### Biodegradable Oxamide-Phenylene-Based Mesoporous Organosilica Nanoparticles with Unprecedented Drug Payloads for Delivery in Cells

<table>
<thead>
<tr>
<th>Item Type</th>
<th>Article</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authors</td>
<td>Croissant, Jonas; Fatieiev, Yevhen; Julfakyan, Khachatur; Lu, Jie; Emwas, Abdelhamid; Anjum, Dalaver H.; Omar, Haneen; Tamanoi, Fuyuhiko; Zink, Jeffrey; Khashab, Niveen M.</td>
</tr>
<tr>
<td>Citation</td>
<td>Biodegradable Oxamide-Phenylene-Based Mesoporous Organosilica Nanoparticles with Unprecedented Drug Payloads for Delivery in Cells 2016 Chemistry - A European Journal</td>
</tr>
<tr>
<td>Eprint version</td>
<td>Post-print</td>
</tr>
<tr>
<td>DOI</td>
<td>10.1002/chem.201601714</td>
</tr>
<tr>
<td>Publisher</td>
<td>Wiley</td>
</tr>
<tr>
<td>Journal</td>
<td>Chemistry - A European Journal</td>
</tr>
<tr>
<td>Rights</td>
<td>This is the peer reviewed version of the following article: Croissant, J., Fatieiev, Y., Julfakyan, K., Lu, J., Emwas, A., Anjum, D., Omar, H., Tamanoi, F., Zink, J. and Khashab, N. (2016), Biodegradable Oxamide-Phenylene-Based Mesoporous Organosilica Nanoparticles with Unprecedented Drug Payloads for Delivery in Cells. Chem. Eur. J., which has been published in final form at <a href="http://doi.wiley.com/10.1002/chem.201601714">http://doi.wiley.com/10.1002/chem.201601714</a>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for self-archiving.</td>
</tr>
<tr>
<td>Download date</td>
<td>2024-01-30 11:01:55</td>
</tr>
<tr>
<td>Link to Item</td>
<td><a href="http://hdl.handle.net/10754/612998">http://hdl.handle.net/10754/612998</a></td>
</tr>
</tbody>
</table>
Title: Biodegradable Oxamide-Phenylene-Based Mesoporous Organosilica Nanoparticles with Unprecedented Drug Payloads for Delivery in Cells

Authors: Jonas Croissant; Yevhen Fatieiev; Khachatur Julfakyan; Jie Lu; Abdelhamid Emwas; Dalaver Anjum; Haneen Omar; Fuyuhiko Tamanoi; Jeffrey Zink; Niveen Khashab

This manuscript has been accepted after peer review and the authors have elected to post their Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: Chem. Eur. J. 10.1002/chem.201601714

Link to VoR: http://dx.doi.org/10.1002/chem.201601714
Biodegradable Oxamide-Phenylene-Based Mesoporous Organosilica Nanoparticles with Unprecedented Drug Payloads for Delivery in Cells


Abstract: We describe biodegradable mesoporous hybrid NPs in the presence of proteins, and its application for drug delivery. We synthesized oxamide-phenylene-based mesoporous organosilica nanoparticles (MON) in the absence of silica source which had a remarkably high organic content with a high surface area. Oxamide functions provided biodegradability in the presence of trypsin model proteins. MON displayed exceptionally high payloads of hydrophilic and hydrophobic drugs (up to 84 wt%), and a unique zero premature leakage without the pore capping, unlike mesoporous silica. MON were biocompatible and internalized into cancer cells for drug delivery.

Biodegradable nanomaterials have attracted a lot of attention in biomedical research for their higher potential to reach the emerging industrial market of nanomedicine. Indeed, the uncertainty of the toxicity and the known limited biocompatibility of nanomaterials greatly hinder their successful clinical translation as therapeutic pharmaceutical products. Biodegradable liposome[2] and micelle[4] NPs have been prepared, as well as polymeric NPs such as polyactic acid, polylactide-co-glycolide, poly-ε-caprolactone,[5] and copolymer-liposome nanosystems.[6] Biodegradable inorganic NPs[7] are much rarer but include silicon NPs,[8] calcium and manganese phosphate NPs.[3, 9] However, biodegradable organic-inorganic hybrid NPs are especially desirable since they may garner the biodegradability features of organic moieties as well as the mesoporous framework and properties of an inorganic matrix. Lin et al. reported biodegradable non-porous bridged silsesquioxane (O1.5Si-R-SiO1.5, R= organic group) NPs via disulfide groups for magnetic resonance imaging.[10] We recently reported the first example of biodegradable non-porous bridged silsesquioxane NPs via the incorporation of oxamide cleavable groups within the matrix and the addition of trypsin.[11] Nonetheless the previous NPs were not porous (< 25 m² g⁻¹) due to extensive H-bonding between oxamide functions throughout the entire diameter of NPs, thus restricting significantly their application. De Cola et al. recently reported trypsin-biodegradable lysine-bridged silsesquioxane nanodonuts for delivery applications.[12] The drawback of this system lies again in the absence of mesopores, which limited the loading doxorubicin (DOX) anticancer drugs (2 wt%).

Mesoporous organosilica nanoparticles (MON) solely designed from bridged organoalkoxysilanes[14] with two or more alkoxyyls[15, 16] (e.g. (EO)OSi-R-Si(EO)3) with R= CH₂–CH₂, C₆H₄)[17] are a rapidly emerging field in nanomaterial research.[18] The use of bridged organoalkoxysilane is generally required in templated aqueous solutions since pending organic R groups in (EO)OSi-R molecules tend to favor materials with medium to low microporosities.[19] When organized mesopores are obtained in MON, they are also called periodic mesoporous organosilica (PMO) NPs. Unlike mesoporous silica nanoparticles (MSN) and organically-doped MSN (SiO₄/R-SiO₃),[20] MON based on organically-bridged silsesquioxanes frameworks (O1.5Si-R-SiO1.5) are not synthesized from silica precursors (e.g. tetraethoxysilane) which makes them hybrid NPs with unusually high organic contents ranging from 20 to 80 wt% depending on the size of the organic bridge. The pore walls of MON are thus extensively covered with organic moieties which dramatically affects their physico-chemical and mechanical properties.[21] For instance, the high hydrophobicity of the pores of ethylene-bis-propyldisulfide-based PMO allowed much higher DOX payloads than mesoporous silica in similar conditions (29 vs 8 wt%).[22] Besides, ethylene and phenylene-based MON were found to have very low hemolytically behaviour up to 2000 g mL⁻¹, unlike silica NPs and MSN.[23] Hence, the unique properties of MON have attracted pioneering works over the past four years in drug,[1, 22, 32] gene,[33] and protein delivery,[34] photodynamic therapy,[35] catalysis,[14, 36] separation,[36, 37] and energy[38]

Biodegradable PMO have been reported by Croissant et al. via the incorporation of disulfide bridges into the pore walls.[22] biodegradation hollow MON were also designed by Chen et al. using thioether bridges in the NPs framework.[22] Both systems relied on the redox cleavage of the sulfide bridges by intracellular bioreducing agent such as glutathione tripeptides. The drug loading capacities of the systems ranged from 148 mg/g (14.8 wt%)[22] to 290 mg/g (29.0 wt%).[1] To date, no proteins has been reported to cleave specific bridges in the framework of MON so as to induce the biodegradation the organosilica mesoporous framework.

[a] Smart Hybrid Materials Laboratory, Advanced Membranes and Porous Materials Center, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia
E-mail: Niveen.khashab@kaust.edu.sa
[b] Department of Chemistry and Biochemistry, California NanoSystems Institute, Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles, California, United States
[c] Department of Microbiology, Immunology and Molecular Genetics, California NanoSystems Institute, Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles, California, USA.
[d] Imaging and Characterization Laboratory, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia.

Supporting information for this article is available on the WWW under.....
Herein, we describe MON based on oxamide-phenylene bridges which are biodegradable in the presence of trypsin proteins, and showed unprecedentedly high drug payloads up to 84 wt% (Figure 1). MON were synthesized from the co-condensation of 1,4-bis(triethoxysilyl)benzene and (N,N'-bis(3-(triethoxysilyl)propyl)oxamide). This strategy allowed the homogeneous distribution of oxamide functions without H-bonding in the NPs, hence affording 100 nm MON spheres with a high surface area of 850 m² g⁻¹. The mechanism of the selective cleavage of the amino-acid-like oxamide moieties inducing the degradation was demonstrated. Additionally, MON were composed of 50 wt% of organic content allowing unprecedentedly high loading capacities of both hydrophilic DOX hydrochloride (65 wt%) and hydrophobic camptothecin (CPT, 84 wt%) drugs, without the requirement of pore capping to prevent the cargo leakage, which stands in stark contrast with pure silica analogues and most porous nanocarriers. Unlike previously reported MON,1,18,33 drugs remained encapsulated inside the oxamide-phenylene pores from pH 7.4 to 5, and could be released upon the degradation. The hybrid nanovehicle was then used for drug delivery in cancer cells.

The oxamide bridged alkoxysilane was first synthesized and fully characterized via ¹H and ¹³C nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) and mass spectrosopies (Figures S1-S3, ESI).¹⁵ The synthesis involved the quantitative coupling of oxaly chloride with aminoproptyltriethoxysilane (Figure S1). The porous nanomaterial was then synthesized via a templated sol-gel method. Briefly, 4-bis(triethoxysilyl)benzene and (N,N'-bis(3-(triethoxysilyl)propyl)oxamide) were co-condensed in 10 to 1 molar ratio in an aqueous cetyltrimethylammonium-templated solution for 2 h at 80 °C with sodium hydroxide as catalyst, then, the surfactant was extracted from NPs (ESI). Transmission electron microscopy (TEM) micrographs displayed monodisperse spherical 100 nm particles (Figure 2A-B), as confirmed by scanning electron microscopy (SEM) imaging (Figure 2C), and statistical TEM analysis (140 NPs, Figure 2D). The non-aggregation and monodispersity of MON were validated from the dynamic light scattering (DLS) analysis with a narrow hydrodynamic diameter distribution centered at 180 nm (Figure S4). The porosity of the material was assessed by nitrogen-sorption leading to a BET (Brunauer-Emmett-Teller) surface area of 850 m² g⁻¹, the BJH (Barrett-Joyner-Halenda) average pore size was 2.1 nm (Figure S5) with a minor pore size distribution at 3.7 nm, which was consistent with the worm-like mesoporosity observed by TEM (Figure 2B).

Figure 1. Representation of the sol-gel synthesis of MON, their pore structure before and after the protein-mediated degradation (top), or after high drug loadings, affording non-leaky NPs with uncapped pores (bottom).

Figure 2. TEM (A-B) and SEM (C) micrographs, and TEM statistical size distribution of MON (D). STEM-EELS elemental mapping (silicon, oxygen, nitrogen, carbon) of a representative MSB NP spectrum image (SI, top left). Fast Fourier transfrom (FFT) of the SI image (bottom left) (E).
The composition of MON was then investigated to verify the incorporation of both phenyl and oxamide groups. UV-visible spectroscopy showed the expected absorption band of phenylene at 270 nm (Figure S6), which formed 7.2 nm domains of molecularly-bridged bridges according to wide angle x-ray diffraction (XRD, 7.6 Å peak) and the Scherrer’s equation (Figure S7). FTIR spectroscopy showed the phenylene aromatic C-H stretching (3100-3050 cm⁻¹) and the \( \nu_{\text{C}sp^2\text{H}} \) modes (520 cm⁻¹, Figure S8). The high condensation degree of the siloxane network was supported by the shift of the \( \nu_{\text{SiO}} \) mode from 1080 cm⁻¹ in the precursor to 1090-1140 cm⁻¹ in NPs. The successful incorporation and preservation of propyloxamides was evident from the aliphatic \( \nu_{\text{C}sp^3\text{H}} \) stretchings from 2990 to 2890 cm⁻¹, and the carbonyl stretching and N-H bending modes at 1670 and 1521 cm⁻¹, respectively. It is noteworthy that for the latter two vibrations, the absence of sharpness and intensity strongly suggests the absence of H-bonding, hence promoting the formation of porosity. We then performed scanning transmission electron microscopy (STEM) combined with electron energy-loss spectroscopy (EELS, see a typical spectrum in Figure S9) to investigate the composition homogeneity (Figure 2E). The homogeneous distribution of phenylene and oxamide groups within MON was demonstrated by STEM-EELS elemental mappings of silicon, oxygen, nitrogen and carbon atoms. This conclusion is particularly important as the efficient degradation of particles depends on the homogeneous dispersion of oxamides within the NP matrix. The absence of pattern in the Fast Fourier Transform (FFT, bottom left in Figure 2E) correlated the worm-like porosity. EELS quantifications provided the following weight percentages: 7.7% of Si, 32.9% of O, 56.9% of C, and 2.5% of N which corresponds to a 23.8% content of O\(_3\)Si-(CH\(_2\))\(_3\)NH-CO-CO-NH-(CH\(_2\))\(_3\)SiO\(_3\) fragments. Thermogravimetric analysis (TGA) correlated these results with a weight decomposition of nearly 45% (Figure S10).

The biodegradability of the hybrid MON was first studied in simulated biological media. Nanomaterials were placed in phosphate buffered saline (PBS) and were exposed to trypsin model proteins (Figure 3A), known to cleave specific amino-acids into carboxylate and ammonium derivatives. After 24 h of mixing, aliquots were centrifuged at (14 k rpm), analyzed by TEM, and revealed the initiation of degradation (Figure 3B). FTIR supported the surface adsorption of trypsins (4 nm wide) on the surface of NPs (Figure S11), a phenomenon well-known for MCM-41 mesoporous silica which were reported to immobilize various proteins including enzymatically-active trypsins. After 48 h, NPs were collected by centrifugation, washed with water and analyzed via TEM and DLS (ZetaPALS Brookhaven). TEM micrographs confirmed the degradation of NPs into 20±15 nm nano-fragments (Figure 3C, see statistical analysis in Figure S11). Note that the low-hydrolitic degradation of phenylene-bearing silsesquioxane and the homogeneous oxamide distribution implied that the degradation occurring in the presence of proteins would be limited to oxamide moieties and produce few nanometer object of P-bridged organosilicas, which is consistent with our findings. In order to demonstrate (i) the trend of the NPs size decrease in the entire solution, and (ii) the key roles of oxamides and proteins, we performed multiple DLS measurements comparing MON and control NPs composed only of phenylene bridges (P PMO, Figure 4A). DLS measurements of degraded MON unequivocally displayed the sharp decrease of the hydrodynamic diameter of degraded objects (Figure 4B). Note that, slightly lower diameters were measured with the same trend with a Zetasizer Nano ZS Malvern instrument (Figure S13). The protein-mediated degradation was further demonstrated via a control involving the use of denatured trypsins (heated at 95 °C for 7 min), which did not induce a significant size change of NPs (Figure 4C). This result strongly suggests the enzymatic mechanism of the degradation. Besides, the necessary role of oxamides was validated by the absence of degradation of P PMO NPs (Figure 4D). Solid state NMR studies were carried out to investigate the structure of degraded NPs. First, the \(^{13}\)C CP-MAS spectrum of non-degraded MON displayed the typical carbon environments of the phenylene (134.9 ppm), propyl (18.4, 31.1, 43.9 ppm), and oxamide moieties (162.0 ppm, Figure S14). Degraded MON, however, displayed the appearance of the carboxylate carbon environment (173.2 ppm, Figures 4E, S15) generated by the protein-mediated cleavage. The \(^{29}\)Si CP-MAS spectra of MON and degraded MON were unchanged (Figure 4F), as expected from the stability of the siloxane network. Note that the absence of Q environments (~100 ppm) supports the silsesquioxane network, and validates the proposed degraded structure. Zeta potential analysis revealed a change reversal from -19 to +19 mV after degradation (Figure S16), which likely resulted from the complete cleavage of oxamides on the surface of NPs, hence generating two propyl-ammoniums per cleaved oxamide. This was also supported by the 13 to 17 cm⁻¹ \( \nu_{\text{C}sp^2\text{H}} \) shift in the FTIR spectrum (Figure S17). A control experiment involving a mixture of oxamide precursors with trypsin yielded the same shifts, hence supporting the protein role in the
decomposition of the oxamide group (Figure S18). Note that, fluorescein-grafted NPs (MON-FITC) could also be prepared (Figures S19-S21) and degraded in a similar manner (Figures S22-S23). The degradation percentages of MON and P PMO NPs were estimated by weighing the nanomaterials before and after 3 days of degradation, and were of 68 and 2 %, respectively (Figure S24).

The loading capacity of MON nanocarriers was then tested by encapsulating hydrophobic (CPT) and hydrophilic (DOX) drugs. We first loaded DOX (11 wt%) and CPT (31 wt%) into MSB NPs, as confirmed by UV-visible spectroscopy (Figures S25-S26). However, using more concentrated drug solutions (NP mass/Drug mass ratio of 1:2 instead of 1:0.5 or 1:1), we could reach unprecedented loadings in MON (mass of loaded drug/mass of loaded drugs+mass of NPs)*100) of 65 wt% of DOX, and 84 wt% of CPT (Figure S27, ESI). Higher payloads were obtained for MON than for P PMO (Figure 5A), with the absence of any premature leakage after 4 days (Figure S28) and without degradation after 1 month (Figure S29). Surprisingly, unlike DOX-loaded P PMO NPs, DOX was not released at pH 5.5 from MON (Figure S30). These observations suggest that oxamide-DOX interactions both enhanced the drug loading and prevented a pH-triggered release. Upon the initiation of the degradation, however, we observed the partial release of both drugs (Figures 5B, S31-S32). The MON internalization into A549 lung cancer cells was demonstrated via confocal images of incubated NPs loaded with non-membrane permeable propidium iodide nuclear staining dyes (Figures S33-S34). MON-FITC-Phosphonate NPs were also internalized (Figure S35). Finally, NPs were biocompatible at 50 µg mL⁻¹ in cells, and we obtained 65 to 85 % of cancer cell deaths via DOX- and CPT-loaded NPs respectively (Figure 5D).

**Experimental Section**

**Synthesis of MON.** The synthesis was performed according to a modified procedure of Croissant et al. A mixture of CTAB (250 mg, 6.86 10⁻¹ mmol), distilled water (120 mL), and sodium hydroxide (875 µL, 2 M) was stirred at 80 °C during 50 minutes at 700 rpm in a
The sample was collected and centrifuged, washed three times with ethanol, water, and ethanol. Each washing was followed by centrifugation collection in propylene tubes during 15 minutes at 21 k rpm. The as-prepared CTAB-free oxime-phenylene MON nanomaterial was dried under vacuum for few hours.

Synthesis of MON-FITC and MON-FITC-Phosphonate NPs.

Following the synthesis of MON (120 mL, non-extracted NPs), fluorescein alkoxysilane (70 µL in EtOH, 1.1 10⁻¹ mol) and 3-triethoxysilylpropyl methylphosphonate for MON-FITC-Phosphonate, 70 µL, 3.7 10⁻¹ mol) was added and the solution was stirred 20 minutes at 70 °C. Afterwards, the solution was cooled to room temperature while stirring; and the sample was collected and extracted as described for MON.

MON degradation with trypsin.

MON (1 mg) were dispersed in PBS (0.75 mL) in an Eppendorf tube at pH 7.4, trypsin was added (0.25%, 0.75 mL), and were stirred gently for 48 h. The final concentration of trypsin was 0.05 mM. The sample was then centrifuged, washed with deionized water, and dispersed in deionized water for TEM and DLS analyses.

Acknowledgements

The authors gratefully acknowledge King Abdullah University of Science and Technology and the University of California, Los Angeles for the support of this work.

Keywords: mesoporous organosilica nanoparticles, bridged silsesquioxanes, drug delivery, biodegradable, enzymes
Biodegradable mesoporous organosilica nanoparticles incorporating bio-responsive organic functions are described and degraded the first time in the presence of proteins. Such nanomaterials displayed exceptionally high drug loading capacities with a unique zero premature leakage without the need for pore capping, and were applied for drug delivery in cancer cells.

Jonas G. Croissant, Yevhen Fatieiev, Khachatur Jullfakyan, Jie Lu, Abdul-Hamid Emwas, Dalaver H. Anjum, Haneen Omar, Fuyuhiko Tamanoi, Jeffrey I. Zink, and Niveen M. Khashab.*