Peer-reviewed version available at *Pharmaceutics* **2018**, *10*, 242; doi:10.3390/pharmaceutics10040242

# Diatoms Green Nanotechnology for Biosilica-Based Drug Delivery Systems

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## Abstract

Diatom microalgae are the most outstanding natural source of porous silica. Diatom cell is enclosed in 3-D ordered nanopatterned silica cell wall, called frustule. The unique properties of diatoms frustule, including high specific surface area, thermal stability, biocompatibility, tailorable surface chemistry, make them really promising for biomedical applications. Moreover, diatoms are easy to cultivate in artificial environment and there is a huge availability of diatom frustules as fossil material (diatomite) in several areas of the world. For all these reasons, diatoms are an intriguing alternative to synthetic materials for the development of low-cost drug delivery systems. This review article focuses on the possible use of diatoms derived silica as drug carrier systems. The functionalization strategies of diatom micro-/nanoparticles for improving their biophysical properties, such as cellular internalization and drug loading/release kinetics, are described. In addition, the realization of hybrid diatom-based devices with advanced properties for theranostics and targeted or augmented drug delivery applications, are also discussed.

**Keywords:** nanotechnology, diatom, biosilica, drug delivery, hybrid devices

## 1. Introduction

Drug delivery systems (DDSs) have the capability to amend the limitation of conventional pharmaceuticals administration such as poor solubility, short half-life circulation, systemic toxicity and degradation [1]. Development of new drug molecules is expensive and time consuming. Hence, the application of nanotechnology to medicine allowed the realization of DDs able to improve the performance and efficacy of existing pharmaceutic compounds, as well as quality life and longevity of patients [2]. Among all of available nanomaterials for drug delivery applications (e.g. liposomes,

dendrimers, polymer, micelles, nanogels, carbon nanotubes, porous silicon (PSi)/silica-, gold-nanoparticles (NPs), etc.) numerous studies have investigated porous silica NPs due to their unique properties such as large specific surface area and pore volume, controllable particle size, good biocompatibility, easy functionalization chemistry [3]. Compared with other synthetic porous silica nanocarriers, mesoporous silica NPs (e.g. MCM-41, SBA-15, M41S) with a pore size ranging from 2 nm to 50 nm resulted to be valid candidates for drug delivery applications [4]. Since their production route is laborious, expensive and involving toxic materials, there is a massive demand to replace these synthetic materials with a valid natural surrogates. Surprisingly, nature has provided exciting porous material with 3-dimensional (3D) porous structures: the single cell photosynthetic diatom algae [5–8]. Diatom microshells, characterized by unique pill-box micro structures having porosity in the micro/nanoscale range, high surface area and great biocompatibility, have been shown to be a promising and low-cost biomaterial for drug delivery applications [9,10]. Diatoms as source of natural silica can be generated inexpensively and in enormous amount through biological algae replication. Diatomite, also known as diatomaceous earth, is a fossil material formed by skeletons of diatoms dead and accumulated on the bottom of lakes or oceans over millions of years [8, 9]. Diatomite is the most abundant source of biosilica and it is largely used as inexpensive biosilica mineral in several industrial applications (e. g. food industry, agriculture, pharmaceutics, and so on) [5,11]. Despite the great development in the field of nanotechnology, diatoms' architecture can actually compete with man-made fabricated devices [12–14]. Due to the high surface area (up to 200 $m^2/g$), thermal stability, easily modification through genetic manipulation or chemical modifications, mechanical resistance, optical and photonic properties, non-toxicity and biocompatibility, diatom frustules are potential scaffolds for the development of nanostructured devices for a variety of applications ranging from liquids filtrations, DNA purifications, immunoprecipitations, photonics, sensing, biosensing, and drug delivery [14–22][i].

This review article presents the recent progress on applications of diatom biosilica-based systems for drug delivery applications. The properties of diatom silica used as whole microfrustules or reduced

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to NPs as non-toxic drug carriers, are described. The functionalization of diatoms based- micro/nano-particles that improves their physicochemical properties and drug delivery behavior are also discussed. In addition, the advantage of using hybrid nanodevices obtained by the combination of diatoms biosilica and other inorganic NPs (e.g. iron oxide-, gold-NPs, graphene oxide nanosheets) for advanced medical applications is described.

## 2. Diatoms: a natural source of nanostructured biosilica

Diatoms are unicellular photosynthetic algae which colonize every aquatic environments, having an essential impact on the maintenance and development of planet life [23]. Diatom cell walls (called frustules) are the most impressive example of 3-D architectures occurring in nature [24]. There are more than 200 living diatom genera with more than 100 000 estimated species classified by their typical morphologies and size (from 2 µm to 2 mm) (**Figure 1, a**). With respect to frustules symmetry, diatoms are divided into two groups: the centrics are radially symmetrical about an axis that passes through the center of the cell; the pennates are bipolar symmetrical with the longitudinal axis running parallel to the plane of symmetry [25,26]. Despite the difference in shape, diatom frustules are typically bipartite structures, with two overlapping valves called thecae. The upper part (epitheca) and lower part (hypotheca), characterized by a series of linking bands (girdle bands or cingula), are often likened to a petri dish or pill-box [27]. The hierarchical organization of porous elements (e. g. cribellum, cribrum, foramen) contains pore diameters with various patterns ranging from nanometers to micrometres (**Figure 1, b**) [28]. These unicellular algae are the prevalent organisms engaged in the biosilicification process, both in terms of the number of silicified structures they can produce, and in global production of biogenic silica [29]. The biomineralization process occurs in specialized intracellular compartments, named silica deposition vescicles (SDVs), where the silicon uptaken from the environment in soluble form as orthosilicic acid $Si(OH)_4$ is converted into silica network ($SiO_2$) [30,31]. Diatoms can be easily cultivated in large quantities: the cells culture route consist in supply the inorganic salts as nutrient and sun light to grow [32,33]. Diversely, a less expensive source of diatom silica is the diatomite formed by million years of fossilisation process of dead algae and

currently mined. Diatom frustules retain impurities (such as inorganic oxide $Al_2O_3$, $Fe_2O_3$, $CaCO_3$, CaO) mainly due the local environment and aging conditions [34]. Thus, diatoms biosilica could be obtained after proper purification treatments (e.g. sulfuric acid-, hydrochloric acid-based solutions) in order to remove impurities, making diatom frustules suitable and safe for biomedical applications [35–37]. The diatoms biosilica structure after acid/oxidative cleaning can be easily manipulated as micro or nano multifunctional scaffold by various chemical modifications, opening the way to new class of bioengineered nanostructured materials for biomedical applications [18,38,39]. The common strategy to develop engineering devices with diatoms is to use the chemistry of silica, which has been highly evolved during the last decades [40,41]. Frustules surface can be chemically modified by targeting free reactive silanol (SiOH) groups, thus improving drug loading/release properties and adding other reactive groups (−NH2, −COOH, −SH, and −CHO), which can be useful for the conjugation of biomolecules (e.g., enzymes, proteins, antibodies, peptides, DNA, aptamer).

## 3. Diatom-based smart drug delivery systems

### 3.1 Micro-nano carriers for delivery of therapeutics

Over the last decades, the use of diatom frustules in controlled drug delivery applications has greatly increased due to their distinct properties [10,42–45]. Starting from 2010, the first studies on the potential use of bare (i.e. without any surface modification) diatom frustules in drug delivery were based mainly on the encapsulation of therapeutics molecules for oral drug delivery. Losic and co-workers explored the application of diatoms microshell for drug delivery of the hydrophobic molecule indomethacin [46]. Results showed the great potentiality and efficacy of diatoms frustule to deliver the drug with about 22 wt % drug loading capability and sustained drug release over two weeks. Principally, two steps of drug release from the frustules were observed: the first over 6 h was rapid due to the surface deposition of the drug, and the second was slow and sustained over two weeks with zero order kinetics ascribed to the release from the internal hallow structure. Zhang et al. investigated the potential of diatom microparticles for the delivery of mesalamine and prednisone under simulated gastrointestinal conditions, as well as the permeability through Caco-2/HT-29 co-culture monolayers

[47]. The results demonstrated the sustained and controlled release of both drugs. Moreover, low toxicity of diatoms (up to 1000 μg/mL) against colon cancer cell lines Caco-2/HT-29 confirmed the safaty of diatoms frustule for drug delivery applications. The possibility to chemical modify the frustules surface opens the way to improve drug loading/release properties and, moreover, to functionalize their surface with organic-, inorganic- and bio-molecules obtaining advanced nanostructured devices. Aw et al. described, for the first time, the impact of organosilanes functionalization on diatom silica microcapsules on drug loading and release of water insoluble drug indomethacin [48]. Different compounds 3-aminopropyltriethoxysilane (APTES) and N-(3-(trimethoxysilyl)propyl)ethylene diamine (AEAPTMS), and phosphonic acids (2-carboxyethyl-phosphonic acid and 16-phosphono-hexadecanoic acid) were used to give hydrophilic and hydrophobic features to the frustules. The results showed that an appropriate surface diatoms functionalization is able to tune drug loading (15−24 wt%) and release (6−15 days). In particular, the hydrophilic functionalization increased the drug loading and prolong the drug release, whereas the hydrophobic modification created lower loading and a fast drug release. Milovic and co-workers reported, for the first time, the application of diatom as a solid carrier for water insoluble carbamazepine drug (CBZ) applied in oral drug delivery system based on the self-emulsifying drug delivery system (SEDDS) [49]. Different solid samples of CBZ suspension in SEDDS, were prepared using two methods. The first method was based on adsorption of CBZ dispersion in SEDDS by gentle mixing with diatoms; the second method was based on diatoms dispersion in ethanol solution of CBZ/SEDDS components, followed by ethanol evaporation. The dissolution of CBZ from SSEPS sample prepared using the second method was faster and better than the sample prepared by the first approach. Higher dissolution was attributed to the loading procedure which was carried out in liquid and hence a partial adsorption of drug molecules might have occurred inside the pores of diatoms. Moreover, stability studies under accelerated conditions for 10 weeks demonstrated that diatoms with adsorbed liquid CBZ-loaded SEDDS maintain polymorphic form of CBZ without significant influence on the drug dissolution rate and crystallinity, contrary to conventional solid dispersion.

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Many reports in the literature have demonstrated the great advantages resulting from the use of polymers in the preparation of nanostructured devices for drug delivery applications [50–55]. Vasani et al. have demonstrated a controlled drug delivery of antibacterial agent levofloxacin from modified-diatom microcapsules obtained by grafting thermo-responsive oligo(ethylene glycol) methacrylate copolymers on their surface using surface-initiated atom radical polymerization (ATRP) [56]. Drug release experiments from the copolymer modified microcapsules showed strong temperature dependence of drug release when comparing release kinetics below and above the lower critical solution temperature (LCST) of the grafted copolymer (**Figure 2**). The antimicrobial action of the released drug was confirmed against two common wound pathogens, proving diatom frustules as inexpensive source for the scalable production of drug carriers facilitating controlled therapeutics delivery. Terracciano et al. reported, for the first time, a study on a biofunctionalization process of diatomite NPs based on PEGylation and cell penetrate peptide biocongiugation (CPP) in order to enhance aqueous stability, improve biocompatibility, reducing cytotoxicity, and increasing the solubility of insoluble anticancer drug sorafenib [57]. Diatomite NPs (DNPs) were obtained through mechanical crushing, sonication of diatom microfrustules and acid solution purification [17]. After that, surface functionalization was carried out with APTES followed by PEGylation via covalent bond between the carboxyl groups (–COOH) of the PEG chains and the amino groups (–$NH_2$) of silanized DNPs using carbamide chemistry. The amino-terminal-PEG-modified DNPs were then conjugated with the carboxyl groups of CPP-peptide, by using the same chemistry (**Figure 3**). The obtained NPs were stable in aqueous solution and resulted biocompatible when tested on breast cancer cell lines (MCF-7 and MDA-MB-231), and red blood cells (RBCs). Moreover, the results confirmed that PEGylation and CPP bioconjugation improved the loading/and release kinetics of anticancer drug sorafenib and NPs' cellular uptake, making them suitable for intracellular drug delivery. Recently, diatom-based nanostructures were explored for gene therapy applications. The first successful attempt to use diatomite NPs for small interfering RNA (siRNA) delivery has been demonstrated by Rea et al. [58]. In this study, siRNA molecules were electrostatically linked to poly-D-arginine peptide

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covalently bond to APTES surface-modified DNPs (**Figure 4**). At first, it was demonstrated the safety of drug free DNPs (up to 200 μg/mL) after 72 h of incubation with epidermoid carcinoma cells (H1355). Effective delivery of siRNA into cytoplasm with efficient gene silencing was demonstrated. These results suggest DNPs as innovative nanocarriers for siRNA transport inside the cancer cells, highlighting the non-toxicity of the material, the efficient cellular uptake and the gene silencing capability in cancer cells. In similar frame, Martucci et al. described a new personalized B-cell lymphoma therapy based on a site-specific receptor-mediated diatomite NPs used as drug delivery systems [59]. Natural silica-based NPs were silanized with APTES and modified with siRNA/poly-D-Arg peptide to actively target antiapoptotic factor B-cell lymphoma/leukemia 2 (Bcl2). The effectiveness of siRNA targeting Bcl2 modified DNPs in downregulation of gene expression was evaluated by quantitative real-time polymerase chain reaction and Western blot analyses. The resulting gene silencing observed was of significant biological importance and opened to new possibilities for the personalized treatment of lymphomas by using DNPs as nanocarriers. Recently, Rea and co-workers investigated the internalization kinetics and intracellular spatial distribution of non-targeting siRNA-loaded DNPs in H1355 lung cancer cells up to 72 h by using label-free Raman spectroscopy [60]. Raman spectra revealed specific bands assigned to DNPs and cellular components, providing evidence that the NPs were internalized and located in endocytic vesicles in the perinuclear region. The analyses demonstrated a considerable NPs cellular uptake within 6 h, with equilibrium being achieved after 18 h leading to an efficient distribution of DNPs within the cell cytosol. Moreover, the results showed the presence of DNPs up to 72 h in the cells without damage their viability or morphology. These data were also confirmed by confocal microscopy and photoluminescence analyses, proving the potentiality of using not conventional Raman spectroscopy to study internalization and fate of NPs in nanomedicine.

### 3.2 Hybrid multifunctional devices

A major goal in nanomedicine is the implementation of multifunctional platforms within a single targeted nanodelivery system that would simultaneously perform diagnosis, targeted delivery and

efficient therapy [61–64]. Great efforts have been made in the nanotechnology research field to design systems that integrate multiple components and different materials at the nano scale into a single nanodevice, the so-called hybrid nanodevices [65]. Nanosized materials such as magnetic and gold NPs, semiconductor quantum dots and so on, have been proposed for magnetic resonance imaging (MRI), fluorescent and photoacoustic imaging, contrast agent, drug delivery carrier, cell sorting, and labelling [62–67]. Recent literature reported promising results obtained by using hybrid based-porous silica NPs as theranostic nanodevices for both imaging and drug delivery. Fine tuning surface chemical modifications are required to incorporate diatoms biosilica with inorganic (graphene oxide, titanium dioxide, etc), semiconducting (Si–Ge), metal (Au) scaffolds, thus obtaining hybrid-diatoms based devices [68–71]. Losic et al. fabricated magnetically guided drug carriers by functionalizing diatoms with dopamine-modified iron-oxide NPs [72]. Dopamine was used to modified iron oxide NPs (DOPA/Fe3O4) thus forming a stable and robust cationic complexes. The magnetized diatoms were obtained by an electrostatically interaction between the cationic magnetic complexes and anionic diatom frustules. The drug release study demonstrated the possibility of obtaining sustained release of a poorly water soluble drug indomethacin within 2 weeks, opening the way for magnetically guided target cancer drug delivery by using hybrid diatom silica shells. For the first time, Todd et al. reported iron oxide NPs (IONPs) encapsulated onto diatom frustules (10 μm size) surface as magnetically active devices for *in vivo* delivery of anticancer small molecules [73]. The magnetic resonance and fluorescence imaging were used to investigate the *in vivo* fate of magnetized diatom frustules. The results demonstrated a significant particles accumulation at tumour site, 6 times higher than the control, when a magnetic field was applied. This, together with the low toxicity and biodegradability of IONPs and diatoms frustule, suggest the great potentiality of this hybrid nanocomplex for drug delivery field.

Kumeria et al. reported, for the first time, the realization of hybrid microcapsules decorated with 2D graphene oxide (GO) nanosheets thus imparting to the systems pH dependent triggered release [74]. Two different approaches for the realization of the nano-hybrids were used: a covalent coupling of

GO sheets onto the diatoms surface and electrostatic attachment. Diatoms structures were silanized using APTES in order to introduce positively amine groups to covalently or electrostatically attach negative GO nanosheets to frustules surface (**Figure 5**). The application of the obtained nano-hybrids (GO-DE hybrids) as smart pH sensitive drug carriers at pH 7.4 and pH 3.5 was demonstrated using a non-steroidal anti-inflammatory drug model indomethacin. The covalently attached GO-DE hybrid devices showed a better drug loading/release capability then electrostatically ones. The results indicated a sustained drug release at pH 3.5 prolonged up 37 days in comparison to 14 days of the control (APTES-DE). This phenomenon was explained by the enhanced interaction of drug with GO through H-bonding and hydrophobic interactions to the diatom surface. The release behaviour was regulated by the changes of loaded drug and GO nanosheets interactions at different buffer pH. Recently, Terracciano et al. proposed hybrid gold-diatomite NPs as multifunctional innovative devices for imaging (e.g. photoacoustic, X-ray) and drug delivery purposes [75]. The hybrid complexes (average size of 400 nm) were obtained by decorating polyethylene glycol (PEG)-modified diatomite NPs with gold NPs by a non-conventional one-pot liquid phase synthesis (**Figure 6**). The inner surface of DNPs was modified by APTES and PEGylated by $NH_2$-PEG-COOH in order to improve DNPs aqueous stability and biocompatibility. Moreover, this procedure provided positive amine (-$NH_2$) groups on DNPs' surface able to adsorb electrostatically the $AuCL_4^-$ ions of gold precursor. PEG-DNPs@AuNPs were obtained by chemical reduction of oxidized $Au^{3+}$ species to metallic $Au^0$ particles by using reducing agent $NaBH_4$ in presence of PEG diacid as stabilizer. The presence of PEG diacid onto AuNPs hindered particles aggregation through electrostatic repulsion between its carboxylic groups (-COOH) and the gold surface, conferring greater stability, as well as biocompatibility, more than conventional stabilizing agents. The formation of PEG-DNPs@AuNPs was macroscopically observed as a colour change of the sample solution from pale yellow to deep purple after the addition of $NaBH_4$. The nanostructures showed optical properties, displaying strong surface plasmon resonance band in the UV-vis spectra with peak absorbance at 550 nm. The *in vitro* cytotoxicity and cellular uptake analysis on human cervix epithelioid carcinoma (HeLa) demonstrated

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the safety of the nanocomplexs with concentration up 400 μg/mL for 72 h and an efficient cytoplasmic localization. These preliminary results suggested the suitability of plasmonic PEG-DNPs@AuNPs nanocomplex as potential theranostic devices in advanced nanomedicine applications.

## 4. Conclusions

Diatoms possess an intricate 3-D biosilica porous structures much more advanced and complex than costly man-made fabricated porous materials. Characterized by hierarchical pore structure, great surface area, easily modifiable surface chemistry, good permeability, non-toxicity, high biocompatibility, diatom frustules have been exploited as low-cost scaffold for the preparation of innovative devices for drug delivery applications. The present review article outlines the applications of diatom biosilica-based micro-/nano-devices in drug delivery field. The preparation of diatom micro-/nanocarriers, surface chemical modifications, biocompatibility tests, cellular uptake, drug loading/release capability, as well as targeted therapeutics transport inside cells and advanced drug delivery applications, were discussed. The presented results would emphasize the great advantage of using diatom biosilica as inexpensive alternative to synthetic porous silica for the preparation of the next generation of DDSs. It is important to note that the amorphous silica of diatoms frustule has been yet authorized by the Food and Drug Administration as Generally Recognized as Safe (GRAS, 21 CFR Section 573.340) for the production of food and pharmaceutics, and classified as not carcinogenic by the International Agency for Research on Cancer. Nevertheless, more studies should be carried out to evaluate the *in vivo* safety as drug carriers on animal models [25]. After this last effort, the hypothesis to concretely commercialize the diatoms frustules in the drug delivery area could come true.

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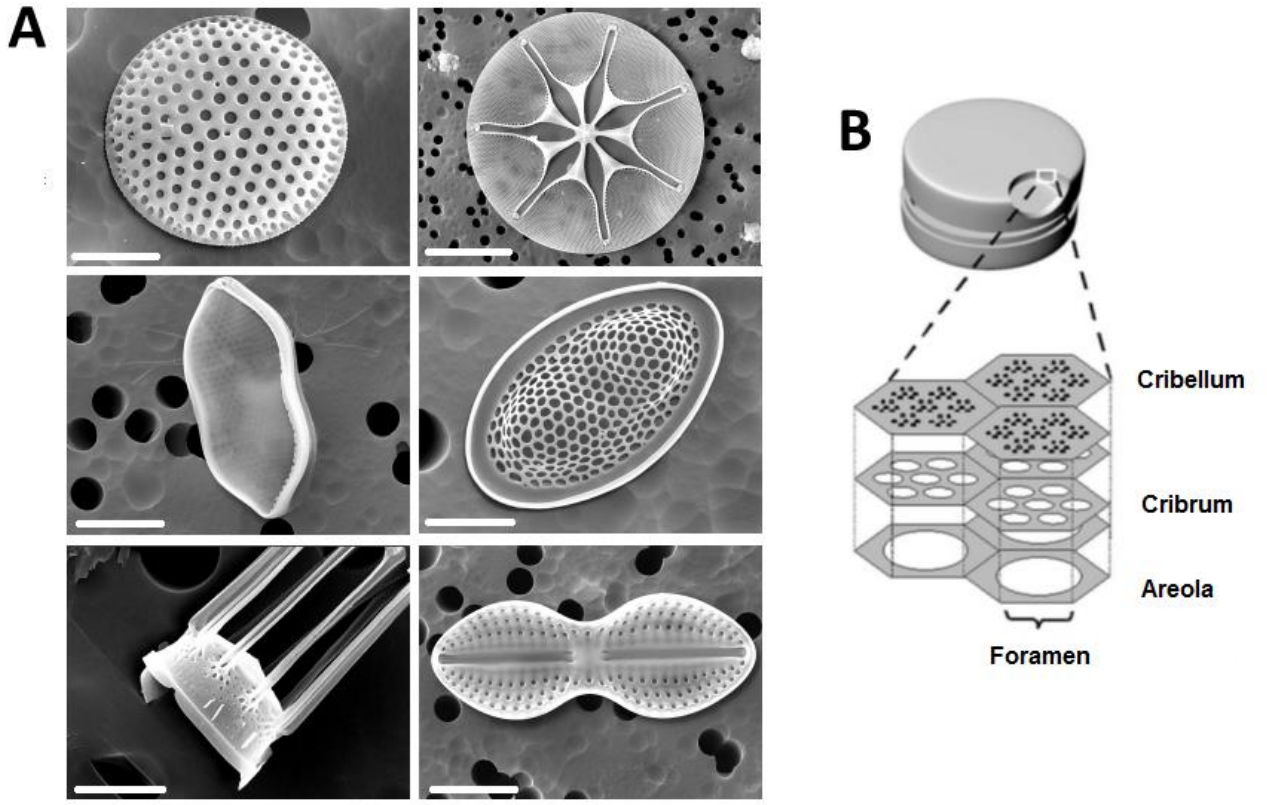


**Figure 1. a**) Scanning electron microscopy (SEM) images of the cell walls of different diatom species. **b**) Illustration of a centric diatom frustule with cross-sectional profile of the silica wall typically formed by three overlapping porous layers: cribellum, cribrum and areola. Reproduced with permission from [27].

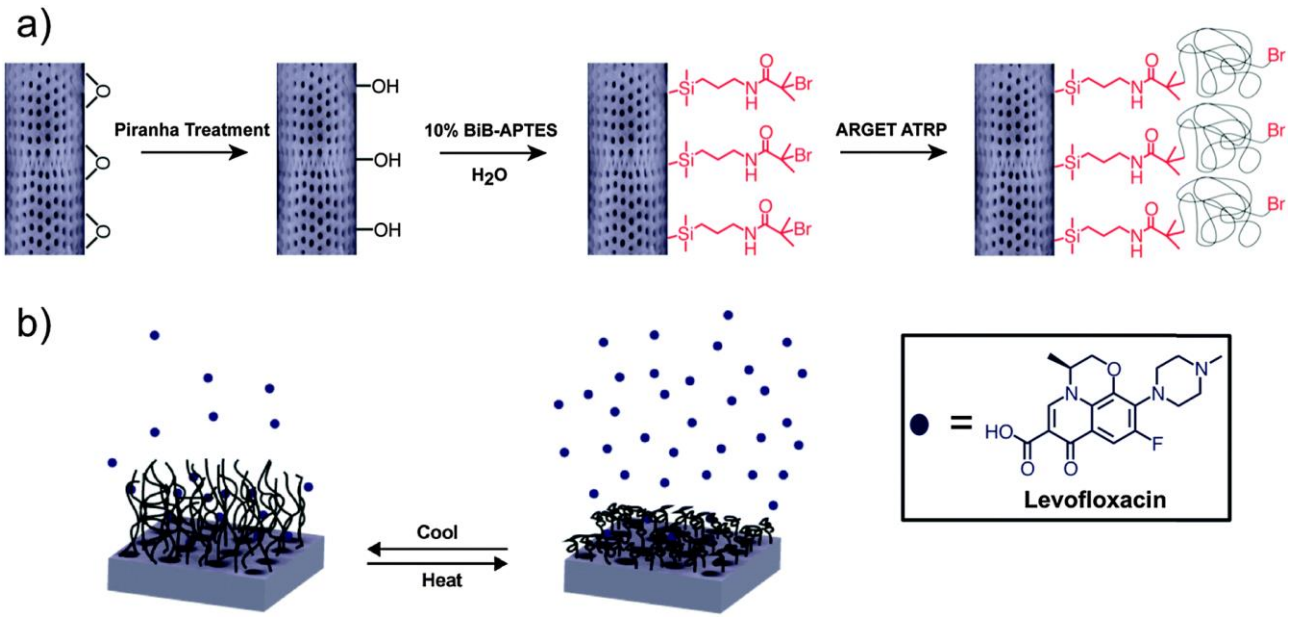


**Figure 2. a)** Schematic representation of diatom frustule functionalization with thermo-responsive polymer and **b**) levofloxacin drug release from thermo-responsive polymer-grafted biosilica frustule. Reproduced with permission from [55].

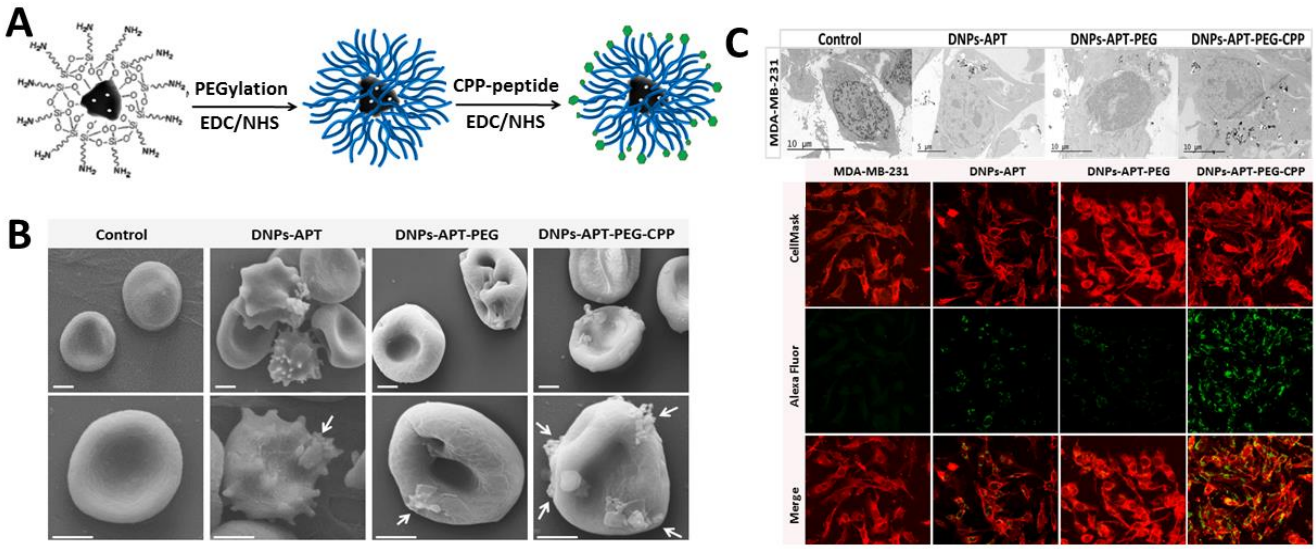


**Figure 3. a)** Schematic representation of DNPs functionalization with PEG and CPP-peptide. **b**) SEM pictures of the red blood cells morphological modification after the exposure to the modified-DNPs. **c**) Transmission electron microscope (TEM, upper panel) and confocal (lower panel) imaging of MCF-7cells treated with 50 µg/ml of DNPs-APT, DNPs-APT-PEG, and DNPs-APT-PEG-CPP for 12 h at 37°C. Adapted with permission from [56].

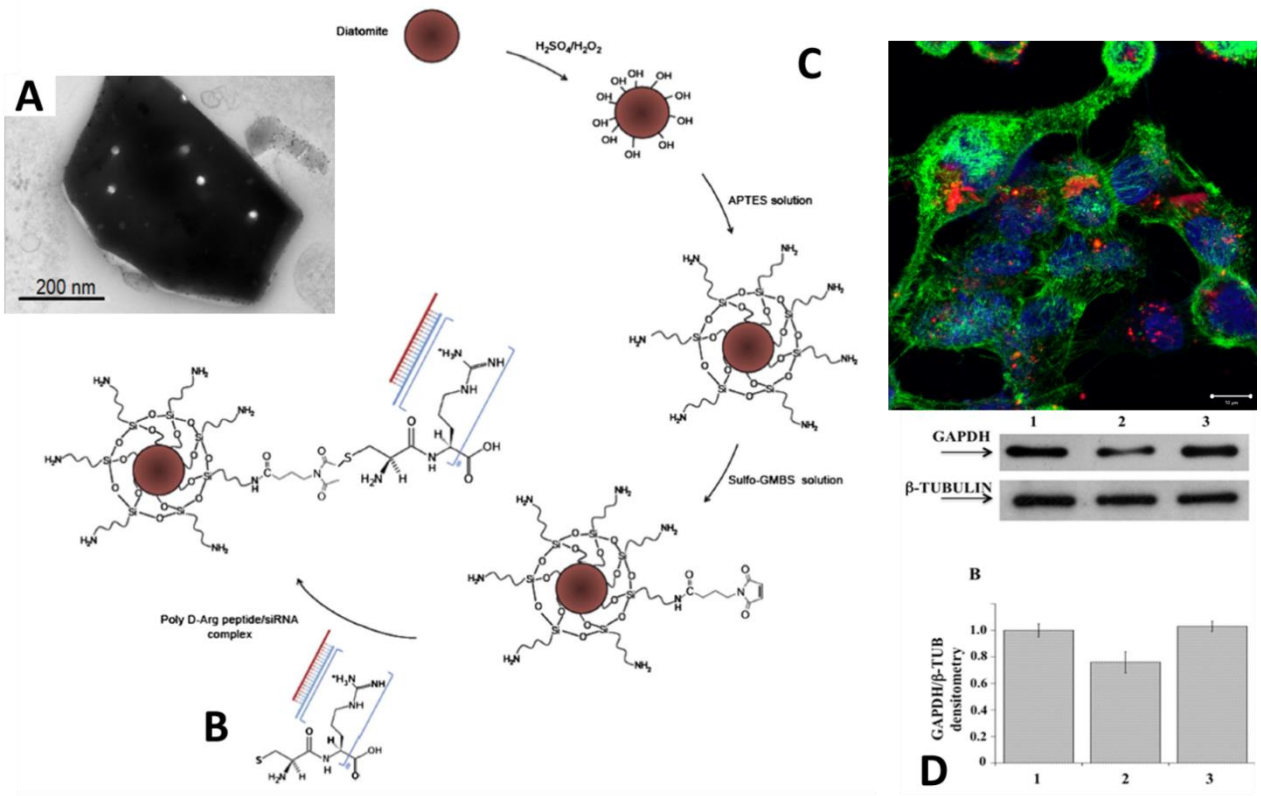


**Figure 4. a)** Transmission electron microscope (TEM) images of diatomite NPs (DNPs). **b**) Schematic representation of DNPs functionalization with siRNA. **c**) Representative confocal microscopy image of cells treated with Dy547-labelled siRNA-DNPs for 12 h at 37 °C. Cell nuclei and membranes were stained with Hoechst 33342 and WGA-Alexa Fluor 488, respectively. **d**) Immunoblotting analysis of GAPDH (upper gel) and of β-tubulin (lower gel) of protein expression in DNPs-siRNA treated cells. Lanes: (1) control cells; (2) DNPs-GAPDH-siRNA; and (3) DNPs-SCR-siRNA. Adapted with permission from [57].

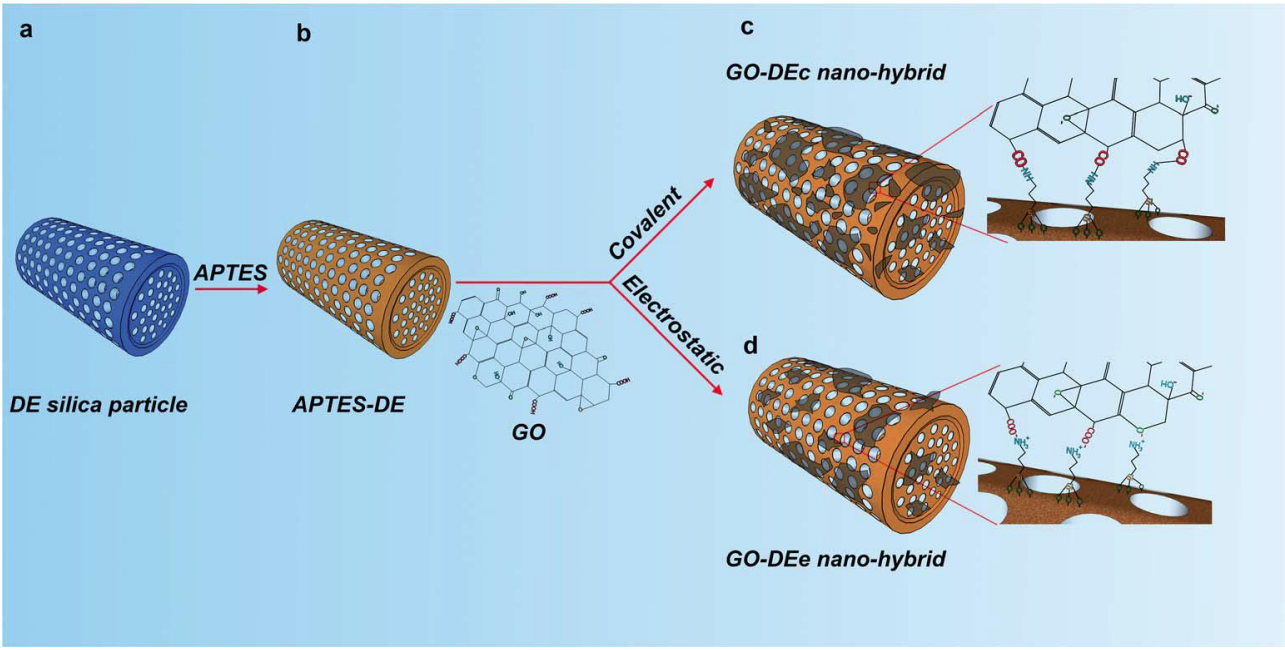


**Figure 5.** Schematic representation of GO nanosheets functionalization of diatom frustules' surface via electrostatic or covalent chemical approach for the realization of nano-hybrid drug delivery devices. Reproduced with permission from [74].

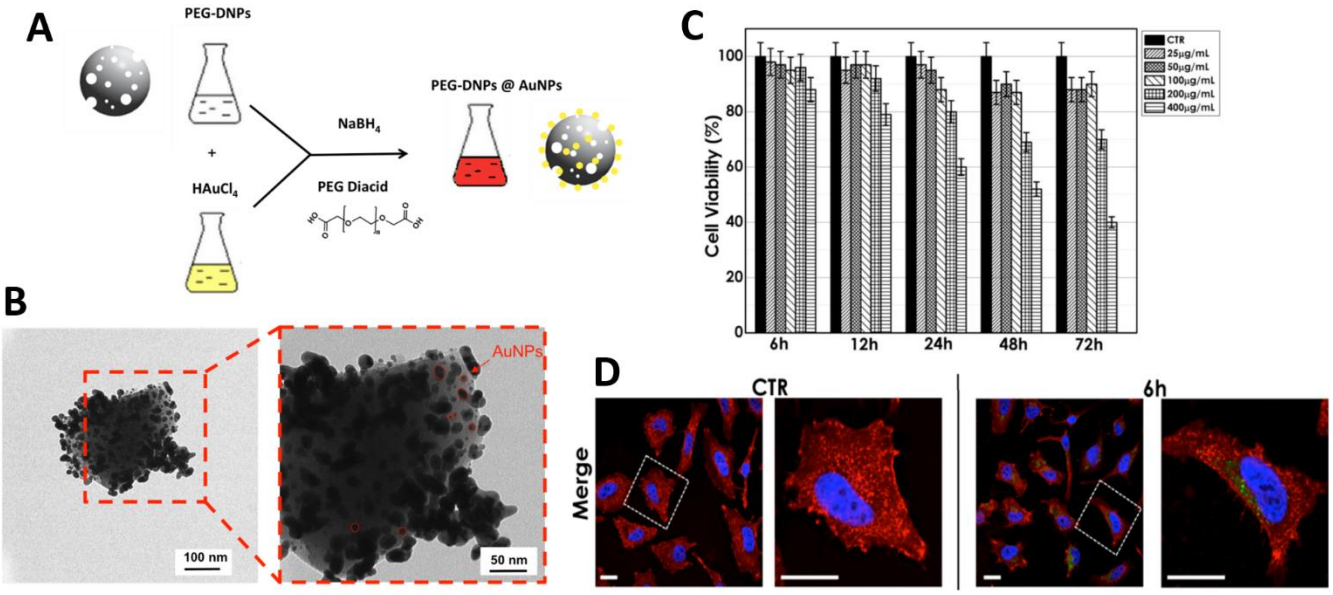


**Figure 6. a**) Scheme of one-pot liquid phase synthesis method used for decorating diatomite NPs' surface with gold NPs (PEG-DNPs@AuNPs). **b**) TEM images of hybrid PEG-DNPs@AuNPs **c**) Cell viability of HeLa cells after exposure to 25, 50, 100, 200 and 400 μg/ml of the PEG-DNPs@AuNPs for 6, 12, 24, 48, and 72 h at 37 °C. **d**) Representative confocal microscopy image of PEG-DNPs@AuNPs (100 μg/ml) labelled with Alexa Fluor® 488 (green) internalized into HeLa cells at 6h. Cell nuclei and membranes were stained with Hoechst (blue) and WGA-Alexa Fluor® 555 (red), respectively. Adapted with permission from [75].

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