From Living Light to Living Materials

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Living Light: Uniting biology and photonics – A memorial meeting in honour of Prof Jean-Pol Vigneron

From living light to living materials

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Abstract

Diatom cells are enclosed within porous silica walls that exhibit a wide variety of forms. Some of them exhibit photonic crystals properties arising from a periodic distribution of pores along these walls. Diatoms then play with solar light to protect the cell and improve its photosynthetic properties. These micro-algae also suggest that life is possible inside a glass box leading to the bio-inspired synthesis of living materials via the so-called sol-gel process.

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Keywords: diatoms, photonic crystal, silica, bio-encapsulation

1. Introduction

Phytoplankton has a major role to play in the ecological equilibrium of our planet. Through photosynthesis alone, by using the sun's energy, it transforms more than 50% of the carbon dioxide gas present in the atmosphere into useful organic products and oxygen.

Phytoplankton includes a wide variety of species such as cyanobacteria, dinoflagellates, coccolithophores and diatoms, the latter of which have been of particular interest for scientists because of their protective silica armour called frustule (Fig.1). Charles Darwin was seduced by their beauty, mentioning them in his work on the origin of

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species: *'few objects are more beautiful than the minute siliceous cases of diatoms, were they only created to be admired under the microscope?'*.[1] We now know the answer to his question; this silica shell plays an essential role in the photosynthetic abilities of diatoms. Their frustules are not only hard and transparent; they also exhibit a photonic crystal type of behaviour, thus enabling diatoms to enhance their photosynthetic capabilities by playing with the solar irradiation.

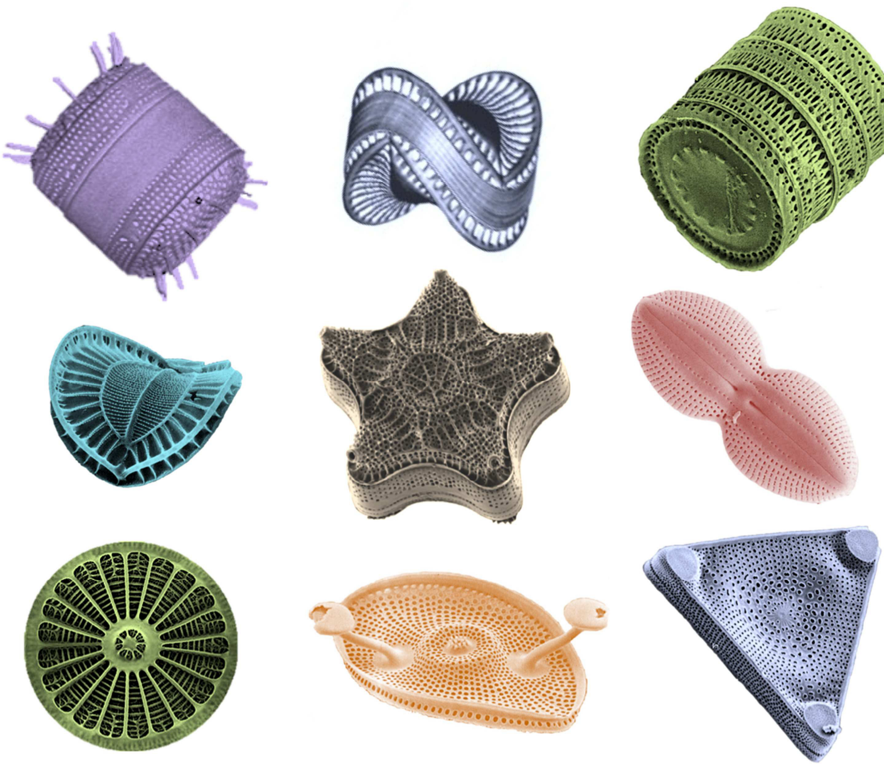

Fig.1 Diatom cells are encased within a silica shell (frustules) in order to protect themselves from predators. These frustules exhibit an incredible variety of structures and more than 100,000 different species have been described.

Diatoms can be found anywhere in nature as long as some degree of moisture is present, whether it be seawater or freshwater, flowing or stagnant [2,3]. Observing them is easy but reproducing their structure can prove to be a complex procedure, since for thousands of years we have produced glass by melting silica sand at temperatures over 1000°C. These microalgae are able to synthesise their silica shell at room temperature by using dissolved silica from their environment. Thus, diatoms show us quite clearly that life is possible inside a glass box leading to the formation of living materials! [4–7]

2. Diatoms as living photonic crystals

Diatom biosilica gathers at the bottom of the oceans as sediments (during a fossilisation process) subsequent to the micro-organisms death. These deposits are a useful raw materials often referred to as diatomite or diatom earth, and are widely used for insulation or as absorbents in filters Moreover, Alfred Nobel invented dynamite by soaking diatomite with nitro-glycerine.

The silica shell is built by the diatom using dissolved silicic acid $Si(OH)_4$ which penetrates into the cell through ionic canals and is stored in the silicon deposition vesicles (SDV) until the next cell division. At this point, a polycondensation reaction takes place, leading to the formation of silica nanospheres, which can assemble outside the cells' cytoplasm to build the new frustules. Such frustules have to be porous to ensure metabolic exchanges between the cell and its environment. However it has to be pointed out that the pore sizes are not distributed at random. Indeed, a hierarchical 3D distribution is observed, with pores at 3 different scales approximately ranging from 10 nm to 1 μm (Fig.2.). In our modern nanotechnology such a 3D distribution can only be obtained via a layer-by-layer deposition process.

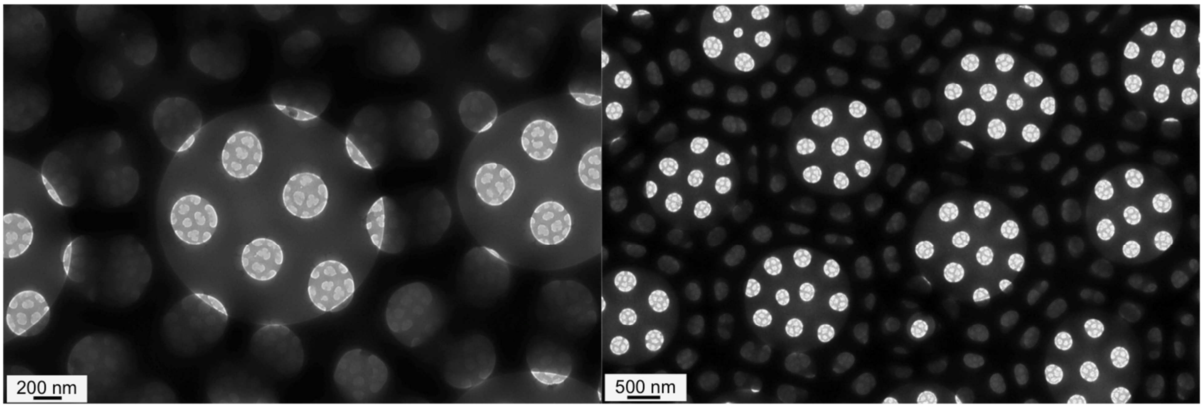

Fig.2 Transition electron microscopy (TEM) images of frustule pores of the *Coscinodiscus Wailesii* diatom with increasing details exhibiting an intricate 3D hierarchical organisation. They range from around 10 nm to 1 micron in diameter. In certain cases, the pores are arranged in a periodic manner, enabling them to exhibit living photonic crystal properties.

Moreover, the frustules of some diatoms present a noteworthy property: they behave as photonic crystals. Indeed, their arrays of pores are distributed in such regular patterns that they make up a 2D network of hexagonal or tetragonal geometries over several tens of micrometres, with repeating units of similar dimensions as visible light wavelengths [8]. Thus, a number of diatoms exhibit iridescence properties and are often referred to as 'sea opals' (Fig.3). Moreover, the silica frustules can also focus light and act as filters with a focal length interrelated to the wavelength of light. Frustules absorb light mainly in the blue wavelength region, focussing the remainder of the visible spectrum and consequently providing diatoms with an effective way to concentrate the biologically useful wavelengths' of light inside the diatom's protoplasm [9]. Chloroplasts are close to the silica wall so that the photonic properties of the frustule could improve the transmission and collection of light by the photoreceptors.

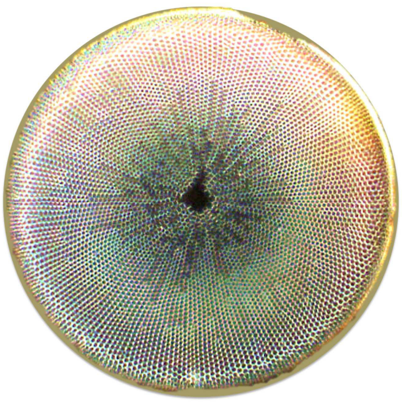

Fig.3. Diatoms are often called 'Sea opals' due to the iridescence arising from the photonic crystal structure of their silica frustule.

Therefore the mesoporous silica shell protects the cell from the damaging effects of UV light while favouring the transmission of useful visible light to the chloroplasts. It has been shown that the frustule of *Coscinodiscus wailesii* diatoms can focus incoming light into a spot of few micrometres. This leads to the partial extinction of excessive irradiation of UV light and enhances the photosynthesis. Diatoms appear to play with solar light to protect the cell and improve their photosynthetic properties but more work would be required to fully understand the underlying mechanisms of such a behaviour! This suggests to use diatoms in photovoltaic devices as waveguides, for example (Fig.4).

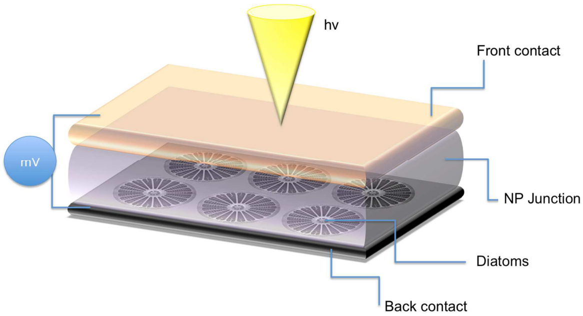

Fig.4. Photovoltaic device based on diatom frustules

3. Diatom nanotechnology

Diatom frustules offer a range of new and exciting possibilities in the nanotechnology field as they are naturally occurring nanostructured materials that can be used to develop innovative nano-devices. Several years ago, a special issue of the 'journal of nanosciences and nanotechnology' was devoted to the theme 'diatom nanotechnology'[10]. Genetically engineered micro/nanodevices (GEMs) have already been built from diatom silica shells [11]. More than 100,000 diatom species presenting different genetically controlled nanostructures can be found in nature. They can be easily cultivated and a large numbers of frustules can be obtained in a matter of days [12]. It therefore becomes possible to design and produce frustule of specific morphologies that have potential applications in nanotechnology for the realization of solar cells, batteries or electroluminescent devices [13].

The porous structure of frustules possesses a very high surface area which leads to strong gas-solid interactions. Silica's physical properties can therefore be modified by the adsorption of gases, leading to the design of micro-sensors. The silica frustule exhibits visible photoluminescence (PL) emission properties, the peak optical intensity and position are affected by surrounding gases. Some of them quench the luminescence, while others enhance it. This could be used for making opto-chemical transduction based sensors as this process is highly sensitive and detection limits as low as 0.1 ppm can be reached with an oxidizing gas such as NO_2 [14,15]. Because of their small sizes, frustules can be directly fixed on the end of an optical fibre.

Diatom frustules can be used as templates for the production of porous materials (oxides, gold, polymers) via replica molding. Polymer, oxide and gold replica possessing the same nanostructure as the silica frustule have thus been obtained [16–18]. They can also be chemically converted into another oxide materials without changing their shape via the so-called BaSIC process (Bioclastic and Shape-preserving Inorganic Conversion). Mesoporous MgO frustules have thus been synthesized via the reduction of silica by magnesium vapours around 600°C [19]. The chemical reaction can even lead to the total reduction of all the silica present to silicon. The MgO formed in the process can then be easily dissolved in an acid solution (HCl) leaving behind a silicon phase which has retained the initial nanostructure of the silica frustule [20]. A wide variety of chemical reactions have been described in the literature showing that the frustule can be transformed into a number of materials such as TiO_2 [21], ZrO_2 [22], $BaTiO_3$ [23], BN [19,24,25]. Foreign ions can be inserted within the silica frustule by immersion in a salt's solution leading to some modification of their optical properties. Experiments performed with nickel sulphate show that the photoluminescence of the silica frustule is quenched suggesting that diatom frustules could be used as sensors to detect traces of pollutants [26].

The frustules can also be chemically modified by introducing new elements in the growth media of the diatoms. For instance, addition of $Ge(OH)_4$ has led to the *in vivo* formation of frustules in which some silicon atoms have been replaced by germanium [27]. These frustules exhibit electro- and photo-luminescent properties characteristic of the Ge^{4+} ions and can be incorporated into electroluminescent devices [28,29]. The doping ions are homogeneously distributed when introduced at the start of the operation alongside silicic acid. Similarly, the distribution of the ion can be controlled by introducing it at various stages of the cultivation process. A two-stage bioreactor was used in which titanium was only introduced in the growth medium during the second phase of the operation. Using this method, amorphous titanium oxide is preferentially deposited on the surface of the frustule where it is converted into TiO_2 anatase by heating in air at 720°C [30]. These TiO_2 covered frustules can then be used as electrodes in dye-sensitized solar cells (DSSC) [13].

Similarly, phosphors were recently obtained via the metabolic insertion of europium into live diatom frustules. Red emission at 614 nm was observed upon near-UV excitation suggesting that such a device could be used for white LED lighting applications [31].

3. Living materials

The development of biotechnologies requires the immobilization of active species, enzymes or cells, on solid substrates. For obvious reasons, bio-encapsulation is mainly restricted to organic or biopolymers, but the example of diatoms suggests that silica glasses could also be biocompatible. These glasses would offer many advantages such as mechanical strength and chemical stability. Moreover, they do not swell in water like most biopolymers. However the high temperature processing of glasses and ceramics is not compatible with fragile bio-molecules.

Following the example of diatoms, chemical syntheses have been developed in order to build nanostructured porous silica networks via the inorganic polymerization of solute molecular precursors. This so-called ‘sol-gel’ process is now currently used in industry to make coatings on glasses [32]. The mild conditions associated with these bio-inspired syntheses allow the formation of organic-inorganic hybrid nanocomposites in which both organic and inorganic phases are mixed at a molecular level. Even fragile bio-species such as enzymes, yeasts or whole cells can be trapped within the solid network, opening new possibilities in the field of biotechnology and nanomedicine. Several processes, such as the use of lipases, have already been scaled-up for industrial production. These enzymes are able to catalyse hydrolysis-esterification reactions and play a substantial role in the agrochemical and petrochemical industries as well as for organic synthesis. Esters are usually insoluble in water, so that the reaction occurs in emulsions at the interface between hydrophilic (aqueous) and hydrophobic (ester) phases. The sol-gel process enables to enhance their biocatalytic properties by adjusting the balance between both phases within the hybrid matrix. Enzymes trapped within such sol-gel matrices exhibit much higher activity than those that have simply been dehydrated via freeze-drying [33].

The sol-gel approach for the encapsulation of whole cells is fairly easy to implement, it is only a matter of adding the precursor solution (silicic acid or silicon alkoxide) to the cell culture medium. The silica condensation reaction kinetic is quite fast, trapping the micro-organisms in a porous silica cage. Trapped cells retain their metabolic activity and the main problem with this method resides in the conserving the cells whole and alive for as long as possible. Several methods to achieve this have been described, all of which are inspired by the biomineralization processes observed in diatoms! However, chemical additives such as glycerol are usually required to limit the interactions between the cells and the silica matrix in order to ensure their survival (Fig.5).

A number of micro-organisms such as yeasts, bacteria and micro-algae are currently used in bio-technological processes to produce interesting active chemicals which are often used in the pharmaceutical, cosmetic, medical and food industries. It is therefore very tempting to use silica gels in order to devise innovative bio-reactors. This idea is strongly supported by diatoms, since they are a natural system showing that life can exist within a glass cage. These ‘living materials’ therefore provide a practical basis for the study, understanding and development of biosensors and bioreactors. Recent literature describes in depth numerous examples of sol-gel bioreactors for the sequestration of pollutants (nitrates, pesticides, heavy metals) as well as for the production of active molecules. Thus, astaxanthin, a potent biocompatible antioxidant currently used as a food additive, can be produced within a silica gel by the alga

Haematococcus pluvialis. The chemical is easily extracted using a solvent while the micro-algae are returned to their culture medium in order to ensure a continuous bioproduction process [34].

Trapping bacteria in this way is a particularly interesting method because it is the basis for numerous biotechnology applications. We have shown that these microorganisms can stay viable for periods of several months in the silica gel matrix [35,36]. The bacteria retain their metabolic activity throughout this time period, and it can even be improved by the addition of quorum sensing molecules. These molecules are primarily used by bacteria to activate specific genes in order to control their metabolic behaviour. This chemical communication method only becomes effective when a minimum concentration of bacteria is present in the medium which enables them to behave in a collective manner leading to the formation of biofilms. This is therefore impossible to achieve when the bacteria are immobilised within a silica gel matrix. They cannot communicate with each other, enabling us to study the individual behaviour of a single bacteria rather than that of a whole colony (Fig.4). The role of quorum sensing molecules on the viability of sol-gel trapped bacteria was studied recently by several authors [37,38].

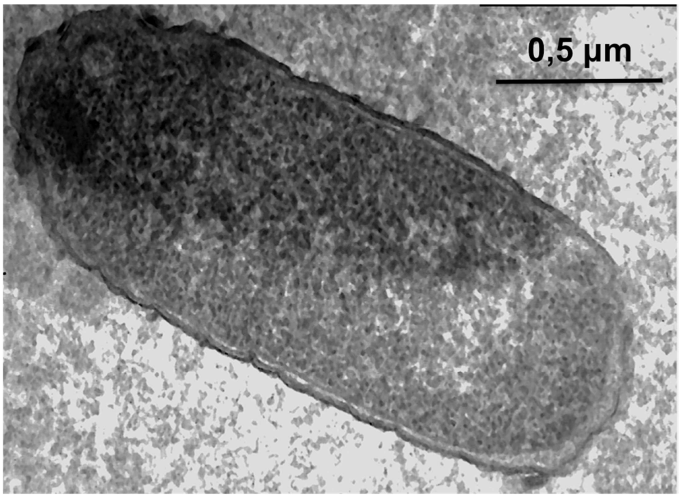

Fig.5. Trapping bacteria (*Escherichia coli*) within a silica matrix preserves their cellular integrity leading to the formation of living materials. Their metabolic activity can be controlled by adding 'quorum sensing' molecules thus offering new opportunities to build efficient biosensors and bioreactors.

Serratia marcescens bacteria are known to produce a red pigment called prodigiosine that possesses therapeutic properties with respect to human colon cancer. These bacteria still produce prodigiosine when trapped within a silica gel, but we showed that adding quorum-sensing molecules to the solution of nutrients significantly increases the production of prodigiosine. The metabolism of trapped bacteria is modified in order to adapt to the inorganic environment, enhancing their resistance to the stresses induced by encapsulation. The bacteria survival rate could then reach 100% with added NHL (N-hexanoyl-homoserine lactone) whereas it is only of 60% when BHL (N-butanoyl-L-homoserine lactone) and less than 40% in the absence of any additives.

A better knowledge of the chemical communication between bacteria could be of paramount importance in the fight against bacterial infections. Bacteria only become harmful once they reach the 'quorum sensing' limit. They can then communicate with each other to achieve a common goal and build a biofilm. The development of 'quorum quenching' molecules devised to block these chemical exchanges could enable us to fight more effectively bacterial infections without having to use antibiotics. This is also a bio-inspired approach since it is the means by which some red algae defend themselves against bacterial aggressions; by producing furanones. It has been shown that these types of molecules help fighting against pulmonary infections caused by the bacteria *Pseudomonas aeruginosa* [39].

4. Conclusion

The frustule of diatoms exhibits a photonic crystal behaviour arising from the periodic distribution of pores along the silica shell. This particular structure is used by these micro-algae for solar protection and photosynthesis optimization. These silica frustules can be used as ready-made nanostructured materials opening new opportunities for the realization of nanodevices. Their chemical composition can even be chemically or biologically modified in order to provide the required properties to be used in batteries, solar cells or luminescent devices. Moreover, the example of diatoms suggest that micro-organisms can be trapped within a glass matrix leading to the bio-inspired synthesis of living materials that can be used as bio-sensors or bio-reactors.

Acknowledgements

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