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REVIEW PAPER

Diversified component incorporated hybrid nanoflowers: A versatile material for biosensing and biomedical applications

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Abstract−Organic-inorganic hybrid nanoflowers (HNFs) have generated widespread research interest owing to their properties to efficiently entrap organic components like protein or enzyme within their nanostructured matrices, yielding high activity, stability, and recyclability. Recently, much effort has been devoted to developing advanced HNFs composed of diversified components, such as multiple proteins, nanoparticles, polymers, and nucleic acids, to achieve different functionalities enabling extended applications. Compared to the conventional HNFs primarily serving as immobilization supports for enzyme, diversified component incorporated HNFs can have unique multiple functionalities, essentially for developing novel biosensing and biomedical strategies. Herein, an overview for the recent advances on diversified components incorporated HNFs is presented with an emphasis on the potential biotechnological applications. Synthetic strategies, structural characteristics, and unique properties of diverse HNFs are discussed with representative studies, demonstrating the versatility of the HNFs. Current challenges and future opportunities of the HNFs are also discussed.

Keywords: Hybrid Nanoflowers, Multiple Proteins, Nanoparticle, Polymer, Nucleic Acid, Biosensing, Biomedical Application

INTRODUCTION

Recently, flower-shaped hierarchically-nanostructured organic-inorganic hybrid microparticles, so called nanoflowers, have gathered intense attention due to their high efficacy to entrap organic components within their matrices. They have been proven to show large surface-to-volume ratio having abundant mesopores, which helps to realize effective confinement of organic molecules, yielding high loading capacity, strong absorption ability, high activity retention, and excellent stability and robustness [1,2]. Since Ge et al. first reported the synthesis of hybrid nanoflowers (HNFs) incorporating organic components, including protein molecules and inorganic components including copper ions [3], much research effort has been directed to clarifying the unique characteristics of HNFs, developing advanced kinds of HNFs, and showing their applicability in diverse biotechnological areas, including biosensing, environmental remediation and monitoring, and catalysis [2]. HNFs were initially considered as one of the enzyme immobilization techniques, since the entrapped enzymes showed better catalytic performance in activity and stability compared with those of conventional immobilization methods [4]. As increasing the numbers of HNFs research, not only protein but also another organic component such as peptides [5], amino acids [6], nucleic acids [7], and different amine-containing compounds [8-10] have been involved to synthesize HNFs.

Although the synthetic strategies can be modified to tailor their performance suitable for specific applications, HNFs have been primarily synthesized via the self-assembly based on co-precipitation method, where organic components and inorganic components are simply co-incubated in phosphate buffered saline (PBS) for a certain period of time, yielding full blooming of the HNFs. The synthesis can be conducted facilely under mild temperature and pH condition, from the coordination interaction between the amine/amide moieties of organic components and metal ions such as Cu, Zn, Fe, Co, Ca, and Mn, which serve as nucleation sites, followed by anisotropic time-dependent growth from the precipitation of metal phosphate crystals, inducing flower-like morphology at nanometer scale (Fig. 1(a)) [11]. In the same year of the first exploration of HNFs, Lee et al. reported another strategy to synthesize a self-assembled RNA microsponge, based on rolling circle transcription process for generating a huge amount of RNA molecules, followed by self-assembly into the flower-shaped structure with the help of magnesium ions (Fig. 1(b)) [12,13]. The resulting material was demonstrated to be effective to deliver RNA with relatively low toxicity compared with that of the other polymer- or lipid-based gene delivery agents. The RNA-entrapping material could be considered as one of the HNFs since there was a coordination between the nucleic acids and magnesium ions; however, several nucleic acid enzymes have to be involved in the synthetic process, hindering its utilizations in extended areas.

After the discovery of HNFs, different types of HNFs composed of diversified components were developed, exhibiting new and multiple functionalities. For example, multiple enzyme incorporated HNFs, where glucose oxidase (GOx) and horseradish peroxidase (HRP)

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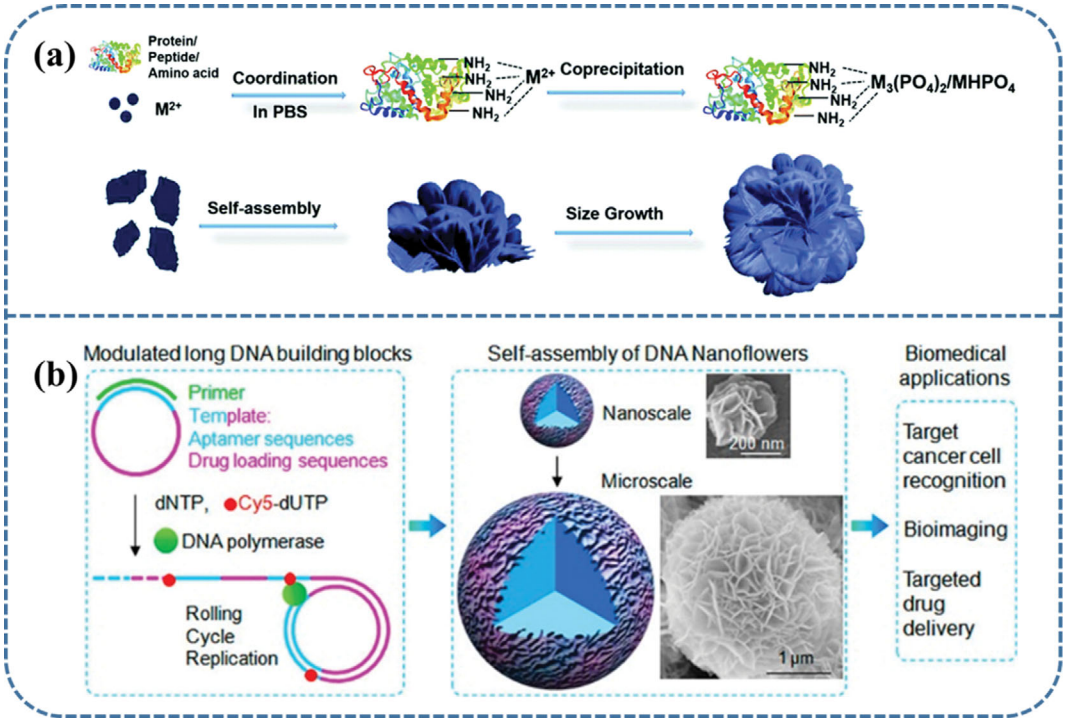

Fig. 1. Synthetic schemes of HNFs using (a) self-assembly based on co-precipitation method and (b) enzymatic method based on rolling circle transcription/rolling circle amplification. Reprinted with permission from [20] and [13], respectively. Copyright 2019 Royal Society of Chemistry and Copyright 2013 American Chemical Society.

were co-entrapped within copper phosphate based HNFs (GOx-HRP-$Cu_3(PO_4)_2$ HNFs), were constructed as a biosensor enabling cascade reaction to detect glucose [14]. Not only multiple enzymes but also different amine/amide containing components such as antibodies, nanoparticles, polymers, and nucleic acids have been involved with metal ions to synthesize the HNFs having different functionalities, allowing extended application of HNFs. Frequently, the extended components within HNFs provide additional binding capacity, magnetic property, conductivity, optic and chemical property, and special absorption property [15-18]. These diversified components incorporated HNFs can be recognized as one of the promising materials meeting the requirements for diverse biosensing and biomedical applications (Fig. 2).

The size of HNFs was considered one of the most important aspects to determine their practical applications [19]. Generally, their size varies from several hundreds of nanometers to micrometer-scale with abundant nanometer-sized petals [20,21]. The biomedical applications of HNFs could be limited due to their difficulty in entering the cell environment by their relatively large size. Interestingly, incorporation of multiple components to synthesize the HNFs was reported to enable size-control of HNFs [11]. For example, the size of HNFs was decreased by the entrapment of certain nanoparticles within their structure [11,16], which could be another advantage of diversified component incorporated HNFs.

Based on the attracting potential of HNFs, there have been several recent reviews; however, most of them focus on the basic HNFs composed of enzymes with metal ions [19,20]. To the best of our knowledge, there have been no reviews primarily discussing diversified component incorporated HNFs. In this review, we describe recent research studies focusing on the advanced HNFs, distributed by the kinds of involved components such as multiple proteins, nanoparticles, polymers, and nucleic acids, with their synthetic methods, unique and versatile characteristics, and applications in bio-

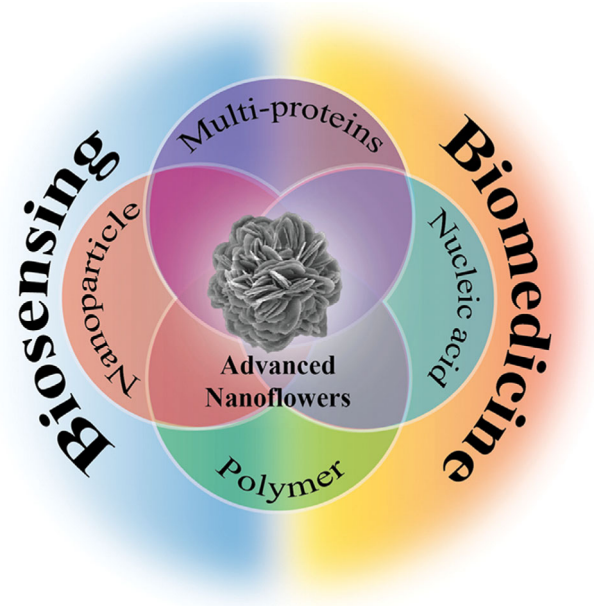

Fig. 2. Schematic illustration of diversified component incorporated HNFs for biosensing and biomedical applications.

sensing and biomedical fields. Current challenges and prospects of the diversified components incorporated HNFs are also discussed.

RESEARCH STUDIES ON DIVERSIFIED COMPONENT INCORPORATED HNFs

1. Multiple Protein Incorporated HNFs

Since the first report by Ge et al. [3], the majority of the developed HNFs have been used to entrap a single kind of protein with crystalline metal phosphate petals [19,21]. Recently, diverse multiple proteins have been incorporated into the HNFs, showing different properties. Multiple protein incorporated HNFs have been synthesized by similar self-assembly, but the strategy can be distributed into two ways: one-step method by all-in-one co-incubation and two-step method inducing spatial co-localization [14]. The all-in-one HNFs were synthesized by just mixing all the employed multiple enzyme molecules and metal salts at appropriate levels in aqueous PBS buffer. The resulting HNFs showed multiple enzymatic activity, and most of them were designed toward cascade reaction. For example, by all-in-one incubation of GOx and HRP with copper sulfate, GOx-HRP-$Cu_3(PO_4)_2$ HNFs were constructed [22]. In the presence of target glucose, entrapped GOx catalyzed the oxidation of glucose to produce H_2O_2, which subsequently induced the oxidation of employed substrate to produce optical responses (Fig. 3(a)). Another oxidative enzyme, such as ChOx [23] or uricase [24], has also been involved with HRP to bloom the dual enzyme incorporated HNFs for detecting their target analytes with the corresponding cascade reactions. Unlike the all-in-one HNFs, the two-step method involves sequential addition of employed enzymes, to yield their pre-defined compartmentalization within the nanoflower matrices (Fig. 3(b)) [14]. To this, the first kind of enzyme molecule is incubated with metal salt, followed by centrifuge-mediated collecting them, which serves as core compartment. It is re-incubated with the second enzyme solution with metal salt, inducing their location at the outer layer of the final HNFs. The spatial co-localized HNFs composed of two different enzymes consequently yields enhanced cascade reaction activity, from the facilitated substrate transport between the enzyme layers. So far, cascade reaction activity from multiple enzymes incorporated HNFs has been mainly applied for detecting the substrate of entrapped oxidative enzyme [14,25]; besides, the GOx-HRP-$Cu_3(PO_4)_2$ HNFs are applied for electrochemically detecting *Escherichia coli* (*E. coli*), based on their ability to produce electrons during the cascade reaction, monitored by the increase of current signals [26].

Multiple enzyme incorporated HNFs have been utilized not only in biosensing but also other biocatalytic applications [27-32]. Interestingly, (*S*)-1-(2,6-dichloro-3-fluorophenyl) ethyl alcohol, an intermediate of anti-cancer drug Crizotinib, was efficiently synthesized using aldehyde ketone reductase-alcohol dehydrogenase-$Ca_3(PO_4)_2$ HNFs [29]. Rai et al. also prepared dual enzyme incorporated HNFs, entrapping β-galactosidase and L-arabinose isomerase, for the transformation of lactose into a high-value sugar D-tagarose with excellent efficiency up to ~25% conversion [32]. Notably, HNFs composed of three different enzymes were also reported [33,34]. Wu et al. demonstrated the effective conversion into 2,5-diformylfuran from hydroxymethylfurfural (HMF), with the triple enzyme incorporated HNFs, including galactosidase, catalase, and HRP. The HNFs exhibited high tolerance toward the oxidative damage caused by high initial level of HMF as well as H_2O_2 released during the conversion of HMF by galactosidase. As a result, higher conversion was achieved compared to that of separately immobilized ones [33].

Protein molecules possessing specific binding affinity toward target analytes, including antibody, streptavidin (SA), and concanavalin A (Con A), have also been involved as organic components to bloom HNFs. By entrapping both the target-recognizing protein and enzyme, dual functions, including target recognition and signal production, were realized, which can potentially replace the existing enzyme-labelled immunosorbent assay (ELISA) techniques. For example, Wei et al. reported an efficient strategy to detect *E. coli O157:H7* using antibody-HRP-$Cu_3(PO_4)_2$ HNFs [15]. The entrapped antibody had a role to specifically capture the *E. coli*, while HRP served as a signal transducer by oxidizing 3,3′,5,5′-tetramethylbenzidine (TMB) substrate to generate blue color in the presence of H_2O_2. Analogously, other HNFs comprising both HRP and SA mol-

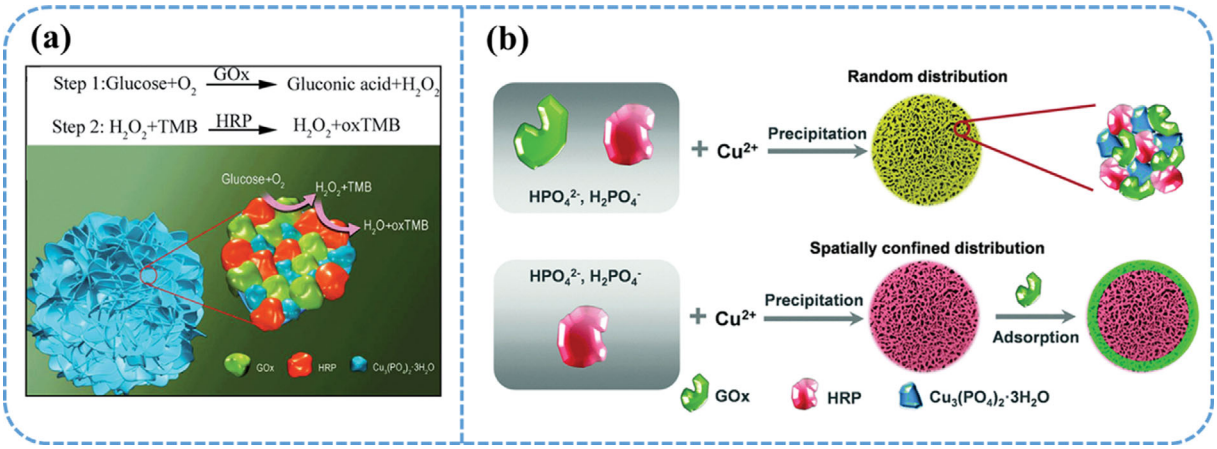

Fig. 3. Schematic illustration of (a) cascade reaction using all-in-one GOx-HRP-$Cu_3(PO_4)_2$ HNFs for glucose detection and (b) two-step synthesis of GOx-HRP-$Cu_3(PO_4)_2$ HNFs yielding spatial compartmentalization. Reprinted with permission from [22] and [14], respectively. Copyright 2014 Royal Society of Chemistry.

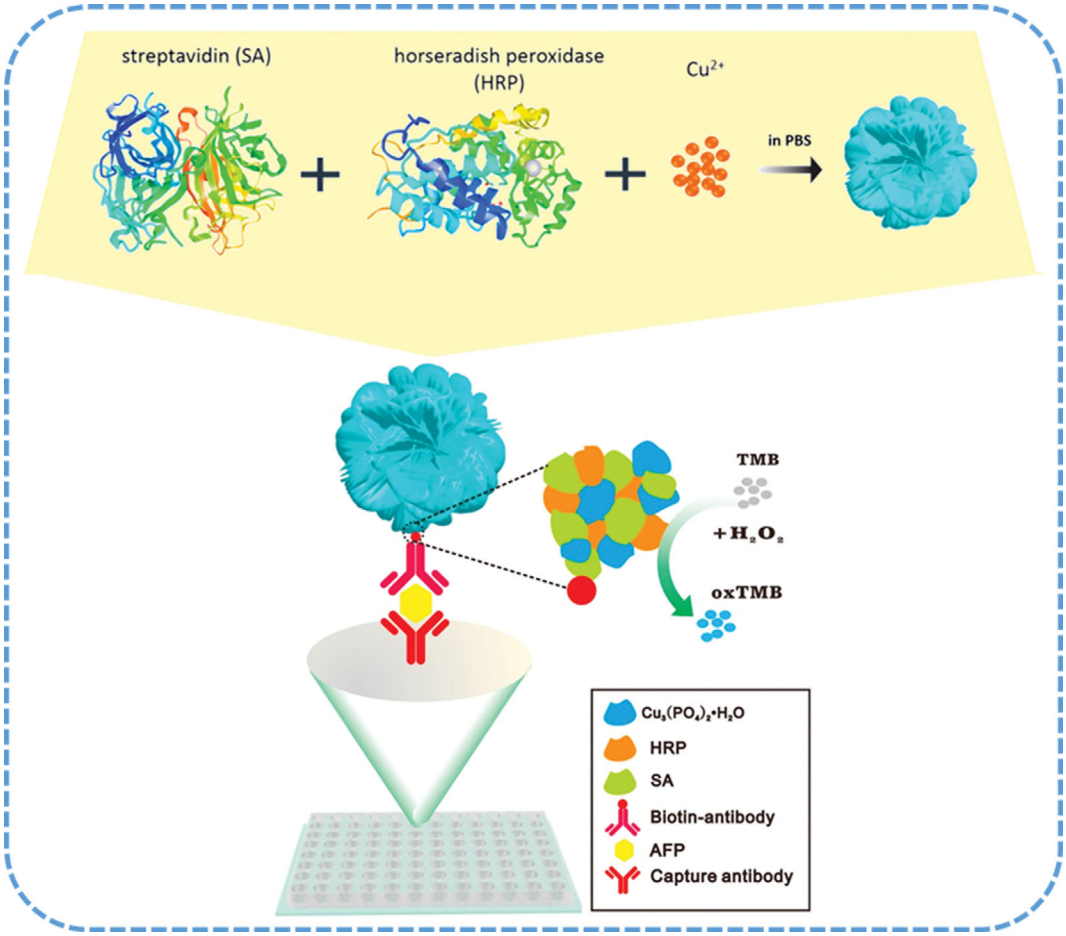

Fig. 4. Representative model of multiple proteins incorporating HNFs conducting dual functions of target recognition and signal production. SA-HRP-$Cu_3(PO_4)_2$ HNFs were developed and used for detecting fetoproteins. Reproduced with permission from [35]. Copyright 2017 Elsevier.

ecules for signal amplification and recognition, respectively, were developed for detecting fetoproteins (Fig. 4) [35]. The target recognition occurred based on the excellent affinity between SA on the HNFs and biotin labelled on the secondary antibody. After the binding events, by adding TMB and H_2O_2, HRP entrapped within the HNFs catalyzed the TMB oxidation to generate blue-colored product. With this dual SA-HRP entrapped HNFs, target fetoproteins were quantified down to 78 pg mL^{-1}, which is more sensitive than that of commercial ELISA kits. Another interesting pH meter-based biosensing strategy based on multiple proteins incorporated HNFs was developed for detecting food pathogens [36,37]. The well-known lectin Con A, which has high affinity to lipopolysaccharides O-antigen on the surface of *E. coli*, and GOx, which catalyzes the conversion of glucose to gluoconic acid to yield pH decrease, were co-entrapped within the HNFs (Con A-GOx-$Ca_3(PO_4)_2$ HNFs). In the presence of target *E. coli*, Con A served as recognition unit; at the same time, GOx acted as signal transducing unit inducing pH decrease. With the concept, target *E. coli* was successfully detected with a convenient pH meter readout, yielding the limit of detection (LOD) of *E. coli O157:H7* down to 10 CFU mL^{-1}. The sensing platform is possibly extended, by replacing GOx with another enzymes like xanthine oxidase (XOD), acetylcholine esterase (AChE), or urease. Because these enzymes are capable of catalyzing reactions involving the release of pH-responsive products such as uric acid, acetic acid, or ammonia, further pH alteration-based biosensors based on the multiple proteins incorporated HNFs could be realized.

2. Nanoparticles Incorporated HNFs

Nanoparticles (NPs) with intriguing properties have been widely studied and utilized in many areas. To date, diverse NPs and NPs-incorporated materials have been developed, and among them, NPs incorporated HNFs have attracted recent attention due to their capability to provide additional NPs' characteristics to the HNFs, which is highly beneficial in many biosensing and biomedical applications. Their synthetic strategy can be distributed into three methods. In the first method, NPs are separately prepared and then incubated with proteins in aqueous buffer [38], inducing the interaction between the NPs and proteins, which serve as nucleation sites for further crystallization [39]. Note that most of the NPs used in this method preserve amine/amide moieties on their surface, since the moieties are used to induce coordination interaction with the incubated metal ions to form the HNFs. Using the method, NPs were efficiently entrapped within HNFs; however, tedious procedures for amine/amide functionalization of the NPs with optimiz-

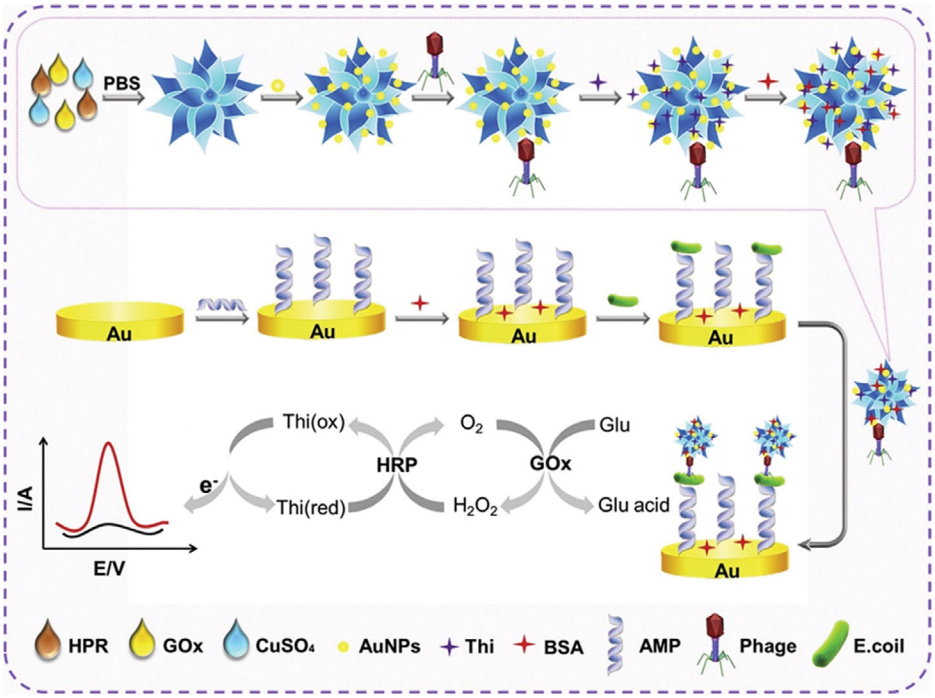

Fig. 5. Au NPs incorporated HNFs for electrochemical detection of *E. coli*. Reprinted with permission from [26]. Copyright 2018 Elsevier.

ing reaction conditions to appropriately induce the coordination of NPs with metal ions are necessary [40]. The second method is intended to decorate NPs on the surface of pre-synthesized HNFs, via an *in situ* reduction [41]. The conventional HNFs are incubated with an aqueous solution containing metal ions, which subsequently reduces to NPs with the help of reducing agents, heating, or radiation. With the method, several NPs are rapidly formed on the surface of HNFs, providing unique optochemical properties, which induce surface-enhanced Raman scattering (SERS) or surface plasmon resonance (SPR) phenomena. Nonetheless, the NPs-decorated HNFs need to be stable during the NPs reduction, to maintain their flower-like morphology. In the third method, HNFs and NPs are separately synthesized, and then, conjugated with each other via physical adsorption or covalent linkage [42]. The strategy is straightforward to prepare HNF-NP hybrid; however, the loading capacity of NPs on HNFs is generally limited. Based on these strategies with marginal modifications depending on application purposes, NPs incorporated HNFs have been constructed.

In general, NPs are entrapped together with proteins within HNFs, to add NPs' unique properties to the conventional protein incorporated HNFs. For instance, Cheon et al. reported the synthesis of magnetic nanoparticle (MNPs)-embedded GOx-$Cu_3(PO_4)_2$ HNFs [16]. In the presence of glucose, entrapped GOx catalyzed the glucose oxidation to produce H_2O_2, which was subsequently reduced to hydroxyl radicals by the HRP-like activity of MNPs and crystalline $Cu_3(PO_4)_2$ petals, yielding the oxidation of employed Amplex UltraRed (AUR) substrate into highly fluorescent product. Using this strategy, glucose was sensitively detected as low as 2.5 μM, with highly improved stability and magnetic reusability. Similarly, MNPs have been widely entrapped within HNFs, to provide magnetism and peroxidase-like catalytic activity [39,43-45]. Noble metal NPs-coated HNFs were also reported for detecting and discriminating pathogenic bacteria. Li et al. fabricated an electrochemical sensor consisting of T4 phages as recognition unit and gold nanoparticle (Au NPs)-thionine (Thi)-GOx-HRP-$Cu_3(PO_4)_2$ HNFs as signal transducer (Fig. 5) [26]. In the presence of target *E. coli* cells, they bound to the receptor antimicrobial peptide (AMP) on Au electrode. The T4 phages loaded on the Au NPs incorporated HNFs could specifically recognize them and, thus, form the sandwich-type connection with electrode. Based on the cascade reaction activity of GOx and HRP entrapped within the HNFs, the binding event was successfully detected with current signals. In particular, the entrapped Au NPs had a role to amplify the current responses due to their excellent conductivity. Based on the Au NPs incorporated HNFs, target pathogenic bacteria were sensitively detected as low as 1 CFU mL^{-1}.

In addition to metal oxide or noble metal NPs, nanocarbons preserving many advantageous features, such as extraordinarily high conductivity, excellent chemical and thermal stability, facile surface functionalization, and high catalytic activity, have been integrated within HNFs [46]. Duan et al. developed graphene oxide (GO)-lysozyme-$Cu_3(PO_4)_2$ HNFs, showing an excellent antibacterial activity from the combined effects of GO, lysozyme, and copper ions [47-49]. The morphology of the HNFs efficiently maintained since GO and $Cu_3(PO_4)_2$ served as "skeletons" to support the structure. Carbon nanotube (CNT) was also incorporated with GO and laccase (Lac) to construct GO-CNT-Lac-$Cu_3(PO_4)_2$ HNFs, showing an efficient catalytic activity and conductivity compared with those of individual components [50]. Another interesting allotrope of graphitic carbon, graphitic carbon nitride (GCN), has also been incorporated to form the HNFs, based on the coordination interaction between the amine moieties of GCN and metal ions [8].

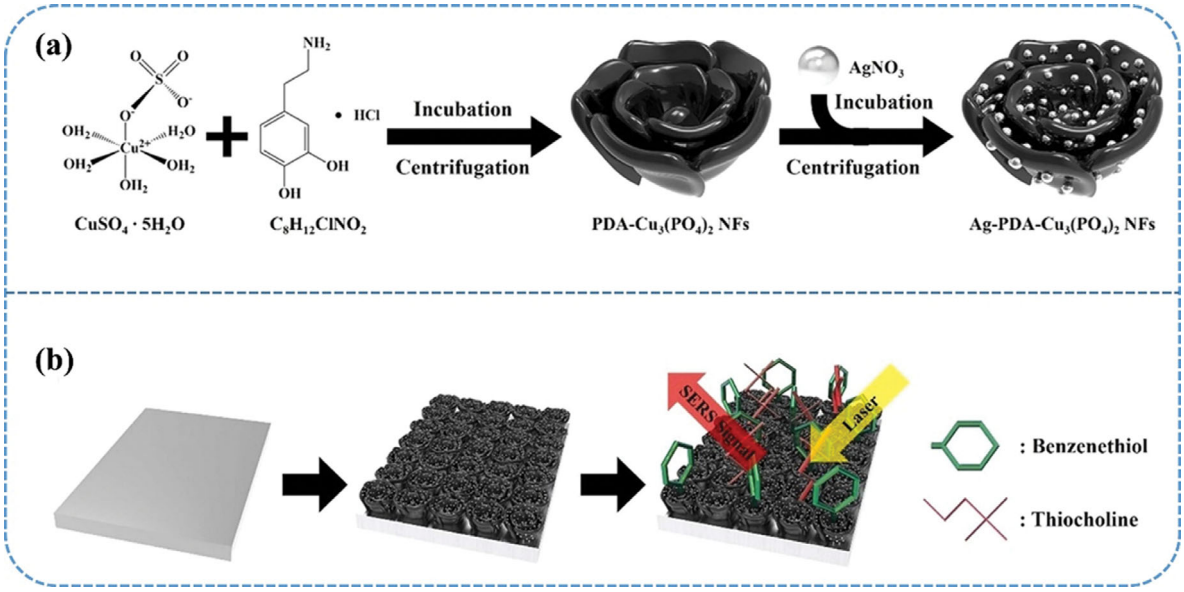

Fig. 6. (a) Construction of Ag NPs-PDA-$Cu_3(PO_4)_2$ HNFs and (b) their application as a new SERS probe for sensitively detecting thiol-containing molecules. Reprinted with permission from [17]. Copyright 2022 Elsevier.

Based on the demonstration, HRP-GCN-$Cu_3(PO_4)_2$ HNFs were constructed for photon-enzyme cascade reaction [51]. In this system, GCN entrapped within the HNFs is stimulated by visible light irradiation, producing electron-hole pairs and leading to facilitated redox reactions. Then, the light-generating electrons interact with O_2 to form H_2O_2, which is broken down into hydroxy radicals by the catalytic activity of entrapped HRP and $Cu_3(PO_4)_2$ petals, consequently degrading phenolic pollutants like bisphenol A. These studies demonstrate the potential of HNFs to incorporate nanocarbons within their matrices, providing new and multiple functionalities and enlarging their applications in diverse areas.

3. Polymer Incorporated HNFs

Polymers are macromolecules generally made up with hydrocarbons, and have diverse physicochemical and biological properties, which play important roles in material engineering [52]. Polymers can be engineered to achieve diverse chemical moieties, including amine/amide moieties, which can be involved to make coordination interaction with metal ions [53,54]. Thus, by just incubating specific polymeric molecules with metal ions, or mixing monomer molecules with metal ions followed by polymerization to produce the amine/amide moieties on the polymer, coordination interactions would occur, to form the corresponding polymers incorporated HNFs. Polydopamine (PDA), which can be prepared from the monomer dopamine (DA) in alkaline conditions [55,56], has been frequently incorporated within HNFs, based on their abundant amine moiety and unique activity. Representatively, Zhang et al. developed silver nanoparticles (Ag NPs)-coated PDA-$Cu_3(PO_4)_2$ HNFs [41]. In this study, DA molecules were incubated in an aqueous solution containing copper ions, inducing the polymerization into PDA and formation of primary complexes of PDA-$Cu_3(PO_4)_2$, which served as nuclei for the formation of HNFs. Furthermore, the prepared PDA acted as a reducing agent, yielding *in situ* reduction of silver ions to prepare Ag NPs without the involvement of further reducing agents. The resulting Ag NPs-PDA incorporated HNFs exhibited high antimicrobial activity toward *E. coli*, while showing negligible cytotoxicity to mammalian cells. In another recent study by Park et al., Ag NPs-PDA-$Cu_3(PO_4)_2$ HNFs were developed as a new surface-enhanced Raman spectroscopy (SERS) probe for sensitively determining thiol-containing molecules (Fig. 6) [17]. By loading the Ag NPs through the similar *in situ* reduction, target molecules such as benzenethiol and thiocholine were detected based on the interaction between the Ag NPs and the thiol groups of the analytes, yielding LOD values as low as 800 and 60 pM, respectively. Kim et al. also reported the attachment of Hg^{2+}-binding DNA aptamer on the Ag NPs-MNPs-PDA-$Cu_3(PO_4)_2$ HNFs [18]. The HNFs were similarly constructed except the involvement of MNPs, which preserved amine moieties on their surface. Then, thiol-modified Hg^{2+}-binding aptamers were conjugated via Ag-thiol interactions. Consequently, the resulting HNFs showed an excellent affinity to Hg^{2+} and served as bioremediation agents to capture and remove the harmful mercury ions.

Different from the HNFs synthesized from the coordination interactions between the amine/amide moieties and metal ions, other HNFs were recently reported, which were synthesized from the different interactions such as hydrogen bonding [57]. Ag-based polyoxometalate ($Ag_3PW_{12}O_{40}$; AgPW) interacted with DA via hydrogen boding, inducing its polymerization to yield AgPW-PDA composites. The composites were then incubated with nisin, which is a well-known antibacterial peptide, and the final AgPW-PDA-nisin HNFs were prepared due to the adhesive ability of PDA with nisin. The HNFs exhibited a high bactericidal ability, based on their constituents such as nisin and Ag ions released from the entrapped AgPW. These approaches provide new opportunities to develop advanced HNFs having enlarged capability, which can highly contribute on developing novel biosensing and biomedical strategies.

4. Nucleic Acids Incorporated HNFs

Nucleic acid incorporated HNFs have gathered recent attention due to their potential as gene delivery carrier, to efficiently encap-

sulate and release the relevant DNA/RNA [58,59]. The HNFs have been synthesized via two primary ways: the first is self-assembly based on the coordination interaction between amine/amide residues of nucleic acids and metal ions, followed by anisotropic precipitation of crystalline metal phosphate, and the second is enzymatic method based on rolling circle transcription or rolling circle amplification to produce a huge amount of nucleic acid, followed by anisotropic precipitation of crystalline metal phosphate. Compared to the conventional protein incorporated HNFs, nucleic acid incorporated HNFs have not been widely studied yet, and thus, many studies are needed to clarify their characteristics and possible applications.

Park et al. reported the development of the first DNA-$Cu_3(PO_4)_2$ HNFs via the self-assembly method by simple incubation of DNA and copper sulfate in PBS at room temperature, which show a high tolerance against nuclease-mediated cleavage action and peroxidase-like activity arising from the $Cu_3(PO_4)_2$ petals [60]. The self-assembly based DNA-$Cu_3(PO_4)_2$ HNFs have recently been demonstrated to have an intrinsic laccase-like activity, which is applied to develop paper microfluidic device to visually identify diverse phenolic compounds [61]. Kim et al. reported an enzymatic method to develop DNA incorporated NFs [59]. In this study, they found that the abundant Mg ions and DNA molecules provided many binding sites for proteins, enhancing the loading capacity and stability of entrapped proteins. Then, by adding protein molecules into the aqueous solution containing DNA and Mg ions followed by rolling circle amplification reaction, HNFs incorporating protein, DNA, and crystalline magnesium pyrophosphate were constructed, consequently showing high toxicity to cancer cells. Wang et al. reported other HNFs, comprised of rolling circle polymerized DNAzyme (DNA-degrading nucleic acid), ZnO NPs, and anticancer drug [62]. Specific DNA tandem was designed to contain sequences corresponding to DNAzyme and aptamer, which help the HNFs to be efficiently delivered into cancer cells. When pH changed into acidic, Zn^{2+} ions were released from the entrapped ZnO NPs, serving as a cofactor for DNAzyme. Subsequently, the DNAzyme catalyzed the cleavage of HNFs' scaffold, inducing drug release. This smart HNFs-based self-driving drug delivery platform could be utilized in many therapeutic systems, expecting wide biomedical applications in the future.

Table 1. Summary of diversified components involved to prepare HNFs

Category of HNFs	Entrapped components	Size (μm)	Ref.
Multiple proteins incorporated HNFs	HRP, GOx	5-30	[14,22,25,26]
	HRP, cholesterol oxidase	~20	[23]
	HRP, uricase	~2	[24]
	Acetylcholine esterase, choline oxidase	4.3-5.7	[27]
	Glucoamylase, GOx	16.2	[28]
	GOx, lipase	20-30	[31]
	HRP, catalase, and galactose oxidase	3-8	[33]
	α-Amylase, lipase, and protease	10-20	[34]
	HRP, antibody	~2	[15]
	HRP, SA	~5	[35]
	Aldehyde-ketone reductase, alcohol dehydrogenase	ND	[29]
	Polyketone reductase, glucose dehydrogenase	ND	[30]
	Invertase, GOx, Con A	~2	[36,37]
	β-Galactosidase, L-arabinose isomerase	3.6-6.4	[32]
NPs incorporated HNFs	MNPs with GOx, keratin, lipase, papain, cholesterol oxidase	2-40	[16,39,43-45,63]
	Au NPs with HRP, GOx	2-5	[26]
	GO with lysozyme	~20	[47]
	GO and CNT with laccase	3.1-3.5	[50]
	GCN with HRP	10.3	[51]
	GO and CNT with HRP	2	[64]
Polymers incorporated HNFs	PDA with Ag NPs	3.5-40	[17,41]
	PDA with MNPs	14-23	[65,66]
	PDA with MNPs, Ag NPs, aptamers	10-20	[18]
	Chitosan with Ag NPs	~30	[67]
	PDA with nisin, AgPW	ND	[57]
Nucleic acids incorporated HNFs	DNA with or without proteins	0.2-30	[7,59-61]
	DNA with ZnO NPs	~0.2	[62]
	DNA and RNA with antigen	1-5	[68]
	RNA with MNPs	~0.5	[69]

ND: Not determined

CONCLUSION AND PROSPECTS OF DIVERSIFIED COMPONENT INCORPORATED HNFs

This review provides an overview on diversified components incorporated HNFs, which have been recently studied for biosensing and biomedical applications. By introducing further components, such as multiple proteins, nanoparticles, polymers, and nucleic acids, the HNFs can obtain new and multiple functionalities, which are essential to realize and extend their applications in diverse biotechnological fields. Diversified components which were involved to prepare the HNFs and their corresponding sizes are summarized in Table 1.

Based on the outstanding properties of diversified components incorporated HNFs, they have promising prospects, but we believe the following technical issues should be resolved: (1) Since multiple components are presented within the nanoflower matrices, optimizing the activity of each component or combined components, by appropriate understanding their reaction mechanisms with controlling the diffusion and decomposition of reactants, is highly required to optimize the synergistic effects. (2) Since the HNFs have a significantly large surface area with high porosity, it is highly beneficial to tailor their surface functionality, which might greatly contribute to achieving high performance in many applications. (3) Further components could be entrapped within the HNFs, making new functional HNFs and consequently realizing new applications. (4) Synthesis of diversified components incorporated HNFs needs to be further optimized, towards the HNFs having narrower size distributions with negligible variation between the synthetic batches. Along with the technological development of the HNFs, we expect that more advanced HNFs will be developed, and their utilization will be realized in many biosensing and biomedical areas.

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