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A luminescent nanoporous hybrid material based drug delivery system showing excellent theranostics potential for cancer†

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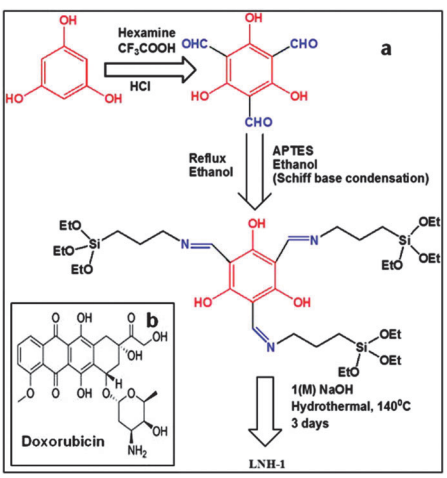
A novel hybrid nanoporous material (LNH-1) bearing a tris(propyliminomethyl)-phloroglucinol fluorescent moiety in the framework has been designed and administration of an LNH-1 based drug delivery system containing doxorubicin to cancer cells showed inhibition of proliferation, suggesting its future potential theranostics application in cancer.

Surfactant-templated synthesis of periodic mesoporous organosilicas (PMOs) has attracted widespread attention for over a decade due to the possibility of grafting a reactive organic functionality in the framework of the nanohybrids, which could lead to many frontline applications.[1] Various bridging organic moieties (R) present in the organosilica precursors [$(OR')_3Si–R–Si(OR')_3$] ranging from aliphatic to aromatic and heterocyclic make them useful for advanced applications, *e.g.* catalysis, gas and metal ion sensing, light harvesting, hole transporting, molecular motors and so on.[2] Although these mesoporous hybrid materials have been studied extensively, very little research has been focused on template-free synthesis of organic–inorganic hybrid nanoporous organosilicas[3] and their potential theranostics application especially in the treatment of cancer.[4] Moreover, the high surface area and internal void space present in the nanoporous material can make it an ideal medium as a drug delivery vehicle[5] and the luminescent framework in this material is highly desirable for the development of fluorescent labels.[6]

In this context, nanotechnology can play a pivotal role in the development of nanomaterials that can be used not only in cancer diagnostics but also in cancer therapeutics and cardiovascular related diseases.[7] Herein, we report a template-free new organic–inorganic luminescent nanoporous hybrid material (LNH-1) bearing an organic fluorophore tris(propyliminomethyl)-phloroglucinol (TPIM-P) moiety in the framework, which is synthesized through self-condensation of 100% organosilane moiety under alkaline pH conditions. Due to the presence of the strongly luminescent TPIM-P moiety inside the framework the material shows fluorescence properties in both lung and breast cancer cells, as observed using fluorescence microscopy. The biocompatibility of LNH-1 materials to both non-cancerous (HUVEC: human umbilical vein endothelial cells; CHO: Chinese hamster ovarian cells) and cancerous (A549: lung carcinoma cells; MCF-7: breast cancer cells) cells was observed by cell viability assay. Additionally, we have designed and fabricated a novel LNH-1 based drug delivery system (DDS) containing doxorubicin (DOX) (FDA approved drug) where LNH-1 acts as a delivery vehicle. The administration of this LNH-1 based DDS to A549 and MCF-7 cells shows excellent therapeutic efficacy compared to free DOX.

TPIM-P has been synthesized from the Schiff base condensation of 1,3,5-triformyl phloroglucinol (TFP) and 3-aminopropyltriethoxysilane (APTES). 1,3,5-TFP was obtained through Duff formylation of phloroglucinol and hexamine.[8] The synthesis of LNH-1 is described in Scheme 1. All the materials and methods, synthesis of Schiff base



Scheme 1 (a) Schematic representation of the synthesis of LNH-1 and (b) structure of doxorubicin.

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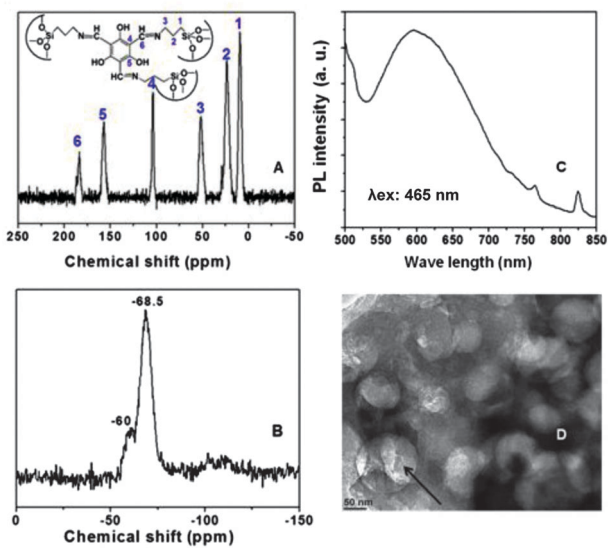

Fig. 1 ^{13}C (A) and ^{29}Si (B) MAS NMR spectra, (C) photoluminescence spectrum and (D) TEM image of LNH-1.

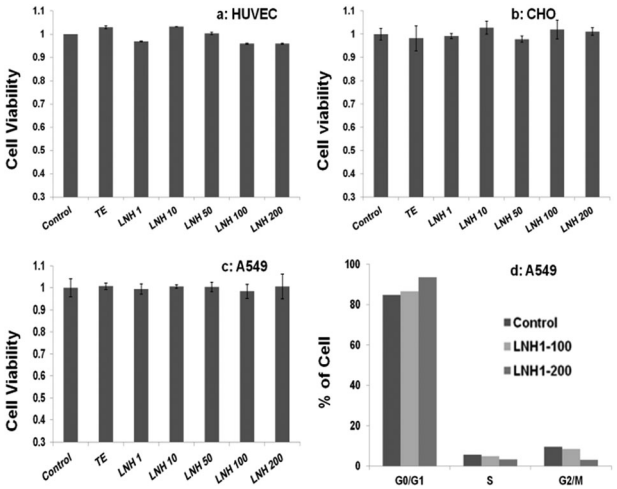

Fig. 2 Biocompatibility of LNH-1 in (a) HUVEC, (b) CHO, (c) A549 cells, and (d) cell-cycle analysis of A549 cells treated with LNH-1 (100–200 μg mL^{-1}).

and LNH-1, conjugation of LNH-1 with DOX and their detailed characterization along with several *in vitro* assays[9,10] are described in detail in the ESI.†

^{13}C and ^{29}Si MAS NMR spectra of LNH-1 are shown in Fig. 1A and B, respectively. The ^{13}C spectrum exhibits several peaks at 8.3, 23.3, 51, 103, 158, 183 ppm corresponding to the alkyl groups of APTES, the quaternary carbon of the phenyl ring attached to the imine CH═N– group, the phenyl ring carbon attached to the –OH group and the imine carbon of the Schiff base. These ^{13}C signals suggest the presence of the TPIM-P moiety in LNH-1 and ^{29}Si NMR exhibits a weak downfield chemical shift at −60 ppm and a very strong and sharp peak at −68.5 ppm, which could be assigned to T^2 and T^3 species.[11] Presence of the T state clearly signifies the complete hydrolysis of the organosilane moiety and absence of any Q states (Q^1–Q^4) ruled out any possibility of the breaking of Si–C bonds, suggesting the direct incorporation of the TPIM-P moiety in LNH-1. This finding clearly demonstrates the hybrid framework. The photoluminescence spectrum of LNH-1 (Fig. 1C) in the solid state at room temperature exhibits a strong red emission band at 595 nm upon excitation at 465 nm corresponding to the presence of the organic fluorophore moiety. In Fig. S1 (ESI†) the powder X-ray diffraction pattern has been shown where in addition to a strong sharp peak in the low 2θ region ($2\theta = 5.96$, $d = 1.53$ nm), a weak peak at 10.6 (d = 0.86 nm) has been observed. This diffraction pattern indicates a new class of porous architecture of LNH-1. The TEM analysis reveals that LNH-1 is composed of self-assembled hollow nanoparticles of dimensions *ca.* 100–150 nm (Fig. 1D). Fig. S2 (ESI†) shows the high magnification TEM image of the marked part of Fig. 1D. CHN analysis of LNH-1 revealed the presence of C: 36.6%, H: 5.12%, N: 7.07%, and FTIR (Fig. S3, ESI†) and UV-vis spectroscopic (Fig. S4, ESI†) analyses further suggested the presence of a fluorophore moiety in LNH-1. The N_2 adsorption–desorption isotherm of LNH-1 (Fig. S5, ESI†) suggested microporosity and its BET surface area was found to be 175 $m^2\ g^{-1}$. Furthermore, substantial entanglement at very high pressure with low hysteresis indicates intermolecular mesoporosity in LNH-1. The pore volume and the average pore diameter was found to be 0.899 cc g^{-1} and 4.5 nm respectively.

The cytotoxicity assay of new kind of materials for any cell is the basic and preliminary criteria for biomedical application. Therefore, in order to investigate the cytotoxicity level of LNH-1, MTT assay was carried out for both non-cancerous (HUVEC: Fig. 2a; CHO: Fig. 2b) and cancerous (A549: Fig. 2c; MCF-7: Fig. S6, ESI†) cells. Results show that the LNH-1 material is highly viable up to 200 μg mL^{-1} for both normal and cancer cells. The biocompatibility of LNH-1 has further been supported by the cell cycle analysis (Fig. 2d) in A549 cells treated with the LNH-1 material where the G2/M phase decreases in a dose dependent fashion indicating that this material is not cytotoxic toward A549 cells. So, the LNH-1 material can be used as a drug delivery vehicle as it is biocompatible in nature. Therefore, we have designed a LNH-1 based DDS containing the anticancer drug doxorubicin (LNH-DOX). Comparing the TGA analyses of LNH-1 (Fig. S7a, ESI†) and LNH-DOX (Fig. S7b, ESI†), we have found that 5.9 wt% binding of DOX in LNH-DOX corresponds to 1.4×10^{15} DOX molecules per mg of LNH-DOX powder. Again, in CHN elemental analysis, 2.51% of C, 0.32% of H and 0.74% of N are increased in LNH-DOX from LNH-1, indicating the binding of DOX. The exact bonding nature of DOX to LNH-1 is unknown at this moment and needs further investigation, which is beyond the scope of our present study. However, it may be electrostatic, covalent, co-ordinate, H-bonding or a combination of them. DLS study shows that all the zeta potential values of LNH-1, DOX and LNH-DOX are positive (5.1 mV, 12.5 mV and 16.9 mV respectively) (Table S1, ESI†), indicating the absence of any electrostatic interaction between LNH-1 and DOX. According to the earlier literature[12–15] we can speculate that the nature of bonding may be covalent, co-ordinate, or H-bonding as DOX molecules contain –OH, $–NH_2$ and ═C═O functional groups and LNH-1 contains –OH, and –N═ functional groups.[12,16,17] Whatever, the weight loss in the TGA profile (Fig. S7a and b, ESI†) indicates the bonding of DOX to LNH-1. ^{13}C CPMAS NMR of LNH-1 and LNH-DOX further supports the bonding interaction between LNH-1 and DOX (Fig. S8, ESI†). The release kinetics study of DOX (Fig. 3a) from LNH-DOX shows the slow release of DOX in DPBS buffer indicating the stability of the nanoconjugate,

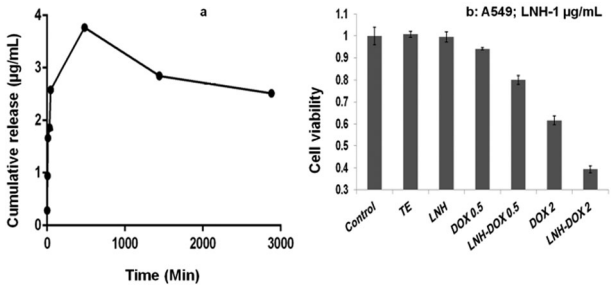

Fig. 3 (a) *In vitro* release kinetics of DOX loaded with LNH-1 materials. (b) The application of LNH-1 as a delivery vehicle in A549 cells where the anticancer drug DOX was attached to LNH-1.

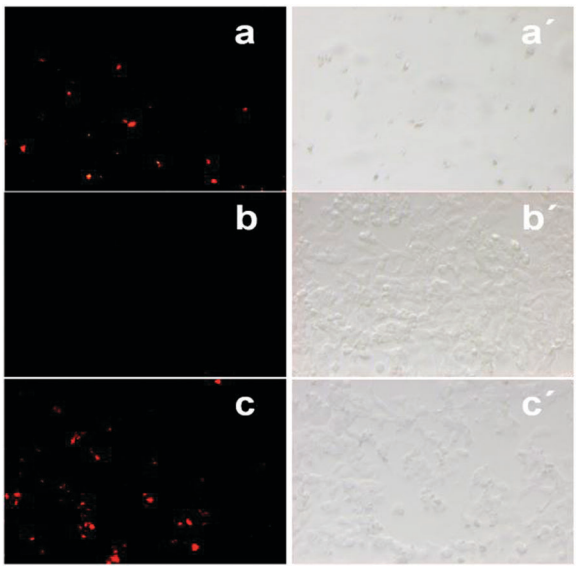

Fig. 4 Fluorescence (a–c) and its corresponding phase images (a′–c′) of LNH-1 nanoparticles (a–a′), untreated MCF-7 cells (b–b′) and MCF-7 cells treated with LNH-1 (c–c′).

which is important for drug delivery. Further *in vitro* stability study (Fig. S9, ESI†) shows a high degree of stability of LNH-1 and LNH-DOX in different biological matrices. Finally, administration of the DDS (LNH-1 μg mL^{-1} DOX) to A549 cells (Fig. 3b) shows significant inhibition of proliferation in a dose dependent manner (w.r.t DOX) (see ESI† for detailed preparation). LNH-50 μg mL^{-1} DOX and LNH-200 μg mL^{-1} DOX also show a similar effect in A549 cells (Fig. S10a and b, ESI†). Similar results were also found in MCF-7 cells (Fig. S11a–c, ESI†). In order to investigate the selective targeting of LNH-1 to the cells, the transfection efficiency of the LNH-1 material towards both cancerous and non-cancerous cells has been studied using fluorescence microscopy. Fig. 4a and a′ show the red fluorescence image (a) and its corresponding phase image (a′) of LNH-1 nanoparticles, respectively. However, we cannot observe any red fluorescence for untreated MCF-7 cells (Fig. 4b), indicating the absence of LNH-1. Fig. 4c and c′ indicate the fluorescence of MCF-7 cells treated with LNH-1 and its corresponding phase image, respectively. The presence of red fluorescence in MCF-7 cells (Fig. 4c and more images in Fig. S12, ESI†) even after extensive washing with DPBS indicates the fluorescence properties of LNH-1 nanoparticles inside the cells. Similarly, we have observed the fluorescence properties of LNH-1 in A549 cells (Fig. S13, ESI†) whereas non-cancerous CHO cells (Fig. S14, ESI†) show very little fluorescence. The results indicate that cells treated with LNH-1 show a higher transfection efficiency of LNH-1 towards cancer cells rather than towards normal cells. Finally these results indirectly prove that the LNH-1 based drug delivery system can selectively target cancerous cells.

In summary, the new luminescent nanoporous hybrid material LNH-1 reported herein is biocompatible and administration of the LNH-1 based DDS containing DOX shows significant inhibition of proliferation in lung and breast cancer cells compared to free DOX. Additionally, *in vitro* fluorescence properties of LNH-1 suggest that this technique might be used for *in vitro* imaging. The results altogether indirectly indicate that the LNH-1 based drug delivery system can selectively target cancerous cells compared to normal cells and the DDS can be useful for future theranostics application in cancer.

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Notes and references

1 S. Inagaki, S. Guan, Y. Fukushima, T. Ohsuna and O. Terasaki, *J. Am. Chem. Soc.*, 1999, **121**, 9611.
2 (*a*) D. Chandra, T. Yokoi, T. Tatsumi and A. Bhaumik, *Chem. Mater.*, 2007, **19**, 5347; (*b*) N. Mizoshita, Y. Goto, Y. Maegawa, T. Tani and S. Inagaki, *Chem. Mater.*, 2010, **22**(8), 2548; (*c*) J. Y. Shi, C. A. Wang, Z. J. Li, Q. Wang, Y. Zhang and W. Wang, *Chem.–Eur. J.*, 2011, **17**, 6206; (*d*) F. X. Zhu, W. Wang and H. X. J. Li, *J. Am. Chem. Soc.*, 2011, **133**, 11632; (*e*) T. Seki, K. McEleney and C. M. Crudden, *Chem. Commun.*, 2012, **48**, 6369.
3 U. Diaz, J. A. Vidal-Moya and A. Corma, *Microporous Mesoporous Mater.*, 2006, **93**, 180.
4 M. Vallet-Regí, M. Colilla and B. González, *Chem. Soc. Rev.*, 2011, **40**, 596.
5 (*a*) S. K. Das, M. K. Bhunia, D. Chakraborty, A. R. Khuda-Bukhsh and A. Bhaumik, *Chem. Commun.*, 2012, **48**, 2891; (*b*) W. R. Zhao, H. T. Zhang, S. Chang, J. L. Gu, Y. S. Li, L. Li and J. L. Shi, *RSC Adv.*, 2012, **2**, 5105.
6 (*a*) A. M. Sauer, A. Schlossbauer, N. Ruthardt, V. Cauda, T. Bein and C. Braeuchle, *Nano Lett.*, 2010, **10**, 3684; (*b*) Q. J. He, Y. Gao, L. X. Zhang, Z. W. Zhang, F. Gao, X. F. Ji, Y. P. Li and J. L. Shi, *Biomaterials*, 2011, **32**, 7711; (*c*) H. Q. Zheng and S. Che, *RSC Adv.*, 2012, **2**, 4421.
7 (*a*) P. Alivisatos, *Nat. Biotechnol.*, 2004, **22**, 47; (*b*) C. R. Patra, J.-H. Kim, K. Pramanik, L. V. d'Uscio, S. Patra, Z. S. Katusic, R. Ramchandran, M. S. Strano and D. Mukhopadhyay, *Nano Lett.*, 2011, **11**, 4932; (*c*) C. R. Patra, R. Bhattacharya, D. Mukhopadhyay and P. Mukherjee, *Adv. Drug Delivery Rev.*, 2010, **62**, 346; (*d*) C. R. Patra, R. Bhattacharya, S. Patra, N. E. Vlahakis, A. Gabashvili, Y. Koltypin, A. Gedanken, P. Mukherjee and D. Mukhopadhyay, *Adv. Mater.*, 2008, **20**, 753.
8 C. V. Yelamaggad, A. S. Achalkumar, D. S. S. Rao and S. K. Prasad, *J. Org. Chem.*, 2009, **74**, 3168.
9 L. Wang, J. S. Lau, C. R. Patra, Y. Cao, S. Bhattacharya, S. Dutta, D. Nandy, E. Wang, C. N. Rupasinghe, P. Vohra, M. R. Speller and D. Mukhopadhyay, *Mol. Cancer Res.*, 2010, **8**, 1591.
10 N. T. Patil, P. G. V. V. Lakshmi, B. Sridhar, S. Patra, M. P. Bhadra and C. R. Patra, *Eur. J. Org. Chem.*, 2012, 1790.
11 A. Modak, J. Mondal, V. K. Aswal and A. Bhaumik, *J. Mater. Chem.*, 2010, **20**, 8099.
12 X. Chao, Z. Zhang, L. Guo, J. Zhu, M. Peng, A. J. M. Vermorken, W. J. M. Van de Ven, C. Chen and Y. Cui1, *PLoS One*, 2012, **7**, e40388.
13 V. Venkatpurwara, A. Shirasb and V. Pokharkar, *Int. J. Pharm.*, 2011, **409**, 314.
14 S. Mukherjee, V. Sushma, S. Patra, A. K. Barui, M. P. Bhadra, B. Sreedhar and C. R. Patra, *Nanotechnology*, 2012, **23**, 455103.
15 R. Bhattacharya, C. R. Patra, A. Earl, S. Wang, A. Katarya, L. Lu, J. N. Kizhakkedathu, M. J. Yaszemski, P. R. Greipp, D. Mukhopadhyay and P. Mukherjee, *Nanomedicine*, 2007, **3**, 224.
16 H. R. Ihre, O. L. P. D. Jesús, F. C. Szoka, Jr. and J. M. J. Fréchet, *Bioconjugate Chem.*, 2002, **13**, 443.
17 H. S. Yoo, K. H. Lee, J. E. Oh and T. G. Park, *J. Controlled Release*, 2000, **68**, 419.

