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Review

Nanoscale MOF–Protein Composites for Theranostics

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Abstract: Nanoscale metal–organic frameworks (nMOFs) have gained increasingly more attention as attractive support materials in the immobilization and delivery of proteins for disease theranostics in recent years owing to their various advantages, such as large specific surface areas, well-ordered pore structures, aperture channel distributions, and ease of functionalization. Here, we present an overview of recent progress in nMOF–protein composites for disease theranostics. First, advantages and construction strategies of nMOF–protein composites as drug carriers are introduced. Then, therapeutic modalities and theranostic nanosystems based on nMOF–protein composites are reviewed. Next, we pay specific attention to their biosafety, biodistribution, and excretion in vivo. Finally, the challenges and limitations of nMOF–protein composites for biomedical applications are discussed, along with future perspectives in the field.

Keywords: nanoscale metal–organic frameworks; protein; theranostics

1. Introduction

Proteins are composed of amino acid sequences with a delicate spatial structure, which determine various biological functions. Protein biomolecules are not only important components of cells, but are also involved in various cellular processes and body metabolism. Specifically, many diseases are induced by the changes in intracellular or extracellular protein molecules, signifying an enormous opportunity for protein therapeutics [1]. Therapeutic proteins have attracted extensive attention in the pharmaceutical industry due to their high specificity and applicability in a broad range of diseases such as infectious diseases, chronic inflammatory diseases, cancers, metabolic disorders, autoimmune diseases, and cardiovascular diseases [2,3]. Protein drugs possess many advantages, among which the most significant are the high bioactivity and specificity when compared to small-molecule drugs. Unfortunately, the structural flexibility and susceptibility to environmental stressors related to protein instability not only lead to decreased bioactivity, but may also potentially elicit undesired immunological responses, hindering the increasing use of therapeutic proteins [4,5]. Therefore, it is particularly important to ensure the stability of protein drugs during production, during transportation, and before reaching the lesion location. In order to overcome these limitations, researchers have been focusing on developing nanocarriers, including liposomes, polypeptide inorganic nanoparticles, polymers, etc., to selectively deliver proteins to lesion locations [6,7].

Porous materials such as mesoporous silica, organic microparticles, sol–gel matrices, and hydrogels, which possess void volume and a large surface area, are competitive candidates for protein drug encapsulation and, thus, have attracted much interest in recent years [8]. Mesoporous silica has attracted much attention due to its large surface area and pore volume. Notwithstanding, the challenges of reasonable structure design, leakage of protein from the mesoporous channel, and surface charges that promote protein denaturation or reduction in protein loading limit the application of mesoporous silica as a protein carrier [9–11]. Sol–gel matrices are intrinsically porous and can prevent protein

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leakage because of entrapment. However, protein immobilization takes place during sol–gel synthesis, which may cause protein molecule denaturation. Moreover, the entry of macromolecular proteins into pores is limited by size mismatch [12,13]. Existing organic microparticles for protein encapsulation are mainly polycation materials which load protein molecules via electrostatic interactions. The hematotoxicity and cytotoxicity of cationic materials limit their application in protein delivery [14,15]. Therefore, it is still very urgent to find new protein drug carriers.

MOFs have drawn much attention due to their unique properties (Figure 1) among nanocarriers. MOFs are a kind of material composed of metal-containing nodes connected via organic ligands, obtaining three-dimensional frameworks with high porosity in the form of, for instance, cavities, channels, and pores [16,17]. MOFs have become highly promising materials in a range of fields including catalysis, environment, energy, and life sciences due to their outstanding features [18]. In the past decades, the research on MOFs has grown exponentially; a great number of MOFs with various structures have been reported and have gained a great deal of attention in drug delivery [19–21]. The potential variation of metal ions and organic ligands and possible postsynthesis modifications endow MOFs with diversified structures and allow researchers to synthesize multifunctional MOFs with a determined shape and size for a particular application [22,23]. Specifically, due to their low biotoxicity and good biocompatibility, as well as their potential to be efficiently internalized by cells, some MOFs have been developed as protein drug delivery vehicles for the theranostics of various diseases, such as cancers and diabetes [24]. These advantages of MOFs make them promising candidates for protein delivery applications in theranostics of different diseases. This paper reviews the vital advances in MOF–protein composites; a large amount of research concerning MOF-based materials as protein drug delivery systems for the treatment of different diseases has been summarized in this comprehensive review.

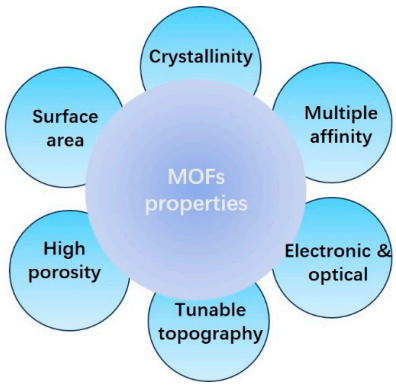

Figure 1. The properties and advantages of MOFs.

2. Construction of nMOF–Protein Composites

MOF–biomolecule composites have been widely applied in bio-related fields, such as biocatalysis, imaging, biosensing, drug delivery, and gene-based therapeutics, because they possess the versatile functionalities of biomolecules, such as nucleic acids, peptides, and proteins [25,26]. Notably, the combination of proteins with MOFs preserves and even enhances the bioactivity of proteins, which has promising prospects in biosensing, catalysis, and protein therapeutics. Proteins' large size and sensitive structure make it a challenge to combine them with MOFs or even encapsulate them into MOFs, which is different from small biomolecules. Special strategies are needed to prepare the MOF–protein composites [27]. Due to the presence of numerous functional groups on the surface of the protein molecules, it is relatively easy to combine them with MOFs via covalent bonds or weak interactions, for instance, π–π interactions, hydrogen bonding, and hydrophobic/hydrophilic interactions [28,29]. These synthesis strategies can be divided into four categories: surface attachment, pore entrapment, covalent linkage, and coprecipitation (Figure 2) [30,31]. Attaching proteins to the surface of MOFs (surface attachment and covalent linkage) is a

straightforward and general method to combine MOFs with proteins, owing to having no special requirements for the composition and internal structure. This method allows MOFs to be presynthesized, which allows synthetic conditions to be outside those of the denaturation ranges of the target protein. Furthermore, the method can preserve the original structure and function of the protein to the greatest extent via immobilizing protein molecules onto the surface of MOFs by weak interactions (i.e., surface attachment) or covalent bonds (i.e., covalent linkage) [32,33].

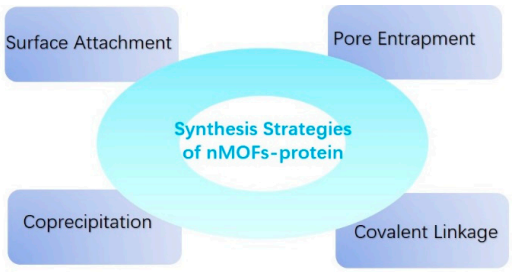

Figure 2. Synthesis strategies of nMOF–protein composites.

Pore entrapment is a vital strategy and has the following advantages in protein delivery by using MOFs with mesoporous cavities [34]: (1) Protein molecules can be physically adsorbed into the cavity instead of adhering to the MOF surface, which helps to reduce protein drug leakage and improve stability in vivo. Physical adsorption of the protein into the pore cavity provides an additional protective layer because substances that cause protein denaturation have to be able to diffuse through the pore channels to access the protein. (2) A high protein loading because of the enhanced pore volume and void space when compared with microporous MOFs. (3) The pore size of the frameworks can provide size selectivity for specific substrates, which is difficult to achieve with surface immobilized proteins (i.e., enzymes).

Proteins also can be covalently anchored on the surface of MOFs, which is typically achieved by the free amino groups on the proteins or MOF surface forming peptide bonds with carboxylate groups on the MOFs or enzyme surface, respectively [35]. The linkage is commonly conducted by carboxylate activating catalytic agents, such as N,N′-dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). In order to meet the requirements of in vivo application, by-products must be removed after the reaction. Amidation and subsequent treatment may lead to partial inactivation of proteins. Moreover, chemical derivatives that cannot be removed may cause serious adverse reactions. These are the shortcomings of surface chemical bonding when compared to other methods.

Coprecipitation is an important method for protein coating using MOFs. In this strategy, the most commonly used protein coating MOF material is a zeolitic imidazolate framework (ZIF) [36–38]. Proteins can be coated in situ during the synthetic process via producing defects in the ZIF crystals. The strategy allows for the inclusion of a guest protein molecule, whose size is larger than the pore openings of the MOFs, which acts as a protective coating. As such, the MOFs can prevent the leakage of protein molecules from the pores and also protect the proteins from being degraded by digestive enzymes.

3. nMOF–Protein Composites for Diseases Theranostics

3.1. nMOF–Enzymes

In human history, we have always learned from nature to solve complex problems, such as self-healing, solar energy harvesting, aerodynamics, and catalysis. Enzymes, nature's catalysts, are one class of biomacromolecules of interest from a biomimetic standpoint. Regulating the amount of enzymes in cells or tissues by biological methods is an important means for the treatment of some diseases due to their efficient catalytic ability [39–43]. In addition, we can also cure a disease by delivering enzymes to tissues or cells. However, the strategy is limited in application owing to the fact that most enzymes in organisms

are proteins, which are easily degraded by protease and easy to deactivate in vivo. On the other hand, the lack of long-term storage stability also limits their application in pharmacy. Immobilization can lead to increased enzyme handling, stability, and recoverability, which in turn reduce costs. As mentioned above, existing protein encapsulation methods have enormous challenges in application, and the immobilization of enzymes also has similar problems. Therefore, it is still urgent to develop enzyme carriers which can prevent enzyme degradation and denaturation.

MOFs offer many outstanding properties that have received a lot of attention in enzyme immobilization and delivery. The structures of MOFs are highly tunable, such as their surface area, and their pore size, volume, and shape can be optimized for the encapsulation and/or immobilization of specific enzymes [44,45]. Moreover, MOFs can be reasonably designed to be robust under harsh thermal, physiological, and chemical conditions, which is vital for immobilization and subsequent protection of enzymes under challenging catalytic conditions [46,47]. Lastly, different targeted ligands can be modified on the surface of MOFs, and this is of great significance for targeted therapy.

The therapeutic effect of traditional chemodynamic therapy (CDT) agents is severely limited by glutathione (GSH) overexpression and the weakly acidic pH in the tumor microenvironment (TME) [48,49]. To combat this challenge, Zhao et al. [50] developed a fusiform-like copper(II)-based tetrakis (4-carboxy phenyl) porphyrin (TCPP) nanoscale MOF (Figure 3). In order to construct the intelligent anti-tumor nMOFs, firstly, glucose oxidase (GOD) was linked to the surface of PCN-224(Cu) MOFs by an amide bond via EDC catalysis. The reaction product (PCN-224(Cu)-GOD) was then coated with MnO_2 after purification. Thus, PCN-224(Cu)-GOD@MnO_2 was obtained. The MnO_2 layer prevented the damage of GOD in PCN-224(Cu)-GOD@MnO_2 to normal cells and also increased the O_2 content by decomposition of MnO_2 in the TME. Meanwhile, the generated O_2 promoted the oxidizing reaction of Glu via the enzyme catalysis of conjugated GOD of PCN-224(Cu)-GOD, which elevated the H_2O_2 concentration in the tumor cells. Moreover, the depletion of GSH in the TME could reduce the Cu^{2+} in PCN-224(Cu) into Cu^{+}, and the combination of Cu^{+} and H_2O_2 generated ·OH due to a Fenton-like reaction. Additionally, $^1O_2$ could be produced by the Russell mechanism via the combination of Cu^{+}, O_2, and H_2O. In vivo fluorescence and MRI confirmed the rapid accumulation of PCN-224(Cu)-GOD@MnO_2 nMOFs in tumor sites. Cell and in vivo experiments showed the good biosafety and antitumor effect of the nMOFs via the combination of CDT and starvation, which was consistent with the hypothesis of the researchers.

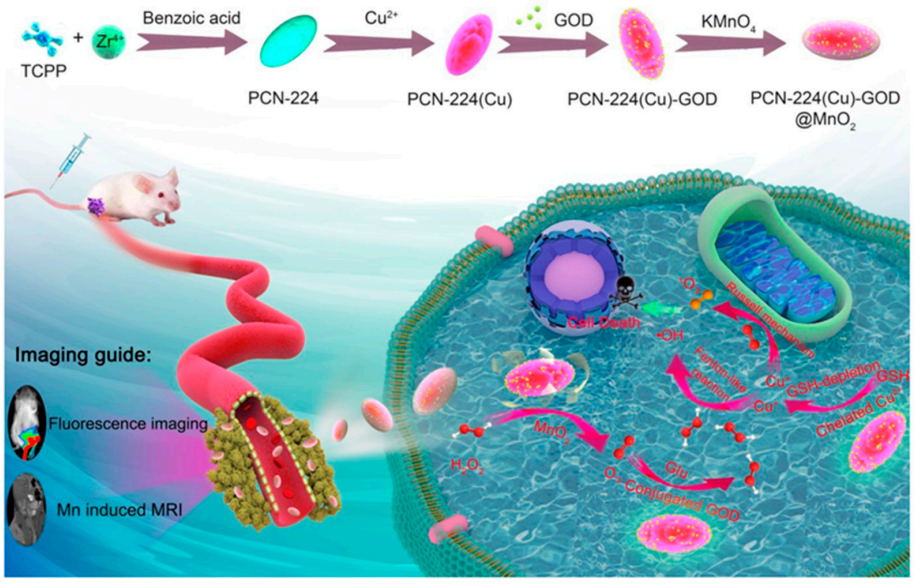

Figure 3. Schematic illustration of the main synthesis procedures and antitumor mechanism of PCN-224(Cu)-GOD@MnO2 nMOFs. Reproduced from [50], copyright 2020 American Chemical Society.

Multidrug resistance (MDR) is a primary reason for poor chemotherapy outcomes in both clinical and experimental trails [51]. In order to overcome MDR in chemotherapy, a similar study was conducted by Xu et al. [52]. Their group designed a Cu^{2+}-based metal–organic framework (COF) and employed it as a carrier to deliver glucose oxidase (GOx) and doxorubicin (Dox) (COF/GOx/Dox) to treat MDR lung cancers. They expected the GOx to catalyze glucose and produce H_2O_2. Meanwhile, the Cu^{2+} of COF/GOx/Dox can react with GSH and then be reduced into Cu^{+}, which would result in GSH depletion. Afterwards, the produced Cu^{+} and H_2O_2 generate ROS to damage the redox equilibrium of cancer cells via a Fenton reaction. They attempted to integrate starvation and chemokinetic therapy organically to overcome MDR. In the experiments, they firstly synthesized the COF via a facile one-pot approach. GOx and Dox were then encapsulated into COF via incubation. COF/GOx/Dox nanoparticles were obtained after centrifugal purification. They used the optimal charge ratios to finally obtain a loading content of 13.6% to Dox and 3.38% to GOx. The TEM images of COF/GOx/Dox revealed that the nanoparticles were spherical with a size of around 80 nm. The H_2O_2 generation capacity of COF/GOx/Dox was confirmed by incubating it with different concentrations of glucose; the concentration of H_2O_2 increased with the introduction of glucose in a positive dependent manner. The gluconic acid produced from the GOx-mediated glucose catalysis reduced the pH of the incubation solution and the results also demonstrated that the COF was an excellent carrier of GOx. The anticancer profile of the COF/GOx/Dox was explored and the results showed it had good anticancer properties in vitro and in vivo.

3.2. nMOF–Antibody

nMOFs have provided an effective platform for macromolecule loading, drug encapsulation, photodynamic therapy, and other biomedical applications. nMOFs are excellent radiosensitizers for radiotherapy–radiodynamic therapy (RT-RDT) [53,54]. In order to augment nMOF-mediated RT-RDT, Ni et al. [55] developed a kind of nMOF to co-deliver anti-CD47 antibodies (αCD47) and TLR-7 agonists (imiquimod, IMD) to modulate macrophages and orchestrate cancer immunotherapy (Figure 4). They synthesized IMD@Hf-DBP/αCD47 (DBP = 5,15-di(pbenzoato)porphyrin) via sequential Hf-DBP surface modification, IMD loading, and αCD47 adsorption. The addition of αCD47 to a PBS suspension of IMD@HfDBP with vortexing afforded IMD@Hf-DBP/αCD47 with 7.5 wt% αCD47 loading. Further studies indicated that IMD@Hf-DBP/αCD47 activates innate immunity to orchestrate adaptive immunity and effectively modulates the immunosuppressive tumor microenvironment when synergized with an anti-PD-L1 immune checkpoint inhibitor, leading to complete eradication of both primary and distant tumors in a bilateral colorectal tumor model. Herein, nMOFs provide a splendid platform to co-deliver multiple immunoadjuvants for macrophage therapy to induce systematic immune responses and excellent antitumor effects.

Cherkasov et al. [56] engineered antibody-directed nMOFs which were capable of specific targeting and killing of cancer cells in vitro. They firstly synthesized Fe_3O_4 nanoparticles with a general method. Then, the growth of the MIL-100 shell on the surface of the previously obtained Fe_3O_4 nanoparticles was initiated. Next, the nMOF (Fe_3O_4@MIL-100(Fe)) was capped with carboxymethyl-dextran and doxorubicin was loaded via incubation with Fe_3O_4@MIL-100. Anti-HER2/neu antibodies were conjugated with the nMOF via an amide reaction. They studied the specificity of immobilized antibodies for cell targeting via performing imaging flow cytometry on HER2/neu-positive BT-474 and SK-BR-3 cells, using CHO HER2/neu-negative cells as a negative control. The results demonstrated the trastuzumab-guided selective targeting and killing of HER2/neu-positive breast cancer cells in vitro. This approach expands the scope of nMOF applications and shows promise for the development of potent theranostic nanoagents.

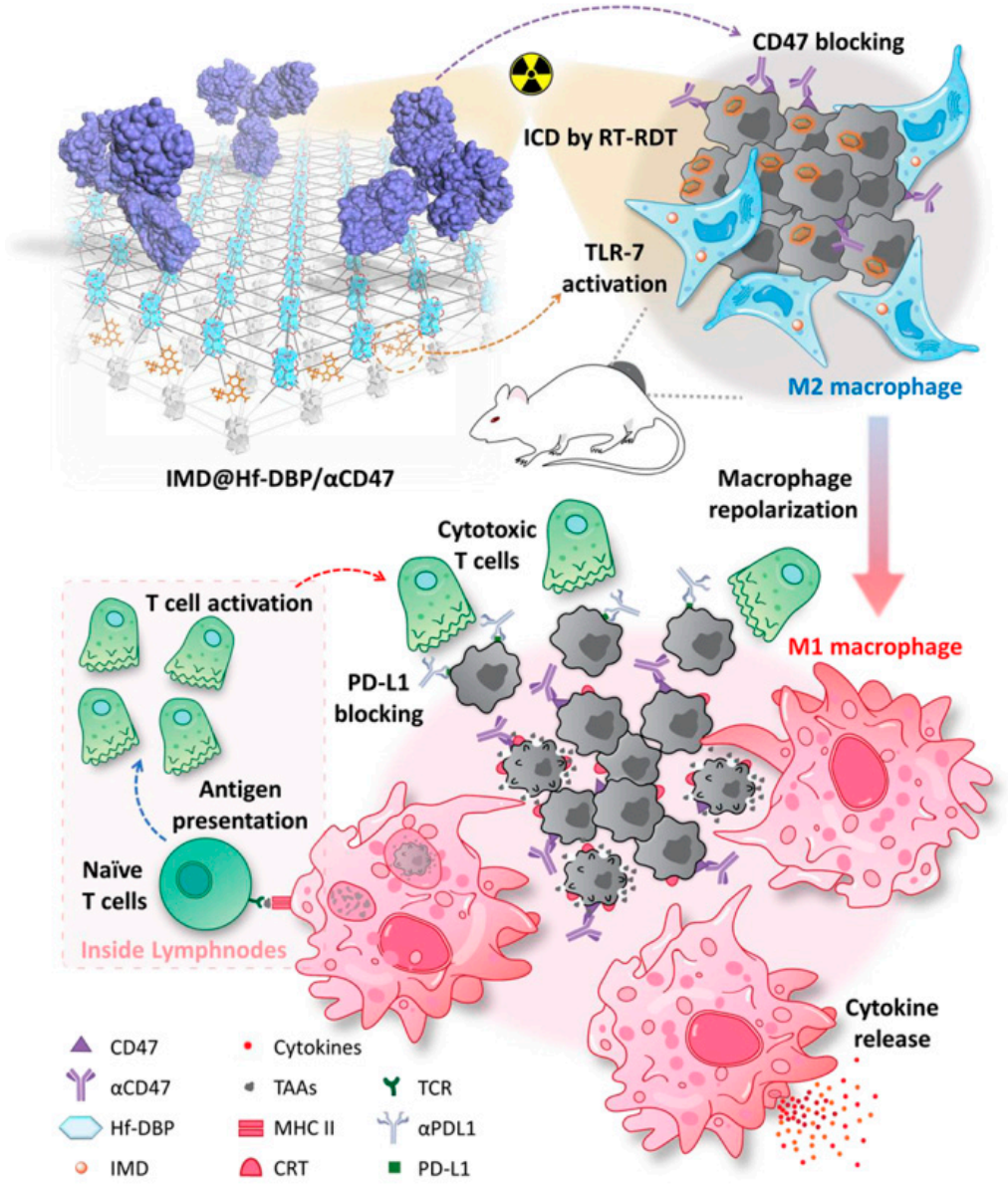

Figure 4. Schematic figure showing repolarization of M2 to M1 macrophages and promotion of phagocytosis by blocking the "don't eat me" signal on tumor cells by IMD@Hf-DBP/αCD47 plus X-ray radiation. This macrophage therapy synergized with αPDL1 CBI to systemically eradicate tumors. Reproduced from [56], copyright 2020 American Chemical Society.

3.3. nMOF–Insulin

Millions of people suffer from diabetes worldwide, and the number of diagnoses continues to increase annually. This metabolic disease leads to chronic organ injury, and in some cases, death. Diabetes induces excessive glucose contents in the bloodstream of affected individuals, which is the direct reason for many complications in diabetes [57]. Under normal physiological conditions, the pancreas regulates the concentration of glucose in blood plasma by producing insulin. At present, direct insulin injections remain the only effectual treatment for insulin-resistant patients, although several therapies have been designed to treat type I (T1DM) and type II (T2DM) diabetes mellitus [58]. The oral route can imitate the dynamics of endogenous insulin, which is concentrated in the liver via the portal vein. Additionally, insulin in the liver can facilitate the storage of glycogen and reduce blood glucose, while subcutaneous injection of insulin fails to satisfy these requirements [59–61]. Therefore, the development of an oral insulin preparation is

necessary to reduce the inconvenience and pain inflicted on patients due to routine insulin subcutaneous injections.

The instability of insulin caused by proteolytic enzymes in the gastrointestinal tract has hindered the development an oral insulin delivery agent [62]. In the gastrointestinal tract, the disulfide bonds in insulin are first cleaved by gastric acid, which induces its denaturation. Unfolded chains of the denatured insulin are then broken into short multipeptide segments by pepsin. All these factors lead to unsuccessful transport of insulin across the intestinal epithelium into the bloodstream. Thus, an acid-stable, highly porous material may protect insulin from degradation and exhibit a high insulin loading capacity. Chen et al. [63] published one of the earliest insulin encapsulation strategies via using an MOF (Figure 5). They immobilized insulin in a crystalline mesoporous MOF, NU-1000, and a high loading of ~40 wt% was obtained in only 30 min. They found the acid-stable MOF capsules could effectively protect insulin from degradation in the presence of stomach acid and the digestive enzyme, pepsin. Furthermore, the loaded insulin can be released from NU-1000 under simulated physiological conditions.

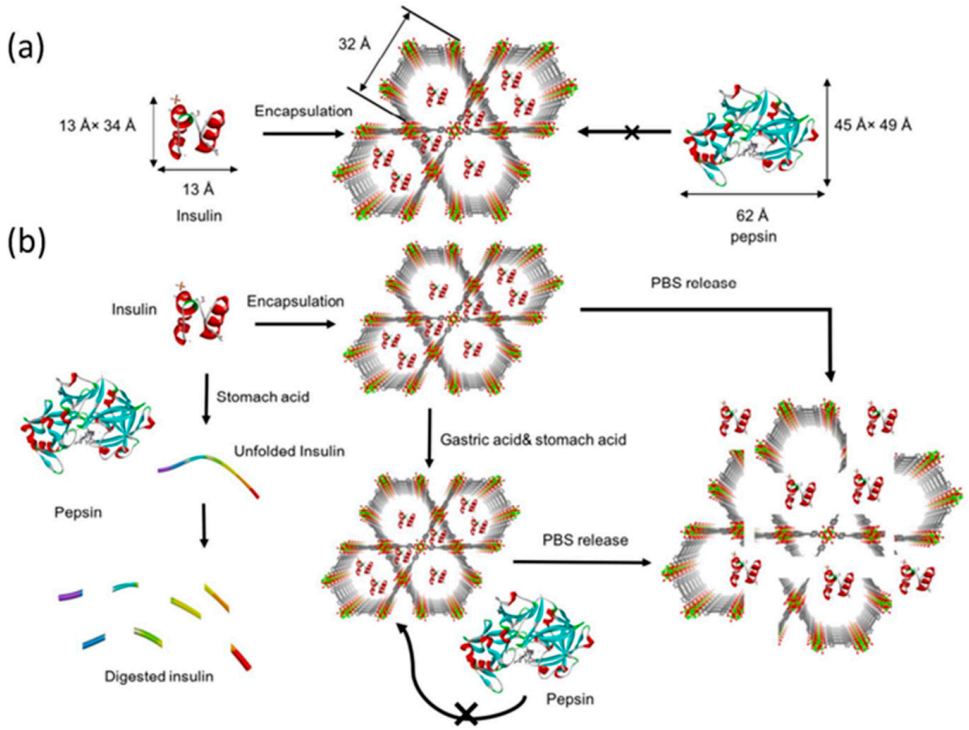

Figure 5. Schematic representation of (**a**) encapsulation of insulin in the mesopores of NU-1000 and exclusion of pepsin from the MOF framework and (**b**) exposure of free insulin and insulin@NU-1000 to stomach acid. Free insulin denatures in stomach acid and is digested by pepsin. Insulin@NU-1000 releases insulin when exposed to a PBS solution. Insulin@NU-1000 withstands exposure to gastric acid and stomach acid and releases encapsulated insulin in PBS. Reproduced from [63], copyright 2018 American Chemical Society.

In order to overcome barriers such as insulin degradation in the gastrointestinal environment and low permeation across the intestinal epithelium, Zhou et al. [64] developed a novel biodegradable nanocomposite microsphere embedded with nMOFs. Their team first synthesized an iron-based nMOF (MIL-100) as a carrier with an insulin loading capacity of 35%. To promote the insulin permeation across the intestinal epithelium, the insulin-loaded MIL-100 nanoparticles were then modified with sodium dodecyl sulfate (Ins@MIL100/SDS). Lastly, Ins@MIL100/SDS nanoparticles were embedded into a biodegradable microsphere to construct the nanocomposite delivery system (Ins@MIL100/SDS@MS) to improve the resistance to the gastric acid environment. They investigated the release profiles of the

insulin-loaded nMOFs at physiologically relevant pHs via fluorescence methods. The results demonstrated that the microspheres could release insulin-loaded nMOFs in simulated intestinal fluid and effectively protect the nMOFs from rapid degradation under acidic conditions. Intestinal absorption of the insulin was further detected, and they found increased intestinal absorption of the insulin in the oral administration of Ins@MIL100/SDS@MS to BALB/c nude mice compared to the oral administration of free insulin or Ins@MIL100/SDS. Apparently increased plasma insulin levels were observed for over 6 h after oral administration of Ins@MIL100/SDS@MS to diabetic rats, resulting in a remarkably enhanced effect in lowering blood glucose levels with a relative pharmacological availability of 7.8%. The study shows the great application prospect of MOFs in oral protein delivery.

4. Biosafety, Biodistribution, and Excretion

4.1. Biosafety

Biosafety issues hinder the biomedical application of many nanomaterials and have attracted particular attention. Although many nanomaterials, such as graphene oxides and gold nanoparticles, have showed superior properties in drug delivery, their potential long-term cytotoxicity brings a lot of challenges to clinical translation [65]. MOFs are formed from metal ions and organic ligands through simple coordination, which makes the synthesis of MOFs easier compared to other nanomaterials. Many toxic substances derived from the complex synthesis process, such as organic solvents and toxic reaction by-products, are avoided in the synthesis of MOFs [66]. Therefore, MOF–protein composites have certain advantages in clinical application. On the other hand, metal ions (e.g., Fe^{3+}, Mn^{2+}, and Zn^{2+}) are important nutrient elements and show minimal acute toxicity and long-term toxicity. For instance, Singamaneni et al. [31] reported a facile approach using a nanoporous material, zeolitic imidazolate framework-8 (ZIF-8), as a carrier for preserving the prototypic protein therapeutic insulin. In order to evaluate the biocompatibility of insulin-embedded ZIF-8, they sacrificed mice treated with ZIF-8-encapsulated insulin and PBS 5 d after insulin administration for histological analysis. The hematoxylin and eosin (HE)-stained images of major organs in the two groups showed similar structures. No apparent histopathological abnormalities or lesions were observed in the heart, liver, spleen, lung, or kidney. In addition, there was no weight loss in either group after 5 d od administration. The results demonstrated the excellent biocompatibility of insulin-embedded ZIF-8. Considering repeated drug administration, as is the case with insulin, the feasibility of removing dissolved ZIF-8 residues was tested. The ZIF-8-encapsulated insulin was first released by adding EDTA and then filtered to remove any ZIF-8 byproduct by centrifugation through a 3 kDa filter. After washing three times, HPLC mass spectrometry analyses showed that more than 99% of 2-methylimidazole can be removed. The purification step mitigates the toxicity concern and the results further proved ZIF-8 as a safe carrier of insulin.

Zhang's team [52] reported a glucose-oxidase-loaded, Cu^{2+}-based metal–organic framework (COF/GOx/Dox) for glutathione depletion/reactive oxygen species elevation enhanced chemotherapy in 2021. The effective anticancer performance of COF/GOx/Dox was proven in vivo. They conducted a biosafety assay to confirm the biocompatibility of COF/GOx/Dox. The liver function of the mice was evaluated via testing alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels after treatment. The liver function of the mice was also assessed via testing creatinine (CREA), uric acid (UA), and blood urea nitrogen (BUN). No significant differences were found between control and COF/GOx/Dox groups, which suggested the high biocompatibility of the formulation. HE staining of major organs also revealed similar results. Although most of the data reported by previous researchers confirm the biocompatibility of MOF–protein composites, it is still difficult to conclude the biosafety of MOF–protein composites without strict toxicology research performed in a standard GLP lab.

4.2. Biodistribution

In order to obtain better therapeutic effects and reduce nMOF–protein composite aggregation in non-focal sites, researchers have designed many nMOF–protein composites with tissue selectivity, especially for cancer theranostics. On the one hand, nano MOF–protein composites can be selectively enriched in tumors through the EPR effect. On the other hand, targeted ligand modification on the surface endows nano MOF–protein composites with active targeting tumor capabilities. Biodistribution is an important parameter to assess the therapeutic index and targeted effects of nanosystems. Yang's team [50] designed fusiform-like copper(II)-based MOFs (PCN-224(Cu)-GOD@MnO_2) for synergetic cancer therapy and achieved remarkable antitumor efficacy in U14 tumor-bearing Kunming mice. They examined the biodistribution of tetrakis (4-carboxyphenyl) porphyrin-labeled PCN-224(Cu)-GOD@MnO_2 nMOFs via an in vivo fluorescence imaging system using cervical cancer cell (U14) tumor-bearing Kunming mice. Gradual accumulation of the nMOFs in tumor areas was found, reaching a maximum after 4 h of intravenous injection. They speculated that the enhanced permeability and retention (EPR) effect induced the accumulation of nMOFs. Such a passive targeting effect has also been observed for many other nMOF-based drug delivery systems. The effect is highly influenced by particle size and cancer type. However, a recent report has questioned the EPR effect due to its low tumor targeting efficiency. Additionally, the fluorescence signal continually decayed in the tumor, and the regions of the liver and kidney emitted strong fluorescence with prolonged time, which suggested the nanocomposites are mainly metabolized by the liver and kidneys. This phenomenon is also common in other nano drugs, which may cause hepatorenal toxicity. These studies point the way for later research into nMOFs clinical application and have spurred more elegant designs to solve the nMOF-based protein delivery problem.

Chen's team [64] reported a nanocomposite vehicle based on MOF nanoparticle-incorporated biodegradable microspheres (Ins@MIL100/SDS@MS) for enhanced oral insulin delivery. They detected the insulin distribution via a Maestro In Vivo Imaging System and CLSM using RhoB-Ins as model insulin after oral administration of the nanocomposites. Intestinal villi were sectioned and visualized at 4 h post-administration to investigate the intestinal absorption of insulin. The intestinal villi of the mice orally administered with Ins@MIL100/SDS@MS showed a higher fluorescence intensity of RhoB-Ins than those treated with free insulin or Ins@MIL100/SDS nanoparticles, which demonstrated that the microspheres containing Ins@MIL100/SDS NPs could effectively promote the transportation of the insulin-loaded systems into the intestine and improve their subsequent permeation across the mucus and epithelium. The biodistribution of RhoB-Ins fluorescence varied in different organs. Strong fluorescence signals were observed in the liver and kidneys, while those in the heart, spleen, and lungs were relatively weaker. The stronger insulin fluorescence in the liver indicated that insulin released from Ins@MIL100/SDS@MS may initially circulate through the portal veins to the liver, followed by entry into cardiac tissue. Insulin leads to glucose storage as glycogen in the liver, which is vital for glucose metabolism in type 1 diabetic patients. Thus, oral insulin delivery systems based on MOF-NP-incorporated microspheres show great potential for lowering the levels of blood glucose post-meal. This study suggests that we should pay more attention to the physiological characteristics of the gastrointestinal tract when designing oral insulin preparations, which are different from intravenous preparations. The above two studies also suggest that the design of MOF-based protein delivery systems needs to pay more attention to the purpose of treatment of different diseases. As the biodistribution results show above, diabetics may benefit from the aggregation of nMOF-based insulin delivery systems in the liver, but this is a disadvantage in nMOF-based antitumor drug delivery due to possible hepatotoxicity.

4.3. Excretion

Excretion is a vital index to evaluate the biocompatibility and biosafety of NPs. Ideally, nMOF–protein composites can be degraded and release drug molecules at the target site, and the degraded MOF materials can then be excreted by the liver or kidney. Theoreti-

cally, the clearance of nMOF-based protein delivery systems can be directly studied via measuring metal concentrations [67]. To achieve high biocompatibility and biosafety, the nMOFs should be completely cleared from the body within a reasonable period of time. Typically, renal excretion has an advantage over hepatic clearance due to faster elimination. The nMOFs should have a suitable size that can pass through the glomerular filtration membrane to improve the renal clearance rate. For example, Wang et al. [68] developed renal excretory Fe(III)–GA networks (namely Fe-CPNDs) with a 5.3 nm diameter, which could be rapidly excreted by the kidney in the body of tumor-bearing mice after tail vein injection, with a blood elimination half-life ($t_{1/2\beta}$) of 5.5 ± 1.9 h. Meanwhile, notable tumor suppression was observed after photothermal therapy under 808 nm NIR laser irradiation.

However, although such ultra-small-sized nanoparticles (<6 nm) benefit from rapid renal excretion, a weakened EPR effect for tumor accumulation was reported [69]. In order to balance the therapeutic requirements and the biosafety concerns for clearance, Chen et al. [70] designed a multifunctional MOF-based nanoplatform (FeAP-NPs) synthesized by using ACN, Fe^{3+}, and PLG-g-mPEG, which had a particle size of 65 nm for selective enrichment in MCF-7-bearing nude mice. In order to reduce accumulation of the nanoplatform in the liver because of high reticuloendothelial system (RES) retention, deferoxamine mesylate (DFO, a strong chelator of iron) was used to dynamically disassemble FeAP-NPs in vivo. The results showed the Fe content was markedly increased in the kidney, while it was significantly decreased in the liver upon injection of DFO, which switched the NP elimination pathway from hepatic excretion to renal excretion. The study provides a general solution to enhance the in vivo clearance of nMOF-based protein delivery systems to combat their potential toxicity. In addition to mechanical barriers, electrical barriers of glomerular filtration membranes also affect the excretion of nMOFs [71,72]. In general, neutral and positively charged particles are more likely to pass through the glomerular filtration membrane than negatively charged particles due to the intrinsic electronegativity of the membrane. Therefore, we believe that developing charge transformation nanosystems is another effective strategy to increase renal clearance to enhance the biosafety of nMOF-based protein delivery systems in the future.

5. Conclusions and Perspectives

In recent years, nMOFs have been recognized as a class of promising nanomaterials for the delivery of functional proteins due to their abundant porous framework architectures, allowing not only high protein loading but also improving the stability of the encapsulated proteins [70]. nMOF-based protein composites show great potential in the clinical treatment of different diseases. We summarized some advantages of the MOFs in protein delivery and general methods for protein encapsulation. The applications of nMOF-based protein composites in treatment of different diseases were reviewed. The biosafety, biodistribution, and excretion of nMOF–protein composites were also reviewed. Published works have demonstrated that MOFs can prevent protein degradation and preserve the bioactivity of proteins to achieve drug delivery of protein therapeutics in vivo.

Although the relevant reports showed the advantages of nMOFs for enzyme, insulin, and antibody delivery in cancer and diabetes therapies, we should realize that this area of research is still in its preliminary stages, and some challenges and deficiencies in their application remain to be solved. For instance, proteins loaded via surface attachment may suffer significant leaching under physiological conditions due to the weak noncovalent interactions between proteins and MOFs, and this method may not protect proteins from degradation due to direct exposure to the environment [72,73]. Thus, surface attachment encapsulation via MOFs is not suitable for the delivery of oral protein drugs such as insulin, which is easily degraded by digestive enzymes in the digestive tract. In addition, targeted ligands are usually modified on the surface of MOF carriers to achieve targeted therapy, which inevitably increases the complexity of the synthesis of nMOF–protein composites. This increases the possibility of protein molecules being destroyed, which may cause serious side effects in clinical applications [22]. This suggests that we should simplify the

synthesis process of nMOF–protein composites. Last but not least, in vivo studies on the degradation mechanism, stability, and side effects of nMOF–protein composites have not been systematically carried out, and the practical therapeutic effects need to be evaluated comprehensively. Olesya et al. [73] found a strong correlation between the amount of escaped cargo from ZIF-8 and the total concentration of amino acids in the environment, which reminds us that some nMOFs may not be stable in plasma. Finally, we believe that increasingly more applications of nMOF–protein composites for disease diagnosis will be discovered in further studies.

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