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Synthesis, characterization and perspectives of mesoporous silica-based nanoplatforms as drug delivery systems

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***Abstract*— Mesoporous silica-based nanomaterials have been considered as potential carriers for drug delivery applications. Their main advantages are: *i)* tunable pore size, *ii)* high surface area, *iii)* functional surface chemistry and *iv)* biocompatibility. The internal and external domains of mesoporous silica allow encapsulating a wide variety of drugs and functionalizing its surface with specific molecules in the perspective of targeting medicine. In this work, we synthetized and characterized the silica-based mesostructures known as SBA-15 and MSF. We highlighted their pore size distribution, functional surface chemistry and loading capacity using valproic acid as probe. We observed that the SBA-15 sample has an average pore size of about 6 nm, while the modified MSF sample has an average pore size of 13 nm. These are encouraging results demonstrating that the use of swelling agents allows increasing the pore size distribution and thus the applicability of MSF as drug carries. The surface functionalization, using liposomes, was successfully achieved. This last may play a key role in the presence of living cells, enhancing the uptake. The more efficient material was the MSF, which encapsulated a higher VPA amount. Hence, the loading capacity was found pore size dependent. This work is the proof-of-concept of the use of MSF as biocompatible and effective drug delivery system.**

Keywords—mesoporous silica; drug delivery systems; SBA-15; mesoporous silica foams; nanomedicine; pore size

I. Introduction

Since their creation in 1992, mesoporous silica-based materials have been the object of numerous studies in order to improve their performance in fields such as catalysis, water remediation, food and pharmacology. The first silica mesostructured compounds, known as Mobil Composition of Matter (MCM), were synthetized at alkaline pH using the tetramethylammonium silicate (TMAS) as silica precursor and the hexadecyltrimethylammonium bromide (CTAB) as cationic surfactant. As a result, ordered structures of about some hundreds nanometers and a pore size distribution from 2 to 5 nm were obtained [1,2]. Even though, the pore size distribution was found heterogeneous, these materials opened new horizons in emerging fields such as material science, biotechnology and nanomedicine. The main advantage of using mesoporous materials rests on their tunable pore size, which increases the range of choice for drug encapsulation. However, the MCM compounds were limited by their pore size that allowed only enclosing small molecules. In 1998, at the University of California in Santa Barbara, the research group leaded by Prof. Dongyuan Zhao, developed a new mesoporous silica-based material known as Santa Barbara Amorphous (SBA) [3]. The SBA material overcame the pore size limitation by changing the synthetic route. This material presented a unique hexagonal pattern “the honeycomb like arrangement”, a wider pore size distribution (2 – 50 nm), a higher surface area and a variable particle morphology [4]. More interestingly, this new material attracted a lot of attention due to its i) mechanical resistance, ii) chemical and thermal stability, iii) surface area (>1000 m2.g-1), iv) pore volume (>1 cm3.g-1) and arrangement, v) surface chemistry (functionalization) and vi) biocompatibility and harmlessness. Indeed, the main advantage of the SBA lies in its external and internal surface domains, which amplified the applicability of the material in medicine. Hence, the physicochemical characteristics of the SBA allow the physisorption or chemisorption of a wide variety of molecules [5].

In the perspective of nanomedicine, the mesoporous silica-based materials are considered as promising biocompatible drug delivery systems [6]. These materials allow developing new therapeutic platforms by i) loading relevant drugs inside the pores and ii) by functionalizing their surface with specific molecules to enhance drugs solubility and selectivity. For instance, the silanol groups (Si-OH) located at the materials’ surface, enhance its interaction with the phospholipid layer of human cells, which might induce a higher internalization and thus a targeted drug release. Additionally, small molecules can be adsorbed on the particle surface due to the presence of the Si-OH groups, which confer the possibility of having 2 to 4 anchorage sites per nm [7]. Targeting molecules can be also added to the surface by its functionalization with amino or carboxyl groups that may enhance the material interaction with other biological

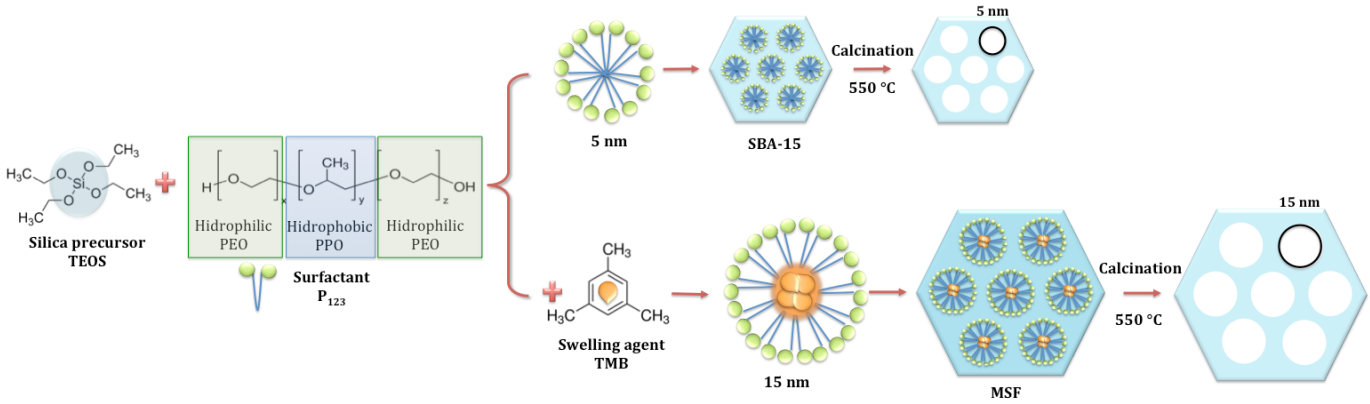

Fig. 1 Schematic representation of the SBA-15 and MSF material's preparation.

molecules such as proteins and enzymes [8]. The inside domain (porosity) can be modified using swelling agents [9], which are capable to increase the pore size; a bigger pore size a wider drug loading range. One of the most common swelling agent is the 1,2,4 Trimethylbenzene (TMB) that reaches the micelle hydrophobic core allowing the pore enlargement [10,11]. In this work, in the perfective of developing efficient nanoplatforms for drug delivery, a TMB modified SBA material was developed and characterized. The structural characteristics were compared to the standard SBA-15 and the loading capacity, using valproic acid, was evaluated.

II. MATERIALS AND METHODS

A. *Synthesis of the SBA-15 and MSF*

The SBA-15 was synthetized by sol-gel according to procedures previously reported [12]. Pluoronic P-123 ($EO_{20}PO_{70}EO_{20}$, MW= 5800, Sigma Aldrich) was used as non-ionic surfactant. In brief, 4.8 g of P-123 were dissolved in a 4 $mol.L^{-1}$ HCl solution at room temperature. Then, 8 ml of the tetraethylorthosilicate (TEOS ≥99.0%, Sigma-Adrich) precursor were added dropwise to the solution and kept under stirring for 24 h at 45ºC. Finally, the mixture was transferred to a polypropylene container and heated up to 80 °C to promote the mesostructure formation. The material was filtered, dried and calcinated at 550°C for 6 hours.

In order to enlarge the SBA-15 pore size, trimethylbenzene (Sigma Aldrich) was used as expander. Indeed, it has been reported that the TMB micelle-swelling agent interacts directly with the P-123 formed micelles increasing significantly their size [13]. After solubilizing P-123, 5.8 ml of TMB were added. This solution was stirred during 45 min and the synthesis proceeds as already described for the SBA-15. The modification leads to obtain mesoporous silica foams (MSF). The methodology is illustrated in Fig. 1.

B. *Functionalization of the SBA-15 and MSF*

Prior to the functionalization, the lipid bilayer was prepared using DOTAP and phosphatidylcholine in a 70/30 molar ratio. The selected lipids were dissolved and mixed in a chloroform-methanol solution to assure homogeneity. The solvent was removed under nitrogen flow. The formed liposomes were purified by micro-filtration using a 400-450 nm pore size membrane and kept under inert atmosphere at 4°C.

Then, the SBA-15 and MSF powders were dispersed in ultra-pure water by sonication and rinsed by centrifugation. Afterwards, SBA-15 and MSF suspentions followed basic and acid rinsing cicles using 4% NH4OH - 4% H2O2 and HCl 0.4 M - 4% H2O2 solutions, respectively. Finally, the SBA-15 and MSF were functionalized incubating their aqueous suspentions with the hydrated liposomes.

C. *Loading of the SBA-115 and MSF with VPA*

A 10 mg.mL-1 aqueous solution of valproic acid (VPA) was prepared. To assure complete solubilization, 50 µL of acetic acid (1 mol.L-1) were added to a 25 mL total volume VPA solution. Afterwards, 125 mg of each functionalized SBA-15 and MSF, were added and kept under stirring for 4 hours. The solvent was then evaporated at 100°C. The dried samples were thus used to perform the gravimetric analysis and determine the VPA loading percentage.

D. *Physicochemial characerization*

The morphology, particle size, pore size distribution and chemical composition were determined by Scanning Electron Microscopy (SEM) using a JEOL JSM -6060LV. The SBA-15 and MSF powders were dispersed in ethanol, placed on top of the aluminium cylinder and dried for observation.

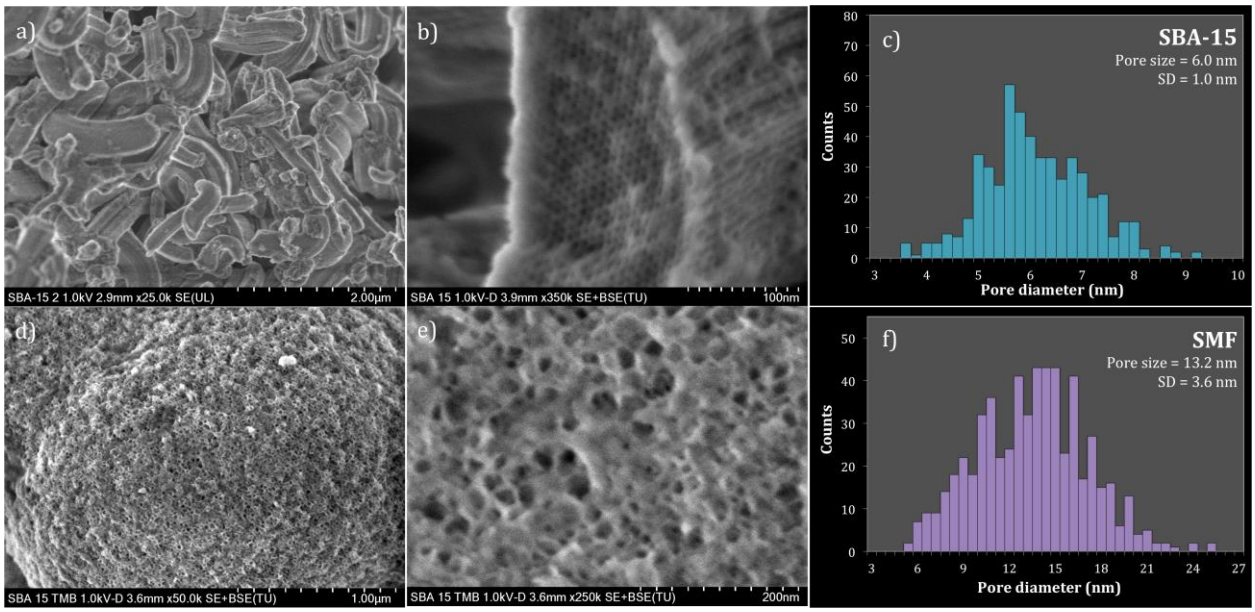

Fig. 2 SEM micrographs of the SBA-15 (a and b) and MSF (d and e) obtained materials at two different magnifications. Pore size distribution histograms of the SBA-15 (c) and MSF (f).

The pore size distribution histograms were built by analyzing the recorded images and by measuring close to 600 pores using the ImageJ 1.46r software. The elemental analysis and mapping were performed using the Energy-Dispersive X-ray Spectrometer (EDS - Bruker Nano Analytics) coupled to the microscope. The samples were as well analyzed by Transmission Electron Microscopy (TEM) using a Hitachi SU8230 microscope. The materials were dispersed by sonication in deionized water and small drops were deposited on formvar/carbon-coated copper grids (200-mesh) and observed after natural drying.

A homemade volumetric adsorption apparatus equipped with precision capacitance manometers (Baratron MKS Instruments, Inc.) was used to determine the textural properties of the silica-based materials *via* nitrogen physisorption at 77 K. The samples were degassed at 523 K for at least 3 h under high vacuum (10^{-3} Torr) to ensure a clean dry surface, free of any loosely bound adsorbed species. The surface areas of the materials were calculated by BET standard procedure using nitrogen adsorption data collected in the relative equilibrium pressure interval of $0.03 < P/P^0 < 0.3$. The total pore volume (Vtotal) was estimated from the amount of nitrogen adsorbed at a relative pressure of 0.99.

The porosity pattern arrengement was evaluated by low-angle X-Ray Diffraction (XRD) using an Ultima IV diffractometer (Rigaku) working at 35 kV with a CuKα source (1.5406 Å). Diffraction intensities were measured at the 2θ angle range between 0 and 2.5 degrees.

III. RESULTS AND DISCUSSION

Fig. 2 shows the scanning electron micrographs of the obtained materials, the SBA-15 (a and b) and the modified MSF (d and e). The obtained SBA-15 (Fig. 2a and b) is, as expected, an amorphous material (mainly elongated particles) with a hexagonally ordered pore arrangement and an average particle size of about 800 nm. In agreement with previous works, the SBA-15 has a pore size of 6.0 ± 1.0 nm [14]. On the other hand, the modified MSF shown in Fig. 2d and e, consists on round-like particles with sizes ranging from 600 nm up to 2 µm, aproximately. The MSF presents a disordered mesoporous structure and a wider pore size distribution with an average value of 13.2 ± 3.6 nm. The increased pore size, observed in the modified MSF, is attributed to the presence of the swelling agent during the synthesis, which also changed the mesoporous structure [15]. The morphology and particle size was confirmed by TEM as shown in Fig. 4 (a to d).

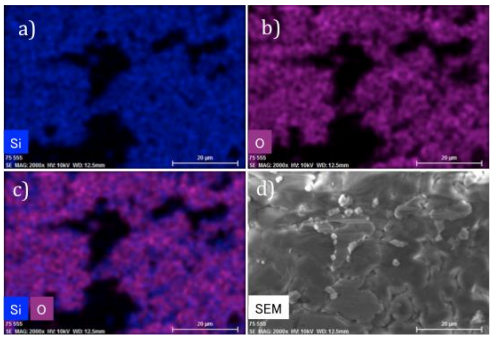

Fig. 3 EDS elemental mapping of the MSF obtained material: a) silicon, b) oxigen, c) merged image representing silicon and oxigen sites and d) SEM micrograph of the analized area.

Fig. 3 shows the EDS elemental mapping of the MSF sample. In panel (a), we observe the sites containing silicon. Panel (b) corresponds to those areas with oxygen. The merge image in Fig. 3c clearly reveals the Si-O composition of the sample. The Si-O proportions, for the SBA-15 and MSF materials were found 59.7-O% / 40.3-Si% and 60.9-O% / 39.1-Si%, respectively.

The functionalization of the SBA-15 and MSF samples with liposomes was characterized by TEM. For instance, Fig. 4e and f show the MSF post-functionalization. The impregnation of liposomes, on the MSF surface, is clearly evidenced by the appearance of electronically denser zones around the material. It is observed (Fig. 4f) that the MSF keeps its mesopourosity after impregnation, leaving the pores for their loading. Hence, we demonstrated the feasibility of the functionalization method, which allows covering biocompatible MSF materials with cell-recognition molecules to enhance their cell uptake.

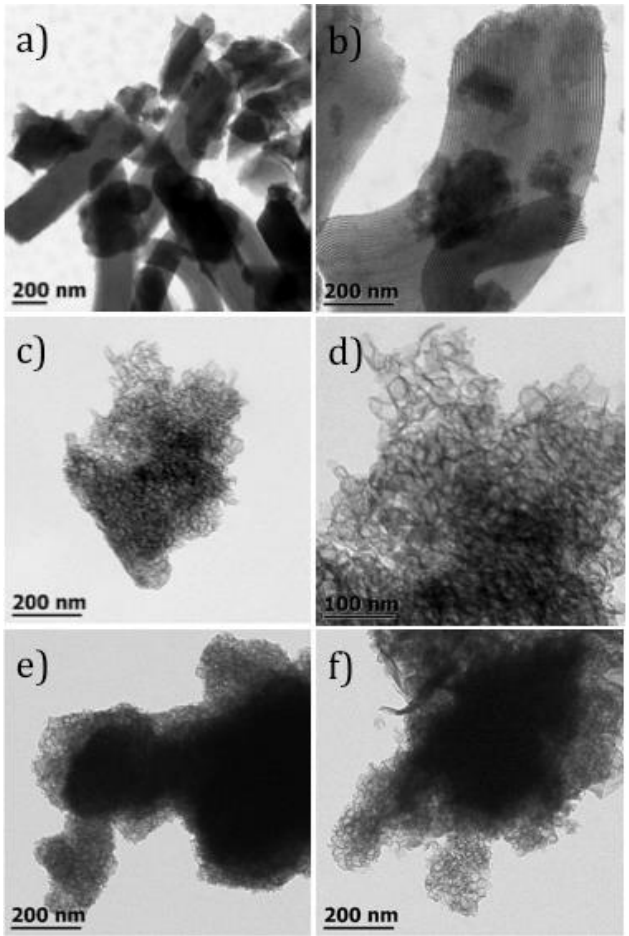

Fig. 4 TEM micrographs of the SBA-15 (a and b) and MSF (c and d) samples before functionalization. MSF material post-functionalization with liposomes (e and f).

The N_2 adsorption isotherms of the SBA-15 and MSF are shown in Fig. 5a and b, respectively. The isotherms can be classified as type IV according to the IUPAC convention, which is typical for SBA-15 mesoporous material. The surface area of the silica-based materials was determinated by the B.E.T. (Brunauer-Emmet-Teller) equation.

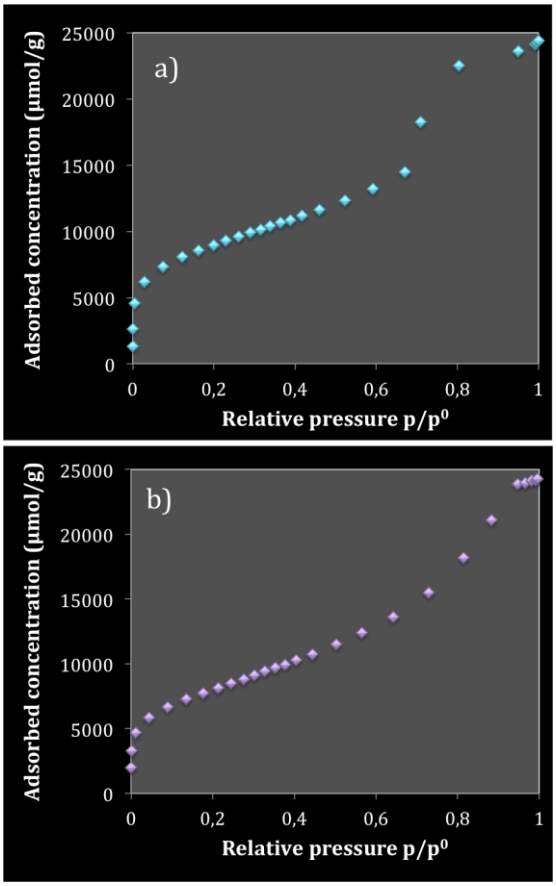

Fig. 5 N_2 adsorption isotherms of SBA-15 (a) and MSF (b).

The main textural properties of the SBA-15 and MSF are listed in Table 1. As expected, both SBA-15 and MSF materials have high textural properties, reason why they are considered suitable materials to participate such as a support in processes of controlled release of drugs.

Table 1. Textural properties of the SBA-15 and MSF.

Sample	S_{BET} (m^2/g)	V_{total} (cm^3/g)	*d*p (nm)
SBA-15	691	0.9	6.0
MSF	630	1.1	14.0

The diffractograms show in Fig. 6 display the (100) reflections of the p6mm hexagonal symmetry space group characteristic on mesoporous ordered materials such as the SBA-15 [3]. The high intensity of the (100) reflection indicates the abundance of a well-organized hexagonal structure in the

SBA-15 sample. On the other hand, a broadened peak (MSF sample) means less arrangement of the hexagonal pattern. These findings are in good agreement with the obtained SEM and TEM micrographs. Furthermore, the small shift between the SBA-15 and MSF samples, respectively from 0.33 to 0.36, might be associated to a wider pore diameter distribution on the MSF.

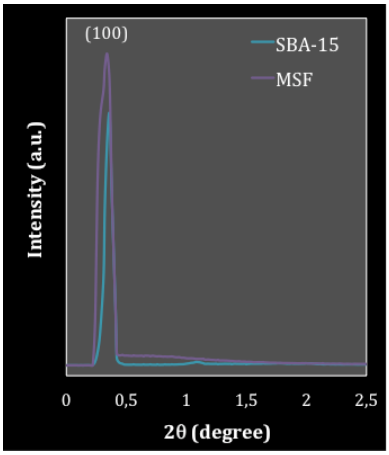

Fig. 6 Low angle X-ray diffraction pattern of the SBA-15 and MSF materials.

The loading capability of the SBA-15 and MSF materials was determined by a simple gravimetric analysis using **VPA**. Fig. 7 shows, for each material, their normalized initial material amount and their proportional drug loading. These results indicate that the weight of the SBA-15 and MSF increased in about 25% and 43%, respectively. Thus, we demonstrate that the bigger pores of the MSF the higher the amount of VPA encapsulated. Indeed, the MSF enhanced in about 42% the VPA loading, which might be attributed to both: the pore size and to the presence of silanol groups (Si-OH) that promote a better pore-drug interaction. A short-term experimental perspective consist in determining *in vitro* the VPA kinetic release, which may summarize and give answers to the motivation of using MSF materials as controlled drug delivery systems.

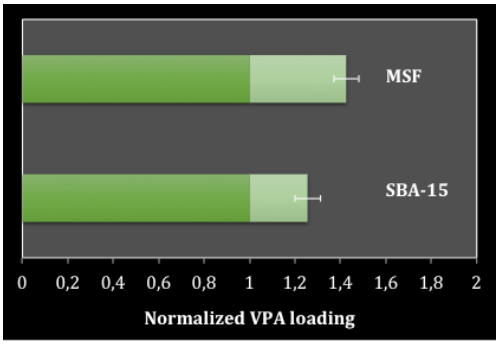

Fig. 7 Gravimetric analysis of the PVA loading in the SBA-15 and MSF. The loading proportion is represented by normalized the initial materials amount.

IV. CONCLUSIONS

Porous materials have many applications in diverse areas. Because of its porosity, MSF are likely to be more useful for drug delivery because of its wider pore diameter, compared with SBA-15, allowing a diverse compound storage of molecules. We structurally characterized the SBA-15 sample and determined the average pore size of about 6 nm, while the modified MSF sample has an average pore size of 13 nm. The results demonstrate that the use of swelling agents allows increasing the pore size distribution and thus the applicability of MSF as drug carries. Also, the surface functionalization using liposomes was successfully achieved. The more efficient material was the MSF, which encapsulated a higher VPA amount. Hence, the loading capacity was found pore size dependent. This work is the proof-of-concept of the use of MSF as biocompatible and effective drug delivery and release system, as is shown in the model proposed in Fig. 8. Furthermore, their extensive surface area will allow diverse functionalization processes that will confer new properties and targets. Some more specific biological assays are required for these materials, to complete a deeper study of its properties.

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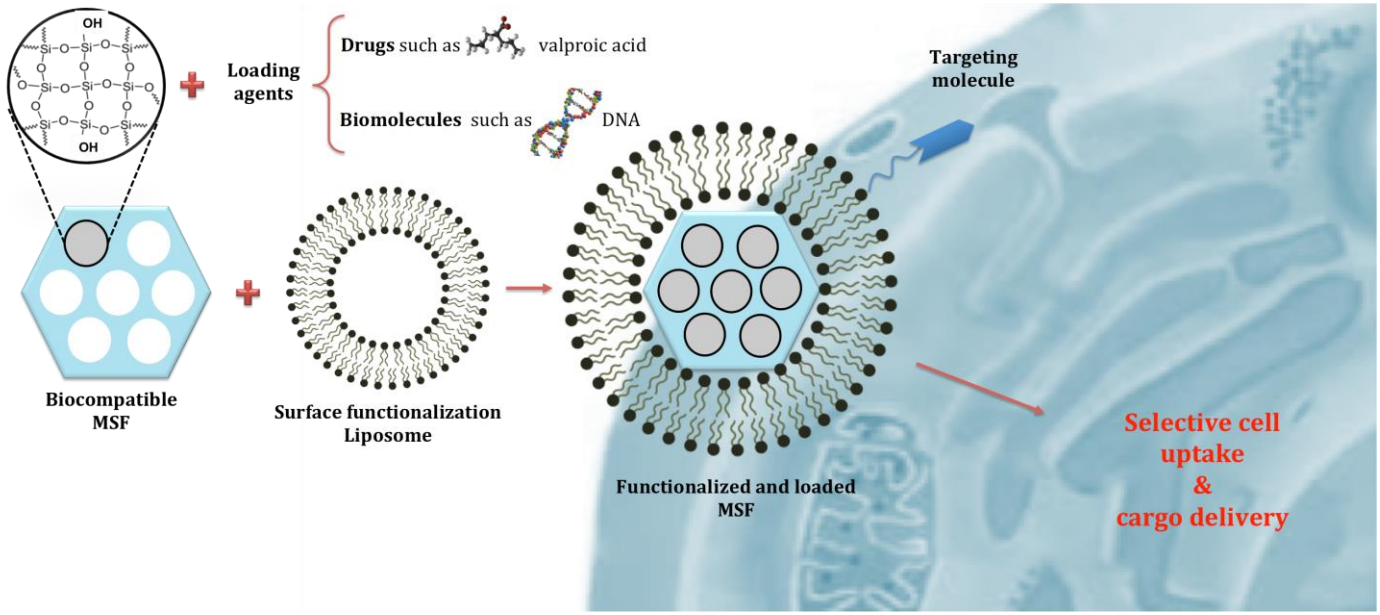

Fig. 8 Schematic representation of the drug loading, biological functionalization and perspectives of a MSF as vehicle for drug delivery.

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