Diatom-Derived Silica for Biomedical Applications

Subjects: Materials Science, Biomaterials
Contributor: Angela Sardo

Diatoms are unicellular eukaryotic microalgae widely distributed in aquatic environments, possessing a porous silica cell wall known as frustule. Diatom frustules are considered as a sustainable source for several industrial applications because of their high biocompatibility and the easiness of surface functionalisation, which make frustules suitable for regenerative medicine and as drug carriers. Frustules are made of hydrated silica, and can be extracted and purified both from living and fossil diatoms using acid treatments or high temperatures. Biosilica frustules have proved to be suitable for biomedical applications, but, unfortunately, they are not officially recognised as safe by governmental food and medical agencies yet.

Keywords: biosilica ; diatom frustule ; sustainable production ; drug delivery

1. Introduction

Diatoms are an extremely diverse group of algae, comprising more than 100,000 different species [1]. They are able to colonise a large plethora of aquatic environments, and play a significant role on a global scale in the biogeochemical cycles of carbon and silicon in the water column. Two diatom species, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, have been employed as model species for studies of gene expression and regulation, since they were the first species for which the whole genome was fully sequenced [2][3]. Subsequently, genomes have been sequenced from a number of diatoms possessing specific metabolic or physiological features, such as oleaginous (*Fistulifera solaris*), psicrophylic (*Fragilariopsis cylindrus*), araphid (*Synedra acus* subsp. *radians*), oceanic (*Thalassiosira oceanica*), biofilm-forming (*Seminavis robusta*), and heterotrophic (*Nitzschia* sp.) species [4][5][6][7][8][9]. Apart from their ecological role, diatoms are also suitable for several biotechnological applications. They can be cultured in the laboratory under sterile conditions and controlled temperatures, light irradiance and nutrient concentrations in order to achieve faster growth rates and to promote the accumulation of specialty products. Diatoms have been employed during the last decades for the production of metabolites exhibiting different biological activities and used as sources for cosmetic ingredients [10], food or feed supplements [11][12][13], fertilizers [14], and sorbents or accumulators for the bioremediation of aquatic environments [15][16]. Microalgae other than diatoms, especially freshwater green algae, also exhibit a great potential in one or more of the abovementioned fields of research.

The true distinctive feature that makes diatoms more suitable than other taxa for biotechnological purposes, is the high proportion of amorphous silica within their cell wall. This natural source of silicon has already shown several advantages, such as its high surface area and biocompatibility, and can be employed for various research fields, especially for biomedical applications after in vitro or in vivo treatments [17]. Diatom-derived silica is also available in huge amounts in aquatic benthic environments, as a consequence of the sedimentation of dead diatom cells.

Currently, diatom biosilica is considered as a suitable biomaterial for metal removal from aquatic environments, as a catalyst support, in optical devices, as a microsensor, and other kinds of applications [18][19]. Since its presence on the market as a device for aquatic remediation and as food-grade products is a pledge of its effectiveness in these fields, the present review is mainly focused on evaluating the potential of diatom biosilica for biomedical applications.

Diatom biosilica is actually exploited, indeed, for its potential as a drug carrier [20] and as a scaffold for bone tissue regeneration [21]. Biosilica-based processes can be considered as low-cost and environmentally friendly alternatives to processes based on artificial structures. While the production of synthetic materials requires the implementation of specific protocols, biosilica carries the advantage of triggering natural and sophisticated structure formation. For example, the employment of diatom-derived biosilica for the development of optical sensors may turn out to be, in the future, more attractive than using synthetic crystals, since it allows control and manipulation of light in a cost-effective way [22]. Biotemplated-based silica can be synthesized by rapid environmentally sustainable methods (solvent-free procedures), thus avoiding the use of hazardous chemicals, and allowing a good control of condensation rates [23].

2. Diatom Biosilica Sources

Diatom-derived silica can be obtained either from living cultures or fossil diatoms (diatomite, e.g., chalky deposits of skeletal remains). The energy required for diatom growth is sustained by either led-based (i.e., low energy demanding) artificial light or sunlight. Furthermore, the nutrients required for algal growth, such as nitrates, phosphates, silicates, vitamins, and some trace elements, can be purchased for a relatively cheap price or even obtained from wastewaters. To avoid both the costs of artificial illumination and the seasonal variability of sunlight, cells can also be grown heterotrophically [24][25][26][27], although organic substrates are to be supplied in this case. However, only a small number of species are able to grow in the dark [28][29], and organic compounds can promote bacterial growth leading to culture contaminations and to a decrease in cell growth. Biosilica is obtained after cell dewatering (i.e., centrifugation or filtration of the whole culture), followed by a purification process that is usually based on treatments with strong acids and/or high temperatures (see below). Besides, the limited motility of diatoms (due to the lack of flagella) and the “heavy” cell wall (due to the presence of a high silicon amount) enhance the spontaneous sinking of cells, limiting the volume to harvest and, thus, costs of biomass collection.

Diatoms generally exhibit fast growth rates and high lipid and biomass productivities, [30] which can be further enhanced by tuning growth conditions [31][32], making diatoms promising candidates for mass culturing. However, to the best of our knowledge, no diatom-based industrial plants (i.e., indoor or outdoor systems of algal culturing) are focusing on biosilica production as their main activity. Follow-up studies are thus required to lay the foundations for the industrial production of silica-based biomaterials.

The most abundant source of biosilica that does not foresee the induction of living cultures is diatomite, which can be easily crushed into a fine powder to become a marketable product, namely, diatomaceous earth (DE). Diatomite is made of frustules of dead diatom cells, usually found in benthic environments. The harvesting of fossil frustules, which are naturally present in benthic environments, is cost-effective and makes diatomite a promising starter for the industrial production of biosilica. However, the composition of DE is variable and the purity is often lower than that of living culture-derived frustules. The quality and abundance of these impurities vary upon environmental and aging conditions [18]. DE, generally made of ca. 80–90% of silicon and of clay minerals [33], is used as a raw material for different kinds of applications, such as agricultural fertiliser, sorbent for pollutants, and filler in plastics and paints to improve the strength of construction materials. In addition, DE is also employed to filter impurities and as an abrasive agent in cleaning and polishing products.

3. Frustule Cleaning/Purification: Main Techniques and Technical Issues

Frustules can be thus purified from both living culture-derived algal biomass and diatomite stocks. The impurities of diatom frustules mainly consist of organic matters adhered to their surface [34]. In the case of diatomite samples, impurities are present in larger amounts, and can vary in relation to the local environment and aging conditions of these natural stocks [18]. Diatomite impurities typically contain also clay and metallic oxides, such as aluminium and ferric oxides [35]. Before cleaning procedures, diatomite particles usually undergo a first step of pulverization, in which micrometric powder is grinded to nanoparticles by mechanical crushing and sonication. However, apart from a few exceptions, most studies report purification protocols based on raw material derived from living cultures rather than diatomite, which is currently the only diatomic silica-based marketable product.

Organic impurities can be removed from the silica frustule by either a chemical pre-treatment with acids or other oxidative agents, or by exposing the frustules to high temperatures. Some studies, aimed at assessing the efficacy of preliminary hydrochloric acid treatments for organic mass removal, showed that acid concentration greatly influenced both the removal rate of impurities and the state of preservation of the frustule shape, with strong acidic pre-treatments causing frustule erosion [36]. Potassium permanganate can be also used to pre-treat frustules for organic compound removal [37][38]. However, this procedure is essentially limited to remove impurities outside the frustule, and pre-treatments with acidic solutions are usually applied (even if they are not mandatory) when purification protocols do not foresee acid-based cleaning procedures, such as baking-based purifications [39]. Some preliminary oxidations with acid solutions do not exclude the employment of both acids and high temperatures. Treatment of diatom frustules with sodium permanganate and oxalic acid, for example, is followed by perchloric acid treatments at 100 °C [37].

Baking (i.e., strong heating of silica cell walls) of diatom frustules at 400–800 °C is the simplest and least expensive method to remove organic components. However, high-temperature treatments can alter diatom architecture and pore size [40]. Oxygen plasma etching, a procedure consisting of the removal of impurities using ionised gases, was found to be effective to preserve the frustule structure, with a negligible loss of material and without shape alterations [41][42].

The most commonly used procedure for the removal of organic matter and the purification of diatom biosilica is, however, an oxidative washing treatment. Some protocols require the use of 30% [34][43][44][45][46][47] or 15% [48] hydrogen peroxide solutions.

The most common washing solvents used in acid-based treatments of diatom frustules are sulphuric [49][50] and nitric [48][51] acids. Sulphuric acid treatment is rapid (10–30 min) and revealed successful even on small amounts of biosilica [35]. Despite the rapidity of this strong acid-based method, cleaning procedures are time-consuming, since several washes with distilled/deionised water are required for a complete acid removal. However, the effect of acid strength needs to be evaluated in each case, since silica nanostructures can be damaged by the action of acids. For example, frustules from poorly silicified diatom species can be dissolved in strong acid cleaning solutions [50].

To improve the efficiency of biosilica purification, Wang and co-workers [52] set up a vacuum cleaning method in which all the cleaning steps, which are cell extraction, acid treatment and washing, are carried out on polytetrafluoroethylene (PTFE) filter cloths, thus decreasing the processing time. This allows the recycling of the sulphuric acid used for cleaning, decreasing the amount of both the reagent needed for purification and the liquid wastes. The main drawback of the vacuum cleaning method is that it depends on the mechanical properties of the raw material, and cannot be applied on poorly silicified diatoms.

Some purification methods combine the use of both sulphuric acid and hydrogen peroxide in a strong oxidizing agent (2 M H_2SO_4, 10% H_2O_2) called Piranha solution [53][54]. The purification process is relatively fast, while post-treatment washes can be time-consuming. The removal of Piranha solution requires, indeed, an overnight treatment with HCl (5 M, 80 °C) and two further washes with distilled water to eliminate the HCl residuals [20]. The main treatments for frustule separations, the tested diatom silica sources, and the main bottlenecks of each cleaning technique are summarized in Table 1.

Table 1. Pre-treatments and treatments for diatom frustule cleaning and their main advantages and drawbacks.

	Treatment	Principle for Organic Matter Removal	Diatom Species	Diatom Silica Source	Advantages	Drawbacks	Reference(s)
Pre-treatments	**HCl**	**oxidizing washing**	***Nitzschia closterium*, *Thalassiosira* sp.**	**freeze-dried samples**	**high purity of frustules**	**possible frustule erosion depending on acid strength**	[36]
	$KMnO_4$ + $C_2H_2O_4$	**oxidizing washing**	***Fragilariopsis cylindrus, Fragilariopsis kerguelensis, Pseudonitzschia seriata, Thalassiosira nordenskioeldii, Thalassiosira aestivalis, Thalassiosira pseudonana, Thalassiosira weissflogii***	**wet pellets washed with sodium lauryl sulfate**	**no frustule erosion**	**removal of the only external organic matter**	[37][38]

	Treatment	Principle for Organic Matter Removal	Diatom Species	Diatom Silica Source	Advantages	Drawbacks	Reference(s)
	baking	high temperature	*Navicula* sp.	APS-fuctionalised diatoms on a mika surface	reduction in hazardous chemicals	possible alterations of pore size, possible post-treatments with acid solutions	[40]
	low-temperature plasma ashing	ionised gas	*Navicula, Amphora, Cocconeis, Planothidium* spp.	desalted drops of cultures, freeze-dried samples	no frustule dissolution	unsuitable for saltwater species, expensive, post-treatments with hazardous chemicals	[41][42]
Treatments	H_2O_2	oxidation	DE, *Ni tzschia frustulum, Pinnularia* and *Coscinodiscus* spp., *Thalassiosira pseudonana, Cylindrotheca closterium*	desalted and freeze-dried cultures, diatom composites	less dangerous than strong acids	long incubation, high-temperature post-treatments needful to increase efficiency	[34][42][43][44][45][46][47]
	H_2SO_4	strong oxidation	*Thalassiosira rotula, Coscinodiscus wailesii*	living cultures	high efficiency in organic matter removal	hazardous chemicals, dissolution of thin frustules, time-consuming post treatments	[49][50]
	H_2SO_4 + PTFE filters	strong oxidation under vacuum	*Nitzschia, Ditylum, Skeletonema, Coscinodiscus* spp.	living cultures on a filter cloth	reduced acid amounts	unsuitable for thin frustules	[52]
	HNO_3	strong oxidation	*Pinnularia* sp., *Coscinodiscus concinnus*	harvested cells	high efficiency in organic matter removal	high-temperature treatments needful to increase efficiency	[48][51]
	Piranha solution (H_2SO_4 + H_2O_2)	strong oxidation	*Thalassiosira pseudonana*	PBS-washed cells	high efficiency in organic matter removal	time-consuming post-treatments	[54]

4. Silica for Biomedical Applications: Advantages

The main benefits of biosilica for biomedical purposes are as follows: plasticity of frustules for functionalization, biocompatibility, possibility of genetic transformation of living cultures for protein immobilization, and high availability of silica-derived diatoms. The biosilica derived from diatoms requires cheap synthesis processes [53], and is also characterised by chemical inertness, low or null toxicity, thermal stability and high availability [48]. Silica has been widely investigated in drug delivery systems because of its high robustness and versatility compared to other materials [55], and frustules derived from both living cultures and diatomite particles have successfully been employed as drug carriers [54][56].

4.1. Surface Functionalization for Drug Loading and for Biosensing Chips for Biomedical Applications

References

1. Mann, D.G.; Vanormelingen, P. An inordinate fondness? The number, distributions, and origins of diatom species. J. Eukaryot. Microbiol. 2013, 60, 414–420.
2. Armbrust, E.V.; Berges, J.A.; Bowler, C.; Green, B.R.; Martinez, D.; Putnam, N.H.; Zhou, S.G.; Allen, A.E.; Apt, K.E.; Bechner, M.; et al. The genome of the diatom Thalassiosira pseudonana: Ecology, evolution, and metabolism. Science 2004, 306, 79–86.
3. Bowler, C.; Allen, A.E.; Badger, J.H.; Grimwood, J.; Jabbari, K.; Kuo, A.; Maheswari, U.; Martens, C.; Maumus, F.; Otillar, R.P.; et al. The Phaeodactylum genome reveals the evolutionary history of diatom genomes. Nature 2008, 456, 239–244.
4. Lommer, M.; Specht, M.; Roy, A.S.; Kraemer, L.; Andreson, R.; Gutowska, M.A.; Wolf, J.; Bergner, S.V.; Schilhabel, M.B.; Klostermeier, U.C.; et al. Genome and low-iron response of an oceanic diatom adapted to chronic iron limitation. Genome Biol. 2012, 13.
5. Galachyants, Y.P.; Zakharova, Y.R.; Petrova, D.P.; Morozov, A.A.; Sidorov, I.A.; Marchenkov, A.M.; Logacheva, M.D.; Markelov, M.L.; Khabudaev, K.V.; Likhoshway, Y.V.; et al. Sequencing of the complete genome of an araphid pennate diatom Synedra acus subsp. radians from Lake Baikal. Dokl. Biochem. Biophys. 2015, 461, 84–88.
6. Tanaka, T.; Maeda, Y.; Veluchamy, A.; Tanaka, M.; Abida, H.; Marechal, E.; Bowler, C.; Muto, M.; Sunaga, Y.; Tanaka, M.; et al. Oil accumulation by the oleaginous diatom Fistulifera solaris as revealed by the genome and transcriptome. Plant Cell 2015, 27, 162–176.
7. Mock, T.; Otillar, R.P.; Strauss, J.; McMullan, M.; Paajanen, P.; Schmutz, J.; Salamov, A.; Sanges, R.; Toseland, A.; Ward, B.J.; et al. Evolutionary genomics of the cold-adapted diatom Fragilariopsis cylindrus. Nature 2017, 541, 536–540.
8. Osuna-Cruz, C.M.; Bilcke, G.; Vancaester, E.; De Decker, S.; Bones, A.M.; Winge, P.; Poulsen, N.; Bulankova, P.; Verhelst, B.; Audoor, S.; et al. The Seminavis robusta genome provides insights into the evolutionary adaptations of benthic diatoms. Nat. Commun. 2020, 11, 3320.
9. Pendergrass, A.; Roberts, W.; Ruck, E.C.; Lewis, J.A.; Alverson, A.J. The genome of a nonphotosynthetic diatom provides insights into the metabolic shift to heterotrophy and constraints on the loss of photosynthesis. BioRxiv 2020.
10. Mourelle, M.; Gómez, C.; Legido, J. The Potential Use of Marine Microalgae and Cyanobacteria in Cosmetics and Thalassotherapy. Cosmetics 2017, 4, 46.
11. Cui, Y.; Thomas-Hall, S.R.; Schenk, P.M. Phaeodactylum tricornutum microalgae as a rich source of omega-3 oil: Progress in lipid induction techniques towards industry adoption. Food Chem. 2019, 297, 124937.
12. Pudney, A.; Gandini, C.; Economou, C.K.; Smith, R.; Goddard, P.; Napier, J.A.; Spicer, A.; Sayanova, O. Multifunctionalizing the marine diatom Phaeodactylum tricornutum for sustainable co-production of omega-3 long chain polyunsaturated fatty acids and recombinant phytase. Sci. Rep. 2019, 9, 11444.
13. Tiwari, A.; Melchor-Martinez, E.M.; Saxena, A.; Kapoor, N.; Singh, K.J.; Saldarriaga-Hernandez, S.; Parra-Saldivar, R.; Iqbal, H.M.N. Therapeutic attributes and applied aspects of biological macromolecules (polypeptides, fucoxanthin,

Frustule functionalization consists of modifying its surface to enable the formation of stable covalent bonds with proteins or DNA [53][57], by introducing chemically reactive species functioning as cross-linkers. This step is crucial to improve the quality of the resulting material for specific applications. Chemical modification of biosilica can be critical, for example, to regulate the kinetics of drug release, and the high surface-to-volume ratio makes this raw material particularly suitable for drug delivery. Diatom frustules are characterized by precise and species-specific cell morphologies, and both the size and shape can highly differ among distinct diatom taxa. It has been estimated that the surface area ranges between 1.4 and 51 m^2 g^{-1} [58][59][60][61]. The size and the architecture of the pores are likely to influence drug release [56].

Drug release in biosilica-based systems is usually characterized by the following two phases: a first phase of fast release, due to the detachment of drug molecules weakly bound to the frustule surface, and a slow releasing phase, due to drug delivery from the internal pore structure of diatom frustules [62]. Chemical modifications of diatom-derived biosilica allow their use as a carrier of both soluble and insoluble drugs.

The effectiveness of DEs as delivery systems for the drugs gentamicin (soluble) and indomethacin (insoluble) was demonstrated in previous studies [47], in which DE was modified with self-assembling monolayers (SAM) including organosilanes and phosphonic acids, thus rendering the diatom frustules hydrophilic or hydrophobic, respectively, before drug loading. A sustained release of indomethacin, which has been exploited as a model drug for silica-based devices, was also demonstrated with DE particles functionalised by dopamine-modified iron oxide nanoparticles (DOPA/Fe_3O_4 nanoparticles). Diatom-derived silica was employed, in this case, as a magnetically guided micro-carrier for drug delivery, since dopamine amino groups on the diatom surface allow the attachment of targeting biomolecules [59].

Another kind of functionalization can be obtained by combining the frustule with graphene oxide (GO) sheets through covalent bindings. These nano-hybrid composites are suitable drug microcarriers. GO sheets enhanced, indeed, drug-surface interactions, improving the kinetics of drug release [63].

Silica functionalization was also used to counteract cancer progression, through the delivery of water-insoluble antitumor drugs. A recent study showed that DE particles coated with vitamin B12 allowed better delivery of cisplatin and 5-fluorouracil (5-FU), two anticancer agents effective against colorectal cancer cells [64]. Silicon nanoparticles (SiNPs) were also functionalized with 5-FU and the chemopreventive agent curcumin, and then encapsulated into acid-resistant microspheres to show the effectiveness of oral administration of these chemotherapeutics against colorectal cancer [65].

DE particles were also used as a solid drug-carrier in phospholipid suspensions for new oral formulations of non-anticancer water-insoluble drugs, such as the anticonvulsive carbamazepine [66].

While the abovementioned applications of biosilica were all based on the employment of fossil sources, other studies were focused on culture-derived biosilica. Functionalised frustules of the diatom *Nitzschia palea* have been successfully exploited as carriers for the antibacterial complex tyrosine-Zn(II); zinc ions covalently bounded to the frustule surface showed, indeed, a toxic effect on bacteria, thus reducing their concentration [67]. Esfandyari et al. [68] exploited the potential of *Chaetoceros* sp. frustules to detect circulating tumour cells. Diatoms were magnetized with iron oxide nanoparticles, and then conjugated with the monoclonal antibody Trastuzumab; this system was effective in selectively targeting and separating breast cancer cells, SKBR3 cells (HER2 positive cells), from HER2-negative cells under a magnetic field. The optical properties of these diatoms allowed to detect this specific binding ability by fluorescence microscopy thanks to the optical properties of the silica.

Similar studies on antibody-functionalized nanoparticles deriving from living cultures were already performed more than ten years ago, and they exploited the potential of two modified centric diatoms as photoluminescent biosensors. Functionalization of *Coscinodiscus wailesii* frustules was one of the pioneer studies highlighting antigen recognition from antibodies that had been covalently bound to frustules [69]. Gale and co-workers [70] succeeded in transforming *Cyclotella* sp. frustules with the model rabbit IgG antibody, showing a correlation between the photoluminescence associated with the frustule/antibody complex and the antigen (goat anti-rabbit IgG) concentration.

The main types of diatom silica functionalization are summarized in Table 2.

Table 2. Sources, type of functionalization and biomedical applications of diatom-derived biosilica.

Diatom Source	Type of Functionalization	Main Application	Aim	Reference(s)
Coscinodiscus wailesii	Silanization and antibody conjugation	Biosensor	Specific recognition antigen–antibody (murine monoclonal antibody)	[69]

sterols, fatty acids, polysaccharides, and polyphenols) from diatoms—A review. Int. J. Biol. Macromol. 2021, 171, 398–413.
14. Grzesik, M.; Romanowska-Duda, Z.; Piotrowski, K.; Janas, R. Diatoms (Bacillariophyceae) as an effective base of a new generation of organic fertilizers. Przemys Chem. 2015, 94, 391–396.
15. Hedayatkhah, A.; Cretoiu, M.S.; Emtiazi, G.; Stal, L.J.; Bolhuis, H. Bioremediation of chromium contaminated water by diatoms with concomitant lipid accumulation for biofuel production. J. Environ. Manag. 2018, 227, 313–320.
16. Mojiri, A.; Baharlooeian, M.; Zahed, M.A. The Potential of Chaetoceros muelleri in Bioremediation of Antibiotics: Performance and Optimization. Int. J. Environ. Res. Public Health 2021, 18, 977.
17. Lomora, M.; Shumate, D.; Rahman, A.A.; Pandit, A. Therapeutic applications of phytoplankton, with an emphasis on diatoms and coccolithophores. Adv. Ther. 2019, 2, 1800099.
18. 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.
19. Uthappa, U.T.; Sriram, G.; Arvind, O.R.; Kumar, S.; Ho-Young, J.; Neelgund, G.M.; Losic, D.; Kurkuri, M.D. Engineering MIL-100(Fe) on 3D porous natural diatoms as a versatile high performing platform for controlled isoniazid drug release, Fenton's catalysis for malachite green dye degradation and environmental adsorbents for Pb2+ removal and dyes. Appl. Surf. Sci. 2020, 528, 146974.
20. 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, 7.
21. Dalgic, A.D.; Atila, D.; Karatas, A.; Tezcaner, A.; Keskin, D. Diatom shell incorporated PHBV/PCL-pullulan co-electrospun scaffold for bone tissue engineering. Mater. Sci. Eng. C Mater. Biol. Appl. 2019, 100, 735–746.
22. Kong, X.; Squire, K.; Li, E.; LeDuff, P.; Rorrer, G.L.; Tang, S.; Chen, B.; McKay, C.P.; Navarro-Gonzalez, R.; Wang, A.X. Chemical and Biological Sensing Using Diatom Photonic Crystal Biosilica With In-Situ Growth Plasmonic Nanoparticles. IEEE Trans. Nanobiosci. 2016, 15, 828–834.
23. Albert, K.; Huang, X.C.; Hsu, H.Y. Bio-templated silica composites for next-generation biomedical applications. Adv. Colloid Interface Sci. 2017, 249, 272–289.
24. Pahl, S.L.; Lewis, D.M.; Chen, F.; King, K.D. Heterotrophic growth and nutritional aspects of the diatom Cyclotella cryptica (Bacillariophyceae): Effect of some environmental factors. J. Biosci. Bioeng. 2010, 109, 235–239.
25. Pahl, S.L.; Lewis, D.M.; Chen, F.; King, K.D. Growth dynamics and the proximate biochemical composition and fatty acid profile of the heterotrophically grown diatom Cyclotella Cryptica. J. Appl. Phycol. 2010, 22, 165–171.
26. Khan, M.; Karmakar, R.; Das, B.; Diba, F.; Razu, M.H. Recent advances in microalgal biotechnology. In Heterotrophic Growth of Micro Algae; Jin, L., Zheng, S., Henn, G., Eds.; OMICS Group eBooks: Foster City, CA, USA, 2016; pp. 1–18.
27. Mao, X.M.; Chen, S.H.Y.; Lu, X.; Yu, J.F.; Liu, B. High silicate concentration facilitates fucoxanthin and eicosapentaenoic acid (EPA) production under heterotrophic condition in the marine diatom Nitzschia laevis. Algal Res. Biomass Biofuels Bioprod. 2020, 52.
28. Chen, G.Q.; Chen, F. Growing phototrophic cells without light. Biotechnol. Lett. 2006, 28, 607–616.
29. Morales-Sánchez, D.; Martinez-Rodriguez, O.A.; Kyndt, J.; Martinez, A. Heterotrophic growth of microalgae: Metabolic aspects. World J. Microbiol. Biotechnol. 2015, 31, 1–9.
30. D'Ippolito, G.; Sardo, A.; Paris, D.; Vella, F.M.; Adelfi, M.G.; Botte, P.; Gallo, C.; Fontana, A. Potential of lipid metabolism in marine diatoms for biofuel production. Biotechnol. Biofuels 2015, 8, 28.
31. Botte, P.; D'Ippolito, G.; Gallo, C.; Sardo, A.; Fontana, A. Combined exploitation of CO2 and nutrient replenishment for increasing biomass and lipid productivity of the marine diatoms Thalassiosira weissflogii and Cyclotella cryptica. J. Appl. Phycol. 2017, 30, 243–251.
32. Orefice, I.; Musella, M.; Smerilli, A.; Sansone, C.; Chandrasekaran, R.; Corato, F.; Brunet, C. Role of nutrient concentrations and water movement on diatom's productivity in culture. Sci. Rep. 2019, 9, 1479.
33. Lutyński, M.; Sakiewicz, P.; Lutyńska, S. Characterization of diatomaceous earth and halloysite resources of poland. Minerals 2019, 9, 670.
34. Qin, T.; Gutu, T.; Jiao, J.; Chang, C.H.; Rorrer, G.L. Photoluminescence of silica nanostructures from bioreactor culture of marine diatom Nitzschia frustulum. J. Nanosci. Nanotechnol. 2008, 8, 2392–2398.
35. Wang, Y.; Cai, J.; Jiang, Y.S.; Jiang, X.G.; Zhang, D.Y. Preparation of biosilica structures from frustules of diatoms and their applications: Current state and perspectives. Appl. Microbiol. Biotechnol. 2013, 97, 453–460.

Diatom Source	Type of Functionalization	Main Application	Aim	Reference(s)
Coscinodiscus wailesii	Silanization and antibody conjugation	Biosensor	Tethering and detecting antibodies (mix of normal rabbit serum and purified Ig-Y)	[44]
Cyclotella sp.	Silanization and antibody conjugation	Biosensor	Selective and label-free photoluminescence-based detection of antigen–antibody (IgG-rabbit) complex formation	[70]
Chaetoceros sp.	Iron oxide nanoparticles and antibody conjugation	Biosensor (with magnetic properties)	Selective targeting of SKBR3 cancer cells through the employment of antibody (Trastuzumab) bioconjugation	[68]
Thalassiosira weissflogii	Nitroxide 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) conjugation	Drug carrier	Ciprofloxacin delivery in fibroblasts and osteoblasts	[71]
Aulacoseira sp.	Silanization, and oligo (ethylene glycol) methacrylate copolymers addition	Drug carrier	Improvement of levofloxacin delivery	[56]
Nitzschia palea	Amino acid (Tyr-Zn^{II}) conjugation	Drug carrier	Inhibition of bacterial growth	[67]
Diatomaceous earth	Silanization and phosphonic acids conjugation—self-assembling monolayer	Drug carrier	Improvement of indomethacin and gentamicin delivery	[47]
Diatomaceous earth	Silanization and phosphonic acids modifications	Drug carrier	Improvement of indomethacin delivery	[72]
DE mineral rocks	Graphene oxide, silanization	Drug carrier	Improvement of indomethacin delivery	[63]
Diatomaceous earth	Dopamine modified iron-oxide nanoparticles (DOPA/Fe_3O_4)	Drug carrier (with magnetic properties)	Improvement of indomethacin delivery	[59]
Diatomaceous earth	Vitamin B12 and ruthenium (II) complex	Drug carrier	Improvement of the anticancer tris-tetraethyl [2,2′-bipyridine]-4,4′-diamine–ruthenium (II) complex delivery (tested on HT-29 and MCF-7 cancer cells)	[64]
Calcined diatomite	Silanization and siRNA conjugation	Drug carrier	Vehiculating siRNA into tumour cells to downregulate the expression of cancer-associated genes (tested on murine A20 lymphoma cells)	[73]
Calcined diatomite	Silanization and siRNA conjugation	Drug carrier	Vehiculating siRNA into tumour cells to downregulate the expression of cancer-associated genes (tested on H1355 cancer cells)	[74]

4.2. Biocompatibility

Diatom-derived biosilica has several advantages compared to other porous materials, in terms of high compatibility with biological systems [18][53]. Biocompatibility tests were performed on various tumour cells, and some significant examples are reported below. An ATP-based luminescent assay aimed at detecting the short-time (6–24 h) detrimental effects on cells showed that DE particles had very low toxicity on the following three colon cancer cell lines: Caco-2, HT-29, and HCT-116 [75]. The effect of amino-modified DE nanoparticles on human lung epidermoid carcinoma cells (H1355) was evaluated by the MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Different concentrations of diatom particles were tested for 24, 48 and 72 h, and the results showed very low cytotoxicity against the abovementioned tumour cells. This feature made functionalized DE particles useful carriers to transport small interfering ribonucleic acid (siRNA) inside human lung epidermoid carcinoma cells (H1355), silencing gene expression [74]. Biocompatibility was also assessed on bone cells, such as normal human dermal fibroblasts (NDHS) and Saos-2 osteoblasts, by the functionalization of *Thalassiosira weissflogii* frustules with 3-mercaptopropyl-trimethoxysilane (MPTMS). The mercapto-coated biosilica successfully stimulated the growth of both cell lines, even more than bare cells [71].

36. Qi, Y.R.; Wang, X.; Cheng, J.J. Preparation and characteristics of biosilica derived from marine diatom biomass of Nitzschia closterium and Thalassiosira. Chin. J. Oceanol. Limnol. 2017, 35, 668–680.

37. Horn, M.G.; Robinson, R.S.; Rynearson, T.A.; Sigman, D.M. Nitrogen isotopic relationship between diatom-bound and bulk organic matter of cultured polar diatoms. Paleoceanography 2011, 26.

38. Mejia, L.M.; Isensee, K.; Mendez-Vicente, A.; Pisonero, J.; Shimizu, N.; Gonzalez, C.; Monteleone, B.; Stoll, H. B content and Si/C ratios from cultured diatoms (Thalassiosira pseudonana and Thalassiosira weissflogii): Relationship to seawater pH and diatom carbon acquisition. Geochim. Cosmochim. Acta 2013, 123, 322–337.

39. Jiang, W.K.; Luo, S.P.; Liu, P.W.; Deng, X.Y.; Jing, Y.; Bai, C.Y.; Li, J.B. Purification of biosilica from living diatoms by a two-step acid cleaning and baking method. J. Appl. Phycol. 2014, 26, 1511–1518.

40. Umemura, K.; Noguchi, Y.; Ichinose, T.; Hirose, Y.; Kuroda, R.; Mayama, S. Diatom Cells Grown and Baked on a Functionalized Mica Surface. J. Biol. Phys. 2008, 34, 189–196.

41. Watanabe, T.; Kodama, Y.; Mayama, S. Application of a novel cleaning method using low-temperature plasma on tidal flat diatoms with heterovalvy of delicate frustule structure. Proc. Acad. Nat. Sci. Phila. 2010, 160, 83–87.

42. Saad, E.M.; Pickering, R.A.; Shoji, K.; Hossain, M.I.; Glover, T.G.; Krause, J.W.; Tang, Y.Z. Effect of cleaning methods on the dissolution of diatom frustules. Mar. Chem. 2020, 224.

43. Jeffryes, C.; Solanki, R.; Rangineni, Y.; Wang, W.; Chang, C.H.; Rorrer, G.L. Electroluminescence and photoluminescence from nanostructured diatom frustules containing metabolically inserted germanium. Adv. Mater. 2008, 20, 2633–2637.

44. 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.

45. Abramson, L.; Wirick, S.; Lee, C.; Jacobsen, C.; Brandes, J.A. The use of soft X-ray spectromicroscopy to investigate the distribution and composition of organic matter in a diatom frustule and a biomimetic analog. Deep Sea Res. Part II Top. Stud. Oceanogr. 2009, 56, 1369–1380.

46. Lin, K.C.; Kunduru, V.; Bothara, M.; Rege, K.; Prasad, S.; Ramakrishna, B.L. Biogenic nanoporous silica-based sensor for enhanced electrochemical detection of cardiovascular biomarkers proteins. Biosens. Bioelectron. 2010, 25, 2336–2342.

47. 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. 2013, 443, 230–241.

48. Van Eynde, E.; Lenaerts, B.; Tytgat, T.; Verbruggen, S.W.; Hauchecorne, B.; Blust, R.; Lenaerts, S. Effect of pretreatment and temperature on the properties of Pinnularia biosilica frustules. RSC Adv. 2014, 4, 56200–56206.

49. Lettieri, S.; Setaro, A.; De Stefano, L.; De Stefano, M.; Maddalena, P. The gas-detection properties of light-emitting diatoms. Adv. Funct. Mater. 2008, 18, 1257–1264.

50. De Stefano, L.; Rendina, I.; De Stefano, M.; Bismuto, A.; Maddalena, P. Marine diatoms as optical chemical sensors. Appl. Phys. Lett. 2005, 87.

51. De Stefano, L.; Rotiroti, L.; De Stefano, M.; Lamberti, A.; Lettieri, S.; Setaro, A.; Maddalena, P. Marine diatoms as optical biosensors. Biosens. Bioelectron. 2009, 24, 1580–1584.

52. Wang, Y.; Zhang, D.Y.; Cai, J.; Pan, J.F.; Chen, M.L.; Li, A.B.; Jiang, Y.G. Biosilica structures obtained from Nitzschia, Ditylum, Skeletonema, and Coscinodiscus diatom by a filtration-aided acid cleaning method. Appl. Microbiol. Biotechnol. 2012, 95, 1165–1178.

53. Tramontano, C.; Chianese, G.; Terracciano, M.; de Stefano, L.; Rea, I. Nanostructured biosilica of diatoms: From water world to biomedical applications. Appl. Sci. 2020, 10, 6811.

54. 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.

55. Abdelhamid, M.A.A.; Pack, S.P. Biomimetic and bioinspired silicifications: Recent advances for biomaterial design and applications. Acta Biomater. 2021, 120, 38–56.

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. 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; Pathak, Y.V., Ed.; Springer: Berlin/Heidelberg, Germany, 2019.

The biological compatibility of silica derived from diatoms was also assessed in studies aimed at targeting the antiapoptotic factor B-cell lymphoma/leukemia-2 (Bcl2) with small interfering RNA (siRNA). Specifically, the amino groups of silanized silica particles were complexed with siRNA to downregulate the expression of tumour-associated genes. The target line was the A20 murine lymphoma, and no differences in cytotoxicity between the functionalised frustules and controls (e.g., untreated cells) were observed by applying the following three different methodologies: MTT, Cell-Titer GLO and propidium iodide assays [73].

Biocompatibility between functionalized DE particles and breast cancer cells (lines MCF-7 and MDA-MB-231) has also been proven. In this case, amino-modified particles were further improved by PEGylation (i.e., diatom-coating with polyethylene glycol) and cell-penetrating peptide (CPP) bioconjugation, to promote cell internalization through physical and biological changes in the silicon source. The biological compatibility was also evaluated with a luminescent cell viability assay based on the adenosine triphosphate concentration, and the results showed that the cytotoxicity of biosilica that underwent a double modification with PEG and CPP was lower than that of the bare material, as well as that of diatoms that had been amino-modified only [76].

Most cytotoxicity assays mentioned above were performed on short timescales. The effect of longer exposure times (21 days) was assessed on human embryonic kidney cells (HEK-293) and MDA-MB-231 breast cancer cells exposed to synthesized (e.g., fused at high temperature) diatoms. Biocompatibility was tested through viability assays with the dye Calcein-AM (its fluorescence intensity depends on the activity of cellular esterases, and thus of viable cells), and the results confirmed that natural silicon is not toxic. This suggested that fused diatom frustules could be a suitable alternative for synthetic bone graft substitutes [77]. In order to foresee the effects of long-term exposure of silica-based devices on biological systems, Terracciano and co-workers [78] investigated the in vivo impact of diatomite particles on the model organism *Hydra vulgaris*. Untreated specimens and animals exposed to bare frustules and to diatom nanoparticles modified with the cell-penetrating peptide [(aminooxy)acetyl]-Lys-(Arg)9 (to enhance cellular uptake) were monitored for 14 days, and no detrimental effects in terms of growth rates and apoptosis were observed in all conditions.

In our opinion, further studies on living organisms are mandatory to definitely ascertain the lack of toxicity of biosilica, especially in the perspective of concrete biomedical applications for drug loading and as scaffolds for bone regeneration.

4.3. Employment of Genetically Engineered Diatom Frustules for Protein Immobilization

Diatom particles can be considered as useful scaffolds for enzyme immobilization that could enhance protein properties. Genetic engineering represents a viable alternative to in vitro immobilization systems, as it does not require protein purification and is carried out under physiological conditions [55]. Since silaffins and cingulins are involved in silica condensation becoming part of diatom frustules, the fusion of an exogenous protein to these frustule-associated proteins can result in the strong binding of exogenous proteins to the silica cell wall.

Transformation of diatom genomes with recombinant genes is a useful tool to allow the fusion between enzymes and cell wall proteins. This technology is mentioned in a recent study as living diatom silica immobilization (LiDSI), and has been mostly performed on the model species *T. pseudonana* [79][80]. To our knowledge, the pioneer studies focused on enzymes immobilised on diatom biosilica were aimed at inserting and blocking the bacterial enzyme hydroxylaminobenzene mutase (HabB) on the silaffin tpSil3 of *T. pseudonana* frustule [79]. Aside from the potential of this specific genome modification, this study paved the way for the genetic manipulation of diatom species to enhance protein immobilization on frustules for biomedical purposes.

The genome of *T. pseudonana* has been recently modified with the insertion of exogenous genes encoding the fusion of two enzymes, glucose oxidase and horseradish peroxidase, with cell wall proteins, enabling a regioselective functionalization, and suggesting that silica morphology could influence the effectiveness of the enzymes reactivity [80]. The frustule of this species has been also antibody-functionalised, in order to test its effectiveness in binding large and small antigen molecules [81].

4.4. Availability of Biosilica Feedstocks

In contrast with other synthetic materials, diatom biosilica is already available in huge amounts as diatomite. Moreover, diatom-derived silica feedstock could be easily obtained by culturing these microalgae in open ponds or enclosed systems, and separating them from the organic matter after culture dewatering.

58. Bao, Z.; Weatherspoon, M.R.; Shian, S.; Cai, Y.; Graham, P.D.; Allan, S.M.; Ahmad, G.; Dickerson, M.B.; Church, B.C.; Kang, Z.; et al. Chemical reduction of three-dimensional silica micro-assemblies into microporous silicon replicas. Nature 2007, 446, 172–175.
59. Losic, D.; Yu, Y.; Aw, M.S.; Simovic, S.; Thierry, B.; Addai-Mensah, J. Surface functionalisation of diatoms with dopamine modified iron-oxide nanoparticles: Toward magnetically guided drug microcarriers with biologically derived morphologies. Chem. Commun. 2010, 46, 6323–6325.
60. Jantschke, A.; Fischer, C.; Hensel, R.; Braun, H.G.; Brunner, E. Directed assembly of nanoparticles to isolated diatom valves using the non-wetting characteristics after pyrolysis. Nanoscale 2014, 6, 11637–11645.
61. Diab, M.; Mokari, T. Bioinspired hierarchical porous structures for engineering advanced functional inorganic materials. Adv. Mater. 2018, 30, e1706349.
62. 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.
63. 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.
64. 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.
65. 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.
66. Milovic, M.; Simovic, S.; Losic, D.; Dashevskiy, A.; Ibric, S. Solid self-emulsifying phospholipid suspension (SSEPS) with diatom as a drug carrier. Eur. J. Pharm. Sci. 2014, 63, 226–232.
67. Singh, R.; Khan, M.J.; Rane, J.; Gajbhiye, A.; Vinayak, V.; Joshi, K.B. Biofabrication of Diatom Surface by Tyrosine-Metal Complexes: Smart Microcontainers to Inhibit Bacterial Growth. Chemistryselect 2020, 5, 3091–3097.
68. Esfandyari, J.; Shojaedin-Givi, B.; Hashemzadeh, H.; Mozafari-Nia, M.; Vaezi, Z.; Naderi-Manesh, H. Capture and detection of rare cancer cells in blood by intrinsic fluorescence of a novel functionalized diatom. Photodiagn. Photodyn. Ther. 2020, 30, 101753.
69. De Stefano, L.; Lamberti, A.; Rotiroti, L.; De Stefano, M. Interfacing the nanostructured biosilica microshells of the marine diatom Coscinodiscus wailesii with biological matter. Acta Biomater. 2008, 4, 126–130.
70. Gale, D.K.; Gutu, T.; Jiao, J.; Chang, C.-H.; Rorrer, G.L. Photoluminescence Detection of Biomolecules by Antibody-Functionalized Diatom Biosilica. Adv. Funct. Mater. 2009, 19, 926–933.
71. Cicco, S.R.; Vona, D.; Gristina, R.; Sardella, E.; Ragni, R.; Lo Presti, M.; Farinola, G.M. Biosilica from Living Diatoms: Investigations on Biocompatibility of Bare and Chemically Modified Thalassiosira weissflogii Silica Shells. Bioengineering 2016, 3, 35.
72. 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.
73. Martucci, N.M.; Migliaccio, N.; Ruggiero, I.; Albano, F.; Calì, G.; Romano, S.; Terracciano, M.; Rea, I.; Arcari, P.; Lamberti, A. Nanoparticle-based strategy for personalized B-cell lymphoma therapy. Int. J. Nanomed. 2016, 11, 6089–6101.
74. Rea, I.; Martucci, N.M.; De Stefano, L.; Ruggiero, I.; Terracciano, M.; Dardano, P.; Migliaccio, N.; Arcari, P.; Tate, R.; Rendina, I.; et al. Diatomite biosilica nanocarriers for siRNA transport inside cancer cells. Biochim. Biophys. Acta Gen. Subj. 2014, 1840, 3393–3403.
75. Zhang, H.; Shahbazi, M.A.; Makila, 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.
76. 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.
77. Amoda, A.; Borkiewicz, L.; Rivero-Müller, A.; Alam, P. Sintered nanoporous biosilica diatom frustules as high efficiency cell-growth and bone-mineralisation platforms. Mater. Today Commun. 2020, 24, 100923.
78. Terracciano, M.; De Stefano, L.; Tortiglione, C.; Tino, A.; Rea, I. In Vivo Toxicity Assessment of Hybrid Diatomite Nanovectors Using Hydra vulgaris as a Model System. Adv. Biosyst. 2019, 3, e1800247.
79. Poulsen, N.; Berne, C.; Spain, J.; Kroger, N. Silica immobilization of an enzyme through genetic engineering of the diatom Thalassiosira pseudonana. Angew. Chem. Int. Ed. 2007, 46, 1843–1846.

80. Kumari, E.; Görlich, S.; Poulsen, N.; Kröger, N. Genetically Programmed Regioselective Immobilization of Enzymes in Biosilica Microparticles. Adv. Funct. Mater. 2020, 30, 2000442.

81. Ford, N.R.; Hecht, K.A.; Hu, D.H.; Orr, G.; Xiong, Y.J.; Squier, T.C.; Rorrer, G.L.; Roesijadi, G. Antigen Binding and Site-Directed Labeling of Biosilica-Immobilized Fusion Proteins Expressed in Diatoms. ACS Synth. Biol. 2016, 5, 193–199.