversible addition-fragmentation chain transfer polymerization (RAFT) and thiol-ene click reaction. After the aminolysis of the RAFT end group, the resulting thiol terminated lipid-p(NIPAM)$_n$ was reacted with allyl folic acid to yield lipopolymer hybrid with specific cell targeting ligand for KB cells. The temperature responsive amphiphilic behavior of the p(NIPAM) allowed the self-assembly of the lipid hybrids and doxorubicin (DOX) was encapsulated for the evaluation of drug loading and temperature sensitive drug release. Cell specific temperature responsive anticancer effect was assessed by blocking the folate receptor in KB cell lines. The folate receptor-mediated intracellular uptake of DOX loaded nanoparticles is depicted in Fig. 1.

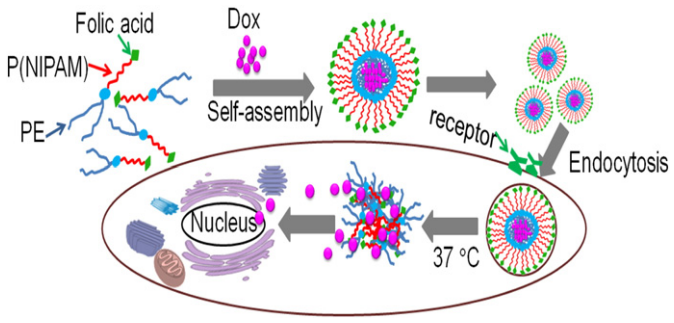

Fig. 1. Receptor mediated delivery of Dox by using PE-p(NIPAM)$_{60}$ nanocarriers.

Keywords: nanocarriers, phosphatidylethanolamine, RAFT, click chemistry, drug delivery

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References

[1] R.P. Johnson, Y.-I. Jeong, J.V. John, C.-W. Chung, D.H. Kang, M. Selvaraj, H. Suh, I. Kim, Dual stimuli-responsive poly(*N*-isopropylacrylamide)-*b*-poly(L-histidine) chimeric materials for the controlled delivery of doxorubicin into liver carcinoma, Biomacromolecules 14 (2013) 1434–1443.
[2] P. De, S.R. Gondi, B.S. Sumerlin, Folate-conjugated thermo responsive block copolymers: highly efficient conjugation and solution self-assembly, Biomacromolecules 9 (2008) 1064–1070.

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Effects of protein transduction domain combination for intracellular recombinant protein delivery

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Advances and popularization of recombinant DNA technology have produced promising therapeutic protein candidates. However, the effectiveness of proteins has been limited by lack of efficient methods and tools to achieve intracellular protein delivery and poor stability of the delivered proteins. PTDs, also known as cell penetration peptides (CPPs), are short sequence cationic peptides that efficiently transduce the cell membrane with various cargos such as proteins, genes and particles [1]. Most of efficient PTDs are composed of positive-charged amino acids such as arginine and lysine [2]. Previously, we have reported high transduction efficiencies of oligo-arginine such as 9-mer arginine [3].