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Nanoengineering of Poly(ethylene glycol) Particles for Stealth and Targeting

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ABSTRACT

The assembly of particles composed solely or mainly of poly(ethylene glycol) (PEG) is an emerging area that is gaining increasing interest within bio-nano science. PEG, widely considered the "gold standard" among polymers for drug delivery, is providing a new platform for exploring fundamental questions and phenomena at the interface between particle engineering and biomedicine. These include the targeting and stealth behaviors of synthetic nanomaterials in biological environments. In this feature article, we discuss recent work in the nanoengineering of PEG particles and explore how they are enabling improved targeting and stealth performance. Specific examples include PEG particles prepared through surface-initiated polymerization, mesoporous silica replication via post-infiltration, and particle assembly through metal–phenolic coordination. This particle class exhibits unique in vivo behavior (e.g., biodistribution and immune cell interactions) and has recently been explored for drug delivery applications.

INTRODUCTION

A central challenge in the nanoengineering of particles is to endow particles with specific properties to control and tailor their interactions with the surrounding environment.[1,2] In biomedical applications, such as drug and gene delivery, vaccination and immunostimulation, and biosensing, the ability of particles to selectively interact with specific biomolecules, cells, or tissues governs their performance.[3–7] "Targeting" is the term often used when particles interact with the intended target, whereas the term "stealth behavior" or "stealth effect" is used when particles show minimal-to-zero interactions with nontarget biological sites/species.[8–11] While the concept of targeting is pervasive in the literature, it has recently come under scrutiny, especially in the field of tumor targeting and cancer nanomedicine.[12] This discussion gained momentum with a meta-analysis, which showed that tumor accumulation of particles (as measured by the percentage of injected dose, %ID, in tumors) has remained stagnant and below 1% when comparing hundreds of particle systems developed over 2005–2015.[13] This has raised questions if nanomedicine has

a “delivery problem”.[14] Treating targeting as a monolithic concept that can be captured by a single metric (such as %ID in tumor) also raises concerns.[15,16] For example, some antibodies that now form part of the backbone in the treatment of cancer[17–19] show similar tumor accumulation (%ID in tumor) to what is observed for many particle systems,[15] highlighting that a holistic perspective is crucial when considering clinical potential.

In parallel with this ongoing discussion on targeting in the clinic and its connection to translational research,[20] tremendous advances are occurring in the fundamental understanding of bio–nano interactions that govern targeting performance. These include the transition of the concept of the “protein corona” to the “biomolecular corona”[21–23] and the emerging understanding of the complex interplay between particle properties, particle ligands, and the dynamic biological environment.[24–27] Though these advances have increased our understanding, they have also introduced new challenging questions. Poly(ethylene glycol) (PEG)—one of the most commonly used “stealth” coatings—has been widely used to functionalize nanocarriers (e.g., PEGylated micelles,[28] liposomes,[29] and poly(lactic-*co*-glycolic acid) particles[30,31]) for reducing nonspecific interactions. However, molecular weight and grafting density have been shown to directly affect targeting outcomes such as biodistribution and cellular uptake.[32–34] For example, particles can display reduced uptake by immune cells when functionalized with a dense PEG brush structure where the Flory radius of the PEG coils is twofold higher than the grafting distance between two neighboring PEG anchors.[34–36] For targeting ligands, such as antibodies and antibody mimetics,[37–39] grafting density and orientation can also critically affect performance.[25,40] Additionally, firmly grafted coatings and ligands may degrade in vivo,[41] adding additional, dynamic complexity to this interface. As stealth and targeting ligands often exist on the same particle surface and can have competing functions (e.g., grafting more PEG onto a particle surface may increase its stealth behavior, however, at the cost of reduced interactions with target cells[24]), the question then becomes how to engineer particle surfaces that can balance these properties. Interestingly, recent studies have shown that there may be a potential to

reduce—or even sidestep—this complex issue,[24] for example by engineering particles directly out of these functional materials instead of grafting them onto pre-prepared particles.

In this feature article, we will discuss our recent work in the nanoengineering of particles with improved targeting and stealth performance. The focus will be on PEG particles: particles that are composed entirely or mostly of PEG. Our work complements other approaches that have been developed for preparing PEG-based particles. For example, DeSimone and coworkers developed a particle replication in nonwetting templates (PRINT) method for the preparation of PEG-based hydrogel particles with different shapes and sizes,[42–44] and Doyle and coworkers used microfluidic devices to generate PEG-based particles through continuous-flow lithography[45] and stop-flow lithography.[46–48] Much research in the engineering of PEG particles is also enabled by template assembly approaches.[49] This includes the formation of PEG particles via the mesoporous silica (MS) templating method and hollow capsules assembled using metal–phenolic networks (MPNs),[50] and layer-by-layer assembly[51,52] using technologies such as flow-based devices[53,54] and fluidized beds.[55,56] Much of our recent work in the area of PEG particles has been conducted using the MS replication approach, where MS is used as template particles (in contrast to the solid, nonporous template particles commonly used for MPN and layer-by-layer assembly of capsules). The resulting "replica particles" consist of PEG hydrogel networks templated by the MS structure, thus enabling innovative inorganic chemistry approaches to guide the development of new organic materials and hydrogel particles. Taken together, these emerging strategies can endow PEG particles with unique properties, for example targeting and stealth behaviors, providing new opportunities at the interface between particle engineering and biomedical applications.

NANOENGINEERING OF PEG PARTICLES

Three main strategies are used for the preparation of PEG particles: (i) surface-initiated polymerization, (ii) MS replication via post-infiltration, and (iii) MPN-based assembly.

Surface-Initiated Polymerization (SIP). Surface-initiated polymerization (e.g., atom transfer radical polymerization (ATRP)[57,58] or reversible addition fragmentation chain transfer (RAFT)[59]) on colloidal templates followed by template removal has been widely used to prepare polymer particles or capsules with tailored properties.[60] PEG particles have been prepared via one-pot SI-ATRP in MS particles using oligo(ethylene glycol) methyl ether methacrylate (OEGMA) as the monomer and poly(ethylene glycol) diacrylate (PEGDA) as the crosslinker (**Figure 1a**).[61] Functional monomer (e.g., 2-hydroxyethyl methacrylate, HEMA) can be added for post-labeling. The molar ratio of OEGMA, PEGDA, and HEMA was typically set as 87:10:3 to obtain stable particles. The zeta-potential of the obtained PEG particles was close to neutral, which suggests reduced nonspecific interactions with proteins and cells.[34,62] Negligible (~4%) cell association with HeLa cells was observed for these PEG particles. This cell association was about 15-fold lower than what was observed for poly(methacrylic acid) (PMA) particles prepared using the same SI-ATRP approach.

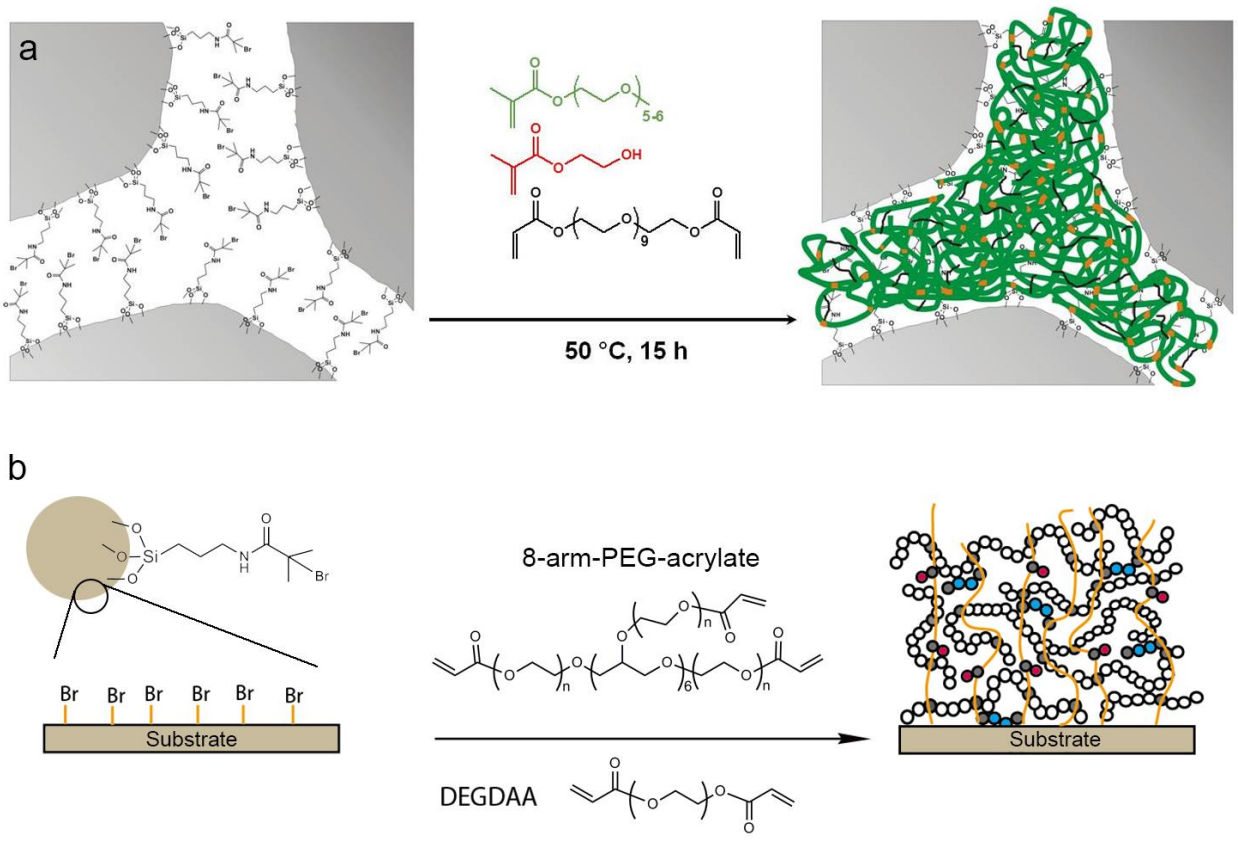

Figure 1. Reaction schemes for the assembly of PEG particles via (a) SI-ATRP and (b) CAP_{ATRP}. Adapted with permission from Ref. 61 (Copyright 2014 American Chemical Society) and Ref. 63 (Copyright 2017 American Chemical Society).

Generally, PEG with a molecular weight greater than 2000 Da is used for particle modification, as this has been observed to increase hydrophilicity and improve stealth properties.[64,65] Atom transfer radical polymerization-mediated continuous assembly of polymers (CAP_{ATRP}) provides an alternative way to assemble PEG capsules using 8-arm-PEG-acrylate (20 kDa) and di(ethylene glycol) diacrylate (DEGDA) as building blocks (**Figure 1b**).[66,67] The obtained PEG capsules showed negligible association with cancer cells (i.e., HeLa), macrophages (i.e., RAW 264.7), and monocytes (i.e., THP-1) when compared to particles prepared with poly(glutamic acid) and PMA using the same approach.[66]

For biological applications, particles with mean diameters of less than 200 nm can exhibit prolonged blood circulation and increase tumor accumulation based on effects such as the enhanced permeation and retention (EPR) effect.[68,69] It is important to note that the EPR effect is not universal, but highly tumor-specific.[15,70,71] MS particles with an average diameter of 110 nm have been used as templates to engineer PEG particles via CAP_{ATRP}, similar to the preparation of PEG capsules (**Figure 1b**).[63] In vitro assays based on monocytes and granulocytes and ex vivo assays based on human whole blood showed that the stealth properties of PEG particles was better than those of PMA and poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) particles, as the interactions of PEG particles with monocytes and granulocytes were fourfold lower than those of PMA and PHPMA particles. Pharmacokinetic studies in rats revealed that PMA particles were rapidly eliminated from circulation: within 5 min from plasma with high particle accumulation in the liver. Although PEG and PHMA particles had similar pharmacokinetic behaviors, the PEG particles had a twofold lower accumulation in the liver (~20% of injected dose) compared to PHMA particles.

Polymerization methods (i.e., SI-ATRP and CAP_{ATRP}) provide an easy way for the preparation of PEG particles, where low molecular weight OEGMA or high molecular weight 8-arm-PEG-acrylate can be used as building materials, and functional monomers (e.g., HEMA) can be introduced for further functionalization (e.g., labeling). However, the non-reacted acrylate groups exposed on the particle surfaces could increase the hydrophobicity of the particles, which could be a disadvantage when attempting to avoid nonspecific interactions. In addition, short crosslinkers with reduced hydrophilicity are typically involved to make the particles stable, which may also influence the stealth properties of the PEG particles.

MS Replication via Post-Infiltration. For particles intended for cancer therapy or imaging, longer blood circulation times can increase their tumor accumulation.[15,72] Although PEG particles engineered by the SIP method could largely avoid nonspecific interactions with cells in vitro and ex vivo, the circulation times of these PEG particles were not long enough, with the amount of PEG particles in blood decreasing below 1% of injected dose in the first 5 h post-injection. A reason for this may be the hydrophobicity of the short crosslinker (DEGDA) and the non-reacted double bonds on the particle surfaces. In an attempt to overcome this limitation, highly hydrated PEG particles mainly composed of 8-arm-PEG were prepared by a MS templating method.[73] 8-Arm-PEG-NH_2 was first loaded into the MS templates through electrostatic interactions, followed by crosslinking with 8-arm-PEG functionalized with succinimidyl carboxyl methyl ester (8-arm-PEG-NHS).[49] Non-reacted NHS groups hydrolyze in aqueous solution to form carboxyl groups, which may form zwitterionic structures together with the non-crosslinked amine groups within the PEG particles, therefore resulting in a zeta potential close to 0 mV at physiological pH. The versatile MS templating method allows the formation of PEG particles with different diameters and good dispersity in aqueous solution (**Figure 2**). It should be noted that the thickness of the PEG particles after air-drying is ~1/100 of the diameter of the PEG particles (AFM measurements in **Figure 2**), indicating that PEG particles are highly hydrated in aqueous solution. Phagocytic blood cell association of the PEG particles decreased when the PEG molecular weight increased (from 10 to 40 kDa) and when the

PEG particle size decreased (from 1400 to 150 nm). Longer in vivo circulation times (from 0.5 h to >12 h) and reduced accumulation in major organs of the mononuclear phagocyte system (MPS) were observed for smaller PEG particles (~150 nm) compared with larger PEG particles (>400 nm). However, phagocytic blood cell association of MS@PEG particles (with the template particle remaining) and their nonspecific accumulation in MPS organs were much higher than that of PEG particles (with template removed). This is possibly due to the difference in mechanical properties between these two particle types.

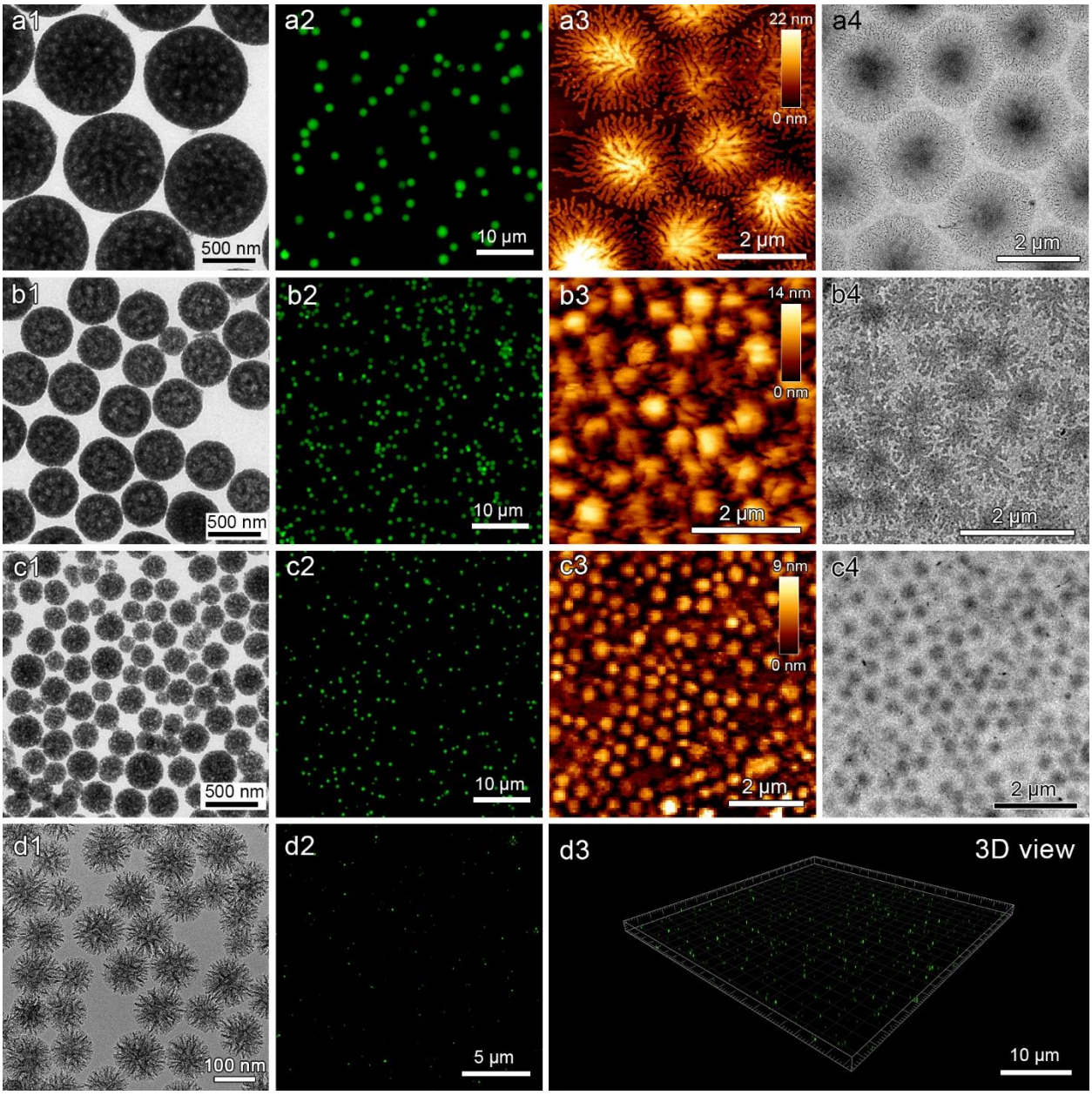

Figure 2. Characterization of MS templates and PEG particles. (a1–d1) TEM images of MS templates with average sizes of 1000, 500, 280, and 110 nm, respectively. (a2–d2,d3) Fluorescence microscopy, (a3–c3) AFM, and (a4–c4) TEM images of PEG particles templated from the corresponding MS particles shown in a1–d1. Adapted with permission from Ref. 73. Copyright 2015 American Chemical Society.

Many mammalian cells are flexible and have a remarkable capacity for reversible deformation. For example, human red blood cells (RBCs) with a diameter of 6–9 μm and a thickness of 1.5–2.5 μm can

traverse blood capillaries with diameters smaller than 5 μm.[74–76] Very recently, Betzig and coworkers visualized extensive deformation and migration of immune cells and metastatic cancer cells in vivo using lattice light-sheet microscopy equipped with adaptive optics.[77] This further highlights that flexibility and deformability are key properties that enable cells to function in their microenvironment. Microfluidic devices have been widely used to generate polymer particles[78,79] and to mimic in vivo microenvironments to investigate the biological behaviors of polymer particles.[80] For example, we have studied PEG particle deformability in a microfluidic blood capillary model (**Figure 3**).[81] The PEG particles were engineered via the MS templating method and had an average diameter of 8 μm. The Young's modulus of the PEG particles could be tuned—by changing the crosslinking density during particle preparation—from 0.2 to 3.3 kPa (stiffness from 0.3 to 3 mN m^{-1}), which is lower than what has been reported for human RBCs (~26 kPa).[82] The PEG particles with lower crosslinking density could easily deform and pass through the microfluidic channels (diameter of 5 μm) when physiologically relevant pressure differentials were applied (equivalent to pressure drops in blood capillaries) across the microchannels. However, substantially higher pressure differentials were required to deform PEG particles with the higher crosslinking densities to pass through the microchannels. The deformability of PEG particles could be tuned to be similar to that of human RBCs, and as rigidity is an important particle design parameter,[83] this may be helpful for reducing nonspecific interactions with MPS and for increasing circulation times of PEG particles.

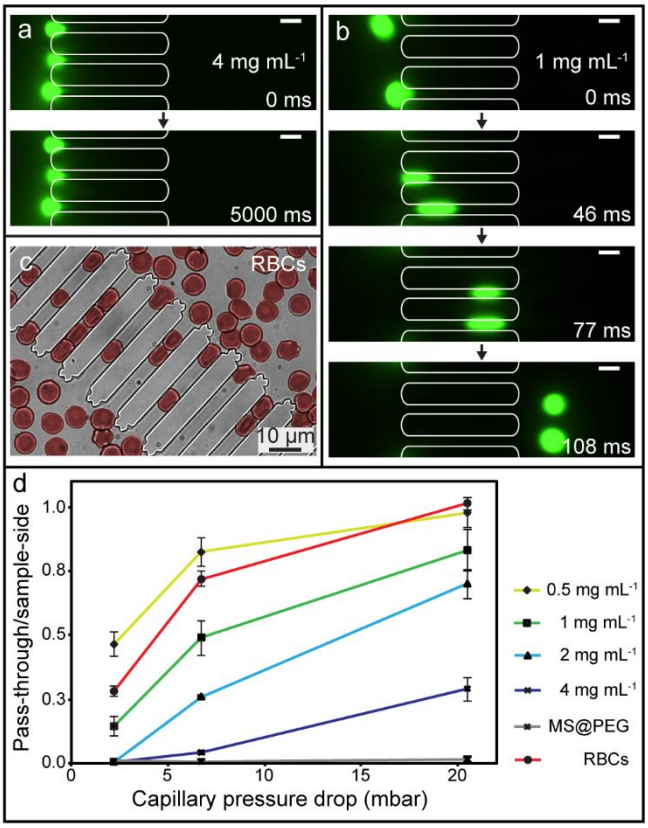

Figure 3. (a,b) Fluorescence microscopy images of PEG particles in microfluidic capillaries. Crosslinker concentrations for the preparation of PEG particles are (a) 4 and (b) 1 mg mL^{-1}, respectively. Scale bars are 10 μm. (c) Bright-field microscopy image of RBCs passing through capillaries. (d) The number ratio of the samples passing through the capillaries and the outlet on the same side. Adapted with permission from Ref. 81. Copyright 2014 John Wiley and Sons.

MPN-Based Assembly. MPNs have proven to be an easy, fast, and robust strategy to engineer films on various substrates based on the near-universal adherent properties of polyphenols containing catechol and/or galloyl groups coordinated with metal ions.[50,84] The dynamic bonds between metal ions and phenolic ligands can provide additional functionality, for example stimulus-responsiveness to assemble degradable films. Polyphenol-modified polymers can endow engineered materials with a range of functionalities, combining the strengths of rationally designed polymers with phenol-based coordination chemistry for diverse biomedical applications[85] such as self-sealing hemostatic needles.[86] For example,

polymer-based MPN hydrogels with responsiveness and self-healing properties can be prepared by simply mixing phenol-modified polymers and metal ions.[87,88] pH-Induced PEG–MPN hydrogels that have been prepared via coordination of metals ions and polyphenols[89] showed near-covalent elastic moduli and self-healing properties.

Similar to the formation of thin MPN films on various substrates, MPN capsules have been prepared by coating colloidal templates followed by a template removal step.[50] By using catechol-modified 8-arm-PEG and iron ions (Fe^{III}) as components, PEG–MPN capsules can be engineered through a templating method (**Figure 4**).[90] The introduction of PEG into the capsules substantially reduced nonspecific protein adsorption and cell association compared to MPN capsules composed of tannic acid (TA) and Fe^{III} (i.e., without any PEG). Additionally, PEG–MPN capsules showed faster disassembly at pH 5 than TA–Fe^{III} capsules (80% in 5 h vs 30% in 10 days). The PEG–MPN system combines the rapid assembly process and controlled disassembly associated with MPNs, with the stealth properties associated with PEG, thus providing a promising platform for biomedical investigations.

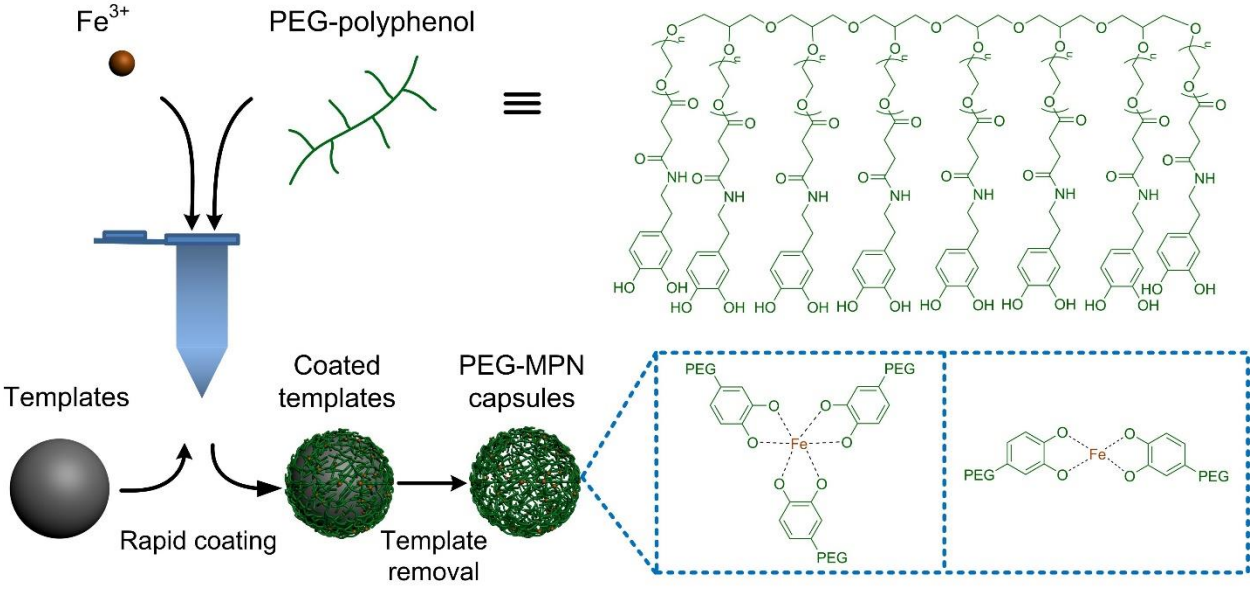

Figure 4. Schematic illustration of the engineering of PEG–MPN capsules through the coordination of PEG–polyphenol and Fe^{III} in the presence of $CaCO_3$ templates and subsequent template removal. Adapted with permission from Ref. 90. Copyright 2015 American Chemical Society.

For systemic drug delivery, stealth particles that efficiently avoid nonspecific interactions with phagocytes and have reduced accumulation in MPS organs (e.g., spleen or liver)[91] can exhibit improved circulation times and biodistribution profiles.[92,93] However, stealth particles may also have reduced interactions with target cells (e.g. tumor cells for cancer nanomedicine), which can limit efficacy (e.g., cell uptake and intracellular drug delivery). This balance (targeting vs stealth) is central to the design of many drug delivery particle systems.[24,94] Surface functionalization of targeting molecules on stealth particles can increase specific interactions and improve biological outcomes, for example through receptor-mediated cell uptake.[95] However, balancing stealth and targeting properties to reduce nonspecific interactions while increasing specific targeting remains challenging. MPNs have provided a facile strategy (**Figure 5**) to prepare MPN capsules composed of PEG and hyaluronic acid (HA) by coating $CaCO_3$ templates with PEG–polyphenol (PEGp) and HA–polyphenol in the presence of Fe^{III}, followed by template removal.[96] Incorporation of HA (which binds to the cell receptor CD44) improves targeting of PEG–MPN capsules to CD44-overexpressing breast cancer cells (i.e., MDA-MB-231). Nonspecific association with a non-CD44 overexpressing cell line (BT-474) and association with the MDA-MB-231 cell line could be tuned by varying the ratio of HA and PEG in the capsules. When an HA-to-PEG ratio of 1:10 was used to assemble MPN capsules, the resultant capsules exhibited low nonspecific association (~10%) with BT-474 cells and high specific targeting (~75%) to MDA-MB-231 cells. HA-functionalized PEG–MPN capsules also showed targeted delivery of the anticancer drug doxorubicin, with the cell viability of targeted MDA-MB-231 cells being more than twofold lower than that of nontargeted BT-474 cells after administration of an equivalent dose of drug-loaded particles.

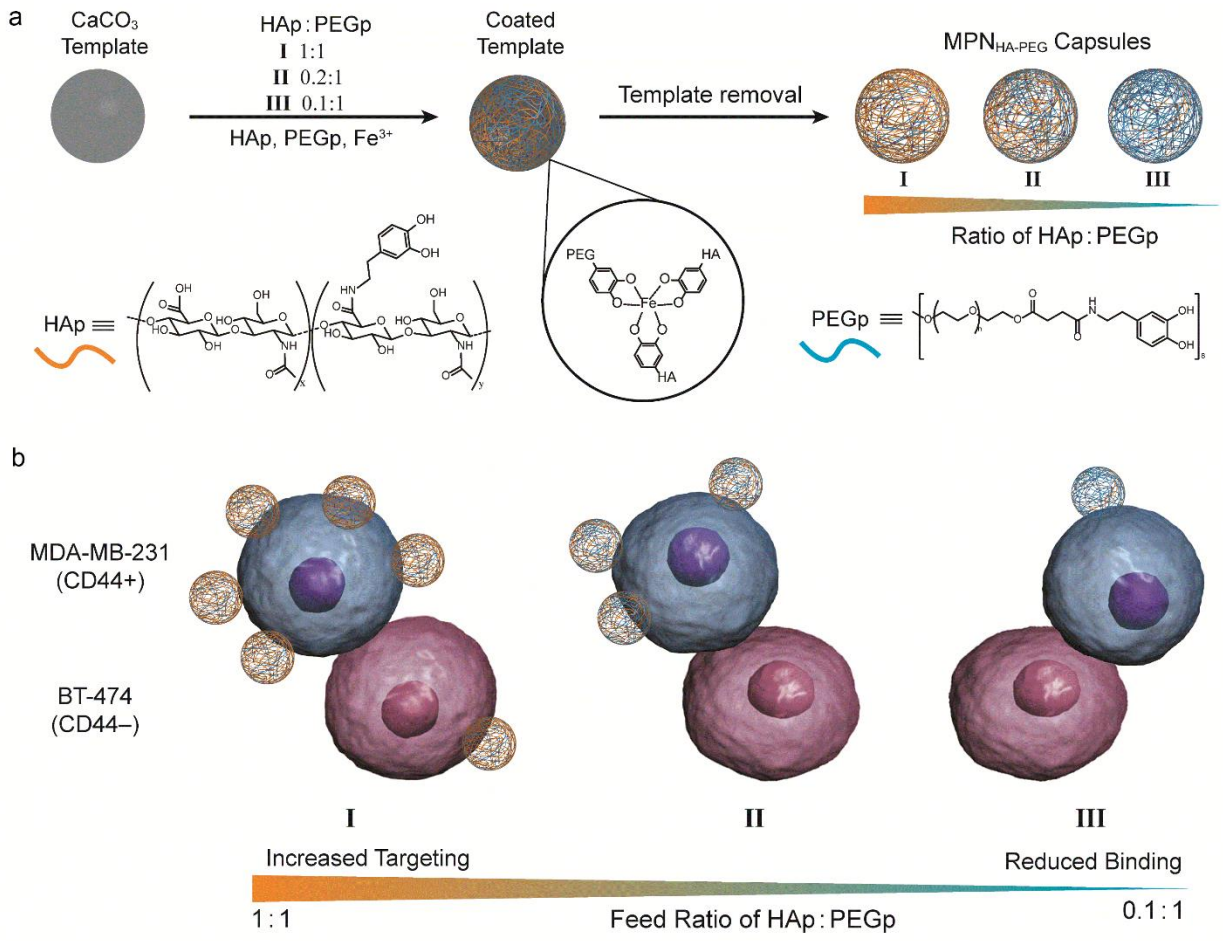

Figure 5. (a) Schematic illustration of the engineering of the HA-functionalized PEG–MPN capsules. Three different capsules were prepared by simultaneously adding HA–polyphenol (HAp, orange) and PEG–polyphenol (PEGp, blue) at three different ratios to a suspension of $CaCO_3$ particles, followed by adding Fe^{III}, increasing the pH, and dissolving the templates. (b) Schematic illustration showing the tunable targeting of the capsules to CD44-overexpressed MDA-MB-231 cancer cells (blue) and nonspecific binding to non-CD44-expressed BT-474 cancer cells (red) cells. Adapted with permission from Ref. 96. Copyright 2016 American Chemical Society.

While PEG–MPN capsules have shown promise, submicrometer-sized particles are desired for some drug delivery applications. However, it is challenging to control particle sizes smaller than 200 nm using $CaCO_3$ as template particles. Water-in-oil nanoemulsification provides a versatile approach to assemble polymer particles consisting of metal ions and ligands with tunable compositions, sizes, drug loading,

biodegradability, and ease of surface functionalization.[97] PEG–MPN nanoparticles (~100 nm) can also be engineered by the nanoemulsification method by mixing one nanoemulsion containing PEG–polyphenol, phenol-modified platinum (Pt) prodrug, and Fe^{III} with another nanoemulsion containing pH 8.5 buffer (**Figure 6**).[98] These PEG–MPN nanoparticles show a drug loading capacity of ~0.15 pg Pt per particle and a circulation half-life of ~18 h in mice. Using these Pt-loaded PEG–MPN particles, a fourfold improved inhibition of tumor growth was observed compared to free prodrug (or cisplatin with a fixed dose). Co-encapsulation of doxorubicin and phenol-modified Pt prodrug in the PEG–MPN nanoparticles can activate nicotinamide adenine dinucleotide phosphate oxidases to generate superoxide radicals and the subsequent reactive oxygen species (i.e., toxic $HO^{\cdot}$ free radicals), which can synergize the chemotherapy to inhibit tumor growth.[99] In addition, phagocytic enzyme myeloperoxidase (MPO) and phenol-modified Pt prodrug has also been loaded into the PEG–MPN nanoparticles, where Pt hydrolyzed from the phenol-modified Pt prodrug to produce H_2O_2 in cells and MPO converted H_2O_2 into highly toxic HOCl.[100]

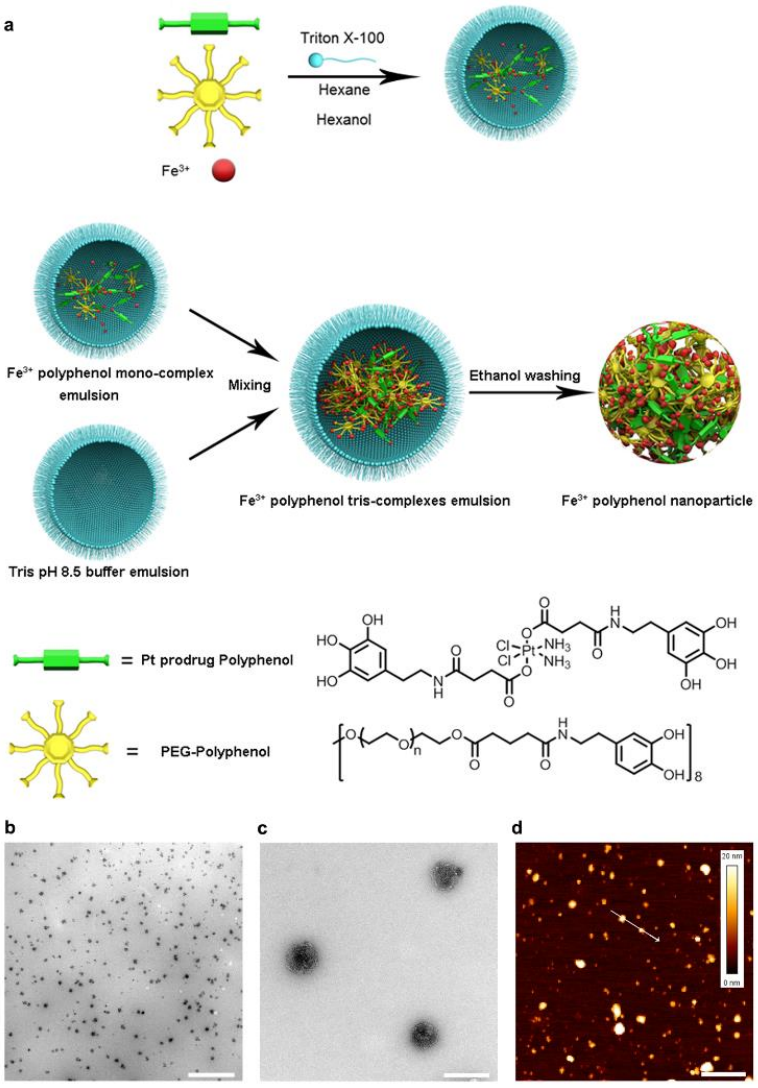

Figure 6. (a) Schematic illustration showing the assembly of the Pt-loaded PEG–MPN particles. (b,c) TEM and AFM images of the obtained PEG–MPN particles. Scale bars are (b) 2 μm, (c) 200 nm, and (c) 1 μm. Adapted with permission from Ref. 98. Copyright 2017 John Wiley and Sons.

SUMMARY AND OUTLOOK

In this feature article, we have discussed our recent work in the nanoengineering of PEG particles based on the MS templating method combined with SI-ATRP and CAP_{ATRP} technique, as well as metal–phenolic networks (MPNs) toward achieving improved targeting and stealth performance, and we have highlighted examples from the field that are helping to shape future research directions. While the

concepts of targeting and stealth behavior are often useful for facilitating discussions about the behavior and performance of nanomaterials in various biological environments, it is important to remember that these are simplifications of a highly dynamic and complex bio–nano interface.[15,101] Complementing current assays used for the evaluation of targeting and carrier efficacy with new and emerging strategies (e.g., microfluidics[80,102] and functional, receptor-based assays[103,104]) is therefore a promising avenue for advancing fundamental understanding of bio–nano interactions.[24]

In our ongoing work, we are exploring a range of targeting ligands with various PEG particle systems. Preliminary results with peptide-based targeting ligands comparing ex vivo, flow-based methods (using spheroids) with in vivo assays show complex relationships between the two approaches, similar to what has been reported using a "tumor-on-a-chip" approach.[105] In a separate ongoing study, we are using antibody constructs with PEG particles, and preliminary results suggest that there is an "optimal" functionalization density above which off-target accumulation increases, similar to what has recently been reported for gold nanoparticles.[40] These results further underscore the care needed when designing and interpreting studies on particle targeting and stealth behavior.

One exciting and related area that is receiving increasing attention is new strategies for quantifying bio–nano interactions such as particle–cell interactions.[106] By considering dispersion states, sedimentation and diffusion rates, and inter-particle forces, quantitative and general information about biological interactions can be uncovered.[107] We have recently reported a framework that can facilitate this process.[108] As these types of approaches are becoming more widespread—combined with increasing capability and interest to share data[109]—this could help accelerate and streamline current research. This is especially important in the field of quantitative particle–cell interaction, for example, for studying particle targeting and stealth behavior.[107]

PEG particles form a distinct group to many conventional particle types as they are composed of what is largely regarded as the "gold standard" among stealth polymers for drug delivery.[110–112] As PEG particles

are directly assembled out of PEG, many of the challenges associated with PEGylation (i.e., coating or grafting particles with PEG after assembly) can potentially be avoided.[113,114] These challenges include controlling grafting density, stability, polymer orientation, and introduction of reactive (but unreacted) functional groups during PEGylation steps. PEG particles may therefore enable new fundamental investigations into bio–nano interfacial phenomena largely uncoupled to external effects associated with PEGylation which—if not controlled for—may compound outcomes and complicate interpretations. This in turn may facilitate the establishment of particle design–biological performance relationships that would advance our fundamental understanding of the exciting interface between particle engineering and biomedicine, and accelerate the development of the next-generation of particle-based therapeutics.

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Notes

The authors declare no competing financial interest.

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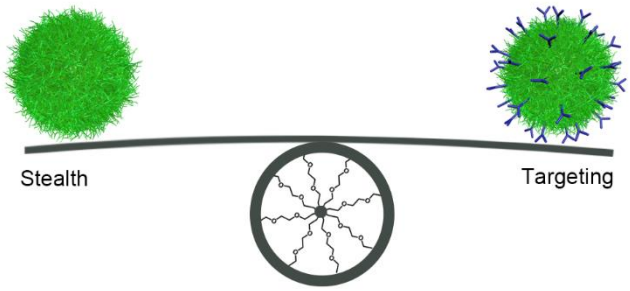
Table of Content Graphic



Biographies

Jiwei Cui is a professor at Shandong University (China). He received his Ph.D. in Colloid and Interface Chemistry from Shandong University in 2010 and worked as a research fellow in Frank Caruso's group at The University of Melbourne (Australia) from 2010 to 2016. He was awarded an Australian Research Council Super Science Fellowship in 2011 and was selected into the Thousand Young Talents Program in 2016. His research interests include colloidal assembly, interface engineering, polymer hydrogels, and therapeutic delivery.

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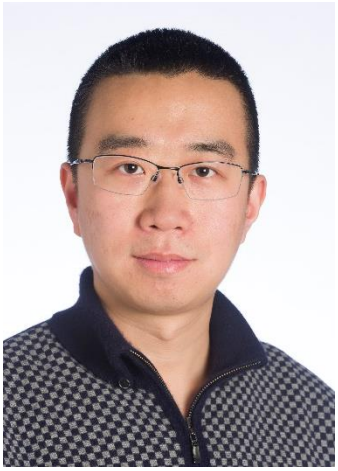

Yi Ju is a research fellow at The University of Melbourne (Australia). He completed his Ph.D. in 2016 under the supervision of Frank Caruso at The University of Melbourne. His current research focuses on investigating how complex biological environments influence bio–nano interactions of drug delivery systems.

Frank Caruso is a professor and an NHMRC Senior Principal Research Fellow at The University of Melbourne (Australia). He is also Deputy Director of the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology. He received his Ph.D. in 1994 from The University of Melbourne, and from 1994 to 1997 was a research fellow at the CSIRO Division of Chemicals and Polymers (Melbourne,

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