Treatement of hyperphosphatemia based on specific interactions between phosphorus and Zr(IV) active centers of nano-MOFs†

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Hyperphosphatemia is closely associated with the occurrence of multiple organ dysfunctions in patients with end-stage renal disease (ESRD). The application of phosphorus binders as an effective clinical approach for such diseases still suffers from serious side effects. Therefore, development of new phosphorus binders for the treatment of hyperphosphatemia remains a great challenge. Herein, we describe a kind of zirconium (Zr(IV))-based nano-MOF that is well suited for specific adsorption and selective fluorescence sensing of phosphate, and is based on the particular interactions between Zr(IV) and phosphate. The reduced levels of phosphate were quantitatively monitored using the MOF-based fluorescence nanosensor. Notably, the MOFs exhibit a greater reduction in phosphate levels than commercially available phosphorus binders, and comparable therapeutic effects in the treatment of hyperphosphatemia of a mice model. Hence, the MOF acts as a promising medication for hyperphosphatemia by directly adsorbing phosphorus in the blood, which offers new perspectives in future applications of MOFs.

Introduction

Chronic kidney disease, especially renal failure, has dramatically increased over the past few years as a serious global public problem which threatens humans. Currently, hemodialysis as an effective renal failure therapy still experiences limitations in the removal of toxins that leads to the presence of metabolites in the body. Moreover, hyperphosphatemia, considered to be a common metabolic complication in hemodialysis patients, was found to be key for raising serum phosphorus levels in chronic kidney disease-mineral and bone disorder (CKD-MB), thereby further increasing the occurrence and development of CKD-MB. The main clinical manifestations of CKD-MB are hyperparathyroidism, abnormal mineral and bone metabolism, and calcification of blood vessels and other soft tissues. Such a disease not only increases the patients' morbidity and seriously influences their quality of life, but also increases the incidence of cardiovascular diseases and eventually death. Hence, curing hyperphosphatemia has become quite crucial for the prevention and control of complications in hemodialysis patients, and urgently needs to be solved.

The phosphorus binders currently used for hyperphosphatemia treatment are aluminum, calcium and iron based binders, as well as La2(CO3)3 etc. However, these binders' side effects and their impact on the patient's prognosis remain a serious concern. Take aluminum-based phosphorus binders for example: their toxicity for the central nervous system, blood system, and bones significantly limits their clinical applications. On the other hand, La accumulates in many organs such as the liver and kidneys, which causes adverse reactions like encephalopathy. Therefore, the development of new types of phosphorus-reducing drug with fewer side effects faces severe challenges.

In order to improve the materials' therapeutic effects, metal organic frameworks (MOFs) as a new kind of porous complex, which have been widely used in the fields of biosensing, catalysis, and photodynamic therapy due to their controllable synthesis, structural diversity and high specific surface area, have raised our interest. Recently, our group designed nano-MOFs with Cu(II) as the active center to reduce glutathione levels and increase the concentration of ROS, thus strengthening the photodynamic efficiency and achieving a synergistic antitumor effect. In this regard, we hypothesized that a Zr(IV)-based nano-MOF with an active center prone to specific phosphorus reactions would have the advantages of a large surface area and enhanced chemisorption abilities, as well as selective fluorescence sensing of phosphate to monitor...
the process. Zr(IV) as the active center of the nano-MOF can specifically bind and absorb in vivo phosphate, thus directly decreasing the blood phosphorus levels and leading to a remission of hyperphosphatemia (Scheme 1). Notably, compared to commercially available phosphorus binders, the nano-MOF exhibits a greatly enhanced decrement in phosphorus levels. Finally, the hyperphosphatemia mice model with the MOF treatment further proved there was less in vivo toxicity and a better curing effect. This work provides strong evidence for MOFs as promising drugs for the treatment of hyperphosphatemia by directly adsorbing phosphorus in the blood, which will further optimize the clinical applications in the future.

**Results and discussion**

**Characterization**

Typically, porphyrin-inlaid Zr(IV)-based nano-MOF UiO-66 materials were prepared and characterized. The mechanical stability of the nano-MOFs was first investigated (Fig. 1a), where the PXRD patterns of the nano-MOF treated with PBS were consistent with those of the as-prepared MOFs, which proves dect crystalline stability in biological environments. By comparison with the FTIR absorptions of UiO-TCPP and UiO-66-NH3, identical results were obtained (Fig. S1†) which suggests that porphyrin does not affect the crystal structure of the MOFs. The morphology and particle size of the MOFs were acquired through dynamic light scattering (DLS), SEM and TEM (Fig. 1b–d), which demonstrated the fine size control of the particles, which had a uniform distribution of 120 nm.

**Fluorescence response**

The fluorescence response of the MOFs to phosphate was studied via the two largest fluorescence emission peaks at 440 nm (2-aminoterephthalic acid) and 650 nm (porphyrin) (Fig. S2†). By interacting with phosphate, the fluorescence intensities of MOFs were significantly enhanced due to the charge transfer interruption between the ligand molecule and the metal junction of the MOFs, and thus the ligand molecule’s fluorescence was restored. To further explore the mechanism of the fluorescence intensity change caused by MOFs and phosphate, the fluorescence response of the MOFs to phosphate in the presence/absence of Zr(IV) ions was examined (Fig. S3†). Interestingly, no significant fluorescence change was observed when adding different concentrations of phosphate, which was attributed to Zr(IV) ions’ preferential interactions with the phosphate. The Zr(IV) of the MOFs does not interact with phosphate, so there are no obvious changes in fluorescence

**Scheme 1** Schematic diagram for the treatment of hyperphosphatemia based on Zr(IV)-MOFs. (a) Proposed mechanism of the fluorescence nanosensor for phosphate; (b) reduced levels of phosphate by MOFs for highly efficient treatment of hyperphosphatemia.

![Scheme 1](image1)

**Fig. 1** (a) PXRD patterns for the as-synthesized samples of MOFs and the stability of the nano-MOFs in biological environments (red line, green line, blue line, light blue line and orange red line are the PXRD patterns for MOFs treated with PBS (2 mM) of pH 3, 5, 8 and 10 in 72 hours, respectively). (b) DLS image of the MOFs; (c) TEM image of the MOFs, scale bar = 100 nm; (d) SEM image of the MOFs, scale bar = 200 nm.

![Fig. 1](image2)

**Fig. 2** (a) Fluorescence responses of MOFs with phosphate and other reactive small species. Relative fluorescence intensity \( F - F_0 \) upon the addition of \( \text{PO}_4^{3-} \) (200 μM), I− (100 mM), GSH (9.8 μM), SO\(_4^{2-}\) (100 mM), SO\(_4^{2-}\) (1.0 mM), Br− (1.0 mM), HCO\(_3^-\) (1.0 mM), CO\(_3^{2-}\) (1.0 mM), Cl− (1.0 mM), NO\(_2^-\) (1.0 mM), ATP (1.0 mM), and L-Cys (200 μM); (b) comparison of phosphate adsorption capacity by the MOF and other phosphorus binders measured using an ICP emission spectrometer. Experimental details: \( C_{\text{MOF}} = 1 \text{ mg mL}^{-1}\), other phosphorus binder concentrations: 1 mg mL\(^{-1}\), fetal bovine serum (10-fold dilution, 10 mL).
Based on the hypothesis, Zn(II)-based MOFs and phosphate, UV-vis spectroscopy was performed to confirm the hypothesis by measuring the concentration of phosphate after incubating with MOFs (Fig. S7†), and 92.4 μg mg⁻¹ was achieved, demonstrating a significant decrease from the phosphate solution, which means that the MOFs exhibit a significant adsorption capacity for phosphate.

Fig. 3 (a) Fluorescence emission spectra of the MOFs upon the addition of a solution of the phosphate for the normal (red line) and model (blue line) groups; (b) data output of (a). P value less than 0.001, indicating significant differences between the two groups of data.

Selectivity

The MOFs’ phosphate absorption selectivity was studied with the interference of common in vivo small molecular species. Fig. 2a shows that the fluorescence responses are significantly weaker for the ions even with higher concentrations than phosphate, representing a high selectivity of the MOFs towards phosphate. By further verification of the relationship between Zn(II)-based MOFs and phosphate, UV-vis spectroscopy was carried out and an enhanced absorption at 420 nm was obtained after 1 h of incubation (Fig. S6†). This is most probably because phosphate, when binding to the MOFs’ network architecture, influences the coordination between the metal center and the ligands, thus enhancing the absorption intensity of the porphyrin. UV-vis spectroscopy was performed to confirm the hypothesis by measuring the concentration of phosphate after incubating with MOFs (Fig. S7†), and 92.4 μg mg⁻¹ was achieved, demonstrating a significant decrease from the phosphate solution, which means that the MOFs exhibit a significant adsorption capacity for phosphate.

Adsoption capacity

To improve the biocompatibility of the MOFs, the BSA protein was used to modify the MOFs’ surface. The surface charge intensity, which further demonstrated the important role of Zr(IV) of MOFs for identification and fluorescence sensing of phosphate.

Table 1. Baseline characteristics of the mouse model treated with the nano-MOF phosphorus binders versus the control.

<table>
<thead>
<tr>
<th>Index</th>
<th>Cystatin C (mg L⁻¹)</th>
<th>Creatinine (μmol L⁻¹)</th>
<th>Phosphorus (mmol L⁻¹)</th>
<th>Weight (g)</th>
<th>GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.25 ± 0.00</td>
<td>50.83 ± 2.80</td>
<td>1.37 ± 0.08</td>
<td>43.56 ± 2.88</td>
<td>15.29</td>
</tr>
<tr>
<td>Model</td>
<td>0.50 ± 0.00</td>
<td>58.33 ± 2.80</td>
<td>2.15 ± 0.32</td>
<td>32.68 ± 3.23</td>
<td>9.79</td>
</tr>
<tr>
<td>Therapy</td>
<td>0.25 ± 0.00</td>
<td>47.50 ± 2.50</td>
<td>1.26 ± 0.45</td>
<td>35.56 ± 2.25</td>
<td>17.73</td>
</tr>
</tbody>
</table>

*GFR (estimated glomerular filtration rate) = 186 × (creatinine/88.14)⁻¹⁻⁰⁵ × (weight/1000) was used to evaluate the renal function indicators of the mice.*

The phosphorus levels was measured using an ICP emission spectrometer. The cystatin C and creatinine levels were evaluated using an automatic biochemical analyzer from Qilu Medical Research Institute.
changes of MOFs before and after modification with BSA were measured using the zeta potential. The potential increase from $-13.7$ mV to $-3.5$ mV (Fig. S8†) indicated that the BSA protein was successfully attached onto the surface of the MOFs (BSA-MOFs). Then, the hyperphosphatemia serum model was used to test if BSA-MOFs can reduce the levels of phosphate using UV-vis spectroscopy and an ICP emission spectrometer (Fig. S9†), and good efficacy was obtained in both characterizations. Subsequently, other commercially available phosphorus binders were compared with MOFs (Fig. 2b), but the MOFs exhibit obviously better adsorption capacities than all of the others. In addition, the MTT test showed the excellent biocompatibility of the MOFs in vivo (Fig. S10†). These results demonstrated that the MOFs directly adsorb phosphate, which remarkably reduces the phosphate levels.

**Treatment of hyperphosphatemia in a mouse model**

Then, a mouse model for chronic renal failure was established using adenine chemical induction. By measuring physiological indicators such as renal function, blood phosphate levels, kidney tissue sections, body weight changes, and imaging of the mouse kidney tissue in normal and model mice, these results showed that all physiological indicators of the model mice were demonstrated that the MOFs directly adsorb phosphate, which remarkably reduces the phosphate levels.

Using adenine chemical induction. By measuring physiological indicators such as renal function, blood phosphate levels, kidney tissue sections, body weight changes, and imaging of the mouse kidney tissue in normal and model mice, these results showed that all physiological indicators of the model mice were remarkably reduced, and the physiological indicators of the mouse models were basically consistent with the normal mice, indicating that the hyperphosphatemia mouse model was effectively treated using the MOFs. In addition, the in vivo toxicity for the organs was tested to prove the biosafety of the MOF material as a phosphorus binding agent (Fig. S11†). Experiments on tissue slides (heart, liver, spleen and lung, respectively) were performed. Although the nano-MOFs enter the bloodstream via stomach feeding, the images showed the MOF material has less effect on other organs, which indicated that the nano-MOF has less in vivo toxicity.

**Conclusions**

In summary, as chronic kidney disease retains high levels of phosphorus in the blood, we have developed a nano-MOF with Zr(IV) as the active center to reduce the blood phosphorus levels and cure hyperphosphatemia by the direct adsorption of phosphorus. The highly porous MOFs offer significant numbers of Zr(IV) surface active centers for interactions, which dramatically reduce the phosphorus concentrations in vivo. By further comparisons with common in vivo small molecules and other phosphate binders, the MOFs possess high specificity and selectivity for reducing levels of phosphorus. Finally, the hyperphosphatemia mouse model with treatment using MOFs shows identical physiological parameters with the control group, representing promising biocompatibility for future applications. The present work provides not only a fluorescence nanosensor for monitoring blood phosphorus levels, but a new approach for effective hyperphosphatemia treatment in clinical therapies.

**Conflicts of interest**

There are no conflicts to declare.

**Acknowledgements**

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**Notes and references**