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Synthesis, Toxicology and Potential of Ordered Mesoporous Materials in Nanomedicine

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Synthesis, toxicology and potential of ordered mesoporous materials in nanomedicine

Although ordered mesoporous silica materials have been studied for almost 20 years, their utilization within life science applications is relatively new and unexplored. An increasing number of researchers are transcending their respective fields in order to bridge the knowledge gap between materials chemistry and biotechnology, and to exploit the potential of mesoporous materials. Their intricate porosity with order in the nanoscale translates into high surface areas above 1000 m^2/g, high selectivity for the encapsulation of biorelevant molecules as well as controlled surface chemistry. Their uses in pharmaceutics to improve drug formulation, drug bioavailability, mitigate drug toxicity and in cellular targeting, through controlled drug delivery strategies, have been shown. The incorporation of a high concentration of fluorescent and nuclear markers within their pores, whilst retaining good diffusion through their porous matrix, has shown them to be ideal candidates for sensing devices, in immunoassays such as flow cytometry and for their use in novel theranostic applications. This article aims to bring to the forefront some of the most important properties of mesoporous materials, which prove advantageous for their use in nanomedical applications and to highlight some of the potential areas into which the field may now emerge.

KEYWORDS: cellular targeting ■ drug delivery ■ excipients ■ formulation ■ mesoporous materials ■ silica ■ theranostics

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Throughout nature there are numerous examples of the advantages of porous materials versus nonporous, and how these confer more efficient functions to a particular process. Irrespective of the material composition, porosity results in a noticeable increase in surface area with a direct effect in the contact of that surface with its surroundings, which may translate to higher reactivity and/or efficiency of the surface [1]. If the porosity of the material is ordered then selectivity can also be inferred. The intricate porosity of the lungs, the selectivity of callus bone, or the structured surface observed in mitochondrial cristae [2] are but a few examples, of how surface and porosity affect living systems at different length scales. It is hence not surprising that numerous researchers find this concept of great inspiration in order to design synthetic materials with high surface areas and controlled porosity.

Ordered mesoporous materials illustrate this. Discovered in 1992 they represent a family of nanostructured particles characterized by ordered pores or cages in the meso-length scale – that is, between 2 and 50 nm [3]. Their preparation relies on the combination of two well-established fields; surfactant and sol-gel chemistry. The general synthetic concept involves the use of an organic template. Typically, a micelle forming lyotropic liquid crystal [4] is assembled in an aqueous solution. To this, a metal oxide precursor is added, which will subsequently form the inorganic wall of the mesoporous material. If silicon oxide (silica) is desired as the final composition then an alkoxysilane precursor may be used. From the resulting synthesis gel, colloidal silica particles grow surrounding the self-assembled organic template. After a suitable condensation period the particles may be filtered and mesoporosity obtained by removal of the organic template by calcination at high temperatures or extracting it using a suitable solvent, such as ethanol. The resulting structure and porosity is a replica of the assembled organic template in inorganic silica. Hence, if a 3D cubic mesophase assembly of rod type micelles is achieved in the organic template then a 3D cubic cylindrical pore structure will be formed after removal of the template. Figure 1 shows transmission electron microscopy images showing the similarities between mesoscale order in mitochondrial cristae, liquid crystals [5] and mesoporous materials, and summarizes the general synthetic concept behind mesoporous materials.

The design, synthesis, structural and surface characteristics of ordered mesoporous materials have been studied in depth over the recent decade, demonstrating the versatility of the surfactant templating approach and the effectiveness of mesoporous materials in applications

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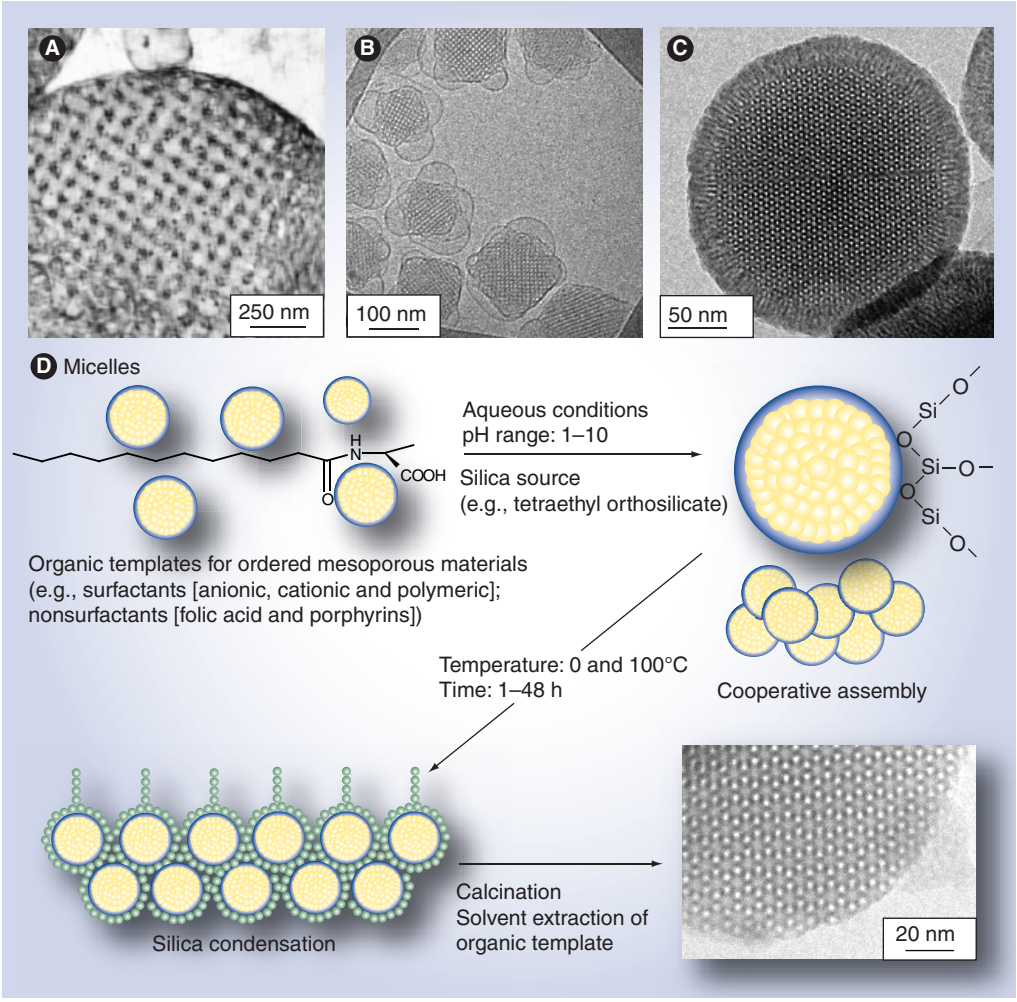

Figure 1. Mesostructures in nature and by design. (A) Electron microscopy image recorded of an amoeba mitochondrion. **(B)** Electron microscopy image of micellar cubic particles, cubosomes, prepared with mixtures of anionic and polymeric surfactants. **(C)** Electron microscopy image of ordered mesoporous silica particle with 3D cubic porous structure prepared using anionic surfactant and recorded prior to removal of the surfactant by calcination. **(D)** Main synthetic steps for the preparation of ordered mesoporous materials including: preparation of pore templating agents; cooperative assembly between organic template and silica; silica condensation and particle growth, followed by template removal.
(A) Reproduced from [2]; **(B)** reproduced from [3].

such as catalysis, sensors, as gas adsorbents and in separation media [6–9]. The fundamental properties of mesoporous materials can in general be summarized as: high surface areas over 1000 m^2/g, large pore volumes, sharp pore size distributions (as measured through sorption studies), chemically and thermally stable compositions, and tailored functionalized surfaces with controllable particle sizes and shapes. It is only recently that the fundamental properties of mesoporous silica materials have been discovered and applied to biomedical research, enabling us to:

- Load large pay loads of single or multiple active molecules within their pores [10];
- Tailor the pharmacokinetic release profiles as drug-delivery vehicles through diffusion-based or other mechanisms [11];
- Target the release of pharmaceutical products to specific cell types [10];
- Form nontoxic biocompatible or biodegradable composition [12];
- Increase the bioavailability of pH-sensitive drug candidates [10];
- Enhance the solubility of hydrophobic pharmaceutical actives with high partition constants [10];
- Act as adjuvants and/or agents in immunotherapies [13];

- Act as diagnostic and theranostic particles [14];
- Enhance the growth of apatite layers in tissue generation and bone implants [15].

Whilst mesoporous silica materials share the same composition as colloidal or amorphous silica, their high surface areas and its direct effect on surface properties warrants an in-depth assessment of their *in vitro* and *in vivo* toxicological behavior, *de novo*. Furthermore, the need of a devoted interdisciplinary team in order to assess the immunological and pharmacokinetic properties has been one of the major challenges in bringing these materials to the medical field. Unfortunately, up to now this has limited their study to a few interdisciplinary research teams.

Mesoporous materials offer several advantages versus organic (polymeric) based materials, such as dendrimers, monodisperse polystyrene and polymethylmethacrylate spheres, or liposomes. The fundamental advantage comes from the use of inorganic surfaces, which are: compatible with hydrophobic and hydrophilic solvents through facile functionalization, thermally and chemically stable, and do not require complex purification steps in their synthesis. Furthermore, their high and ordered porosity and large internal pore volumes (above 1.00 cm^3/g) result in large loaded amounts of active drugs within their pores, a feature that limits the use of liposomes and dendrimers. The internal and external surfaces of mesoporous materials can be tailored independently, allowing them to conjugate groups with multiple functions, without affecting the materials drug loading and release properties.

Despite the obvious advantages of mesoporous materials versus other known pharmaceutical excipients or diagnostic particles, a whole new set of physicochemical and other problems are encountered when applying mesoporous particles to medical applications. Colloidal stability in a variety of biomedical dispersions, optimization of administration routes, regulatory issues, processing and economic considerations are but a few of the barriers that mesoporous materials face before their use is cemented within the nanomedical field.

Whilst highlighting some of the most relevant properties of mesoporous materials in the area of nanomedicine, this article also aims to give the reader a brief background of the variety of structures and compositions that can easily be prepared. In addition, the author aims to bring to the forefront the most innovative applied studies within the area of nanomedicine in the hope of shining some light towards where the field is heading, and where some obvious barriers exist for the implementation of these materials.

Strategies for the preparation of mesoporous particles

The discovery of mesoporous material has been assigned to two research groups who almost simultaneously developed different methods for their preparation. However, it is without doubt that one of these procedures has proven to be the most facile, versatile and most widely studied. Kuroda *et al.* developed the so-called folded sheet mechanism for the preparation of mesoporous materials [16]. Relying on the use of the mineral kanemite as a silica source, their approach relies on the infiltration of surfactants between kanemite sheets, followed by the thermal re-arrangement of these to form hexagonally ordered pores. The alternate and most versatile synthesis relies on the cooperative self-assembly of amphiphilic surfactants together with an alkoxysilane silica source under aqueous conditions at pH values between 0–2 and 8–12 – that is, below or well above the isoelectric point of silica (1.7–3.5) [17]. In order to control the pH of the synthesis sodium hydroxide, organic amines, hydrochloric or sulfuric acids are typically employed, which in turn catalyze the hydrolysis of the alkoxysilane source, typically tetraethyl orthosilicate [17]. Other alkoxide sources may be used for other metal oxide compositions, such as aluminium-tri-sec butoxide, titanium (IV) butoxide and so on [18]. The use of a combination of metal oxide precursors, such as a metal alkoxide together with its salt, has been shown to extend the composition of ordered mesoporous materials to numerous metal oxides, metal phosphates, metal borates and mixed metal oxide compositions [19].

Zhao *et al.* have recently reviewed the main synthetic strategies for the preparation of ordered mesoporous silica materials under a variety of conditions using cationic, anionic and neutral polymeric amphiphilic surfactants [7]. The preparation and conditions used depend largely on the amphiphilic surfactant's physicochemical properties, as well as the silica source used. Whilst the critical micellar concentration of the amphiphile is important, it is now widely accepted that ordered mesoporous silica materials can be obtained by using surfactant concentrations below the critical micellar concentration [20], giving rise to the notion of a cooperative assembly aided by the hydrolysis and condensation of the silica source. In general, the use of neutral polymeric surfactants, based

on tri-block copolymers such as P123 [21] – that is, polyethylene glycol (PEG)-polypropylene glycol-PEG; $(CH_2CH_2O)_{20}(CH_2CH[CH_3]O)_{70}(CH_2CH_2O)_{20}H$ – afford well-ordered structures with larger pores than mesoporous materials prepared with cationic (e.g., cetyl trimethylammonium bromide; $C_{16}H_{33}NCH_3Br$; CTAB) [22] or anionic surfactants (e.g., palmitic acid; $CH_3[CH_2]_{14}COOH$) [23]. This is due to the larger micelles formed by the larger molecular weight polymeric surfactants.

A large variety of surfactant types exist and numerous mesoporous structures have been prepared with all **subfamilies of amphiphilic surfactants**. Of noteworthy mention are the amino acid-derived surfactants, such as *N*-lauroyl-alanine ($C_{15}H_{29}NO_3$) [24], which are nontoxic amphiphiles commonly utilized in the cosmetic industry due to their excellent physicochemical, nonirritant, nontoxic properties [25]. In addition, amino acid-based surfactants have been shown to display a rich mesostructure diversity with an increasing number of structures being reported, some of which have not been observed in cationic or polymeric surfactant synthesis. The use of anionic surfactants requires the aid of a co-structure directing agent (CSDA) to control the pH of the synthesis as well as to facilitate the electrostatic interaction between the anionic headgroup and the growing positively charged silica species under basic conditions. A common CSDA used is aminopropyl triethoxysilane ($H_2N[CH_2]_3Si[OC_2H_5]_3$), which allows us to functionalize the internal mesopore surface with propyl-amine groups by simple extraction of the surfactant template and their use as sites for further conjugation [26].

Overall, to the best of the author's knowledge, mesoporous structures with some 15 different space group symmetries have been reported. These include lamellar [3], cylindrical pore 2D hexagonal [3], cylindrical pore 2D rectangular [27], cylindrical pore 3D hexagonal structures [28,29], cylindrical pore 3D cubic structures [3,30], cage-type 3D cubic structures [31–33], 3D orthorhombic [34], and 3D tetragonal structures [34,35]. **Figure 2 & Table 1** show representative unit cell models of the surface topology of some of these structures as well some structural details of mesoporous materials.

Recently, a novel approach involving a non-surfactant template has been employed for the synthesis of ordered mesoporous silica materials. The author and colleagues prepared ordered mesoporous silica particles with 2D hexagonal pore structures, termed nanoporous folic acid material (NFM)-1 taking advantage of the self-assembly properties of folic acid [36]. Folates and numerous folate derivatives are capable of forming hexagonal liquid crystal mesophases via the formation of planar hydrogen-bonded tetramers, themselves interacting via π–π stacking interactions into hexagonal ordered rods. The self-assembly occurs in such a way that the glutamate group of the molecule remain exposed towards the exterior of the stack, which enables interaction of this with a suitable silica source under a narrow pH range (approximately between 7 and 9.5). The synthesis requires the use of a CSDA, as in the case of anionic surfactants, to maximize the interaction between the template and the silica wall. This route replaces the surfactant by a functional molecule, folic acid, which is electrostatically bound to the mesoporous wall. The folate stacks, which have been found to arrange as chiral stacks within the pores, are released from the pores at different rates depending on the morphology of the material [37]. Folic acid and folate derivatives have been widely used as targeting agents in cancer therapeutics due to the expression of increased amounts of folate receptors by certain tumor cells. NFM-1 materials (**Figure 3**) are promising candidates for the combined delivery of a targeting agent and pharmaceutical drug since a large amount of drug candidates are known to interact via π–π stacking with folate receptors and may be loaded into mesoporous materials directly during the synthesis of the material. In a similar approach Che *et al.*, have reported the synthesis of ordered mesoporous materials using other functional porphyrins as the template [38].

Properties of mesoporous materials relevant for nanomedicine

Mesoporous materials with extremely large pore volumes up to 3.3 cm^3/g have been reported [39]. The accessibility of this internal pore space for loading different functional molecules together with the reproducibility and specificity imparted by the sharp pore size distributions is arguably the biggest advantage of mesoporous materials. Pore size distributions are easily tailored, either through a variation in surfactant template, conditions or the use of swelling agents [40]. Ordered mesoporous materials are indeed contributing to developments of gas adsorption and electron crystallography analytical tools due to their structured pores and hence are an excellent modeling tool for physicochemical effects that occur in the mesoscale, in a variety of relevant techniques [31,41–43].

Mesoporous surfaces may be functionalized both directly during the synthesis and postsynthetically after removal of the surfactant. Direct synthesis methods, unless a CSDA is employed in the synthesis mechanism, typically afford limited coverage of functional groups after removal of the surfactant and often lead to disordered materials with pore blocking; however, under some conditions levels of up to 2.3 mmol g^{-1} have been reported for large pore 2D cylindrical hexagonal systems (structural name SBA-15) functionalized with propyl amine groups [44]. Using a CSDA, Che *et al.* have produced ordered cubic cage type structures with up to 4.3 mmol g^{-1} [24]. Due to a direct interaction between the CSDA and the surfactant/template headgroup this method of functionalization ensures a maximum number of groups within the internal porosity, without pore blocking or the reduction of diffusion properties through the porous structure. Increased amounts of CSDA added directly to the synthesis may result in mesophase transformations due to changes in pH or neutralization of the surfactant headgroup and its effect on the overall curvature of the surfactant and its packing parameter [45]. Postsynthetic methods of functionalization are versatile. Alkoxysilane chemistry has been exploited to include a library of conjugating groups that may be tethered on the internal or external surfaces, including amines, carboxylic acids, thiols groups and PEG, amongst others [46–50]. In general, postsynthetic methods are more difficult to control if larger amounts of functional groups or material quantities are required, and may lead to pore blocking of pore entrances. Mesoporous surfaces may be rendered hydrophobic through the use of silylating agents on their high internal pore surfaces, ensuring a high coverage of lipophilic methyl groups.

Applications in drug formulation & as drug-delivery vehicles

There is a clear need to develop new excipient technologies to meet the formulation challenges associated with drug discovery and repositioning of pharmaceutical actives. Poorly soluble compounds offer a poignant problem, and their dissolution in suitable media for simple toxicology studies is a major reason for the rejection of lead compounds even before clinical trials. The improvement of bioavailability and subsequent increases in potency or selectivity, the mitigation of toxicity, improving patient compliance through more patient-friendly administration routes or reduce number of doses, improving drug stability or reducing first pass effects are some of the important reasons that drive research within new drug-delivery vehicles. Loading protocols and techniques to characterize loaded materials have been recently reviewed by Vallet-Regi [51], who pioneered the field of pharmaceutical drug release using mesoporous materials [52]. Hence, numerous pharmaceutical actives have been loaded into calcined or extracted, naked or functionalized mesoporous particles with different pore structures and pore sizes. There has been strong focus on the improvement of pharmacokinetic properties of poorly water soluble drugs with large partition constants (with logP larger than 3) [53]. Augustijns *et al.* have demonstrated *in vivo* under supersaturating conditions that drug performance (bioavailability) could be improved with decreasing pore size [54]. Presumably this is due to a slower rate

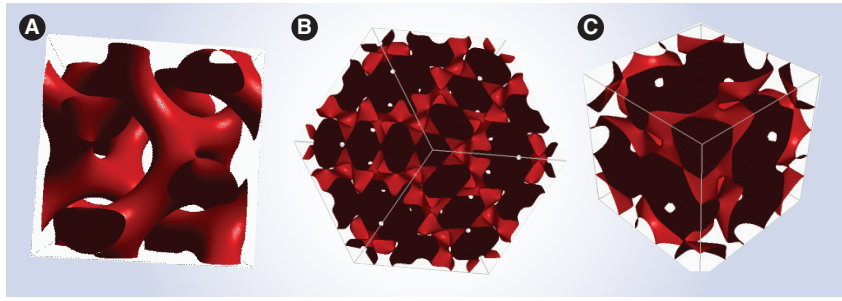

Figure 2. Unit cell models of the pore surface of three mesostructures that may be prepared with surfactants. (A) Cylindrical 3D-cubic *Ia3d*, **(B)** cage-type 3D cubic *Fd3m* and **(C)** cage-type 3D cubic *Pm3n* space group symmetries.

Table 1. Structural range of ordered mesoporous materials.

	Crystal class	Space group symmetry	Structural examples	Ref.
Lamellar	–	–	MCM-50	[3]
2D cylindrical	Hexagonal	*p6mm*	MCM-41	[3]
	Rectangular	*c2mm*	SBA-8	[27]
3D cylindrical	Hexagonal	$P6_3/mcm$	IBN-9	[28]
	Cubic	*Ia3d*	MCM-48	[3]
		Pn3m	AMS-10	[30]
3D cage	Hexagonal	$P6_3/mmc$	SBA-2	[29]
	Cubic	*Pm3n*	SBA-1	[31]
		Fm3m	KIT-5	[33]
		Im3m	SBA-16	[31]
		Fd3m	AMS-8	[32]
	Orthorhombic	*Pmmm*	FDU-13	[34]
	Tetragonal	*P4/mmm*	FDU-11	[34]
		$P4_2/mmc$	AMS-9	[35]

Note that the nomenclature for mesoporous structures has not been determined and example codes typically refer to the institution where the materials were first prepared, or to an acronym representing the synthesis mechanism (e.g., AMS: Anionic mesoporous silica; MCM: Mobile composition of matter).

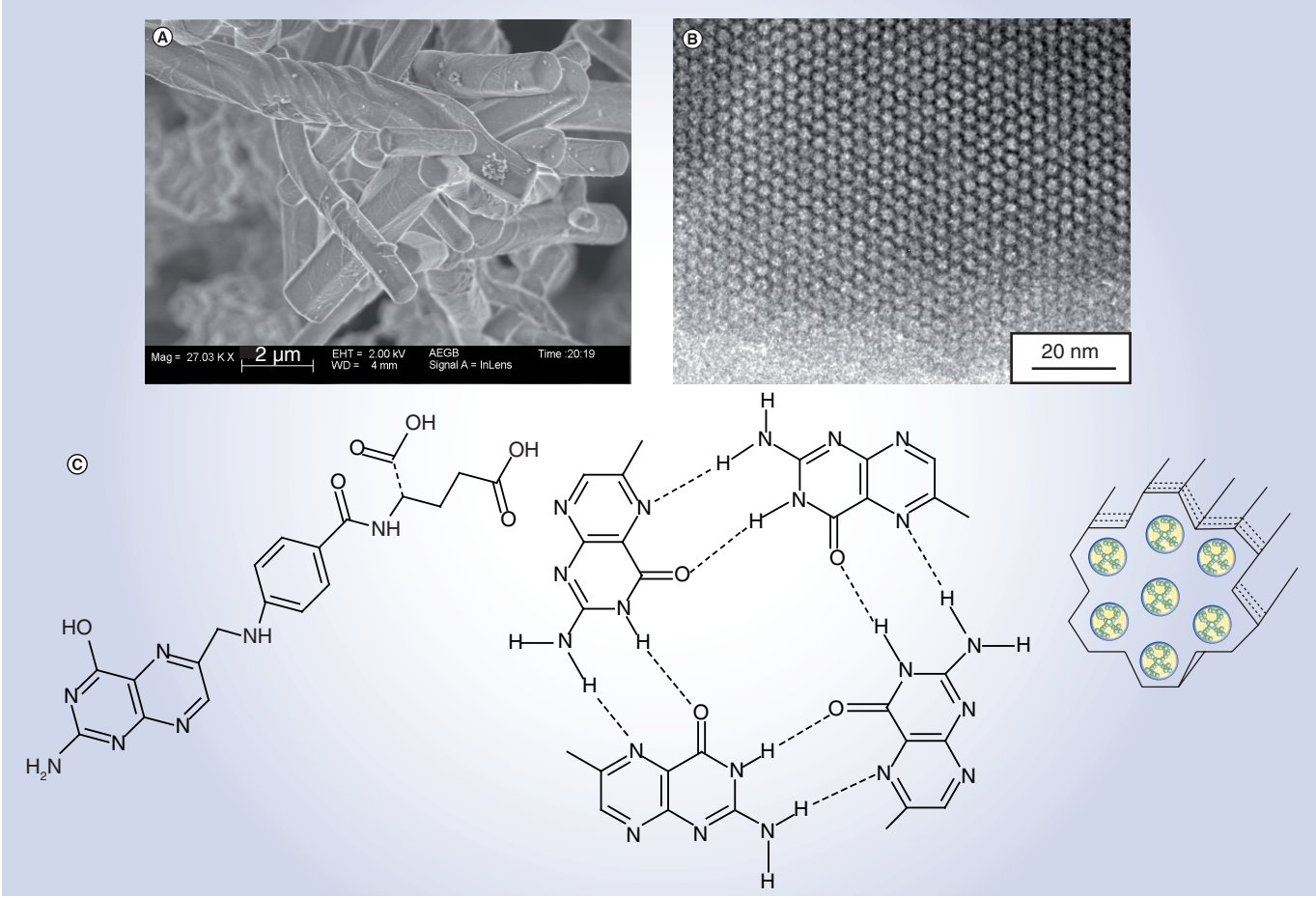

Figure 3. Properties of ordered mesoporous material NFM-1 prepared with folic acid as template. (A) SEM image of chiral motives in NFM-1 particles. **(B)** TEM image showing the hexagonal arrangement of mesoporous in NFM-1. **(C)** The chemical structure of folic acid (left) and its hydrogen bonding tetramer arrangement (only pterine groups are shown) via hydrogen bonding interactions (middle) and how these are stacked within the mesopores (right).
SEM: Scanning electron microscopy, TEM: Transmission electron microscopy.

of drug released from drug-loaded materials with smaller pores size, a result correlated from other studies [55].

Several reliable strategies have emerged to tailor the release kinetics of pharmaceutical drugs, DNA [56], siRNA [57], nutraceuticals, and growth factors from the internal pore space of mesoporous materials. These include structural variations, variations in the pore diameter, electrostatic or covalent interactions between the wall and the drug [58], functionalization of the internal pore space [59], particle PEGylation [60], and external functionalization of the pore entrances [61]. A change in pore structure alone from 2D to 3D pore connectivity was observed to vary the diffusion properties of model drugs from the internal pore space by several orders of magnitude [62]. The desire to tailor release properties via an external (e.g., optical response or magnetic field) or internal stimuli (e.g., gastric changes in pH) has led to several interesting pore gatekeeping strategies [63]. These include the use of photo-switching molecules that release the contents of the pores upon the use of certain wavelengths (e.g., coumarin at 310 nm) [64–66]. Furthermore, intelligent drug delivery vehicles based on mesoporous silica particles that respond *in situ* to changes in blood plasma composition of certain disease markers have already been achieved and represent the next wave of drug-delivery technologies. Lin *et al.*, have demonstrated how release of insulin from mesoporous particles can be regulated in such a fashion in response to saccharide triggers [67]. Finally, some considerable advances have been made in the area of targeted drug delivery. This is of particular importance in oncology where avoiding release of high potency chemotherapeutic agents is necessary in order to mitigate undesired off-target secondary effects, achieve higher efficiencies and reduce dosage. It is well known that nanoparticles undergo an enhanced permeability and retention effect and few studies have focused on this issue with respect to nanoparticles. The

ease of functionalization and compatible surface chemistry allows them to conjugate to the external surfaces of mesoporous particles with cell-targeting conjugates such as folates. Passive and active cellular targeting strategies utilizing nanoparticles have recently been reviewed by Garcia-Bennett and colleagues [68]. In a landmark paper Tamanoi *et al.* [69] reported *in vitro* and *in vivo* results on the cellular targeting abilities of naked mesoporous silica particles, camptothecin-loaded particles of the same, as well as folate-conjugated drug-loaded particles. Their results indicate a strong correlation between tumor volume decrease for drug-loaded mesoporous particles after 48 days of inoculation. This was not observed for the naked particles despite the observation of a certain amount of particle accumulation within tumors after 24 h postinjection (110 ng mg^{-1}). By contrast, folate-conjugated particles showed an accumulation of 170 ng mg^{-1}, indicating a possible accumulation via a passive (enhanced permeability and retention) mechanism as well as an enhanced particle uptake mediated via folate receptors. These results correlate well with other *in vitro* reports demonstrating the efficacy of targeting strategies using DNA aptamers, proteins and antibodies, amongst others [64].

Applications in diagnostics/theranostics

The flexibility in designing the structural, textural (porous) and surface properties of mesoporous materials allows one to apply multiple functions to the internal pores/surface of the solid, and separate functions to the external surface. Linden *et al.*, have demonstrated that functionalization of the external surfaces can be achieved preferentially by the use of alkoxysilanes (and further PEG) on the as-synthesised materials – that is, containing the unremoved surfactant template [65,53]. This is a simple strategy that does not eliminate the possibility of functionalization of the internal pore space or indeed its loading with pharmaceutical drugs, as well as nanoparticles with fluorescent or magnetic properties [70]. The use of mesoporous materials as multifunctional devices opens their use within theranostic applications (therapeutics plus diagnostics). Almost simultaneously, several groups have approached the evaluation of such schemes [65,71]. Lin and coworkers have demonstrated that mesoporous materials can be capped with superparamagnetic iron oxide nanoparticles after loading with guest drugs that are smaller than 3 nm, and that these could be released in the presence of cell-produced antioxidants such as dihydrolipoic acid in the presence of an external magnetic field. Such magnetic-mesoporous silica particles may be used not only for site-specific release of drugs but also to study inter- and intra-cellular chemical mechanisms *in vitro* [71]. Zink *et al.* [72] have elegantly demonstrated that drug delivery, magnetic resonance and fluorescence imaging, magnetic manipulation and cell targeting are simultaneously possible using a multifunctional mesoporous silica nanoparticle.

De novo toxicology

Synthetic amorphous silica is an approved food additive (E551 under EU regulations) [101]. The toxicological behavior of colloidal silica particles has been well reported and assessed, both *in vitro* and *in vivo*, extensively [73]. Although mesoporous silica materials share a similar chemical composition and disordered atomic structure, their high surface area coupled to their ability to adsorb a large amount of biological relevant molecules within their pores, as well as their surface chemistry, warrants a *de novo* evaluation of their immunotoxicological properties. Some of the fundamental studies in this area have been already performed. Rapid and efficient internalization and little attendant toxicity was observed, without impartment of the macrophage function when mesoporous materials were cocultured with primary human monocyte-derived macrophages [74]. Rapid and efficient internalization and little attendant toxicity was also observed in primary human monocyte-derived dendritic cells, where in addition there was a slight upregulation of CD-86 (associated with T-cell activation), which was particle size dependent [75]. It is likely that the mechanism of uptake is an active one for mesoporous silica particles with particle sizes below 300 nm, since a decrease in the uptake was observed at lower temperatures of 4°C, as well as their inclusion within vesicular compartments in the dendritic cells. Reports by us and others hence suggest that naked (unconjugated) silica particles are taken up via classic clathrin-mediated routes, and are energy-dependent (v-ATPase-dependent) [76], demonstrated through the use of endocytic trafficking inhibitors. The slight upregulation of CD-86 is a promising feature to serve as an immune stimulant with possible T-cell modulatory properties, which suggests that some mesoporous materials may behave similar to common adjuvants, such as alum (aluminium oxide), and have enhanced immunogenecity effect. A comprehensive *in vivo*

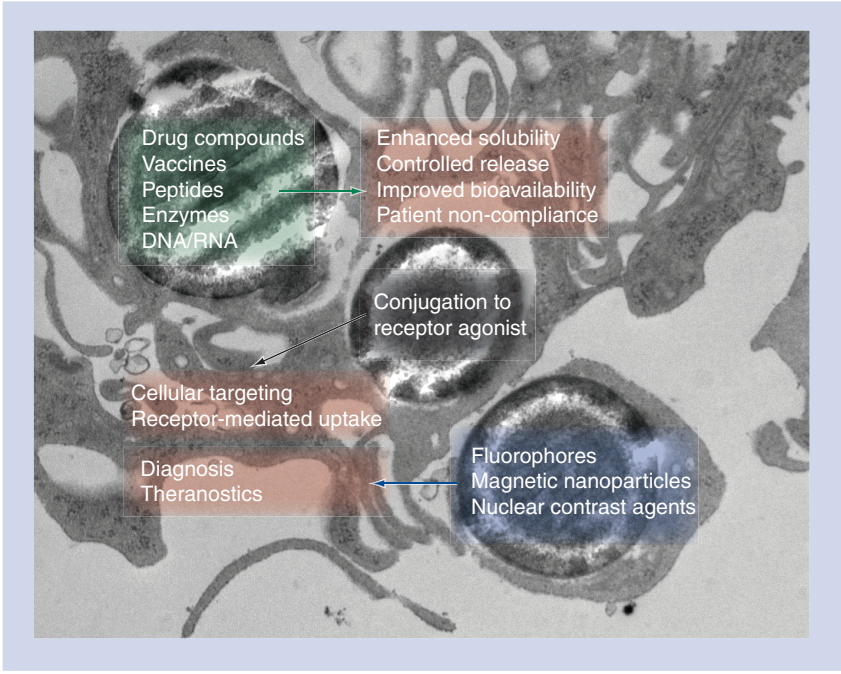

Figure 4. Summary of the potential of mesoporous silica particles in pharmaceutical sciences, nanomedicine and theranostics. Inserted image shows mesoporous nanoparticles (500 nm) engulfed within a macrophage cell.

study has been performed by Tamanoi *et al.* [69], which determined maximum tolerated doses in female nude mice injected intravenously with 2D hexagonal cylindrical pore mesoporous silica at different dose ranges. Mice treated with doses higher than 100 mg/kg showed mild elevation of liver transaminase aspartate aminotransferase, an early signs of toxicity, after dosing once a day for a period of 10 days. In the same study, long-term toxicity was also assessed with administrations at doses of 1 mg mouse^{-1}d^{-1} or saline solution through intraperitoneal injection, twice a week during 2 months. No significant toxicological signs were observed, concluding that mesoporous particles (~100 nm) with structural (2D hexagonal) and surface properties (calcined particles, unfunctionalized) as those represented in the study were well tolerated by mice.

It is still early days in the toxicological evaluation of mesoporous silica particles and nanoparticles. The main concern that the high surface area coupled to their nanoparticle size and surface chemistry may result in a different behavior than conventional colloidal particles appears, at first light, to be unfounded. However, Hudson *et al.* reported that intraperitoneal or intravenous administration of very large doses, 1.2 g/kg, of mesoporous silica particles in SV129 mice was safe when reduced to 40 mg/kg [77]. It is important to note the importance of accurate material characterization and reporting of material properties, such as surface charge, degree of silica condensation, functionalization, particle size, surface area and porous structure, when evaluating toxicological properties of nanoparticles. Hence, each set of mesoporous material groups (e.g., nanoparticles and micron size particles, functionalized and nonfunctionalized, cage type pores and cylindrical pores and so on) should be individually assessed. Furthermore, pulmonary routes of administration, which may be associated with work-place exposure and risk assessment in the large-scale production, must be additionally investigated. Silica and colloidal silica has a strong association with pulmonary diseases [78]. Tailoring the surface properties of mesoporous silica particles may affect not only the colloidal stability and dispersability of the particles, but in addition the degree of opsonization in biological media affecting cellular uptake and potential therapeutic as well as toxicological properties of the particles [76,79].

Finally, silica particle degradation, metabolism and disposal by living organisms is a matter of concern and little *in vivo* work has been conducted thus far. This is likely to depend largely on the methodology used to prepare the particles in the first instance – that is, temperature and time of thermal treatment and calcination steps (if used) to remove the organic template. These processes will determine the degree of condensation of the amorphous silica network forming the pore walls and the dissolution of the particle. Likewise, 3D-connected mesoporous silica materials are more likely to have faster degradation kinetics than 2D-connected porous structures. Shi *et al.* have compared the *in vitro* degradation of 2D-hexagonal mesoporous materials, and found that the degradation was almost completed after 15 days of immersion in 0.5 mg/ml simulated body fluid for the extracted sample, and only to approximately 32% degradation for the calcined sample [80]. Both types of sample showed considerably faster degradation times than nonporous amorphous silica particles, owing to the larger surface area of the mesoporous particles. The degradation mechanism was divided into three stages; a fast, bulk, degradation on an hour timescale followed by the formation of calcium and magnesium deposits and a passivating layer on the particle surface, and finally a slower degradation on a day-time scale. *In vivo* studies conducted on xenograft mice reported that 95% of the silica particles were excreted through urine and feces and showed that the major route for clearance of silica particles is via renal excretion [70]. This is consistent with studies in nonporous

silica, which show that particles of approximately 45 nm in size accumulate mainly in the liver, kidney and urinary bladder after a few hours of intravenous injection, in contrast to nonporous silica particles (3.0–6.0 nm in size), which were found to remain in systemic circulation after 48 h, with minimum retention in any of the major organs [81,82].

Future perspective

Mesoporous materials are rapidly placing themselves at the forefront of new delivery vehicles, allowing multiple functions due to their large surfaces and internal volumes. In formulation science they have potential to deliver both lipophilic and hydrophilic drugs, and solve problems associated with the poor bioavailability of lipophilic drugs, as well as limit the effects of drug–drug interactions. They have shown how facile encapsulation can allow the design of pharmacokinetic profiles for a variety of drug candidates, with release profiles extending to periods of weeks, and potentially months if the drug is covalently bonded to the silica particle. Drug targeting has been proved to be viable using folate conjugation and we anticipate that new schemes will no doubt be reported shortly, since folate conjugation is but one of the available schemes [68,83]. The toxicology of mesoporous materials appears thus far to be closely related to conventional amorphous silica, and *in vivo* murine models suggest that high doses of mesoporous silica are well tolerated in oral and intravenous administration routes. The biocompatibility of mesoporous materials, relevant to bone tissue repair, has been demonstrated. In addition, mesoporous materials naturally lend themselves to theranostics approaches due to the large pore volumes and ability to incorporate a variety of nanoparticles as well as pharmaceutical actives, which are sequentially loaded/encapsulated into the mesopores. FIGURE 4 summarizes the main application areas within the life sciences where mesoporous materials are likely to make an impact.

The potential of mesoporous materials in nanomedine has been demonstrated. It is clear that there is a considerable need to complete the toxicological profile of these materials before they enter a more developed clinical phase. However, having barely scratched the surface of how mesoporous materials may perform within nanomedicine, there is room for quiet optimism.

Financial & competing interests disclosure

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Executive summary

- Silica-based ordered mesoporous materials possess high surface areas and pore volumes capable of encapsulating, loading and releasing a large number of pharmaceutical and biorelevant molecules.
- Mesoporous materials have been demonstrated to be efficient at enhancing the bioavailability of poorly soluble drugs, tailoring pharmacokinetic properties and at cellular targeting, *in vitro* and *in vivo*.
- The large volumes of mesoporous materials allows them to covalently attach fluorochromes, MRI contrast agents and other detection molecules, as well as therapeutic molecules and targeting agents. Hence, mesoporous materials are multifunctional materials.
- The toxicological properties of mesoporous materials appear to be closely related to their compositional equivalent colloidal amorphous silica. More work is clearly needed before mesoporous materials are deemed completely safe and in particular each compositional and structural variant should be assessed individually.
- The low immunotoxicological behavior so far observed in mesoporous silica makes them a good candidate for further advancement to clinical studies.

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