Review

Design of Functional Nanoparticles for Intractable Disease Therapy

Hiroyuki Koide

Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan.

Received September 2, 2020

Protein affinity reagents are widely used for basic research, diagnostics, and disease therapy. Antibodies and their fragments are known as the most common protein affinity reagents. They specifically and strongly bind to target molecules and inhibit their functions. Thus, antibody drugs have increased in the recent two decades for disease therapy, such as cancer. These strong protein–protein interactions are composed of a nexus of multiple weak interactions. Synthetic polymers that bind to target molecules have been developed by the imitation of protein–protein interactions. These polymers show nanomolar affinity for the target and neutralize their functions; thus, they are of significant interest as a cost-effective protein affinity reagent. We have been developing synthetic polymer nanoparticles (NPs) that bind to target peptides and proteins by the inclusion of several functional monomers, such as charged and hydrophobic monomers. In this review, the focus is on the design of synthetic polymer NPs that bind to target molecules for disease therapy. We succeeded in neutralization of toxic peptides and signaling proteins both in vitro and in vivo. Additionally, linear polymers were modified on a lipid nanoparticle surface to improve polymer biodistribution. Our recent findings should provide useful information for the development of abiotic protein affinity reagents.

Key words polymer nanoparticle; peptide; protein; protein–protein interaction; protein affinity reagent

1. PROTEIN AFFINITY REAGENTS

Our biological activities are supported with many protein–protein interactions (PPIs), including antibody–antigen, enzyme–substrate, and ligand–receptor interactions. Protein affinity reagents that bind to target molecules are able to change our biological activities through inhibition of these PPIs. Thus, they are used for disease therapy, diagnostics, and research. Antibodies and their fragments are the gold standards for inhibition of PPIs. Although they specifically and strongly bind to target molecules, their high production cost and low reproducibility are still significant concerns. In nature, biomacromolecules recognize their binding target via the many weak complementary interactions, such as hydrogen bonds and hydrophobic and electrostatic interactions (Fig. 1a). Additionally, typically, PPIs are larger than 1000 Å bonds and involve more than 20 amino acid contacts. Synthetic polymers, such as dendrimers, linear polymers, and polymer nanoparticles (NPs), have the potential to work as protein affinity reagents by mimicking protein–protein interactions (Fig. 1b). For example, the Schrader group designed methacrylamide-based copolymers that bind to a specific epitope of enzyme. The Haag group prepared sulfate-functionalized dendrimers for the inhibition of selectins in vivo. The Shea group synthesized poly N-isopropylacrylamide (pNIPAm)-based NPs that bind to immunoglobulin G (IgG), snake toxin, and lipopolysaccharide.

2. SYNTHETIC POLYMER NPS THAT BIND TO AND NEUTRALIZE TARGETS

To demonstrate that NPs bind to target molecules and neutralize their functions like an antibody, melittin, honeybee venom, was used as a target toxic model. Melittin is a positively charged amphiphilic peptide composed of 26 amino acids. Melittin makes a pore on the cellular membrane after binding to the cell surface. Because melittin is composed of neutral, hydrophobic, and positively charged amino acids, N-isopropylacrylamide (NIPAm, based monomer), N-tertbutoxycarbonyl (TBAm, hydrophobic monomer), N,N'-methylenediacrylamide (Bis, cross-linker), and acrylic acid (AAC, negatively charged monomer) were used for the preparation of the NPs. In the beginning, NPs were prepared by molecular imprinting technology. We found that inclusion of both negatively charged and hydrophobic monomers into the NPs is important for the induction of high melittin affinity. Inclusion of only negatively charged or hydrophobic monomers into NPs is not sufficient for the generation of a high melittin affinity. The optimized imprinted NPs showed a high melittin affinity and inhibited hemolysis induced by melittin in vitro. Additionally, the NPs did not show any affinity for plasma proteins, such as albumin and fibrinogen. To demonstrate the potential of the synthesized NPs in vivo, the NPs were intravenously injected into melittin-treated mice 20 s after melittin injection. The melittin biodistribution significantly changed after intravenous injection of the NPs. Melittin spread in the entire body after its intravenous injection; however, large amounts accumulated in the liver after the NP treatment. These results indicated that the NPs captured melittin in the bloodstream of living mice after intravenous injection. Additionally, more than 60% of the mice were dead after melittin injection with 24 h; however, all mice survived after the NP treatment, indicating that the NPs not only captured melittin but also neutralized it in the bloodstream. This result indicated that NPs have potential as a synthetic antibody.

This review of the author’s work was written by the author upon receiving the 2020 Pharmaceutical Society of Japan Award for Young Scientists.

e-mail: hkoide@u-shizuoka-ken.ac.jp

© 2021 The Pharmaceutical Society of Japan
3. SYNTHESIS OF POLYMER NPS THAT BIND TO AND NEUTRALIZE A TARGET WITHOUT MOLECULAR IMPRINTING TECHNOLOGY

Because the molecular imprinting method for the preparation of NPs that have a high target affinity uses the target molecule as a disposable material, the price of the imprinted NP will not be cheaper than that of the template. Thus, NPs that have a high target affinity need to be prepared without imprinting for mass production. To prepare non-imprinted NPs, we optimized the functional monomer percentage. We found that although a slight decrease in the binding affinity of the NPs for the target was observed compared with that of the imprinted NPs, non-imprinted NPs still had a high affinity for the target. Non-imprinted NPs captured melittin in the bloodstream and improved the survival rate of the melittin-treated mice. Thus, we found that non-imprinted NPs also work in vivo, and thus, non-imprinted NPs were used in the subsequent experiment.

4. ANTI-CANCER THERAPY WITH ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) PLASTIC ANTIBODIES

Melittin has a relatively simple structure because of the negatively charged amino acid-deleted peptide. However, nature proteins, such as signaling proteins, form a more complicated structure. To demonstrate whether NPs that have a high affinity for these signaling proteins can be prepared or not, we used vascular endothelial growth factor 165 (VEGF165) as a target signaling protein. It is well known that VEGF is secreted from cancer cells and increases tumor growth by creating angiogenic blood vessels. Bevacizumab (Avastin®), a monoclonal antibody for VEGF, has made significant contributions to cancer treatment. However, these treatments are very costly and impose a burden on patients and health care systems. Thus, “anti-VEGF NP” will be an attractive protein affinity reagent for cancer therapy. For the development of anti-VEGF NPs, we focused on the fact that VEGF has two different binding domains, a receptor-binding domain and a heparin-binding domain. It is well known that heparin is highly sulfated, suggesting that inclusion of sulfated or heparin-mimicking monomers into NPs is important for the preparation of NPs that have a high VEGF affinity. NPs were prepared with NIPAm, TBAm, Bis, and a heparin-mimicking monomer (3,4,6 trisulfated N-acetylglucosamines monomer (3,4,6S-GlcNAc)) or 2-acrylamido-2-methylpropane sulfonic acid (AS) by modified precipitation polymerization (Fig. 2a). Although AS-containing NPs did not show any affinity for VEGF, 1.7% loading of the 3,4,6S-GlcNAc monomer into the NPs showed a high affinity to VEGF165. An increase in the 3,4,6S-GlcNAc monomer percentage did not increase the affinity for VEGF but caused a decrease. Additionally, a decrease in the TBAm percentage from 40 mol% to 20 or 0%
decreased the VEGF affinity, indicating that optimization of both 3,4,6-S-GlcNAc and the hydrophobic monomer percentage is important for the generation of a high VEGF affinity. The optimized NPs did not show any affinity to heparin-binding domain-deleted VEGF (VEGF 121), indicating that the NPs bind to the heparin-binding domain of VEGF 165.

It is known that VEGF interacts with the receptor (VEGFR-2) that expresses on the cell surface. Then, phosphorylation of VEGFR-2 and cell growth enhancement are induced. Because anti-VEGF NPs bind to the heparin-binding domain of VEGF, binding of NPs to VEGF does not guarantee that NPs inhibit the VEGF–VEGFR-2 interaction. To demonstrate whether NPs inhibit the VEGF and VEGFR-2 interaction or not, human umbilical vein endothelial cells (HUVECs) were incubated with the NPs and VEGF. As a result, NPs dose-dependently inhibited phosphorylation of VEGFR-2 and HUVEC growth (Fig. 2b). From these results, NPs not only were found to bind to VEGF 165 but also inhibit the VEGF–VEGFR-2 interaction (Fig. 2c). To demonstrate whether NPs inhibit tumor growth after intravenous injection through inhibition of the VEGF function, tumor-implanted mice were intravenously injected with NPs. Intravenous injection of NPs into tumor-implanted mice significantly inhibited the tumor growth (Fig. 2d) without any side effects. These results indicated that anti-VEGF NPs have potential as an attractive anti-cancer agent.

5. DESIGN OF POLYMER NPs THAT CAPTURE TARGET MOLECULES IN THE INTESTINES AFTER ORAL ADMINISTRATION

We showed that the synthesized NPs captured the target peptide and protein in the bloodstream of living mice; however, only few have reported that synthetic polymers specifically capture small molecules in the intestine of living mice. For proof of concept, we used indole, an aromatic low-molecular compound, as a model of a toxic small molecule. Indole is synthesized from tryptophan by intestinal bacteria, such as Escherichia coli. After the adsorption of indole from the intestine, indole will be metabolized to indoxyl sulfate (IS), a uremic toxin, in the liver. Although IS is excreted from...
the kidney in healthy subjects, it accumulates in the kidneys and causes damage in patients with chronic kidney disease.\textsuperscript{42)} Thus, inhibition of indole adsorption from the intestine is one critical strategy. Because indole is a hydrophobic and aromatic compound, NPs were prepared with NIPAm, Bis and TBAm, N-phenyacrylamide (PAA), or 2,3,4,5,6-pentafluorophenyl acrylamide (5FPAA)\textsuperscript{43)} (Fig. 3a). We found that an increase of hydrophobic monomer in the NPs also increased the indole capture rate. Additionally, incorporation of two hydrophobic monomers, TBAm and 5FPAA, increased the indole capture rate compared with a single hydrophobic monomer-containing NP. This suggested that TBAm made hydrophobic regions in the NPs and captured indole (Fig. 3b). However, 5FPAA captured indole via quadrupole interactions of the pentafluoro rings (Fig. 3b). These results indicate that incorporation of different binding modes in the hydrophobic monomer enhances the affinity of NPs for the target. The optimized NPs did not degrade by the digestive enzymes. Additionally, the orally administered NPs significantly inhibited orally administered \textsuperscript{14}C-labeled indole, indicating that the NPs captured indole and inhibited indole adsorption from the intestines. These results indicate that synthetic NPs are attractive agents for capturing and inhibiting the adsorption of target molecules in the intestines.

6. NEUTRALIZATION OF A TARGET PROTEIN IN THE BLOODSTREAM BY LIPOSOME ANTIBODIES

We have been developing synthetic polymer NPs that bind to and neutralize target molecules in the bloodstream. How-
ever, the NPs eliminated very shortly from the bloodstream after intravenous injection. The short circulation characteristic of NPs is a significant problem for long-term neutralization in the bloodstream. There are some examples in which the inclusion of polyethylene glycol into NPs reduced the affinity for plasma proteins and increased the circulation time. However, polyethylene glycol (PEG) incorporation into NPs may also reduce the target affinity. Thus, we focused on a lipid nanoparticle (LNP), which is a highly biocompatible drug delivery agent, to improve the polymer circulation time after intravenous injection. For the modification of the polymer to LNP, we synthesized a linear polymer (polymer ligands, PL) by reversible addition–fragmentation chain transfer polymerization and modified it on the LNP surface (Fig. 4a). Histones were used as the target toxic protein in the study. Histones usually packed in the nuclei; however, it was released into the bloodstream from the damaged cell. It is known that histones are major proteins for the induction of sepsis. Thus, histone neutralization reagents will be an attractive medicine for sepsis therapy. For the development of polymer-modified LNPs that have a high histone affinity, we used NIPAm, TBAm, and AAc as functional monomers and optimized the functional monomer percentage and polymer length. We found that a linear polymer composed of NIPAm : TBAm : AAc = 20 : 40 : 40 showed a high histone affinity. Additionally, the 100-mer polymer showed better affinity to the target protein than 30- and 1000-mer polymers. The polymer circulation time significantly increased after intravenous injection compared with that of the polymer alone. Surprisingly, the affinity of the polymer ligand for the target significantly increased upon modification onto the LNP surface because of the corporative effect of the modified ligands. Additionally, the histone neutralization effect in the living mice was significantly enhanced (Fig. 4b). We believe that these results will be useful for increasing polymer circulation time in vivo.

7. CONCLUSION

In this report, I reviewed the design of synthetic polymers that exhibit high affinity for target peptide, protein, and small molecules. Protein affinity reagents can be used in several fields, including diagnostic, research, and medicine. In particular, considerable antibody therapeutics will be developed for disease therapy. However, the therapeutic cost may not be economical for many patients. Although there are numerous issues such as inflammatory induction, carcinogenesis, and metabolism of synthetic polymers for clinical applications, I strongly believe that synthetic polymers can replace antibodies in these fields as an economical protein affinity reagent. Furthermore, some synthetic polymers work in vivo. However, the number of in vivo applications of synthetic polymers is still considerably low. I hope this review will provide useful information for the in vivo application of synthetic polymers.

Acknowledgments I would like to acknowledge Dr. Naoto Oka at Graduate School of Pharmaceutical Sciences, Teikyo University, Tomohiro Asai and Sei Yonezawa at Graduate School of Pharmaceutical Sciences, University of Shizuoka, and Kosuke Shimizu at Department of Molecular Imaging, Hamamatsu University School of Medicine. I wish to thank Dr. Yoshiko Miura and Yu Hoshino, Department of Chemical Engineering, Kyushu University, and Dr. Kenneth J. Shea, Department of Chemistry, University of California, Irvine. I also thank team “Polymer nanoparticles” (Saki Ariizumi, Chiaki Kiyokawa, Hiroki Tsuchida, Naoki Hayashi, Anna Okishima, Kosuke Shimizu, Ayaka Masuda, Yasuko Tempaku, Satoshi Hirano, Kazuhiro Saito, Hickaru Suzuki, Ikumi Yamauchi, Go Yasuno, and Hiroki Ochiai) and laboratory members at the Department of Medical Biochemistry, Graduate School of Pharmaceutical Sciences, University of Shizuoka.

Conflict of Interest The author declares no conflict of interest.

REFERENCES

1) Snider J, Kotlyar M, Saraon P, Yao Z, Jurisica I, Stagljar I. Fundamentals of protein interaction network mapping. Mol. Syst. Biol., 11, 848 (2015).
2) Hardiman G. Next-generation antibody discovery platforms. Proc. Natl. Acad. Sci. U.S.A., 109, 18245–18246 (2012).
3) Janin J, Chothia C. The structure of protein–protein recognition sites. J. Biol. Chem., 265, 16027–16030 (1990).
4) Jones S, Thornton JM. Principles of protein–protein interactions. Proc. Natl. Acad. Sci. U.S.A., 93, 13–20 (1996).
5) Mignani S, El Kazzouli S, Boussina MM, Majoral JP. Dendrimer space exploration: an assessment of dendrimers/dendritic scaffoldings as inhibitors of protein–protein interactions, a potential new area of pharmaceutical development. Chem. Rev., 114, 1327–1342 (2014).
6) You CC, Miranda OR, Gider B, Ghosh PS, Kim IB, Erdogan B, Krovil SA, Bunz UH, Rotello VM. Detection and identification of proteins using nanoparticle-fluorescent polymer ‘chemical nose’ sensors. Nat. Nanotechnol., 2, 318–323 (2007).
7) Sandanaraj BS, Demont R, Aathimanikandan SV, Savariar EN, Thayumanavan S. Selective sensing of metalloproteins from non-selective binding using a fluorogenic amphiphilic polymer. J. Am. Chem. Soc., 128, 10686–10687 (2006).
8) Wada Y, Lee H, Hoshino Y, Kotani S, Shea KJ, Miura Y. Design of multi-functional linear polymers that capture and neutralize a toxic peptide: a comparison with cross-linked nanoparticles. J. Mater. Chem. B, 3, 1706–1711 (2015).
9) Cutivet A, Schembri C, Kovensky J, Haupt K. Molecularily imprinted microgels as enzyme inhibitors. J. Am. Chem. Soc., 131, 14699–14702 (2009).
10) Weisman A, Chen YA, Hoshino Y, Zhang H, Shea K. Engineering nanoparticle antixins utilizing aromatic interactions. Biomacromolecules, 15, 3290–3295 (2014).
11) Mahon CS, Fulton DA. Mimicking nature with synthetic macromolecules capable of recognition. Nat. Chem., 6, 665–672 (2014).
12) Gilles P, Wenck K, Stratmann I, Kirsch M, Smolin DA, Schaller T, de Groot H, Kraft A, Schrader T. High-affinity copolymers inhibit digestive enzymes by surface recognition. Biomacromolecules, 18, 1772–1784 (2017).
13) Renner C, Piefeler J, Schrader T. Arginine- and lysine-specific polymers for protein recognition and immobilization. J. Am. Chem. Soc., 128, 620–628 (2006).
14) Maysinger D, Ji J, Moquin A, Hossain S, Hancock MA, Zhang J, Chang PKY, Rigby M, Anthonsen M, Grütter P, Breitner J, McKinney RA, Reimann S, Haag R, Multhaup G. Dendritic polyglycerol sulfates in the prevention of synaptic loss and mechanism of action on glia. ACS Chem. Neurosci., 9, 200–211 (2018).
15) Derride J, Rausch A, Weinhart M, Enders S, Tauber R, Licha K,
Schirmer M, Zügel U, von Bonin A, Haag R. Dendritic polyglycerol sulfates as multivalent inhibitors of inflammation. Proc. Natl. Acad. Sci. U.S.A., 107, 19679–19684 (2010).

Lee SH, Hoshino Y, Randall A, Zeng Z, Baldi P, Doong RA, Shea KJ. Engineered synthetic polymer nanoparticles as IgG affinity ligands. J. Am. Chem. Soc., 134, 15765–15772 (2012).

O'Brien J, Lee SH, Onogi S, Shea KJ. Engineering the protein corona of a synthetic polymer nanoparticle for broad-spectrum sequestration and neutralization of venomous biomacromolecules. J. Am. Chem. Soc., 138, 16604–16607 (2016).

O'Brien J, Lee SH, Gutierrez JM, Shea KJ. Engineered nanoparticles bind elapid snake venom toxins and inhibit venom-induced dermonecrosis. PLOS Negl. Trop. Dis., 12, e0006736 (2018).

Chou B, Mirau P, Jiang T, Wang SW, Shea KJ. Tuning hydrophobicity in abiotic affinity reagents: Polymer hydrogel affinity reagents for molecules with lipid-like domains. Biomacromolecules, 17, 1860–1868 (2016).

van den Bogaart G, Guzman JV, Mika JT, Poolman B. On the mechanism of pore formation by melittin. J. Biol. Chem., 283, 33854–33857 (2008).

Hoshino Y, Urakami T, Kodama T, Koide H, Oku N, Okahata Y, Shea KJ. Design of synthetic polymer nanoparticles that capture and neutralize a toxic peptide. Small, 5, 1562–1568 (2009).

Chen L, Wang X, Lu W, Wu L, Li J. Molecular imprinting: Perspectives and applications. Chem. Soc. Rev., 45, 2137–2211 (2016).

Hoshino Y, Koide H, Urakami T, Kanazawa H, Kodama T, Oku N, Shea KJ. Recognition, neutralization, and clearance of target peptides in the bloodstream of living mice by molecularly imprinted polymer nanoparticles: A plastic antibody. J. Am. Chem. Soc., 132, 6644–6645 (2010).

Hoshino Y, Koide H, Furuya K, Haberaecker WW 3rd, Lee SH, Kodama T, Kanazawa H, Oku N, Shea KJ. The rational design of a synthetic polymer nanoparticle that neutralizes a toxic peptide in vivo. Proc. Natl. Acad. Sci. U.S.A., 109, 33–38 (2012).

Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell, 86, 353–364 (1996).

Kerbel RS. Inhibition of tumor angiogenesis as a strategy to circumvent acquired resistance to anti-cancer therapeutic agents. BioEssays, 13, 31–36 (1991).

Rak J, Yu JL, Kerbel RS, Coomer BL. What do oncogenic mutations have to do with angiogenesis/vascular dependence of tumors? Cancer Res., 62, 1931–1934 (2002).

Hurwitz H, Fehrenbacher L, Novotny W, Cartron T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabinnavar F, Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. N. Engl. J. Med., 350, 2335–2342 (2004).

Garcia J, Hurwitz HI, Sandler AB, Miles D, Coleman RL, Deurloo R, Chinot O, Bevacizumab (Avastin®) in cancer treatment: a review of 15 years of clinical experience and future outlook. Cancer Treat. Rev., 86, 102017 (2020).

Li F, Subramanian J, Anderson S, Thomas D, McKinley J, Jacobs IA. Development of biosimilars in an era of oncologic drug shortages. Drug Des. Devel. Ther., 9, 3247–3255 (2015).

Zhao W, McCallum SA, Xiao Z, Zhang F, Linhardt RJ. Binding affinities of vascular endothelial growth factor (VEGF) for heparin-derived oligosaccharides. Biotechnol. Rep., 32, 71–81 (2012).

Meneghetti MC, Hughes AJ, Budd TR, Nader HB, Powell AK, Yates EA, Lima MA. Heparan sulfate and heparin interactions with proteins. J. R. Soc. Interface, 12, 0589 (2015).

Koide H, Yoshimatsu K, Hoshino Y, Lee SH, Okajima A, Arizumi S, Narita Y, Yonamine Y, Weisman AC, Nishimura Y, Oku N, Miura Y, Shea KJ. A polymer nanoparticle with engineered affinity for a vascular endothelial growth factor (VEGF165). Nat. Chem., 9, 715–722 (2017).

Leung DW, Cachianes G, Kwong WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science, 246, 1306–1309 (1989).

Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L, VEGF receptor signalling—in control of vascular function. Nat. Rev. Mol. Cell Biol., 7, 359–371 (2006).

Koide H, Yoshimatsu K, Hoshino Y, Arizumi S, Okishima A, Ide T, Egami H, Hamashima Y, Nishimura Y, Kanazawa H, Miura Y, Asai T, Oku N, Shea KJ. Sequestrering and inhibiting a vascular endothelial growth factor in vivo by systemic administration of a synthetic polymer nanoparticle. J. Control. Release, 295, 13–20 (2019).

Niwa T, Miyazaki T, Hashimoto N, Hayashi H, Ise M, Uehara Y, Maeda K. Suppressed serum and urine levels of indoxyl sulfate by oral sorbent in experimental uremic rats. Am. J. Nephrol., 12, 201–206 (1992).

Niwa T, Ise M, Miyazaki T. Progression of glomerular sclerosis in experimental uremic rats by administration of indole, a precursor of indoxyl sulfate. Am. J. Nephrol., 14, 207–212 (1994).

Smith T. A modification of the method for determining the production of indol by bacteria. J. Exp. Med., 2, 543–547 (1897).

Banoglu E, King RS. Sulphation of indoxyl by human and rat aryl (phenol) sulfotransferases to form indoxyl sulfate. Eur. J. Drug Metab. Pharmacokinet., 27, 135–140 (2002).

Vanholder R, Scheppers E, Pletinck A, Nagler EV, Glorieux G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. J. Am. Soc. Nephrol., 25, 1897–1907 (2014).

Duranton F, Cohen G, De Smet R, Rodriguez M, Jankowski J, Vanholder R, Argiles A. Normal and pathologic concentrations of uremic toxins. J. Am. Soc. Nephrol., 23, 1258–1270 (2012).

Okishima A, Koide H, Hoshino Y, Egami H, Hamashima Y, Oku N, Asai T. Design of synthetic polymer nanoparticles specifically capturing indole, a small toxic molecule. Biomacromolecules, 20, 1644–1654 (2019).

Gan DJ, Lyon LA. Synthesis and protein adsorption resistance of PEG-modified poly(N-isopropylacrylamide) core/shell microgels. Macromolecules, 38, 9654–9659 (2002).

Suk JS, Xu Q, Kni B, Hanes J, Turkoglu LM. Pegylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv. Drug Deliv. Rev., 99 (Pt A), 28–51 (2016).

Koide H, Ichitani H, Nakamoto M, Okishima A, Arizumi S, Kiyokawa C, Asai T, Hoshino Y, Oku N. Rational designing of an anidate nanoparticle decorated with abiotic polymer ligands for capturing and neutralizing target toxins. J. Control. Release, 268, 333–342 (2017).

Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, Taylor FB, Esmon NL, Lupu F, Esmon CT. Extracellular histones are major mediators of death in sepsis. Nat. Med., 15, 1318–1321 (2009).