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Biocompatibility and Biodegradability of Metal Organic Frameworks for Biomedical Applications

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1. Abstract

Metal organic frameworks (MOFs) are a unique class of smart hybrid materials that have recently attracted significant interest for catalysis, separation and biomedical applications. Different strategies have been developed to overcome the limitations of MOFs for bio-applications in order to produce a system with high biocompatibility and biodegradability. In this review, we outline the chemical and physical factors that dictate the biocompatibility and biodegradability characteristics of MOFs including the nature of the metal ions and organic ligands, size, surface properties and colloidal stability. This review includes the in-vitro biodegradation and in-vivo biodistribution studies of MOFs to better understand their pharmacokinetics, organs toxicity and immune response. Such studies can guide the design of future bio-friendly systems that bring us closer to safely translate these platforms to the pharmaceutical consumer market.

2. Introduction

Metal organic frameworks (MOFs) are composed of multiple metal ions or metal clusters and organic bridging ligands and are considered as the prime members of inorganic-organic hybrid
MOFs are widely studied for their tunable topologies and functionalities. However, the main incentive leading the exploration of MOFs is their tailorable composition and high and uniform porosity which makes them preferable for various useful applications such as catalysis, gas storage and separation. Modulation of the MOF size into a nano-MOF (nMOF) and the organic/inorganic nature opened the door for unlimited biological applications. Moreover, functionalization of the organic linker or strut during or after synthesis drastically enhanced the physiological properties of these nMOFs. For instance, it can decrease cytotoxicity, improve colloidal stability and promote suitable degradation rates and efficient cellular uptake. Compared to conventional nanocarriers (like inorganic zeolites and silica nanomaterials, and organic nanocarriers such as lipids and polymers), nMOFs possess the right properties that make them promising candidates for biological applications. First, nMOFs can be synthesized using biocompatible components with a tolerable pharmacokinetic profile. Second, large surface area and small pore volume can guarantee a high loading capacity and great biopreservation properties for optimal drug delivery system. Wolfgang and co-worker also studied in detail the benefit of using MOFs for drug delivery in comparison to mesoporous silica and dendrimers. Similarly, Falcaro group compared the protective property of ZIF to the inorganic nanoparticles; CaCO₃ and mesoporous silica. They encapsulated HRP enzyme into the three systems and monitored the activity of the encapsulated enzyme after exposing them to harsh conditions. ZIF-8 showed superior protective properties and retained most of the enzyme activity. Also, it showed a controlled release of the cargo under slightly acidic condition which makes MOFs ideal for drug delivery. Moreover, MOFs need to be stable enough to deliver the molecule of interest to the targeted tissue but also be degraded and readily eliminated from the body without endogenous accumulation. The presence of labile metal ligand bonds endows nMOFs with rapid degradation to release the loaded material. Therefore, nMOFs are widely investigated as delivery vehicle for imaging, diagnosis, treatment of diseases and bio-sensing (Scheme 1).

Many reviews were published on Bio-MOFs or biomimetic MOFs, which included the various applications of these platforms in nanomedicine. In this review, we focus on the different factors that affect or dictate the biocompatibility and biodegradability of MOFs. The biocompatibility depends on the nature of the coordination metal and the organic linker in addition to the overall physical properties including size, shape and surface charge. As for biodegradability, we reviewed the available in-vivo and in-vitro data to conclude the best profiles that have been reported so far. Moreover, we comment on the future directions of these intriguing class of smart materials to speed their translation into actual pharmaceutical and biomedical applications.

3. Biocompatibility of MOFs:

3.1. Nature of the building blocks

The biocompatibility of MOFs precursors is very essential for the overall system to fall within the bioavailability range. The toxicity of the metals and the ligands has been assessed compared to the assembled MOFs where a direct correlation can be deduced. MOFs toxicity depends on several other factors such as the kinetics of degradation, bio-distribution, accumulation in
tissues and organs, excretion from the body, applications and balance between risks and benefits and so on. MOFs that are used for biomedical applications usually consist of metals that are essential to the body such as iron, zinc and magnesium.

3.1.1. Metal ions

The most compatible cations for preparation of biocompatible MOF are simply selected on the basis of lethal dose and daily dose of metal. Lethal dose is considered the median lethal dose (LD50), the amount of compound that kills half the members of a given population after a specific duration. The experimental results of the oral lethal dose to the rats reveal that Ca, Mg, Zn, Fe, Ti, and Zr are appropriate metals for the construction of biocompatible MOFs. However, these doses vary with chemical formulation (counter anion and oxidation state). Another concern is the daily dose as few metals are required by humans in mg/day amounts and recognized as essential trace elements. Metals such as Zr and Ti are poorly absorbed by the body and not considered as toxic for specific applications, such as their use in cosmetics (LD50 > 25 g/kg).

3.1.2. Ligands

A wide range of organic ligands are available for the construction of biocompatible MOFs and could be classified into exogenous and endogenous ligands. Exogenous ligands are synthetic linkers that are not naturally found in the body. Therefore, it is necessary for them to be excreted or metabolized after the in-vivo application. This category includes polycarboxylates, phosphonates, sulfonates, imidazolates, amines, pyridyl and phenolates. Recent biocompatibility data revealed that few polycarboxylate (terephthalic, trimesic, 2,6 napthalenedicarboxylic acid) and imidazolate linkers are not very toxic due to their high polarity and ease of removal under physiological conditions (Scheme 2). Functionalization of exogenous linkers with apolar and polar functional groups such as amino, nitro, chloro, bromo, carboxylate, methyl, perfluoro etc. can tune their ADME (absorption distribution metabolism excretion) behavior. The presence of functional groups not only modulates the host guest interactions but also influences the flexibility of the framework for better absorption and delivery of the cargo biomolecules. There are various examples of functionalized MOFs such as MIL-53(Fe), MIL-88B(Fe), UiO-66(Zr), MIL-125(Ti) decorated with polycarboxylates linkers. In addition, organically modified porous Zn imidazolate solids also can be included in this series.

On the other hand, ligands or linkers that are naturally found in the body such as amino acids, peptides, proteins, nucleobases, carbohydrates and porphyrins are referred to as endogenous linkers. For bio-applications, the use of endogenous molecules in MOFs can reduce the risk of adverse effects as it could be absorbed safely by the body. A notable number of MOFs containing endogenous linkers has been reported so far. The endogenous molecules that have been used as linkers for MOF preparation are aspartate, adenine, fumarate, muconate and cyclodextrin (Scheme 2). However only few of them have been utilized for bio-applications due to the stability and porosity limitations.
3.2. Physiological Properties

3.2.1. Size

The size of nMOFs is an important factor that impacts the bio-distribution, circulatory lifetime in-vivo and targeting abilities. Studies in this direction suggest that the optimal size would be < 200 nm.\textsuperscript{63-66} Controlling the nMOFs size attracted much attention and included various methods such as hydrothermal,\textsuperscript{67} hydrosolvothermal,\textsuperscript{68} reverse-phase microemulsions,\textsuperscript{69} sonochemical,\textsuperscript{70} and microwave assisted synthesis\textsuperscript{71} in addition to the conventional techniques of using growth inhibitors for delaying the nucleation process,\textsuperscript{68} nano template for confining the space\textsuperscript{72} and tuning the ratio of surfactants.\textsuperscript{73} However the exact relationship between the size of nMOFs and their impact on the body is still uncertain.\textsuperscript{74} A size study of the Zr-nMOFs ranging between 30-190 nm was conducted by Zhou and co-workers on cellular uptake by HeLa cells.\textsuperscript{63} The result reveals MOF PCN-224 cell uptake was size dependent in the order of 90 nm > 60 nm > 30 nm > 140 nm > 190 nm. Liu and co-workers studied the size effect of drug loaded MOFs (DOX@AZIF-8) on the in-\textit{vivo} bio-distribution, cellular uptake and killing effect on tumor cells. They found that the 60 nm DOX@AZIF-8 showed prolonged blood circulation and higher tumor uptake compare to the larger size of DOX@AZIF-8 (Figure 1).\textsuperscript{65} Zhu’s group conducted a detailed research to investigate and compare the biosafety of the micron- and nanoscale Mg-MOF74 (m/n-Mg-MOF74) particles.\textsuperscript{66} The study reveals that both micron/nanoscale Mg-MOF74 showed good biocompatibility and n-Mg-MOF74 showed a wider range of safe concentrations compared to the micron-sized particles. Furthermore, the suitable dose of n-Mg-MOF74 achieved early osteogenic promotion and angiogenic stimulation effects suggested nanoscale Mg-MOF74 as a better option over the micron-sized particles. It could be concluded that a size of up to 200 nm has unique physiological properties.\textsuperscript{64} The size of the particles dictate their velocity and diffusion in the body as well influence their response whether to be internalized in tumor cells or cleared from the body by macrophages or renal system to protect the body from their side effects.\textsuperscript{75}

3.2.2. Stability

The nMOFs are constructed with different kinds of metal ions and organic linkers and their structural stability is a potential concern for bio-applications. The structural stability of MOFs in aqueous media is influenced by many factors such as metal–ligand bond strength, basicity of the ligand, coordination number and the oxidation state of the metal center and framework dimensionality.\textsuperscript{76} The stability of MOFs can be improved by introducing the catenation or interpenetration into framework and employing a linker of higher pK\textsubscript{a} value.\textsuperscript{77} For bio-applications, a certain extent of chemical stability is required for approaching the target sites and upon changing the pH and composition of body fluid, degradability of the framework becomes necessary to release the cargo. MOF-5 and MOF-177, which are composed of zinc and poly-carboxylates ions, do not show stability in water and decompose rapidly.\textsuperscript{78, 79} MOF-5 was observed to be very moisture sensitive due to its relatively weak metal–oxygen coordination bonds. On the other hand, some MOFs have been reported to show stability under hydrothermal and humid conditions. MOFs based on Al-carboxylate such as Al-MIL-53, were stable in 50% humidity for 30 days.\textsuperscript{80} Ni-CPO-
27 MOFs were stable in bovine serum at 37 °C for 4 days.\textsuperscript{81} MOF PCN-222 showed exceptional hydrothermal stability owing to the assembled Zr\(_6\) cluster, which is considered as one of the most stable secondary building units (SBUs).\textsuperscript{82} Gassensmith and group studied the stability of ZIF-8 in common laboratory buffers, cell media, and serum and showed surface chemistry changes affecting the interpretation of cellular uptake and cargo release (Figure 2).\textsuperscript{83} Other MOFs, such as MIL-100 (Fe)\textsuperscript{84} and UiO-66 (Zr)\textsuperscript{82} are stable in water. However, UiO-66 (Zr) and MIL-100 (Fe) degrade within a few hours after being dispersed in a phosphate buffer.\textsuperscript{85} MIL- 88A (Fe) full degradation occurred in phosphate buffer after several days.\textsuperscript{86} The MOF stability in body fluid cannot be predicted by their stability in water and further studies are needed in full culture media or simulated body fluids for a better understanding of the degradation mechanism.

3.2.3. Surface

To achieve the bio-adhesive and targeting properties, an appropriate design of the nMOFs system becomes necessary. Along with size and stability, other biophysical properties of nMOFs such as surface hydrophilicity and the nature and density of the ligands at their surface are also important as they regulate the interaction of nMOFs with physiological media components like proteins, lipids, ions etc. The outer surface of nMOFs can be modified to tune the stability and the ability of nMOFs to circulate in the bloodstream till the successful targeted delivery. The most common way discovered to modify the surface properties of nMOFs is post-synthetic modification, which includes coating of a functional layer on the surface. As a functional coating material, organic polymers, silica shells and lipid bilayers have been used and reported as nMOFs surface modifiers. Silica, as a coating material for nMOFs, increases the biocompatibility by improving water dispersibility and reducing the decomposition of nMOFs. H.-L. Zhu reported a dual-responsive ZIF-8 nanoscale drug delivery system by functionalizing the organosilica shell with redox-responsive disulfide bridges in its framework.\textsuperscript{12} As a disulfide bond is relatively stable in plasma and breaks down in the presence of high concentration of glutathione (GSH), a controlled degradation was also established (Figure 3). The nano-carriers maintain their stability under physiological conditions and after internalization into cancer cells, the disulfide linkages are cleaved by the endogenous GSH triggering the release of the encapsulated anti-tumor drug (DOX). Moreover, cell internalization, drug release, cytotoxicity, subcellular localization, and antitumor activity \textit{in-vivo} experiments showed that ZDOS NPs exhibited negligible hemolytic potential and significantly enhanced anticancer efficiencies compared to free DOX.

Organic polymers are another representative coating material. Polyethylene glycol (PEG), polyvinylpyrrolidone, polyacrylic acid (PAA) and hyaluronic acid (HA) have been most frequently used as surface modifiers. PEG is amphiphilic in nature and its hydrogen bonding capability enhances the hydrophilicity of nMOFs. Furthermore, the PEGylation of nMOFs elongates the circulation time \textit{in-vivo} by preventing the aggregation, decreasing the opsonization by blood proteins and uptake by the macrophages of the immune system. Forgan and co-workers modified the surface of UiO-66 nanoparticles with PEG via a mild conjugation reaction.\textsuperscript{11} PEGylation of UiO-66 made them more stable, dispersed, and generally more favored for cellular uptake. Zr-MOFs degrade very fast in a phosphate medium and so PEGylation of UiO-66 improved their stability towards phosphate induced degradation and dispersion in aqueous media. Hyaluronic acid
(HA) is a hydrophilic biopolymer that can easily bind to the cancer cells by HA-receptor mediated interaction between CD44 or RHAMM receptors that are overexpressed on the cancer cell surface.\(^8\) HA is considered as an ideal coating material for surface functionalization of nMOFs due to its ability to overcome the poor bio-distribution, lack of tumor-targeting and serious side effects. Yang and coworkers prepared HA modified nMOFs through supramolecular and coordination interactions of the three building blocks, which showed improved stability in physiological fluids.\(^8\)

Lipid bilayer coating has been also applied to nMOFs, yielding a nano-carrier that can efficiently store dye molecules inside the porous scaffold of the MOF. The lipid bilayer coated MIL-100(Fe) nanoparticles showed incremental increase in the colloidal stability and efficient uptake by the cancer cells.\(^8\) However, no intracellular release was shown for these nanoparticles. H. Engelke and group used the exosome as a coating material for MIL-88A.\(^9\) Exosome are extracellular vesicles present in the body fluid and coating with exosome provide additional advantage compared to artificial lipid coating. The exosome coated MOFs can effectively shield the carriers from the immune system for longer circulation time.

A laser or light responsive pharmaceutical delivery nanoparticles were later on designed by an emulsion approach using the redox responsive selenium (Se) substituted polymer as shell and photosensitive porphyrin zirconium metal–organic frameworks (PCN-224 MOF) as core.\(^9\) The poly(DH-Se/PEG/PPG urethane)@MOF nanoparticles were loaded with chemotherapeutic DOX. A combination of chemotherapy and photodynamic therapy, upon irradiation with laser light, causes the cleavage of poly(DH-Se/PEG/PPG urethane) polymer chain and the release of the encapsulated DOX.

An advanced surface modification based on cancer cell membrane coating technology has been developed by our group to enhance the uptake of nanoparticles, which inherit the antigenic properties of the source cells and can be employed for cancer therapy and vaccination. The zeolitic imidazolate frameworks encapsulating CRISPR/Cas9 (CC-ZIFs) are coated with a cancer cell membrane to enhance cell-specific gene editing selectivity for tumor cells (Figure 4).\(^3\)

4. Biodegradability of MOFs

4.1. In-vitro

Degradation of MOFs in terms of biosafety needs to be studied before using them as carriers in bio-applications. Generally, degradation studies are carried out in water, phosphate buffer (PBS) and cell culture media at 37 °C at different pH. However, delivery to the target cells and the impact of carrier degradation is better analyzed in-vitro using different biological fluids considering the administration route like simulated intestinal fluid (SIF) for the oral route and the simulated body fluid (SBF) for the parenteral route. The degradation of MOFs is influenced by many factors such as metal-ligand strength and environmental condition as discussed in the stability section. The pH of the body fluid is the main factor affects the degradation of MOFs and release the cargo which makes MOFs ideal for drug delivery. Our group have reported the release of CRISPR/Cas9 (CC)
at pH 5, 6 and 7 using ZIF-8 as carrier. ZIF-8 showed great stability at pH 7 and degraded at pH 6 and lower. Also, ZIF-8 showed an enhanced endosomal escape which was promoted by the protonated imidazole moieties (Figure 5). Before using MOFs for biomedical applications, it is essential to test the biocompatibility of the building blocks of MOFs as they might have a toxic effect upon the degradation of nMOFs inside the cells. Also, it is important to test the biocompatibility of MOFs with various cell lines as they might show different effects. The in-vitro biocompatibility of MIL-100 nMOFs based on three different metal systems (Fe, Al, Cr) was analyzed. The cytotoxicity test was performed using four epithelial cell lines: Lung (A549 and Calu-3) and hepatic (HepG2 and Hep3B) considering pulmonary, ingestion or intravenous exposure modes. The MIL-100 (Fe, Al, Cr) NPs did not induce in-vitro cell toxicity even after high dosages in A549 and calu-3 (lung) and HepG2 (liver) cell lines. Only MIL-100(Fe) toxicity was noted in the Hep3B cell line. Hoop et al. checked the biocompatibility of ZIF-8 with respect to six different cell lines, representing different body parts (kidney, skin, breast, blood, bones, and connective tissue). The study revealed that ZIF-8 showed a cytotoxicity above the threshold value of 30µg ml$^{-1}$ due to the effect of the released zinc ions (Zn$^{2+}$) on the mitochondrial ROS production. As mentioned in the physiological property section, the size plays a big role in the biocompatibility and degradability of MOFs. The in-vitro cytotoxicity of micron/nanoscale Mg-MOF74 was evaluated against the HeLa cells with concentration ranging from 50–2000 µg mL$^{-1}$. Both micron and nanoscale Mg-MOF74 showed no significant toxicity to cells below 200 µg mL$^{-1}$. However, µ-Mg-MOF74, cytotoxicity increased above the doses of 500 µg mL$^{-1}$ but for n-Mg-MOF74 the cytotoxicity increased above 1000 µg mL$^{-1}$. The in-vitro cytotoxicity of CAU-7, a biocompatible bismuth-based MOF, was also measured on HeLa cells in the range of 0-1.5 mg mL$^{-1}$. The MTS viability values for CAU-7 MOF showed biocompatibility in the range of the used concentrations and no significant difference compared to the untreated cells was observed. The cytotoxicity of IRMOF 1-3 was tested on HepG2 cells with respect to the five concentrations of IRMOFs (5, 10, 15, 20, 25, 30 and 35 mg mL$^{-1}$). The significant in-vitro cytotoxicity was not observed for different concentrations of IRMOFs (5, 10, 15, 20, 25, 30 and 35 mg mL$^{-1}$) and confirmed the biological safety of IRMOFs. Our group studied the in-vitro uptake of cancer cell membrane coated ZIF-8 with MCF-7, HeLa, HDFn, and aTC cell lines. The cytotoxicity was observed in the concentration range of 50-150 µg mL$^{-1}$ with high uptake for cancerous cell lines and minimum toxicity for all the cell lines tested. The in-vitro study is a fast, low cost and effective method for analyzing the behavior of nMOFs and their potential toxicity. However, it does not provide realistic data on MOF’s physiological interactions in the human body.

4.2. In-vivo

In the last few years, more in-vivo studies have been performed using mice model. Zn, Fe and Zr based MOFs are among the most reported MOFs for biomedical applications. Assessing the biocompatibility of MOFs in-vivo is very essential as it gives a more defined picture of the toxicity of MOFs in the biological system. For in-vivo biocompatibility, many parameters need to be considered such as bio-distribution, pharmacokinetic, organs toxicity and immune response. In
order to have a robust system with minimum side effects, it is crucial to study these parameters. The first parameter is pharmacokinetics, which helps to understand the fate of MOFs once they enter the body until they are excreted (recognition, metabolism, and clearance). Bio-distribution, which is the pattern of MOFs accumulation in different organs in the body, is also crucial for the overall assessment of the delivery system. MOFs usually tend to accumulate in liver\textsuperscript{94, 95} and kidney\textsuperscript{96}, as they are the main organs responsible for NPs clearance. Few have reported high accumulations of MOFs in lungs\textsuperscript{97} and spleen\textsuperscript{98}. Furthermore, in the case of tumor treatment, MOFs tend to accumulate in tumor tissues due to the high permeability of cancer cells\textsuperscript{99}.

Zhu and coworkers, investigated the \textit{in-vivo} biosafety of Mg-MOF74. The \textit{in-vivo} biocompatibility of Mg-MOFs was assessed using the rat model through intraperitoneal injection administration. The doses were calculated based on the body weight and no significant difference was observed between the treated and untreated rats except for the highest dose which confirmed the concentration-dependency of the \textit{in-vivo} toxicity of m/n-Mg-MOF74. The trends of body weight show the less impact of n-Mg-MOF74 on the growth compared to their micron-sized particles. The n/m-Mg-MOF74 did not show significant toxicity or damage for the important organs such as lung, liver and spleen. Mg-MOF74 showed excellent biosafety and high \textit{in-vivo} clearance efficiency with limited myocardial toxicity, which only occurred at very high doses.\textsuperscript{66} The \textit{in-vivo} toxicity of three different porous iron(III) carboxylate MOF NPs MIL-88A, MIL-88B-4CH\textsubscript{3} and MIL-100 was intravenously investigated by evaluating their bio-distribution, metabolism and excretion.\textsuperscript{40, 41, 100} The toxicity of the above mentioned MOFs was assessed by animal behavior, water and food consumption, changes in body and organ weights, biochemical parameters, oxidative stress, oxidative metabolism, macro and microscopic histological observations, as well as some insights of NPs bio-distribution and elimination. During these studies, no death, toxicity and difference in body weight was observed up to 30 days after administration. In the histological examination (lungs, spleen, liver, brain, heart and kidneys) no severe toxicity was observed.

The \textit{in-vivo} biocompatibility is also greatly affected by the physiochemical properties of MOFs. Researchers have found innovative methods to make these MOFs more robust with low \textit{in-vivo} cytotoxicity. For instance, functionalizing the MOFs surface and coating it with more biocompatible materials enhanced the stability and target ability of these systems as mentioned in previous sections. Cell membrane coating is one of the emerging techniques that enable MOFs to exhibit cell-mimicking properties.\textsuperscript{101} For example, our group had reported previously the coating of ZIF-8 with cancer membrane, which enhanced the bio-distribution and improved the target ability to cancer cells significantly\textsuperscript{38}. Coated ZIF-8 with MCF-7 membrane had no significant accumulation in liver and kidney, whereas, it showed selective, prolonged and 2.5-fold high accumulation in MCF-7 tumor cells compared to bare ZIF-8 (Figure 6). Zhuang \textit{et.al} also reported that coating ZIF-8 NPs with RBC had prolonged their circulation in the blood.\textsuperscript{96} They encapsulated uricase enzyme to catalyze uric acid in the plasma.

Further work was done to enhance the therapeutic efficacy of anticancer therapy by combining different anticancer therapies. For instance, Men \textit{et.al} had combined photodynamic therapy with
antiangiogenic drugs by wrapping Zr-MOF with MnO$_2$. This system was also coated with cancer membrane to enhance the bio-distribution. For MOFs immunological response in vivo, few reports have emerged in this area as most metal-based MOFs do not trigger the immune system. Therefore, to use MOFs for immunotherapy applications, other immunogenic materials needs to be incorporated into the system such as antigens or adjuvants. For example, aluminum based MOFs and aluminum incorporated MOFs were reported for vaccines related applications as aluminum is historically used as adjuvant in vaccine. Moreover, using tumor antigen had showed promising results as well. Furthermore, our group developed a biocompatible and biodegradable immunotherapeutic delivery system using ZIF-8 for the controlled delivery of nivolumab (NV), a monoclonal antibody checkpoint inhibitor (Figure 7). The NV-ZIF has shown a higher efficacy than the naked NV to activate T cells in hematological malignancies. We further modified the system by coating NV-ZIF with breast cancer cell membrane (MCF-7) to enable tumor-specific targeted delivery. NV-ZIF$_{MCF}$ showed great tumor inhibition in mice and prolonged retention of NV-ZIF$_{MCF}$ within tumor microenvironment that resulted in efficient NV delivery. Our system showed superior antitumor effects in hematological and solid tumors in comparison with free NV. Furthermore, combining immunotherapy with other anticancer therapies can improve the efficacy of the treatment as already reported by many research groups.

5. Conclusion

As the interest in MOFs as a smart platform for biomedical applications is constantly increasing, the need to optimize the biocompatibility and biodegradability of these systems is a must. In this review, we discussed the most significant findings related to the use of MOFs for biomedical applications specifically for drug delivery. We highlighted the critical aspects that promote the best performance including the nature of the building blocks (metal ions and ligands), size, stability and surface chemistry. Moreover, we summarized some of the most recent findings pertaining to the in-vitro and in-vivo behavior of nMOFs based systems. It can be easily concluded that all the previously discussed factors significantly affect the pharmacokinetic profile and eventual metabolism and consequently the predicted toxicity of these synthetic biomedical platforms. Although not overwhelmingly critical, metal ions and ligands that are endogenous to human bodies are advantageous to a biocompatible design. Platforms with a size <200 nm with a hydrophilic surface and a relatively stable framework under physiological pH, showed the best performances in terms of biocompatibility and bioavailability thus far. Although considerable research is currently being conducted to better understand these platforms, this field is still in its infancy as much work still needs to be conducted especially on the biological interface. From a chemistry perspective, researchers in the MOF field has mastered designing and synthesizing different sizes, morphologies and topologies however, advancement on the biological front is still comparatively shy. More in-vivo work should take place to better assess the pharmacokinetic profile of these platforms especially in terms of degradability and toxicity. It is also important to study the interaction of MOFs with different proteins in the body and to analyze the protein corona after
various proteins attach to the surface, which has been well explored for other nanoparticles. Moreover, colloidal stability is a key factor in pharmaceutical formulations and needs to be very well studied if these platforms should have a real shot in making it to the consumer market. It is very clear that there is much to be done on the forefront of MOFs for biomedical applications but this is exactly what makes this field of research very intriguing. Researches in this field are able to embark on a totally new journey and thus have the chance to be the pioneers in unraveling the story of MOFs in the biological world.

6. References:


**Scheme 1.** Structures of the MOFs [(a) MIL-100 (Fe)]\(^{105}\) Reproduced with permission from ref 105. Copyright 2016 RSC. (b) MIL-88B (Fe)]\(^{106}\) Reproduced with permission from ref 106. Copyright 2013 American Chemical Society. (c) ZIF-8 \(^{107}\) Reproduced with permission from ref 107. Copyright 2018 MDPI (d) PCN-222.\(^{108}\) Reproduced with permission from ref 108. Copyright 2019 American Chemical Society. (e) Uio-66(Zr)\(^{109}\) Reproduced with permission from ref 109. Copyright 2019 Elsevier. (f) Mg-MOF-74.\(^{110}\) Reproduced with permission from ref 110. Copyright 2016 Wiley. ]
Scheme 2. Presentation of the Exogenous and Endogenous Ligands
Table 1. Biodegradability, Biocompatibility and *In vivo* distribution of different MOFs

<table>
<thead>
<tr>
<th>MOF</th>
<th>Biodegradability</th>
<th>Biocompatibility <em>in vitro</em></th>
<th><em>In vivo</em> dose and biodistribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZIF-8</td>
<td>Stable in water, Degradation at pH 6, 38, 44 and in PBS pH 7.4</td>
<td>100 µg/ml</td>
<td>Safe up to 50mg/kg (Mouse, injection) Accumulate in tumor, liver</td>
</tr>
<tr>
<td>MIL-100</td>
<td>Stable in water, Degradation in PBS at pH 7.4</td>
<td>1.1 mg/ml</td>
<td>Safe up to 220mg/kg (Rat, injection) Accumulate in liver</td>
</tr>
<tr>
<td>MIL-88B</td>
<td>Stable in water, Degradation in PBS at pH 7.4</td>
<td>1.26 mg/ml</td>
<td>Safe up to 110mg/kg (Rat, injection) Accumulate in liver, spleen, lung</td>
</tr>
<tr>
<td>Mg-MOF-74</td>
<td>Degrade in water over time</td>
<td>40 µg/ml</td>
<td>Reported 2mg/ml (Mouse, injection)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No biodistribution data</td>
</tr>
<tr>
<td>PCN-222</td>
<td>Stable in water, Degradation at acidic pH</td>
<td>160 µg/ml (IC60)</td>
<td>Reported 5 mg/kg (Mouse, injection) Accumulate in lung, tumor</td>
</tr>
<tr>
<td>Uio-66(Zr)</td>
<td>Stable in water and at acidic pH 2, Degradation in PBS at pH 7.4</td>
<td>Up to 200 µg/ml (100% viability)</td>
<td>Reported 5 mg/kg (Mouse, injection) Accumulates in liver, spleen, tumor</td>
</tr>
<tr>
<td>CAU-7</td>
<td>Stable in water, Degradation in PBS at pH 7.4</td>
<td>Up to 1.5 mg/ml (100% viability)</td>
<td>No <em>in vivo</em> study</td>
</tr>
</tbody>
</table>

- IC50 is used for *in vitro* biocompatibility unless otherwise indicated
**Figure 1:** (a) DOX@AZIF-8 showed size-dependent cell uptake and drug release (b) 60 nm $^{64}$Cu-DOX@AZIF-8 and 130 nm $^{64}$Cu-DOX@AZIF-8 exhibited significant difference in the tumor accumulation. Reproduced with permission from ref 65. Copyright 2018 American Chemical Society.

**Figure 2.** SEM images of ZIF-8 incubated in (a) water pH 7.8, (b) 0.1 M bicarbonate buffer (pH 9.5), (c) 0.1 M KP buffer (pH 7.4), (d) DMEM (pH 7.6), and (e) serum (bovine serum, pH 7.9). (Scale bar: 1 μm.) Reproduced with permission from ref 83. Copyright 2019 Taylor & Francis.
Figure 3: Preparation of the ZIF-8@DOX@organosilica (ZDOS) NPs. Reproduced with permission from ref 12. Copyright 2019 American Chemical Society.

Figure 4: Preparation of C₃-ZIF and cancer cell selectivity of C₃-ZIF. Reproduced with permission from ref 38. Copyright 2020 American Chemical Society.
Figure 5. (a) Cas9/sgRNA within ZIF-8 to form CC-ZIFs. (b) Endosomal escape of CC-ZIFs. (c) CLSM images of cells before and after treatment with CC-ZIFs (d) pH dependent release of AF-Cas9/sgRNA from CC-ZIFs. Reproduced with permission from ref 92. Copyright 2018 American Chemical Society.

Figure 6. (a) ICP-MS analysis of Zn in tumors of mice injected with PBS (control), CC-ZIF, or C³-ZIFMCF (b) Nanoparticles biodistribution in mice 72 h after injection. Reproduced with permission from ref 38. Copyright 2020 American Chemical Society.
Figure 7. (a) The *in vivo* fluorescence images of 4T1 cancer-bearing mice after intravenous injection of NV-ZIF and NV-ZIFMCF. Images were taken at 3 hours (left) and 24 hours (right) post-injection. (b) Representative images of tumors isolated from mice at the end of various treatments (21 days post-injection). (c) Tumor growth curves of different groups of 4T1 cancer-bearing mice after various treatments. (d) Kaplan-Meier survival curve images of 4T1 cancer bearing mice after various treatments. Reproduced with permission from ref 44. Copyright 2021 American Association for the Advancement of Science.