Mesoporous Silica Nanoparticle

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Mesoporous silica nanoparticles (MSNs) have great potential for applications as a drug delivery system (DDS) due to their unique properties such as large pore size, high surface area, biocompatibility, biodegradability, and stable aqueous dispersion. The MSN-mediated DDS can carry chemotherapeutic agents, optical sensors, photothermal agents, short interfering RNA (siRNA), and gene therapeutic agents. The MSN-assisted imaging techniques are applicable in cancer diagnosis. However, their synthesis via a chemical route requires toxic chemicals and is challenging, time-consuming, and energy-intensive, making the process expensive and non-viable. Fortunately, nature has provided a viable alternative material in the form of biosilica from marine resources.

biosilica nanoparticle diatoms Characteristics

1. Introduction

A DDS-based on nanoparticles (NPs) is essential in cancer treatment. Compared to conventional DDS, a NP-based DDS shows enhanced efficacy by increasing the half-life of the drugs or proteins for a slow release from the carrier, improving the hydrophobic drug's solubility and allowing a controlled and targeted drug release at the diseased site [1]. Nanomaterials have found wide applications in synergistic platforms for cancer therapy such as photodynamic, immuno, chemo, and photothermal therapy, and for medical imaging, which includes computed tomography, magnetic resonance imaging, single-photon computed tomography, positron emission tomography, optical imaging, photoacoustic imaging, and ultrasound [2]. The major issues with the traditional pharmacological drug administration are a poor solubility, a short half-life, systemic toxicity, and drug disintegration before reaching the targeted site. The DDS therefore offers great potentials to overcome these constraints. Various types of nanocarriers have been developed for drug delivery, each with its own unique features, advantages, and disadvantages.

Silica and liposomes are the two most common materials used for targeted drug delivery. Mesoporous silica nanoparticles (MSNs) or silica nanoparticles (SiNP) have been applied for cancer treatment and diagnostic/imaging agents for cancer prognosis, such as the MSN-based biosensors and quantum dots, and for applications in tissue engineering, as catalysts in chemical synthesis, and as adsorbents for absorbing toxic substances and waste. Silica is highly porous, has excellent drug-holding capacities, is biocompatible, and is prone to surface functionalization for tunable properties [3][4]. The large surface area and the tunable surface of MSNs can be loaded with drugs and multi-functionalized for targeting purposes, or carry traceable fluorescent or magnetically active agents for therapeutic delivery and medical imaging [5]. At the nano size, with a high pore size and surface area

(often exceeding 1000 m^2/g), the MSNs are superior for adsorption and drug loading [6]. The loading and release kinetics of the drug can be modified by changing the size of the NPs [7]. Surface modification of the MSNs could improve the targeting ability of the NPs, for more effective drug delivery, and for slow release to reduce systemic toxicity [8][9].

2. Diatoms as the Natural Sources of Biosilica

Diatoms are single-celled phytoplankton that contribute to a fifth of all photosynthesis on the planet [10]. Diatoms can build micro- or nanoscale structures by depositing silica with distinctive architectures, a varied aspect ratio, and an ease of chemical functionalization/alteration to confer thermal and mechanical strength, optical/photonic properties, and environmental resilience [11]. These render the biosilica frustules with a wide potential for applications in bioimaging/biosensing, drug/gene therapy, photodynamic and fluid dynamics, biophotonics, and molecular filtrations. The non-porous biosilica generated from genetically modified diatoms has been developed for targeted drug delivery to neuroblastomas and B-lymphocytes, as well as a xenograft mouse model [12]. Mesoporous silica materials such as MCM-41 and SBA-15 have been widely exploited in the drug delivery applications [13], and the naturally engineered biosilica from diatoms offers a more cost-effective material for use as a drug delivery vehicle [14]. This has a superior biocompatibility, and a higher surface area, drug loading capacity, mechanical strength, and feasibility for functionalization in 3D structure [15][16].

For protection, diatoms have a mineral shell, and silica formed on the surface of the cell. Diatom morphology varies based on the species with a rod-like, hexagonal, or circular shape, and each species has its own distinct surface feature [14]. Among the diatom species are *Thalassiosira weissflogii*, *Thalassiosira pseudonana*, *Coscinodiscus* sp., *Nitzschia* sp., and *Aulacoseira* sp. [17]. The biosilica generation takes place in the “silica deposition vesicles” compartment. These vesicles consist of organic macromolecules that aid in the synthesis of silica and also serve as a template for frustule growth and the creation of incisions and dumps [18]. Biosilica frustules can be specific to the diatom species and the environment in which they are developed [19]. The silica morphogenesis can be altered such as by changing the processing of silaffins in *Thalassiosira pseudonana* [20]. A diatom frustule has a distinct nanostructure with diverse features such as spikes, holes, crests, networks, and bristles [21]. Diatoms can be divided into centric and pennate structures. Because of their extensive pore design and distinct porosity, the centric diatoms have a centrifugally proportioned structure. In contrast, the pennate diatoms have a more extended structure. The epitheca is the superior portion of the diatoms, while the hypotheca is the inferior portion [22].

The cultivation conditions of diatoms require the temperature of 20–24 °C, 18 h lights (1000–1200 lux) and 6 h dark, pH 8.2–8.7, and salinity 20–24 g/L, with culture mixing using an aerator [23]. In the natural environment, the microparticles of diatoms may include impurities such as clay, volcanic gas, terrigenous particles, and miscellaneous organic substances. These necessitate the removal of the contaminants from marine biosilica before pure silica micro-shells, can be recovered [24]. The amorphous structure [25], and the nano-pores encased in an ultra-fine polymer layer, render unique characteristics to become an inexpensive carrier of drugs and therapeutics [26]. Pure diatoms can be obtained by utilizing a pulverizer to crush the structure, with a compatible

solution to remove the contaminants [17]. For surface modification, the organic layers can be removed, and the hydroxyl groups added on the surface [27].

3. Characteristics, Biodistribution, and Synthesis of MSNs

3.1. Properties of MSNs

Silica is “Generally Recognized As Safe” (GRAS) by the United States Food and Drug Administration (USFDA, Silver Spring, MA, USA) [3]. The characteristics of the NPs, such as shape, size, and porosity, play an important role in delivering drugs to the target site, in effective protection of the cargoes such as imaging agents, drugs, oligonucleotides, and enzymes, from premature release, and unwanted degradation in harsh environments, such as the stomach and intestines, before reaching the specified target and in subsequent removal from the body [3]. Size is the key factor controlling cellular uptake and biocompatibility of the NPs [28]. Three different forms of the MSNs, such as spherical nanoparticles (NS), long-rod nanoparticles (NLR), and short nanoparticles (NSR), have been analyzed for in vivo oral bioavailability where the NLR shows a longer residence time, a longer blood circulation, and a slower renal clearance as compared to the NS and NSR [29]. The NSR degrades faster as compared to the NS and NLR due to the higher specific surface area [30]. These ordered porous structures allow the precise control of drug loading and release kinetics [3]. The large surface area (>700 m^2/g) and pore volume (>1 cm^3/g) give ample room for particle loading and improved drug solubility. The molecular size of the MSNs from 50 to 300 nm is suitable for easy cellular endocytosis by the living cells [3]. There are two functional surfaces—the cylindrical pore and the exterior particle surface—containing silanol that can be selectively functionalized or engineered with targeting bonds. The tunable diffusion and adjustable drug release make it feasible to deliver effective cell-specific drugs and for the development of a biogenic local concentration in the target area, thus reducing the total dose and preventing severe or chronic complications [3].

3.2. Uptake and Biodistribution

With intravenous delivery, the MSNs are normally distributed in the liver and spleen, and to a lesser extent in the lungs, and a small amount in the kidneys and heart [31]. Effective absorption of the MSNs involves energy-dependent cellular uptake and delivery [32], which can be controlled by the size and morphology of the NPs, the nano-carrier surface functionalization, and the electrostatic interactions between the MSNs and the cell membrane. The most suitable pathway to assimilate the MSNs is through cellular endocytosis, incorporating adherent material into vesicles (endosomes) [33]. For effective therapeutic activity, the MSNs should release the cargo in the cytoplasm via various endosomal escape routes [34]. Particles larger than 1 µm can cause phagocytosis that engulfs the particles into specific cells such as monocytes, neutrophils, macrophages, and dendritic cells. The particle size, shape, and surface charge affect one of the specific endocytic pathways [35]. The smaller particles (<200–300 nm) are absorbed by the endocytosis pathway, in most cases involving mechanisms such as clathrin- and cavulin-dependent cell endocytosis and caffeolin-dependent, based on the types of cells and surrounding conditions. Although the smaller particles may exhibit a longer circulation time, the blood circulation period, in vivo distribution, and biological filtration will further influence the impact of the particle size. The effects of the pore size,

shape, and surface properties of SiNPs on the cytotoxicity against macrophages (RAW 264.7) and epithelial carcinoma (A549) cells have been evaluated [35]. The main pathway of the toxicity associated with SiNPs is attributed to its surface silanol groups, which interact with the membrane components, leading to cell lysis and the leakage of cellular components. The pyrolyzed MSNs for example, show a lesser interaction than the non-porous silica due to the lower density of the silanol groups on the surface. Three different pore sizes, 2.3, 5.4, and 8.2 nm, named MSN2, MSN5, and MSN8, respectively, have been prepared using microemulsion to evaluate the effect of doxorubicin (DOX)-loaded MSN pore sizes on the anticancer efficacy. The MSN2 exhibits the lowest loading capacity (8.2%), while MSN5 shows the strongest cellular uptake and release profile, suggesting the importance of controlling the pore size of the nanocarrier [36]. The NPs synthesized with a high surface area and a small size can improve the biodistribution of cancer drugs and increase the diffusion time in the bloodstream. The drug-loaded NPs can be designed to target and select only tumor cells, and show high permeability and retention prospects in the specific tumor environment [4].

3.3. Chemical and Biosynthesis

The MSNs can be synthesized using Stober's, microemulsion, solution-based, and evaporation-induced self-assembly (EISA) methods. The materials of a mesoporous nature have been synthesized by changing the starting precursors and reaction conditions to result in materials with a varied structural arrangement or pore size. For instance, the Mobil Crystalline Material No.48 (MCM-48) has a cube arrangement, while the MCM-50 has a plate-like arrangement. Tri-block non-ionic copolymers such as poly(ethylene oxide) alkyl (PEO) surfactant and poly(alkylene oxide) block copolymers are also used as template designated as SBA-11 (cubic), SBA-12 (3-d hexagonal), SBA-15 (hexagonal), and SBA-16 (cage-shaped cube) based on the consistency of the mesoporous structure and the tri-block polymers used [37]. The SBA-15 differs from MCM in that it has pores larger than 4.6–30 nm with thicker silica walls [38] suitable for biomedical applications. FSM-16 is a mesoporous structure, prepared from quaternary ammonium surfactant as a template and polysilicate kanemate layers, suitable for pharmaceutical applications rather than as an adsorbent or for catalysis [39]. The time-consuming and energy-intensive processes and the wastes generated are among the major problems that hinder the further development of the chemical synthesis of the MSNs.

Nature has provided promising alternative porous materials from the biosynthesis of the MSNs. During biomineralization, marine organisms make remarkable, well-designed inorganic frameworks and patterns down to the nanometer scale [40]. Silica has important biological functions in higher plants, animals, and humans. The presence of silica in most cellular organisms such as bacteria and fungi proves its essential roles. Silica is actively taken up and transported by diatoms and algae for their replication and survival. The lack of silica in animals may result in the abnormal growth of tissues, especially collagenous tissues such as the skull, hair, peripheral bones, skin, and joints. Hence, the importance of biosilica in medical fields such as in orthopedics [41]. In fact, the compositional control of shape, size, pore pattern, surface area, and pore size of the synthetic MSNs, can be achieved in diatoms by altering the physicochemical parameters, post-harvest modifications, or genetic engineering [40]. Diatoms are considered to be the main biofactories for global silica biogenesis. The application of frustules has led to the creation of a new, rapidly developing field called "Diatom nanotechnology" [40]. The solid

surfaces of frustules consist of particles with a size of approximately 40 nm or 100–200 nm and display a patterned network of highly ordered pores mostly of uniform shape (nano to micrometer in size) with a homogeneous distribution within the surface. These pores contain species-specific patterns, with unique molecular transport properties and mechanical, photonic, and optical properties [42][43].

Bacterial silica production is achieved by the interaction of soluble anionic silicates with amine side chains (the positively charged groups on the peptidoglycan layer). The bacterial membrane demonstrates the deposition of silicates as thin unorganized scales (100 nm) and as a granular covering on the cell wall. The interaction in the peptidoglycan may be based on the hydrogen bond between the polysaccharide hydroxyl groups and silanol groups. The interaction between mostly negatively charged bacterial cell walls and the external cations may provide nucleation sites for mineralization. This is often found in those bacteria living in extreme environments, suggesting their protective properties [42]. Marine sponges form specific types of amorphous silica structures known as spicules (ranging from microscopic to macroscopic structures). These spicules are formed in a separate cell type called sclerotic cells, with the help of proteins (scaffolding silicatein) that catalyze hydrolysis and the polymerization of tetraethoxysilane [44]. Other alternative methods, in addition to chemical- and biosynthesis for the fabrication of MSNs, may involve spray drying [45], pressured carbonation [46], and low-temperature vapor-phase hydrolysis [47], which also involve additional chemicals and energy input.

4. Surface Modification, Functionalization, and Engineering of MSNs

As drugs differ in their chemical and physical properties, it is difficult to load drugs into only one carrier and release them one by one in the body. It is crucial to be able to design a smart-rate and section-controlled multi-drug delivery system to enhance targeted delivery to the diseased sites [48][49]. For this, biosilica modified with different surface and coating materials could provide solutions [50][51]. Surface modification is one of the approaches to fine-tune the pore size and improve the bioavailability and drug control release from a mesoporous silica material [43]. Diatoms can provide solutions to overcome problems associated with water-insoluble drugs and poor bioavailability. The coating of biosilica is done layer by layer using polyacrylic acid or micro-particles of polyallylamine hydrochloride [52][53]. The introduction of cross-linkers to the surface of biosilica creates strong covalent interactions with proteins or nucleic acids, which is critical for some specific applications. Biosilica is resilient and stable, and therefore can be standardized to study drug release kinetics and to meet high surface-to-volume ratio requirements for drug delivery [54][55]. Although the surface area of diatom biosilica varies between 1.4 and 51 m^2g^{-1}, effective drug release is further influenced by the size, layout, and distribution of the pores [56]. The mechanisms may involve a fast release phase from the detachment of the weakly attached drug molecules to the biosilica surface, and a gradual release phase with drug transport from the carrier interior structure to the external environment.

Marine biosilica can be chemically modified to improve the delivery of soluble and non-soluble drugs [43]. The versatility can be improved by incorporating different ligands for tunable properties in biomolecular and cell sensing, intracellular delivery, and disease diagnosis. The high concentration of surface silanol groups of the MSNs

can be modified with diverse organic functional groups. There are three well-defined domains that can be functionalized independently—the silica framework, the mesopores, and the outer surface of the NPs. These allow for nanoscale platforms to integrate multiple functionalities in treating and diagnosing diseases [57]. The functional groups could control the surface charge, chemically bind the functional particles inside or outside the pore, and control the pore size to trap the particles within the nanopores [58]. Some drugs are hydrophobic, which prevent them from penetrating the hydrophilic silica. Functionalization with hydrophobic groups could therefore enhance drug loading and prolong the kinetics of drug release from porous channels into the aqueous medium due to the low wettability of the material surface [59]. Both the internal and external surface of MSNs can be modified with different moieties, affecting the drug loading and release behavior, the tumor-targeting ability, and the imaging effect [59]. Some drugs may be limited to specific channels. In this case, higher loads and slower release kinetics can be achieved if the silica wall is functionalized with specific functional groups to cater for specific drugs, or a specific ligand to target a specific tumor. For instance, folate is affixed to the surface of the MSNs to target cancer cells with increased expression of the folate receptor [59].

Surface functionalization of the MSNs can be attained by surfactant displacement, co-condensation, and post-synthesis grafting methods [28][58]. The co-condensation method entails the presence of modified functional groups even within the pores of the NPs, while post-synthetic grafting involves the coupling of functional groups, often on the surface of the NPs [28]. The advantages of the co-condensation method include a simple process, a standardization in uniformity distribution, and a high achievable load. However, under some conditions, the recovery of the surfactants may not be complete, depending on the solvent used. The degradability and elimination of SiNPs can be carefully designed by changing the size, structure, and functional properties [28]. Factors that may affect the efficiency of the hydrolysis rates of SiNPs include the effect of surface modification, pH, pore size, and structure composition. The molecules with co-condensing functional groups (synthesized in the base state) could degrade at a much faster rate than the purely silicified MSNs. Additionally, when the co-condensation molecules are modified with disulfide-bridged silane on the surface, the degradation rate becomes slower [60].

During the post-synthesis grafting method, the functional groups are introduced after demolding by extraction or calcination [61]. This provides many possibilities for the functional group sites to be grafted with the most chemically sensitive organic functions and can be subjected to hydrolysis and removal reactions [62]. However, the distribution of the functional groups may not be regular if blocking of the nanopore occurs [58]. One of the major issues with the post-synthesis method is the potential for the mesopore openings being blocked by the functional groups, leading to a heterogeneous mesopore matrix [63]. A bi-functional surface modification of the MSNs, by incorporating the co-condensation and post-synthesis grafting method, may provide an alternative route of functionalization [64]. The coating method may have specific control of the stereochemical arrangement of the ligands on the mesopore surfaces [65].

The surfactant displacement method forms a single-layer uniform coverage with finely controlled amounts of functional organic silanes on the surface [58]. It consists of direct surfactant decomposition with simultaneous removal of the surfactant using acidic alcohol as a solvent [66]. This method synthesizes a homogeneous monolayer coating in which the number of functional groups can be precisely adjusted on the mesopore surface

[57]. Lactose targeting is combined with drug delivery and cellular endocytosis of the MSNs to form a new DDS. Docetaxel as the model drug and fluorescein isothiocyante as the dye confirm that the cargo-loaded MSNs functionalization with lactose (hMSNs) attains a higher drug release over a long period of time, as compared to the cargo-loaded MSNs without surface functionalization [67].

Mesoporous NPs at different sizes, volumes, and porosities exhibit different levels of toxicity at single-dose, acute (10 days), and near-chronic (60 and 180 days) dose exposure to mice [68][69]. The smaller non-porous SiNPs (50 nm) and larger mesoporous SiNPs (500 nm) exhibit more severe toxicity than the larger non-porous SiNPs (500 nm) [69]. Through surface engineering or the surface coating of MSNs such as with hyaluronic acid (HA), targeted drug deliveries to cancer stem cells can be attained [60]. Surface coating by positively charged polymers such as polyethylenimine (PEI) could attract negatively charged nucleic acids. The nucleic acid binding can be so strong that it can protect from nuclease activities and this can be useful for the delivery of plasmid DNA or short interfering RNA (siRNA) [59]. MSNs have been used to stabilize lipases, which are the common enzymes for the hydrolysis of triacylglycerol to fatty acids. The MSNs confer the combination of mechanical and thermal stability with controlled compositional properties and abundant surface hydroxyl groups that allow surface engineering for the strong immobilization of lipases with hydrophobic supports [70]. A silica aerogel (SA–OH) nanostructure has been modified by grafting the –COOH group into SA–COOH. The loading amount of celecoxib (CCB) drug is increased with SA–COOH, where the bonding between COO– on the silica with NH_3 of the drug is improved. Both drug-loaded SA–OH and SA–COOH exhibit greater loading capacities and faster drug dissolutions as compared to the pure drug. The controlled release rate is observed in the case of SA–COOH/CCB as compared to the SA–OH/CCB, suggesting a good potential for tunable drug dissolution and biocompatibility [71].

The binding of different antibodies to the surface-engineered diatom silica from *Coscinodiscus wailesii* has been demonstrated where the biological functions of the antibodies are retained. The modified silica exhibits prospective applications in antibody engineering similar to immunoprecipitation [72]. The drug loading and release capacity can be changed from surface-engineered diatomaceous earth (DE) microparticles [13]. Different types of silanes such as 3-aminopropyl triethoxysilane (APTES), methoxy-poly(ethyleneglycol) silane (mPEG-silane), 7-octadecyl trichlorosilane (OTS), 3-glycidyloxypropyl trimethoxysilane (GPTMS), and two phosphonic acids, namely 2-carboxyethylphosphonic acid (2 CEPA) and 16-phosphonohexadecanoic (16 PHA), are attached to the DE surface. The hydrophilic surfaces favour the extended release of the water-insoluble drug indomethacin (IMC) while the hydrophobic surfaces favor the sustained release of water-soluble gentamicin [13]. Novel nanohybrid particles from DE and graphene oxide (GO) have been synthesized where the GO is attached to the DE using covalent coupling and electrostatic attraction. The hybrid material is used as a smart pH-dependent drug microcarrier at pH 7.4 and 3.5, using IMC as the model drug [73].

Biosilica from *Thalassiosira weissflogii* is functionalized with a good reactive oxygen species (ROS) scavenger, 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), and then explored as a carrier for ciprofloxacin, an orthopaedic and dental drug. The antioxidant property of ciprofloxacin is enhanced by silica functionalization with TEMPO [17]. Diatomite nanoparticles between 100 and 300 nm are surface engineered with 3-aminopropyltriethoxysilane and labeled with tetramethylrhodamine isothiocyanate. The confocal imaging analyses suggest that the distribution of

nanoparticles is homogenous in the cytoplasm and the nucleus, which is ideal for DDS [74]. The diatom microcapsules with different hydrophilic and hydrophobic functionalizations have resulted in an increased water-insoluble IMC drug loading capacity by 60% with *N*-[3-(trimethoxysilyl)propyl] ethylenediamine, where the interaction of active polar functional groups on the silica enhance the drug loading capacity and prolong drug release [75]. Natural, mesoporous, and biodegradable silica from diatom *Amphora subtropica* (AMPS) has been extracted and purified as *Amphora* frustules (AF), which are then surface engineered with chitosan and tethered with DOX. The steps involved the demineralization of AMPS (AF) to form Amino-AF (AF-NH_2), where APTES and glutaraldehyde are used as cross linkers to bind AF and Chitosan (Chi) to synthesize Chi@AF. The result suggests an efficient drug loading and release and a high biocompatibility and is therefore a good candidate for DDS [76].

5. Cytotoxicity and Anticancer Activities of Drug-Loaded MSNs

The synthesis of diatom nanoparticles (DNPs) with a functionalized nanocarrier can replace synthetic NPs for their application as an anticancer DDS. Three different surfactant- templated biosilica NPs have been synthesized—Triton X-100 (Triton@MSNs, nonionic surfactant), sodium dodecylbenzene sulfate (SDBS@MSNs, anionic surfactant), and cetyltrimethylammonium bromide (CTAB@MSNs, cationic surfactant)—as carriers of an anticancer drug CPT-11 and their anticancer activities were determined and compared with free drug. The cytotoxic activities are in the order of CTAB@MSNs > SDBS@MSNs > Triton@MSNs. The cationic CTAB@MSNs could have interacted with silanol groups on the silica surface and the drug for more focused actions to produce higher anticancer activities than the other surfactants and the free drug [77]. The anticancer drug, DOX, loaded onto MSNs via a physical absorption process, has been combined with cancer-targeting polypeptide 2,3-dimethyl anhydride (DTCPP) and a therapy peptide 2,3-dimethyl anhydride (DTPP). The pH-sensitive DOX@MSN-ss-DTPP&DTCPP NPs drug carriers exhibit the release behavior influenced by an acidic pH and glutathione (GSH). A high cytotoxicity towards HeLa cells is observed after 48 h treatment where the release of DOX and the therapy peptide stimulates apoptosis, targeting the cell nucleus and mitochondria [78]. A study on the controlled-release mechanism of the drug shows direct correlation of the decomposition of the silica nanostructure with the drug release from the porous structure. This diatom biosilica is biodegradable and cost-effective as compared to the synthetic counterparts, for theranostic applications, and the delivery of chemotherapeutics [79]. Curcumin (CUR) and 5-fluorouracil (5FU) loaded onto the MSNs with magnetic bacterial iron oxide nanowires, and further encapsulated in pH-sensitive hypromellose acetate succinate microspheres using droplet-based microfluidics, have exhibited synergistic effects on SW480 colon adenocarcinoma cells [80]. An advanced dual DDS (dDDS), based on R5 peptide-fused ferritin (R5FT) with biosilica deposition on the ferritin surface, has been developed to overcome the limitation of different physicochemical properties of the different drugs during combination drug therapy. DOX is loaded into the core inside (the ferritin cage), and the monomeric red fluorescent protein (mRFP) or paclitaxel (PTX) is retained by the biosilica matrix. The mRFP or PTX exhibits short-term release, while DOX shows a sustained release. The compartment-based and rate-controlled dDDS with the PTX-DOX combination attains a two-fold higher cytotoxicity than the single DOX [81].

Mesoporous silica (MCM-41, SBA-15, MCF-17, and MCF) synthesized by the hydrothermal method, having different pore sizes, surface areas, and structures, and functionalized with 3-mercapto propyl trimethoxy silane (MPTMS) followed by the oxidation of the thiol groups of the sulfonic group, has shown different loading capacities of tamoxifen citrate (TMXC) and release rates. A higher loading of TMXC is attained in the SO_3H-functionalized SBA-15 and MCM-41. A fast release is however shown by the SO_3H-functionalized MCF in acidic conditions and in the presence of NaCl [82]. The ~300 nm size of MCM-41 silica particles on a magnetic iron oxide template has been modified with NH_2 or COOH groups, before or after the template removal, and then further modified with PEG-chains, for use as a DDS. The amount of TMX drug loaded in the carrier and its release characteristics depend on whether the modification step takes place before or after the template removal step. The TMX interacts more strongly with the silanol groups on the silicate surface than the surface-modified COOH groups, but sustained release is shown by the PEGylated formulation with COOH group modification after the template removal [83]. Mesocellular foam (MCF) with a spherical, continuous 3-D pore structure has been synthesized based on a Pluronic 123 triblock polymer (P123) surfactant and CTAB co-surfactant, for an oral drug delivery study. The MCF exhibits a higher telmisartan loading capacity and a faster release rate than the fibrous SBA-15 [84]. An antipsychotic drug, paliperidone-loaded mesoporous silica foam incorporated into polylactic acid and polylactic (co-glycolic acid), has resulted in enhanced drug solubility, but also prolonged release time [85]. The different performance of these functionalized silica-based drug carriers suggests the need to tune the carrier for a specific environment and to meet the intended purpose of the cargo to be delivered.

Diatom species produce potent bioactive compounds exhibiting anti-cancer, anti-biofilm, anti-inflammatory, and antibacterial activities [86]. Marine diatom *Thalassiosira weissflogii* frustules have been envisaged as a SMART DDS, where CUR is loaded at the capacity of almost 79%. Physiological conditions are found more suitable for faster CUR release, as compared to the acidic conditions. CUR adsorption into the void pores of biosilica is attributed to the surface charge of −3.05. The CUR-loaded biosilica shows cytotoxicity towards human renal adenocarcinoma cells (ACHN), but no toxicity against the human embryonic kidney (HEK293) cells, and with a broad spectrum of antibacterial activities against *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Aeromonas* spp. [87]. Biosilica extracted from *Chaetoceros* sp. diatoms with the surfaces modified with iron oxide NPs and functionalized with Trastuzumab antibody, has successfully exhibited fluorescence emission and could specifically target the SKBR3 (HER2-positive) breast cancer cells and not the normal cells. The *Chaetoceros* sp.-derived biosilica is eco-friendly and a promising biomaterial for biosensing chips and targeted drug delivery [88]. Marine microalgal-based diatomaceous earth microparticles (DEM) coated with vitamin B_{12} (cyanocobalamin) and loaded with cisplatin (CPT), 5-FU, and a tris-tetraethyl [2,2-bipyridine]-4,4-diamine-ruthenium(II) complex, are synthesized for targeted delivery to HT-29 colorectal cancer cells and MCF-7 breast cancer cells. CPT and 5-FU show rapid losses from the carrier, while the ruthenium complex is retained for up to 5 days in aqueous media, but readily released in a lipophilic environment as found in the cell membrane. The B_{12}-modified DEM exhibits a three-fold higher adherence to HT-29 and MCF-7 cells, attributable to increased transcobalamin II receptor expression, and is suitable as a micro-shuttle interacting with the tumor site before unloading the drug [89].

Diatomite biosilica has been developed as a nanocarrier to transport siRNA into the cancer cells. The microporous frustules are crushed, sonicated, and filtered up to a 450 nm size and surface-engineered with siRNA conjugation. The frustule carriers are proven as non-toxic materials for the transportation of siRNA into the tumor cells [90]. Iron oxide nanoparticles (IONP) have been loaded onto diatom frustules (IONP-DTM) and attracted to the tumor site of the in vivo animal model via an external magnetic field. Diatom sizes of 10 µm are used for the IONP and molecule loading, but the size may be large enough to cause blockage in the narrow capillaries of the lung, and the large pore size leads to cargo losses, suggesting the need to optimize the NP sizes. The broad range of diatom functionalities and the low toxicity and biodegradability of the IONP-DTM, suggest the great potential for its application in multimodality imaging and as a therapeutic vehicle in combination therapy [91]. CUR-loaded magnetically IONP-active frustules have exhibited reasonably higher cytotoxicity against the human cervical cancer (HeLa) cell line as compared to the pure CUR [92]. The DNPs nanovectors can be taken up by the cells and acclimatized to the lipid environment of the cell membrane, without damaging the cell morphology and viability [93]. The physicochemical and biological characteristics of the DNPs modified with 3-aminopropyltriethoxysilane (DNP-APT) have been improved using the dual-surface functionalization method by PEG layer and bioconjugation with cell-penetrating peptides (CPP). These are meant to enhance biocompatibility of the DNP-APT and its subsequent intracellular uptake by the cancer cells. The PEG-ylated and CPP-bioconjugated DNP-APT exhibit biocompatibility with erythrocytes, but a reduced cytotoxicity against the MCF-7 and MDA-MB-231 breast cancer cells. However, CPP conjugation enhances the DNP cellular uptake, and the dual biofunctionalization improves sorafenib drug-loading by 22% and the release profiles in aqueous system [94]. The genetically engineered *Thalassiosira pseudonana* diatom displays an IgG-binding domain of protein G on the surface of the biosilica to capture the cell-targeting antibodies. Only specific antibodies are attached to the drug-loaded biosilica NPs to target and selectively kill neuroblastoma and B-lymphoma cells. With similarly designed biosilica treatment, a regression in the tumor growth is also observed in the subcutaneous mouse xenograft model of neuroblastoma [95].

Living diatom cells suspended in buffer in darkness have very little silica dissolved from their cell walls, even if left for many weeks, suggesting the diatom resilience under a harsh environment and that there are intrinsic mechanisms stabilizing the diatom silica walls [96]. Diatom silica microparticles could improve permeation and sustained release following the oral delivery of drugs [97]. However, the biocompatibility and safety on human health upon treatment are of paramount importance, and many in vitro and in vivo tests are designed to test the biocompatibility of the material. The determination of cytotoxicity and mutagenicity during the in vitro analysis requires in vivo animal testing to improve injectable materials before further validation during clinical testing for later human use. This safety aspect is ever so pertinent in the application of siRNA-diatomite nanoconjugate for cancer treatment. The effects of siRNA should be disease site-targeted such that the normal cells will not be damaged. In vitro experiment has shown that diatomite NP concentration up to 300 ug/mL for 72 h shows very low toxicity, but the siRNA-diatomite nanoconjugate exhibits cytoplasmic localization and causes gene silencing in human epidermoid cancer cells (H1355) [90]. Biosilica accumulation has been detected in the kidney and the liver of the BLAB/c mice after 8 days, although no major distortion is observed in major organs [95], suggesting greater caution should be taken should there be the case of overdose and particle accumulation in tissues and organs in the body [4]. Apart from the use to induce cytotoxic and anticancer activities through drug delivery, biosilica can also

be explored for synergistic applications with the drugs and biocompounds for reduced systemic toxicity during treatment [98]. For tissue engineering applications, biosilica from marine sponges has been shown as a promising biomaterial for bone graft due to the great potential to exploit its osteogenic properties. Biosilica has a positive impact on the viability of MC3T3-E1 osteoblast precursor cells, and stimulates Runx2 and BMP4 (Bone morphogenetic protein 4) expressions, the genes responsible for giving instructions to make proteins for the development and maintenance of teeth and bones [99].

References

1. Dang, Y.; Guan, J. Nanoparticle-based drug delivery systems for cancer therapy. Smart Mater. Struct. 2020, 1, 10–19.
2. Siddique, S.; Chow, J.C. Application of nanomaterials in biomedical imaging and cancer therapy. Nanomaterials 2020, 10, 1700.
3. Zhou, Y.; Quan, G.; Wu, Q.; Zhang, X.; Niu, B.; Wu, B.; Huang, Y.; Pan, X.; Wu, C. Mesoporous silica nanoparticles for drug and gene delivery. Acta Pharm. Sin. B 2018, 8, 165–177.
4. Hussein, H.A.; Abdullah, M.A. Anticancer compounds derived from marine diatoms. Mar. Drugs 2020, 18, 356.
5. Rosenholm, J.M.; Sahlgren, C.; Lindén, M. Multifunctional mesoporous silica nanoparticles for combined therapeutic, diagnostic and targeted action in cancer treatment. Curr. Drug Targets 2011, 12, 1166–1186.
6. Hu, Y.; Zhi, Z.; Wang, T.; Jiang, T.; Wang, S. Incorporation of indomethacin nanoparticles into 3-D ordered macroporous silica for enhanced dissolution and reduced gastric irritancy. Eur. J. Pharm. Biopharm. 2011, 79, 544–551.
7. Hu, Y.; Zhi, Z.; Zhao, Q.; Wu, C.; Zhao, P.; Jiang, H.; Jiang, T.; Wang, S. 3D cubic mesoporous silica microsphere as a carrier for poorly soluble drug carvedilol. Microporous Mesoporous Mater. 2012, 147, 94–101.
8. Nadrah, P.; Porta, F.; Planinšek, O.; Kros, A.; Gaberšček, M. Poly(propylene imine) dendrimer caps on mesoporous silica nanoparticles for redox-responsive release: Smaller is better. Phys. Chem. Chem. Phys. 2013, 15, 10740–10748.
9. Sun, L.; Wang, Y.; Jiang, T.; Zheng, X.; Zhang, J.; Sun, J.; Sun, C.; Wang, S. Novel chitosan-functionalized spherical nanosilica matrix as an oral sustained drug delivery system for poorly water-soluble drug carvedilol. ACS Appl. Mater. Interfaces 2013, 5, 103–113.
10. Phogat, S.; Saxena, A.; Kapoor, N.; Aggarwal, C.; Tiwari, A. Diatom mediated smart drug delivery system. J. Drug Deliv. Sci. Technol. 2021, 63, 102433.

11. De Oliveira, L.F.; Bouchmella, K.; Goncalves, K.D.; Bettini, J.; Kobarg, J.; Cardoso, M.B. Functionalized silica nanoparticles as an alternative platform for targeted drug-delivery of water insoluble drugs. Langmuir 2016, 32, 3217–3225.
12. Rabiee, N.; Khatami, M.; Jamalipour, S.G.; Fatahi, Y.; Iravani, S.; Varma, R.S. Diatoms with invaluable applications in nanotechnology, biotechnology, and biomedicine: Recent advances. ACS Biomater. Sci. Eng. 2021, 7, 3053–3068.
13. Bariana, M.; Aw, M.S.; Kurkuri, M.; Losic, D. Tuning drug loading and release properties of diatom silica microparticles by surface modifications. Int. J. Pharm. 2012, 443, 230–241.
14. Uthappa, U.T.; Brahmkhatri, V.; Sriram, G.; Jung, H.Y.; Yu, J.; Kurkuri, N.; Aminabhavi, T.M.; Altalhi, T.; Neelgund, G.M.; Kurkuri, M.D. Nature engineered diatom biosilica as drug delivery systems. J. Control. Release 2018, 281, 70–83.
15. De Stefano, L.; De Stefano, M.; De Tommasi, E.; Rea, I.; Rendina, I. A natural source of porous biosilica for nanotech applications: The diatoms microalgae. Phys. Status Solidi C 2011, 8, 1820–1825.
16. Sprynskyy, M.; Pomastowski, P.; Hornowska, M.; Król, A.; Rafińska, K.; Buszewski, B. Naturally organic functionalized 3D biosilica from diatom microalgae. Mater. Des. 2017, 132, 22–29.
17. Cicco, S.R.; Vona, D.; De Giglio, E.; Cometa, S.; Mattioli-Belmonte, M.; Palumbo, F.; Ragni, R.; Farinola, G.M. Chemically modified diatoms biosilica for bone cell growth with combined drug-delivery and antioxidant properties. Chempluschem 2015, 80, 1104–1112.
18. Yang, X.Y.; Chen, L.H.; Li, Y.; Rooke, J.C.; Sanchez, C.; Su, B.L. Hierarchically porous materials: Synthesis strategies and structure design. Chem. Soc. Rev. 2017, 46, 481–558.
19. Wikfors, G.H. A Taxonomic Guide to Some Common Marine Phytoplankton-Rita A. Horner, Biopress Limited, The Orchard, Clanage Road, Bristol, BS3 2JX, England; Elsevier Science: Amsterdam, The Netherlands, 2003; Volume 2.
20. Poulsen, N.; Kroger, N. Silica morphogenesis by alternative processing of silaffins in the diatom Thalassiosira pseudonana. J. Biol. Chem. 2004, 279, 42993–42999.
21. Almqvist, N.; Delamo, Y.; Smith, B.L.; Thomson, N.H.; Bartholdson, Å.; Lal, R.; Brzezinski, M.; Hansma, P.K. Micromechanical and structural properties of a pennate diatom investigated by atomic force microscopy. J. Microsc. 2001, 202, 518–532.
22. Amato, A. Diatom reproductive biology: Living in a chrystal cage. Int. J. Plant Reprod. Biol. 2010, 2, 1–10.
23. Ashokkumar, S.; Manimaran, K.; Kim, K. Cultivation and identification of microalgae (Diatom). In Marine Algae Extracts: Processes, Products, and Applications; John Wiley & Sons: Hoboken, NJ, USA, 2015; Volume 1–2, pp. 59–78.

24. Rosi, N.L.; Thaxton, C.S.; Mirkin, C.A. Control of nanoparticle assembly by using DNA-modified diatom templates. Angew. Chem. 2004, 116, 5616–5619.
25. Maher, S.; Kumeria, T.; Aw, M.S.; Losic, D. Diatom Silica for Biomedical Applications: Recent Progress and Advances. Adv. Healthc. Mater. 2018, 7, e1800552.
26. Terracciano, M.; De Stefano, L.; Rea, I. Diatoms green nanotechnology for biosilica-based drug delivery systems. Pharmaceutics 2018, 10, 242.
27. Karakoy, M.; Gultepe, E.; Pandey, S.; Khashab, M.A.; Gracias, D.H. Silane surface modification for improved bioadhesion of esophageal stents. Appl. Surf. Sci. 2014, 311, 684–689.
28. Selvarajan, V.; Obuobi, S.; Ee, P.L. Silica nanoparticles—A versatile tool for the treatment of bacterial infections. Front. Chem. 2020, 8, 602.
29. Zhao, Y.; Wang, Y.; Ran, F.; Cui, Y.; Liu, C.; Zhao, Q.; Gao, Y.; Wang, D.; Wang, S. A comparison between sphere and rod nanoparticles regarding their in vivo biological behavior and pharmacokinetics. Sci. Rep. 2017, 7, 4131.
30. Huang, X.; Li, L.; Liu, T.; Hao, N.; Liu, H.; Chen, D.; Tang, F. The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility in vivo. ACS Nano 2011, 5, 5390–5399.
31. He, X.; Zhao, Y.; He, D.; Wang, K.; Xu, F.; Tang, J. ATP-responsive controlled release system using aptamer-functionalized mesoporous silica nanoparticles. Langmuir 2012, 28, 12909–12915.
32. Lu, J.; Liong, M.; Sherman, S.; Xia, T.; Kovochich, M.; Nel, A.E.; Zink, J.I.; Tamanoi, F. Mesoporous silica nanoparticles for cancer therapy: Energy-dependent cellular uptake and delivery of paclitaxel to cancer cells. Nanobiotechnol 2007, 3, 89–95.
33. Trewyn, B.G.; Nieweg, J.A.; Zhao, Y.; Lin, V.S. Biocompatible mesoporous silica nanoparticles with different morphologies for animal cell membrane penetration. Chem. Eng. J. 2008, 137, 23–29.
34. Radu, D.R.; Lai, C.Y.; Jeftinija, K.; Rowe, E.W.; Jeftinija, S.; Lin, V.S. A polyamidoamine dendrimer-capped mesoporous silica nanosphere-based gene transfection reagent. J. Am. Chem. Soc. 2004, 126, 13216–13217.
35. Jafari, S.; Derakhshankhah, H.; Alaei, L.; Fattahi, A.; Varnamkhasti, B.S.; Saboury, A.A. Mesoporous silica nanoparticles for therapeutic/diagnostic applications. Biomed. Pharmacother. 2019, 109, 1100–1111.
36. Li, J.; Shen, S.; Kong, F.; Jiang, T.; Tang, C.; Yin, C. Effects of pore size on: In vitro and in vivo anticancer efficacies of mesoporous silica nanoparticles. RSC Adv. 2018, 8, 24633–24640.
37. Øye, G.; Sjöblom, J.; Stöcker, M. Synthesis, characterization and potential applications of new materials in the mesoporous range. Adv. Colloid Interface Sci. 2001, 89–90, 439–466.

38. Zhao, D.; Huo, Q.; Feng, J.; Chmelka, B.F.; Stucky, G.D. Nonionic triblock and star diblock copolymer and oligomeric surfactant syntheses of highly ordered, hydrothermally stable, mesoporous silica structures. J. Am. Chem. Soc. 1998, 120, 6024–6036.
39. Narayan, R.; Nayak, U.Y.; Raichur, A.M.; Garg, S. Mesoporous silica nanoparticles: A comprehensive review on synthesis and recent advances. Pharmaceutics 2018, 10, 118.
40. Ghobara, M.M.; Gordon, R.; Reissig, L. The mesopores of raphid pennate diatoms: Toward natural controllable anisotropic mesoporous silica microparticles. In Diatom Morphogenesis; Gordon, R., Annenkov, V., Seckback, J., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2021; pp. 10–11.
41. Farooq, M.A.; Dietz, K.J. Silicon as versatile player in plant and human biology: Overlooked and poorly understood. Front. Plant Sci. 2015, 6, 994.
42. Mitra, S.; Kumar, R.; Roy, P.; Basu, S.; Barik, S.; Goswami, A. Naturally occurring and synthetic mesoporous nanosilica: Multimodal applications in frontier areas of science. Int. J. Nanosci. 2019, 18, 1850027.
43. Aw, M.S.; Simovic, S.; Yu, Y.; Addai-Mensah, J.; Losic, D. Porous silica microshells from diatoms as biocarrier for drug delivery applications. Powder Technol. 2012, 223, 52–58.
44. Krasko, A.; Lorenz, B.; Batel, R.; Schröder, H.C.; Müller, I.M.; Müller, W.E.G. Expression of silicatein and collagen genes in the marine sponge Suberites domuncula is controlled by silicate and myotrophin. Eur. J. Biochem. 2000, 267, 4878–4887.
45. Cho, Y. Fabrication of hollow or macroporous silica particles by spray drying of colloidal dispersion. J. Dispers. Sci. Technol. 2015, 37, 23–33.
46. Cai, X.; Hong, R.Y.; Wang, L.S.; Wang, X.Y.; Li, H.Z.; Zheng, Y.; Wei, D.G. Synthesis of silica powders by pressured carbonation. Chem. Eng. J. 2009, 151, 380–386.
47. Yan, F.; Jiang, J.; Chen, X.; Tian, S.; Li, K. Synthesis and characterization of silica nanoparticles preparing by low-temperature vapor-phase hydrolysis of SiCl4. Ind. Eng. Chem. Res. 2014, 53, 1184–11890.
48. Hu, Q.; Sun, W.; Wang, C.; Gu, Z. Recent advances of cocktail chemotherapy by combination drug delivery systems. Adv. Drug Deliv. Rev. 2015, 98, 19–34.
49. Li, J.L.; Cheng, Y.J.; Zhang, C.; Cheng, H.; Feng, J.; Zhuo, R.X.; Zeng, X.; Zhang, X.Z. Dual drug delivery system based on biodegradable Organosilica core-shell architectures. ACS Appl. Mater. Interfaces 2018, 10, 5287–5295.
50. Markman, J.L.; Rekechenetskiy, A.; Holler, E.; Ljubimova, J.Y. Nanomedicine therapeutic approaches to overcome cancer drug resistance. Adv. Drug Deliv. Rev. 2013, 65, 1866–1879.

51. Aftab, S.; Shah, A.; Nadhman, A.; Kurbanoglu, S.; Ozkan, S.A.; Dionysiou, D.D.; Shukla, S.S. Nanomedicine: An effective tool in cancer therapy. Int. J. Pharm. 2018, 540, 132–149.
52. Delasoie, J.; Zobi, F. Natural diatom biosilica as microshuttles in drug delivery systems. Pharmaceutics 2019, 11, 537.
53. Brewer, M.; Zhang, T.; Dong, W.; Rutherford, M.; Tian, Z.R. Future approaches of nanomedicine in clinical science. Med. Clin. N. Am. 2007, 91, 963–1016.
54. Lewin, J.C. Silicon metabolism in diatoms III. Respiration and silicon uptake in Navicula pelliculosa. J. Gen. Physiol. 1955, 39, 1–10.
55. Patel, P.; Hanini, A.; Shah, A.; Patel, D.; Patel, S.; Bhatt, P.; Pathak, Y.V. Surface modification of nanoparticles for targeted drug delivery. In Surface Modification of Nanoparticles for Targeted Drug Delivery; Springer: Cham, Switzerland, 2019; pp. 19–31.
56. Vasani, R.B.; Losic, D.; Cavallaro, A.; Voelcker, N.H. Fabrication of stimulus-responsive diatom biosilica microcapsules for antibiotic drug delivery. J. Mater. Chem. B 2015, 3, 4325–4329.
57. Vallet-Regí, M. Mesoporous silica nanoparticles: Their projection in nanomedicine. ISRN Mater. Sci 2012, 2012, 608548.
58. Wu, S.H.; Hung, Y.; Mou, C.Y. Mesoporous silica nanoparticles as nanocarriers. Chem. Commun. 2011, 47, 9972–9985.
59. Vallet-regi, M.; Tamanoi, F. Overview of Studies Regarding Mesoporous Silica Nanomaterials and Their Biomedical Application, 1st ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2018.
60. Möller, K.; Bein, T. Degradable drug carriers: Vanishing mesoporous silica nanoparticles. Chem. Mater. 2019, 31, 4364–4378.
61. Lim, M.H.; Stein, A. Comparative studies of grafting and direct syntheses of inorganic- organic hybrid mesoporous materials. Chem. Mater. 1999, 11, 3285–3295.
62. Zapilko, C.; Widenmeyer, M.; Nagl, I.; Estler, F.; Anwander, R.; Raudaschl-Sieber, G.; Groeger, O. Advanced surface functionalization of periodic mesoporous silica: Kinetic control by trisilazane reagents. J. Am. Chem. Soc. 2006, 128, 16266–16276.
63. Ritter, H.; Brühwiler, D. Accessibility of amino groups in postsynthetically modified mesoporous silica. J. Phys. Chem. C 2009, 113, 10667–10674.
64. Putz, A.M.; Almásy, L.; Len, A.; Ianăşi, C. Functionalized silica materials synthesized via co-condensation and post-grafting methods. Fuller. Nanotub. Carbon Nanostruct. 2019, 27, 323–332.
65. Elzaki, B.I.; Zhang, Y.J. Coating methods for surface modification of ammonium nitrate: A mini-review. Materials 2016, 9, 502.

66. Liu, Y.H.; Lin, H.P.; Mou, C.Y. Direct Method for surface silyl functionalization of mesoporous silica. Langmuir 2004, 20, 3231–3239.
67. Zhong, J.; Li, L.; Zhu, X.; Guan, S.; Yang, Q.; Zhou, Z.; Zhang, Z.; Huang, Y. A smart polymeric platform for multistage nucleus-targeted anticancer drug delivery. Biomaterials 2015, 65, 43–55.
68. Liu, Y.; Mi, Y.; Zhao, J.; Feng, S.S. Multifunctional silica nanoparticles for targeted delivery of hydrophobic imaging and therapeutic agents. Int. J. Pharm. 2011, 421, 370–378.
69. Mohammadpour, R.; Yazdimamaghani, M.; Cheney, D.L.; Jedrzkiewicz, J.; Ghandehari, H. Subchronic toxicity of silica nanoparticles as a function of size and porosity. J. Control. Release 2019, 304, 216–232.
70. Costantini, A.; Califano, V. Lipase immobilization in mesoporous silica nanoparticles for biofuel production. Catalysts 2021, 11, 629.
71. Jabbari-Gargari, A.; Moghaddas, J.; Hamishehkar, H.; Jafarizadeh-Malmiri, H. Carboxylic acid decorated silica aerogel nanostructure as drug delivery carrier. Microporous Mesoporous Mater. 2021, 323, 111220.
72. Townley, H.E.; Parker, A.R.; White-Cooper, H. Exploitation of diatom frustules for nanotechnology: Tethering active biomolecules. Adv. Funct. Mater. 2008, 18, 369–374.
73. Kumeria, T.; Bariana, M.; Altalhi, T.; Kurkuri, M.; Gibson, C.T.; Yang, W.; Losic, D. Graphene oxide decorated diatom silica particles as new nano-hybrids: Towards smart natural drug microcarriers. J. Mater. Chem. B 2013, 1, 6302–6311.
74. Ruggiero, I.; Terracciano, M.; Martucci, N.M.; De Stefano, L.; Migliaccio, N.; Tatè, R.; Rendina, I.; Arcari, P.; Lamberti, A.; Rea, I. Diatomite silica nanoparticles for drug delivery. Nanoscale Res. Lett. 2014, 9, 329.
75. Aw, M.S.; Bariana, M.; Yu, Y.; Addai-Mensah, J.; Losic, D. Surface-functionalized diatom microcapsules for drug delivery of water-insoluble drugs. J. Biomater. Appl. 2013, 28, 163–174.
76. Sasirekha, R.; Sheena, T.S.; Deepika, M.S.; Santhanam, P.; Townley, H.E.; Jeganathan, K.; Kumar, S.D.; Premkumar, K. Surface engineered Amphora subtropica frustules using chitosan as a drug delivery platform for anticancer therapy. Mater. Sci. Eng. C 2019, 94, 56–64.
77. He, Q.; Shi, J.; Chen, F.; Zhu, M.; Zhang, L. An anticancer drug delivery system based on surfactant-templated mesoporous silica nanoparticles. Biomaterials 2010, 31, 3335–3346.
78. Cheng, Y.; Qin, S.; Ma, Y.; Chen, X.; Zhang, A.; Zhang, X. Super-pH-sensitive mesoporous silica nanoparticle-based drug delivery system for effective combination cancer therapy. ACS Biomater. Sci. Eng. 2019, 5, 1878–1886.
79. Maher, S.; Kumeria, T.; Wang, Y.; Kaur, G.; Fathalla, D.; Fetih, G.; Santos, A.; Habib, F.; Evdokiou, A.; Losic, D. From the mine to cancer therapy: Natural and biodegradable theranostic silicon

nanocarriers from diatoms for sustained delivery of chemotherapeutics. Adv. Healthc. Mater. 2016, 5, 2667–2678.

80. Maher, S.; Santos, A.; Kumeria, T.; Kaur, G.; Lambert, M.; Forward, P.; Evdokiou, A.; Losic, D. Multifunctional microspherical magnetic and pH responsive carriers for combination anticancer therapy engineered by droplet-based microfluidics. J. Mater. Chem. B 2017, 5, 4097–4109.
81. Ki, M.R.; Kim, J.K.; Kim, S.H.; Nguyen, T.K.; Kim, K.H.; Pack, S.P. Compartment-restricted and rate-controlled dual drug delivery system using a biosilica-enveloped ferritin cage. J. Ind. Eng. Chem. 2020, 81, 367–374.
82. Mohebbi, S.; Shariatipour, M.; Shafie, B.; Amini, M.M. Encapsulation of tamoxifen citrate in functionalized mesoporous silica and investigation of its release. J. Drug Deliv. Sci. Technol. 2021, 62, 102406.
83. Popova, M.; Koseva, N.; Trendafilova, I.; Lazarova, H.; Mitova, V.; Mihály, J.; Momekova, D.; Momekov, G.; Koleva, I.Z.; Aleksandrov, H.A.; et al. Tamoxifen delivery system based on PEGylated magnetic MCM-41 silica. Molecules 2020, 25, 5129.
84. Zhang, Y.; Jiang, T.; Zhang, Q.; Wang, S. Inclusion of telmisartan in mesocellular foam nanoparticles: Drug loading and release property. Eur. J. Pharm. Biopharm. 2010, 76, 17–23.
85. Nanaki, S.; Tseklima, M.; Terzopoulou, Z.; Nerantzaki, M.; Giliopoulos, D.J.; Triantafyllidis, K.; Kostoglou, M.; Bikiaris, D.N. Use of mesoporous cellular foam (MCF) in preparation of polymeric microspheres for long acting injectable release formulations of paliperidone antipsychotic drug. Eur. J. Pharm. Biopharm. 2017, 117, 77–90.
86. Lauritano, C.; Andersen, J.H.; Hansen, E.; Albrigtsen, M.; Escalera, L.; Esposito, F.; Helland, K.; Hanssen, K.Ø.; Romano, G.; Ianora, A. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. Front. Mar. Sci. 2016, 3, 68.
87. Saxena, A.; Dutta, A.; Kapoor, N.; Kumar, A.; Tiwari, A. Envisaging marine diatom Thalassiosira weissflogii as a ‘SMART’ drug delivery system for insoluble drugs. J. Drug Deliv. Sci. Technol. 2022, 68, 5–6.
88. Esfandyari, J.; Shojaedin-Givi, B.; Hashemzadeh, H.; Mozafari-Nia, M.; Vaezi, Z.; Naderi-Manesh, H. Capture and detection of rare cancer fluorescence of a novel functionalized diatom. Photodiagn. Photodyn. Ther. 2020, 30, 101753.
89. Delasoie, J.; Rossier, J.; Haeni, L.; Rothen-Rutishauser, B.; Zobi, F. Slow-targeted release of a ruthenium anticancer agent from vitamin B12 functionalized marine diatom microalgae. Dalton Trans. 2018, 47, 17221–17232.
90. Rea, I.; Martucci, N.M.; De Stefano, L.; Ruggiero, I.; Terracciano, M.; Dardano, P.; Migliaccio, N.; Arcari, P.; Taté, R.; Rendina, I.; et al. Diatomite biosilica nanocarriers for siRNA transport inside cancer cells. Biochim. Biophys. Acta 2014, 1840, 3393–3403.

91. Todd, T.; Zhen, Z.; Tang, W.; Chen, H.; Wang, G.; Chuang, Y.J.; Deaton, K.; Pan, Z.; Xie, J. Iron oxide nanoparticle encapsulated diatoms for magnetic delivery of small molecules to tumors. Nanoscale 2014, 6, 2073–2076.
92. Javalkote, V.S.; Pandey, A.P.; Puranik, P.R.; Deshmukh, P.K. Magnetically responsive siliceous frustules for efficient chemotherapy. Mater. Sci. Eng. C 2015, 50, 107–116.
93. Manago, S.; Migliaccio, N.; Terracciano, M.; Napolitano, M.; Martucci, N.M.; De Stefano, L.; Rendina, I.; De Luca, A.C.; Lamberti, A.; Rea, I. Internalization kinetics and cytoplasmic localization of functionalized diatomite nanoparticles in cancer cells by Raman imaging. J. Biophotonics 2018, 11, e201700207.
94. Terracciano, M.; Shahbazi, M.A.; Correia, A.; Rea, I.; Lamberti, A.; De Stefano, L.; Santos, H.A. Surface bioengineering of diatomite based nanovectors for efficient intracellular uptake and drug delivery. Nanoscale 2015, 7, 20063–20074.
95. Delalat, B.; Sheppard, V.C.; Rasi Ghaemi, S.; Rao, S.; Prestidge, C.A.; McPhee, G.; Rogers, M.L.; Donoghue, J.F.; Pillay, V.; Johns, T.G.; et al. Targeted drug delivery using genetically engineered diatom biosilica. Nat. Commun. 2015, 6, 8791.
96. Lewin, J.C. The dissolution of silica from diatom walls. Geochim. Cosmochim. Acta 1961, 21, 182–198.
97. Zhang, H.; Shahbazi, M.A.; Mäkilä, E.M.; da Silva, T.H.; Reis, R.L.; Salonen, J.J.; Hirvonen, J.T.; Santos, H.A. Diatom silica microparticles for sustained release and permeation enhancement following oral delivery of prednisone and mesalamine. Biomaterials 2013, 34, 9210–9219.
98. Hussein, H.A.; Kassim, M.N.I.; Maulidiani, M.; Abas, F.; Abdullah, M.A. Cytotoxicity and 1H NMR metabolomics analyses of microalgal extracts for synergistic application with Tamoxifen on breast cancer cells with reduced toxicity against Vero cells. Heliyon 2022, 8, e09192.
99. Gabbai-Armelin, P.R.; Kido, H.W.; Cruz, M.A.; Prado, J.P.; Avanzi, I.R.; Custódio, M.R.; Renno, A.C.; Granito, R.N. Characterization and cytotoxicity evaluation of a marine sponge biosilica. Mar. Biotechnol. 2019, 21, 65–75.