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Inherent anchorage in UiO-66 nanoparticles for efficient capture of alendronate and its mediated release

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Zr-based MOF nanoparticles were applied as an efficient carrier for alendronate delivery, and an unprecedented drug loading capacity was achieved thanks to the inherent drug anchorages of Zr-O clusters therein. The encapsulated drug featured with a pH-dependent release profile, and inhibited the growth of cancer cells more efficiently than free drug.

The amino-bisphosphonate of alendronate (AL) is widely used for the treatment of osteoporosis, solid tumor bone metastases and myeloma bone disease.1 Although AL is mostly known for its strong anti-resorptive activity, it also demonstrates direct or indirect antitumor effects, notably in the cases of prostate and breast cancers.2 However, its short lifetime and preferential accumulation within bone tissues limit its use as antitumor agents for extra-skeletal malignancies.3 Furthermore, due to its poor bioavailability, administration of over-high doses is usually required for practical clinical applications, which will inevitably cause systemic toxicity. Therefore, a local, sustained AL delivery system that can enhance the loading capacity and delivery efficiency of AL into cancer cells is highly desirable.

It has been well documented that some metal oxides, such as zirconia, can be modified with phosphonates due to the stable M-O-P bonds.4 Thus, introducing metal oxide into nanocarriers was strategically employed to serve as the drug anchorage for AL capture. For example, Colilla and co-workers5 modified the framework of mesoporous silica with zirconia, which not only effectively increased AL loading amount but also mediated its release. However, limited by the small amount of the doped metal oxide, the AL loading capacity was still relatively low.

Porous metal-organic frameworks (MOFs) have been successfully employed as drug delivery vehicles attributing to their enormous porosity, high surface area and versatile framework compositions.6 Several studies confirmed that MOFs exhibited exceptional biocompatibility and biodegradability.6 However, their relatively low chemical and thermal stabilities could be the key limitations to meet the requirements of practical applications.7 Recently, zirconium based MOFs of UiO-66 received great attention due to its excellent stabilities.8 UiO-66 contains linear 1,4-benzenedicarboxylate (BDC) ligand and Zr₆O₄(OH)₄ clusters as 12-connected nodes.9 It possesses a face-centered-cubic arrangement of the Zr-O clusters and thus consists of octahedral (∼11 Å) and tetrahedral (∼8 Å) cages in 1:2 ratio.10 These open cavities and Zr-O clusters make it possible to tailor the controlled capture and release of AL molecules based on the strong complexation of Zr-O-P bonds. These features coupled with its non-toxic nature and easiness for nanoparticle formation11 make UiO-66 an ideal candidate for AL delivery.

Herein, UiO-66 nanoparticles (NPs) with uniform particle size were elaborated and successfully applied as an AL delivery vehicle. To the best of our knowledge, this is the first report on taking advantage of the Zr-O clusters in UiO-66 as natural drug anchorages to realize the effective capture of AL molecules. The AL loading amount was unprecedentedly high up to 1.06 g of drug per gram UiO-66, and their release from the nanocarriers was greatly sustained and featured with a pH-dependent profile.

The obtained NPs exhibited remarkable water dispersity, and could be effectively endocytosed by cancer cells. The encapsulated drug presented enhanced growth inhibition effect than free AL against both MCF-7 and HepG2 cells.

Fig. 1 Typical (a) TEM and (b) FE-SEM images of the synthesized UiO-66 nanoparticles.

The highly crystallized UiO-66 NPs were prepared by a modified solvothermal method (See ESI†).8 TEM was used to investigate their size and morphology. It can be observed that the monodispersed nanocrystals with a mean diameter of ca. 70 nm are mainly cubical in shape (Fig. 1a). FE-SEM further confirms that the isolated UiO-66 crystals with cubical morphology and a diameter range from 50 to 90 nm were obtained (Fig. 1b). This size matches well with the reported suitable nanoparticle diameter for the effective cell uptake.5c Hence, it is anticipated that the obtained UiO-66 NPs could be internalized into cells effectively. Actually, the particle size of UiO-66 NPs could be also determined by dynamic light scattering (DLS) technique thanks to their well water dispersity. The hydrodynamic size is narrowly distributed and peaks at ca. 100 nm (Fig. S1, ESI†), which is slightly larger than the geometric size obtained by either TEM or SEM. When a laser pointer was used to illuminate the NPs
The variations of surface area and pore volume of UiO-66 upon drug loading were analyzed by nitrogen sorption techniques. Both samples before and after drug loading display typical type-I gas sorption isotherms (Fig. S4, ESI†). This agrees well with the fact that the AL loading process did not destroy the structure integrity of UiO-66. AL-UiO-66 exhibits a BET surface area of 45 m² g⁻¹, which is much lower than that of pristine UiO-66 (1136 m² g⁻¹, Table 1) in accordance with an apparent change in the pore volume (from 0.63 cm³ g⁻¹ to 0.07 cm³ g⁻¹). The dramatic decreases in the surface area and pore volume can be attributed to the AL molecule bonding in the cages of UiO-66.

Fig. 2 (A) FT-IR spectra of (a) AL free drug, (b) parent UiO-66 nanocarriers and (c) AL loaded UiO-66. (B) The O1s XPS spectrum of AL-UiO-66.

The interaction of the UiO-66 framework with AL molecules was probed by Fourier transformed infrared (FT-IR) spectra (Fig. 2A). In the spectrum of pristine UiO-66, the intense doublet at 1590 and 1400 cm⁻¹ (labeled with black stars) can be assigned to the in- and out-of-phases stretching modes of the carboxylate groups.⁸ The triplet at 723, 650 and 550 cm⁻¹ (labeled with green arrows) is attributed to Zr-O₂ as longitudinal and transverse modes, respectively.⁹ In contrast, after AL loading, the original bands at 1590 and 1400 cm⁻¹ are blue-shifted to 1630 and 1433 cm⁻¹ (labeled with green stars). Meanwhile, broad P-O stretching bands between 1200 and 900 cm⁻¹ appear in the spectra of AL-UiO-66 with two main characteristic absorption bands at 1150 and 1020 cm⁻¹ and a shoulder at 957 cm⁻¹. These bands, ascribing to the P=O, P-OH and P-O-Zr bonds, respectively, evidence that the drug has been successfully encapsulated into UiO-66.¹⁰ We note that P-O bands in the AL molecule (labeled with blue arrows in Fig. 2A) have been substantially red-shifted, which is plausible because binding of AL molecules to the Zr-O clusters could significantly decrease the frequency of the P-O stretching modes.¹² We also measured the XPS spectra of O, Zr and P of AL-UiO-66 to further confirm the existence of a linkage between UiO-66 and AL molecules (Fig. S5, ESI†). The deconvoluted O1s peak (Fig. 2B) consists of four peaks that are assigned to O in O=C–O (533.5 eV), P-O-H (532.6 eV), Zr-O-Zr (530.4 eV), and in Zr-O-P and P=O (531.6 eV), respectively.¹³ These strongly evidence that the Zr-O clusters in UiO-66 present high affinity towards AL molecules. Thanks to this strong complexation of Zr-O-P, the UiO-66 NPs show a remarkable AL loading capacity, which is extraordinarily high up to 51.4 wt%, or 1.06 g of drug per gram of porous nanocarriers, as measured by ICP-AES. It should be pointed out that the loading amounts of AL in the reported nanocarriers in previous studies have never been higher than 37 wt%,¹⁴ revealing the overwhelming advantage of our strategy by employing UiO-66 as AL delivery vehicles.

The release of AL from UiO-66 NPs was assessed at 37 °C in PBS buffer with pHs of 5.5 and 7.4 (Fig. 3a). The time-dependent drug release profile is characterized by the slow and sustained patterns, which is quite beneficial to prevent the drug dissipation prior to reaching the cancer cells.³⁰ As shown in Fig. 3a, about 42.7 % of AL is released from the UiO-66 NPs in 60 h at pH 7.4, whereas more than 59 % drug releases in the same time interval at pH 5.5, indicative of the sensitivity of AL-UiO-66 to endosome/lysosome pH (ca. 5).¹⁴ The pH-responsive release feature may be attributed to the protonation of phosphate in the acidic environment, which weakens the interaction between AL and Zr-O cluster in UiO-66.¹⁴ These results imply that UiO-66 nanocarriers can diminish premature drug release during circulation but specifically enhance intracellular drug release, which will be definitely useful for effective tumor treatment.¹⁵ It is very interesting to note that the release amount was up to 88.1 % in 108 h at pH 7.4, while at pH 5.5, the amount is less than 76 % within the same time duration. Such a unique change in drug release behavior could be due to the lower degradation rate of UiO-66 in acidic condition than that in neutral and basic condition.³⁰

To test in vitro cytotoxicity of the pristine UiO-66 NPs, cell viability was examined by standard MTT assays against HepG2 and MCF-7 cells (Fig. S6, ESI†). It was found that the changes in cell viability after 24 and 48 h of incubation were negligibly small. The cell proliferation is slightly hindered after incubation at a very high concentration up to 300 ug mL⁻¹ of nanocarrier for 48 h, indicating the relatively good biocompatibility of UiO-66 NPs in vitro.

Given the suitable particle size of UiO-66 nanocarriers, studies on the cellular uptake efficiency were carried out on HepG2 cells. We grafted fluorescent molecule of flavin mononucleotide (FMN, the phosphorylated form of vitamin B2) onto UiO-66 (UiO-66-FMN) by taking advantage of the high affinity between Zr-O clusters and phosphate function in FMN molecule for intracellular imaging.¹⁷ The effective capture of FMN on UiO-66 was verified by fluorescence measurement (Fig. S7, ESI†). To check whether FMN would leak from the nanocarrier, UiO-66-FMN was soaked in water for 24 h and the detached FMN was removed by centrifugation-redispersion cycles. The fluorescence

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Table 1. Texture parameters of UiO-66 and AL-UiO-66

<table>
<thead>
<tr>
<th>Samples</th>
<th>S_BET (m² g⁻¹)</th>
<th>V_g (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UiO-66</td>
<td>1136</td>
<td>0.63</td>
</tr>
<tr>
<td>AL-UiO-66</td>
<td>45</td>
<td>0.07</td>
</tr>
</tbody>
</table>

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15 The X-ray diffraction (XRD) technique was employed to characterize the structural evolvement of the synthesized UiO-66 and AL loaded UiO-66 (AL-UiO-66). The similar Bragg diffraction peaks of both samples indicate that the AL loading did not alter the parent crystalline structure of UiO-66 (Fig. S2, ESI†). A significant decrease in the peak relative intensity is observed for AL-UiO-66, which could be ascribed to the trapping of the AL in the UiO-66 pores and consequently results in the decreased X-ray contrast between porous framework and pore cages. The AL encapsulation did not change the morphology of nanocarriers as shown by TEM and SEM (Fig. S3a and S3b, ESI†).

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enhanced growth inhibition effect of the encapsulated AL with (labeled in Fig. 3d) cell lines, implying the controlled release and those of free AL both in HepG2 (labeled in Fig. 3c) and MCF7.

As shown in Fig. 3b, the NPs are remarkably internalized within a short period and distributed intensively in the cytoplasm as manifested by the appearance of green fluorescence around the nucleus in consistent with the results determined from flow cytometric analysis.

The therapeutic effect of AL loaded nanocarriers was studied through in vitro cytotoxicity measurements against MCF-7 and HepG2 cells using MTT method. The AL-Uio-66 exhibits slightly lower cytotoxicities than that of free AL with an equivalent dose in 24 h incubation for both cancer cells. This may be partially attributes to the different cell uptake processes between free AL and AL-Uio-66. Free AL can passively diffuse through the cell membrane and rapidly accumulate in the nucleus to kill the cancer cells, while the nanocarriers are generally endocytosized into the cells and detained in the endosomes instead of the cytoplasm. However, in 48 h of incubation, the AL-Uio-66 leads to higher amounts of cancer cell deaths than free AL. The IC_{50} values of AL-Uio-66 are much lower than those of free AL both in HepG2 (labeled in Fig. 3c) and MCF-7 (labeled in Fig. 3d) cell lines, implying the controlled release and enhanced growth inhibition effect of the encapsulated AL with prolonged incubation time.

In summary, Zr-based MOF of Uio-66 NPs could be applied as an efficient carrier for AL delivery. The inherent Zr-O clusters in nanocarrier serve as natural drug anchorages for effective AL capture, leading to enhanced loading capacity and its mediated release. The pH-sensitive drug-carrier interaction accelerates the AL release in the acidic milieu of cancer cells, resulting in increased antitumor efficiency against both MCF-7 and HepG2 cell lines. These merits combined with its high stability, make Uio-66 highly promising for therapeutic applications as AL delivery vehicles.

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