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Polyglycerol/Polydopamine-Coated Nanoparticles for Biomedical Applications

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Nanoparticles play an active role in biomedical science due to their unique properties, which cannot be obtained from bulk materials. Therefore, understanding and controlling the physicochemical properties of nanoparticles are gaining increasing importance for their practical applications. Surface coating is an important technique that controls the physical properties of nanoparticles since the coating is the first part of the nanoparticle that is in contact with the environment. Additionally, the coating creates robust targeting, therapy, imaging, and sensing opportunities. This review first introduced two recently developed outstanding coatings, namely, hyperbranched polyglycerol and polydopamine, and the research achieved by the polyglycerol/polydopamine-coated nanoparticles was then highlighted.

Keywords: imaging, nanoparticle, polydopamine, polyglycerol, sensing, targeting, therapy

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INTRODUCTION

Nanoparticles (NPs) are gaining popularity in biomedical applications. They are defined as particles smaller than 100 nm in size, with numerous types reported to date comprising organic, inorganic, and metal NPs (Mcnamara and Tofail, 2016; Maiti et al., 2018; Aflori, 2021). Additionally, NPs show unique chemical, physical, and/or optical properties compared with bulk materials attributed to their nanometer size and large surface-to-volume ratio. These properties vary significantly in size, shape, structure, and composition. For example, the energy level of electrons becomes discrete when they are confined to a nanometric region, revealing peculiar electrical, and optical properties. Also, in the medical field, NPs are useful carriers of biomolecules and/or drugs in certain parts of the body for diagnosis and therapy (Brigger et al., 2012). They also passively accumulate in tumors through their enhanced permeability and retention (EPR) effect (Shi et al., 2020). Recently, crystal defects in inorganic NPs, such as diamond NPs, have attracted new imaging, and sensing applications (Basso et al., 2020). Additionally, small molecules, polymers, and biomolecules modify the surface of NPs. These special properties allow NPs in advanced biomedical applications, such as targeting, therapy, imaging, and sensing (**Figure 1A**) (Davis et al., 2008; Holzinger et al., 2014; Yang et al., 2019; Mitchell et al., 2021).

Surface coating with organic polymers is crucial to controlling the properties of NPs since it determines the interaction between NPs and the environment. For example, the colloidal stability of NPs at different pH values or in the presence of salt greatly depends on the coatings, including an adequate coating that contributes to the aggregation inhibition. Additionally, the coating provides prospects for further functionalization, e.g., imaging probes, targeting agents, biomolecules, and drugs. There are numerous combinations of NPs and surface coatings; thus, the potential applications of hybrids of NPs and polymers are vastly robust. Common approaches applied for surface coating include the atom transfer radical polymerization or reversible addition-

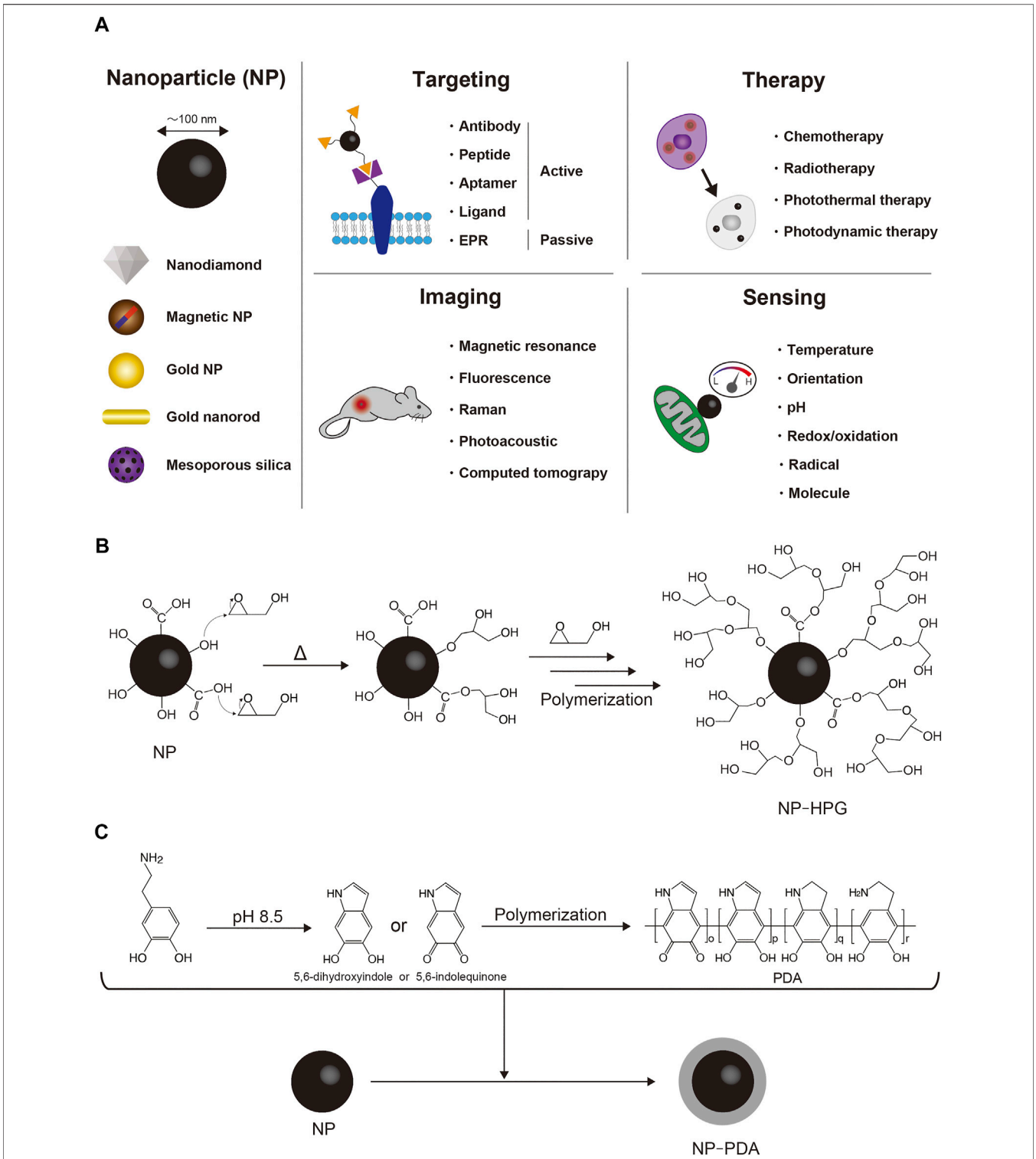

FIGURE 1 | Schematic illustration of **(A)** nanoparticles focused in this review and their biomedical application and synthesis of **(B)** NP-HPG and **(C)** NP-PDA.

fragmentation chain transfer polymerization (Zoppe et al., 2017). However, these methods involve multiple organic synthesis steps, are technically difficult and require transition metal catalysts, such as Cu^{+} ions, and for polymerization. However, their use in synthesis is best avoided due to the toxicity of these transition metal ions to living organisms. Recently, two surface coatings have been developed with simple, versatile, robust, and metal-free methods: hyperbranched polyglycerol (HPG; **Figure 1B**) and

polydopamine (PDA; **Figure 1C**) coatings. This article reviewed the fundamental properties of HPG and PDA and recent advances in the bioapplications of NPs with HPG and PDA coatings. **Supplementary Table S1** summarizes the size, surface functionalization, and applications of NPs coated with HPG and PDA.

SURFACE COATING

Polyglycerol

HPG is obtained through a ring-opening reaction of glycidol, with a structure similar to the branched polyethylene glycol and OH groups as a terminal (Khan and Huck, 2003; Wang et al., 2008). As a result, HPG exhibits excellent hydrophilicity reduces nonspecific absorption of biomolecules (Zhao et al., 2011; Zhao et al., 2012; Sotoma et al., 2015; Jafari et al., 2020; Zou et al., 2020). Additionally, HPG can be modified with various functional groups from the terminal OH groups. Notably, Sandler and Berg (Sandler and Berg, 1966) first attempted the demonstration of the polymerization of glycidol, and later, researchers devoted efforts to polymerizing glycidol using various approaches.

In 2011, Komatsu and colleagues reported a simple method of HPG coating on nanodiamond (ND) surfaces, where the ND and glycidol mixture was heated at 140°C in argon atmosphere for 20 h without a catalyst, producing ND-HPG (Zhao et al., 2011). The ring-opening reaction mechanism is the polymerization reaction between an OH group and a carbon atom in the epoxy group initiated from the nucleophilic groups on the surface of NPs. Hence, nanoparticles must be designed to have nucleophilic groups on their surface. The thickness of the HPG layer is controlled by changing the reaction time and temperature (Zou et al., 2020). The HPG modification altered the hydrophobic nature of the ND material such that ND-HPG showed extremely high solubility not only in pure water but also in buffer solutions. Furthermore, the HPG layer blocks nonspecific absorption, with further functionalization with targeting moieties (antibody, ligand, and among others), specifically targeting NPs to the biomolecules of interest (Hsieh et al., 2019). Besides, Sotoma and coworkers reported one-pot functionalization of COOH, amine, and alkyne (Sotoma et al., 2018; Terada et al., 2018; Hsieh et al., 2019). Zhou *et al.* also revealed that HPG-coated metal quantum dots (QDs) are less toxic than pristine QDs due to the biocompatible envelope of HPG on the QDs (Zhou et al., 2009).

Polydopamine

PDA is a bioinspired polymer similar in capabilities with a mussel's adhesive foot protein that firmly attaches the mussel to a surface, polymerizing on the NP surfaces (Lee et al., 2007). Although the mechanism remains controversial (Liebscher et al., 2013), dopamine self-polymerizes under basic conditions (pH 8.5), creating a layer of PDA adhering strongly to the surface of NPs without pretreatment. The thickness of the PDA layer is easily controlled by changing the dopamine concentration and reaction time. Also, PDA possesses universal adhesion to any material or surface and provides active platforms for further functionalization through catechol/quinone groups (Liu et al., 2014). The notable feature of PDA is its photothermal effect; PDA nanospheres achieved 40% photothermal conversion efficiency, much higher than that of gold nanorods (GNRs) (22%) (Liu Y. et al., 2013; Jung et al., 2018; Harvey et al., 2019). Hence, it is applied in hyperthermia therapy against cancer (Zelasko-Leon et al., 2015; Li D. et al., 2016; Ding et al., 2016; Cheng Y. et al., 2017). Furthermore, PDA can be easily functionalized with metal nanomaterials by reducing metal ions. Therefore, various metal types can be deposited on the surface of PDA, including Au, Ag, Pt, and Cu (Zeng et al., 2018; Lu et al., 2020).

NANOPARTICLES

Diamond

The diamond NP is a carbon-based nanomaterial with a broad prospect for bioapplications. This review classified ND into three types: detonation nanodiamond (DND), nanodiamond without a nitrogen-vacancy center (ND), and ND with nitrogen-vacancy centers (FND). The DND is a synthetic diamond obtained from explosives with a 4–6 nm uniform size (Mochalin et al., 2011; Dolmatov et al., 2020). Additionally, since DNDs exhibit tunable surfaces, excellent biocompatibility, and large areas, they are attracted as drug and gene carriers (Zhang et al., 2009; Huang et al., 2010; Mochalin et al., 2011). However, the NDs produced by chemical vapor deposition or high-pressure high-temperature methods with a size of 30–100 nm are used for bioimaging applications (Hui et al., 2010). Therefore, particular attention is given to NDs containing nitrogen-vacancy centers (NVCs), e.g., FNDs (Yu et al., 2005). The FND fluorescence shows no photobleaching or photoblinking, permitting single-particle tracking and long-term fluorescence imaging (Yu et al., 2005). Also, the magneto-optical property is another notable feature of FND. The quantum states of the electron spins in NVCs are optically read at room temperature *via* optically detected magnetic resonance (ODMR) (Gruber et al., 1997; Degen et al., 2017). The ODMR signal allows nanoscale sensing of an electric field, magnetic field, temperature, and angle with high precision (Wu et al., 2016; Zhang et al., 2021).

Magnet

Magnetic NPs (MNPs), such as Fe_3O_4, are conventional nanomaterials that offer controlled size, size-dependent magnetic property, manipulation externally, paramagnetic properties, and heat generation in an alternating magnetic field associated with hysteresis loss (Holzinger et al., 2014; Ali et al., 2021; Materón et al., 2021). It is possible to synthesize MNP with uniform size distribution using the bottom-up approach and controlled particle size. Therefore, due to these properties, MNPs are used for drug/gene delivery, protein separation, contrast enhancement in magnetic resonance imaging (MRI), and hyperthermia (Materón et al., 2021). It is also necessary to employ a synthesis method that exposes OH groups on the surface of MNPs for HPG coating.

Gold

GNPs are among the most studied nanomaterials, with their unique optoelectronic properties, indispensable for DDS probes, catalysts, and sensors (Yeh et al., 2012; Elahi et al., 2018). Since the physical, chemical, optical, and electrical properties change with size and shape, various GNPs have been developed, including sphere, rod, hollow, star, and cubic (De Berardis et al., 2021). Principally, GNRs have two surface bands, a short-wavelength band (TSPR: transverse surface plasmon resonance) and a long-wavelength band in the near-infrared region (LSPR: longitudinal SPR). The TSPR is generally observed around 525–586 nm, whereas the LSPR is highly dependent on the aspect ratio of the GNR (Nikoobakht and El-Sayed, 2003; Runowski et al., 2019). Additionally, GNRs generate heat used mainly for photothermal therapy of cancer cells after absorbing light in the region of their TSPR or LSPR (Huang et al., 2008; Zelasko-Leon et al., 2015; Du et al., 2017; Riley and Day, 2017). The surfaces of the synthesized GNPs are stabilized with citric acid or cetyltrimethylammonium bromide. Also, the PDA coating applies to GNP even in the presence of these stabilizers. However, HPG coating is not applicable because GNPs do not have nucleophilic groups on the surface. Sotoma *et al.* reported the possibility of growing HPG on NP-PDA since PDA acts as a scaffold for the ring-opening polymerization of glycidol (Sotoma and Harada, 2019). Therefore, subsequent HPG coating overcomes the relatively low dispersity of NP-PDA.

Quantum Dot

QDs are 2–10 nm NPs with a fluorescence property because of the quantum confinement effect. They have numerous potential applications, including bioimaging, displays, solar cells, and quantum communication. Various QDs have been developed, including heavy metals, silicon, and carbon. Among them, QDs made of heavy metals show cytotoxicity, requiring surface coating to reduce the toxicity. Additionally, the wavelength of fluorescence emission of QDs can be tuned by controlling their sizes. These properties often contribute to bioimaging and biodiagnosis (Kairdolf et al., 2013). Also, a recent study reported that QDs serve as a nanometric temperature sensor, with monitoring fluorescence intensity or fluorescence lifetime (Medintz et al., 2005; Kalytchuk et al., 2017). However, since PDA coatings absorb light and considerably reduce fluorescence, limited research has been conducted on PDA QD coating.

Mesoporous Silica

Mesoporous silica NPs (MSNPs) are amorphous silica NPs with numerous pores with sizes ranging from 2 to 50 nm (Narayan et al., 2018) that have gained increasing attention due to their catalytic and biomedical applications. Several types of mesoporous silica depends on the morphology, such as SBA, LMU, FSM, MCM, among others, and including varying particle properties (Moritz and Geszke-Moritz, 2015). Molecules can be loaded into the pores and slowly released, with their properties often used for DDS (Narayan et al., 2018; Niculescu, 2020). Although surface modification of MSNPs is relatively easy through silane reagents, HPG-coated MSNPs have not been reported to date.

APPLICATION

HPG-Coated NPs

HPG coating is used to increase dispersity and actively target DND (Zhao et al., 2014c; Zhao L. et al., 2015; Li et al., 2018; Li et al., 2019; Yuan et al., 2019; Chen et al., 2020), ND (Zhao et al., 2011; Zhao et al., 2014a; Yoshino et al., 2019; Zou et al., 2020; Zou et al., 2021), FND (Boudou et al., 2013; Sotoma et al., 2015; Sotoma et al., 2016; Torelli et al., 2019; Barton et al., 2020; Suarez-Kelly et al., 2021), or MNP (Wang et al., 2008; Wang et al., 2009; Wang et al., 2011; He et al., 2015). Reports have shown that the aggregation property of DND, which is stronger than that of NDs, is overcome by HPG coating (Sotoma and Shirakawa, 2016). Previous MNP experiment with HPG originates from Zhao *et al.*, who revealed that the HPG layer suppresses nonspecific adsorption of proteins on the MNP surface to a level comparable or superior to commonly used polyethylene glycol (Zhao et al., 2012). In the latter study, Zou *et al.* reported that the 30 wt% HPG layer on the surface of NPs prevented protein corona formation, thus indicating its stealth effect (Zou et al., 2020). These properties enable the HPG-coated NPs for targeted therapy, imaging, and sensing.

NP-HPGs are used for selective delivery of dox or cisplatin to A549, U937, U87MG, glioblastoma, and breast cancer cells (Zhao et al., 2014b; Zhao et al., 2014c; Li et al., 2019), indicating the enhanced therapy efficacy. For example, Zhao *et al.* developed ND-HPG further functionalized with RGD peptide as the targeting moiety and platinum as a drug, i.e., ND-HPG-RGD-Pt (Zhao et al., 2014c). Furthermore, the group showed that ND-HPG-RGD-Pt was preferentially assimilated by specific U87MG cells expressing RGD peptide receptors and revealed cytotoxicity, illustrating its potential as a chemotherapy agent.

Additionally, Hsieh *et al.* used FND-HPG for single-particle tracking. The group selectively targeted FND-HPG to membrane glycoprotein through click chemistry and continuously monitored the protein movements on/in live cells for over 2 h (Hsieh et al., 2019). These results affirm the potential for biomedical applications at the single-molecule level. Finally, Arsalani demonstrated the application of the MNP-HPG for MRI of the liver and kidney *in vivo*. They found that the particles produced a strong negative contrast, which persisted in the liver and kidney for 80 and 110 min, respectively (Arsalani et al., 2012).

Igarashi *et al.* reported a unique sensing application by tracking the three-dimensional rotational motion of F1-ATPase using FND-HPG with angular uncertainty of ±3 with a time resolution of 1.7 s (Igarashi et al., 2020).

Additionally, regarding other NPs, HPG coating has been reported for TiO_2 (Qin et al., 2016), CdTe (Zhou et al., 2009), CdSe-ZmS (Panja et al., 2017), Mn-ZnSeS (Panja et al., 2017), silicon NPs (Das and Jana, 2014), and carbon dot (Li et al., 2017). Also, Panja *et al.* used silica coating on CdSe-ZnS, Mn-ZnSeS, γ-Fe_2O_3, and gold particles as a scaffold for HPG coating. These studies reported that HPG modification does not interfere with QD fluorescence, has high dispersibility, reduces QD cytotoxicity,

and improves hemocompatibility. Furthermore, Das *et al.* succeeded specifically targeting glioblastoma and cervical cancer cells with overexpressing $\alpha_v\beta_3$ integrin by modifying the HPG surface on silicon QDs with (cRGDfK) peptide (Das and Jana, 2014).

PDA-Coated NPs

The dispersibility and stealth effect of PDA were not high. However, its surface modification was easy, with its dispersibility, and stealth effect enhanced by modifying it with PEG or other chemicals. Additionally, tumor accumulation due to the EPR effect was observed. The high scalability permits nanoparticles to be conjugated and expands the multifunctional application in therapy, imaging, and sensing.

MNP-PDA has absorption properties used for eliminating metals and dyes (Liu R. et al., 2013; Xie et al., 2014; Zhao Y. et al., 2015; Li J. et al., 2016). Zhang *et al.* reported a protein-imprinted MNP-PDA that improved binding ability of the respective targets using the absorption property, demonstrating high potential for proteomics and drug delivery (Zhang et al., 2012). Moreover, MNP-PDA serves as an imaging/therapy platform (Lin et al., 2014; Zheng et al., 2015; Xue et al., 2017). Lin *et al.* revealed that PDA exhibited strong NIR absorbance, high fluorescence quenching, and high functionality. Additionally, the group illustrated that MNP-PDA act as a multifunctional agent for intracellular mRNA detection and synergistic MRI and photoacoustic dual-modal imaging-guided photothermal therapy (Lin et al., 2014).

PDA coating is reported for GNP (Choi et al., 2015; Wang C. et al., 2016; Xu et al., 2019), GNR (Black et al., 2013; Zelasko-Leon et al., 2015; Wang S. et al., 2016), hollow (Ju et al., 2015), and nanostar (Li D. et al., 2016; You et al., 2019). Liu *et al.* assessed GNP-PDA's cellular uptake and biodegradability *in vivo* and *in vitro* (Liu X. et al., 2013). TEM observation revealed that no significant change was affected by the GNP-PDA structure in the lysosomes or cytosol within cells over a 24 h incubation period and in the liver or the spleen from 1 day to 6 weeks after injection. Zhang *et al.* synthesized GNR-PDA-loaded RGD peptide, cisplatin, and iodine-125 for an image-guided combination chemo-thermal therapy platform (Zhang et al., 2016). The hybrid particles target the tumors, and upon internalization into cells, the particles release cisplatin into endosomes. The particles can be visualized via CT imaging and photoacoustic imaging.

Additionally, MSNPs with PDA shells realize unique DDS applications (Cheng et al., 2017b; Wei et al., 2017; Lei et al., 2019; Shi et al., 2019). Zheng *et al.* developed a system for drug release control through coating drug-loaded MSNPs with PDA (Zheng et al., 2014). Notably, the system uses PDA as a gatekeeper mechanism to inhibit drug release. Once the particles are placed under acidic conditions such as endosomes, the PDA disassembles, gradually release the drugs into the cell. Cheng *et al.* developed a nanocarrier system of PDA-coated MSNPs functionalized with d-a-tocopheryl polyethylene glycol 1,000 succinate (TPGS) (i.e., MNSs-DOX@PDA-TPGS). The groups demonstrated that MSNs-DOX@PDA-TPGS displays exceptional overcomes multidrug resistance with better therapeutic efficacy *in vivo* than free DOX and DOX-loaded NPs without TPGS ligand modification (Cheng et al., 2017a).

FND-PDA functions double-sided since PDA exhibits photothermal properties in nanoheater/thermometer and is also used for intracellular thermal conductivity measurements (Sotoma et al., 2021). Therefore, the measurement of intracellular thermal conductivities of HeLa and MCF-7 cells was examined, revealing a mean conductivity of the two separate cell lines of 0.11 ± 0.04 $Wm^{-1}K^{-1}$ (95% confidence) with similar accuracies, significantly lower than that of water ($0.6\ Wm^{-1}K^{-1}$). Therefore, the latest study group fabricated a composite quantum sensor containing FND, PDA, GNP, and HPG as a dual-purpose, enhanced nanoheater/thermometer (Sotoma and Harada, 2021).

Other interesting bioapplications of NP-PDA have been reported and summarized in excellent papers such as protein sensing (Xia et al., 2013), stimuli-responsive molecule release (Yang et al., 2021), biocatalyst (Liu R. et al., 2013; Xie et al., 2014; Landarani-Isfahani et al., 2015; Deng et al., 2016; Liu et al., 2017; Lu et al., 2020), molecule removal from a solution (Xie et al., 2014; Li J. et al., 2016; Baghban et al., 2018), immune assay (Lai et al., 2013), antibacterial (Fu et al., 2021), wound healing (Zheng et al., 2021), and tissue engineering (Tang et al., 2021).

CONCLUSIONS AND PERSPECTIVES

This review described how HPG and PDA coatings affect the properties of the NPs, including the current applications of HPG/PDA-coated NPs. The ease of coating and functionalization and excellent biocompatibility have greatly improved and broadened the application of the numerous NPs in biomedical science, including targeting, therapy, imaging, sensing, and technologies that combine them.

Nevertheless, HPG and PDA have several hurdles to overcome for future study. For HPG coating, nucleophilic groups on the surface of NPs are necessary for the coating. To overcome this limitation, NPs without nucleophilic groups on their surface must introduce appropriate scaffolds. Also, care must be taken to ensure that this scaffold does not affect the intrinsic properties of the NPs. Despite increasing investigations, PDA's polymerization mechanism remains controversial. Notably, the structure is vital in understanding its physical properties; however, a deeper understanding will better control the strategy for later functionalization.

HPG and PDA are reported to be less toxic and reduce the inherent toxicity of NPs in biological systems. However, there are limited reports on the interaction of HPG and PDA coatings, which affects cellular functions. Additionally, their long-term stability, and biodegradability *in vivo* are not fully understood. The systematic investigation on the HPG-NPs

and PDA-NPs in animal models accumulate safety knowledge and perspectives, thus increasing potential clinical applications.

Once the abovementioned issues can be addressed, HPG and PDA coatings will greatly impact the biomedical field. Furthermore, the trend of future research will be the combination of NP functions with those of polymers to create nanorobots with multifunctional properties that modify their functions according to external signals and/or the local environment, subsequently establishing a new diagnostic system, and tailor-made medicine. Therefore, I believe the interest in these coatings is expected to increase substantially in the next decades.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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SUPPLEMENTARY MATERIAL

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