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Past, present and future of diatoms in biosensing

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ABSTRACT

Diatoms possess natural nanostructures and unique properties of great interest in nanotechnological applications. Among them, their use as nanostructured supports in biosensing platforms, although still in its childhood, is promising. Herein, the works focused on the immobilisation of diatom frustules, the modification of their composition and chemical features, and their functionalisation with biomolecules, are reviewed. The preservation of the three-dimensional nanostructure is almost always pursued and thus, processes are carried out under strict control. Additionally, high immobilisation yields, appropriate spatial distributions, modification or coating with specific components and oriented immobilisation of biomolecules, are also sought. The biocompatibility of diatom frustules, together with the nanofeatures, and the wide variety of processing methods, indicate that their exploitation in biosensing is imminent.

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1. Introduction

The unique properties of diatoms led in 1988 to a new interdisciplinary area of research called diatom nanotechnology [1]. The interest of biologists and materials scientists on exploiting this intricate three-dimensional hierarchically nanostructured material as building blocks for the next generation of nanodevices has been continuously growing since then [2–9]. Diatoms are single cell microalgae with a silica (SiO_2) shell called frustule, which possesses a nanoporous pattern of unparalleled diversity far beyond the possibilities of current micro- and nanofabrication techniques. Even though still being under research, it is commonly accepted that these precise silica micro- and nanostructures have evolved to suit diatoms functionality. Such is the case of their biophotonic properties, which have been related to their capacity to guide light as a way to help photosynthesis [10].

Nature is a continuous source of inspiration for the design of advanced biomaterials at the nanoscale and thus diatom frustules are an excellent example of platforms able to feature multiple functions. Diatoms can be modified to incorporate nanoparticles or

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biomolecules with different functionalities, and their composition and morphology can be easily tuned, overcoming some of the challenges involved in the production of nanomaterials. Their mechanical strength, high surface area and porous three-dimensional micro- and nanopatterns, open up a wealth of possibilities in terms of nanoengineering new attractive functional materials, maximising the potential of ancient nanochemistry based on using glass as a host for functional nanoparticles (e.g. the Lycurgus Cup) [7].

Compared to other recent reviews [11–15], this survey aims to describe the protocols used in each of the three fundamental steps (immobilisation, modification and biofunctionalisation) addressed to rationally design the use of diatoms as building blocks in biosensing systems. We describe in detail the methods used to assemble diatoms onto solid supports, to modify their properties by using physical, chemical or culture methods producing composite frustules or replicas, and/or to functionalise them with biomolecules. Most of the works described below are summarised in Table 1. The exploitation of the knowledge acquired on the advantages and limitations of these fundamental steps will provide researchers with the capacity to harness the unique properties of diatom frustules to rationally design diatom-based building blocks for biosensing applications.

2. Diatom immobilisation

Methods for the immobilisation of diatom frustules on transducers are needed to ensure the exploitation of these natural nanostructures for biosensing purposes. The nature of the substrate may constrain the method to use, which will dictate the diatom orientation, immobilisation yield and spatial distribution that can be achieved. Although it may depend on the application, morphological integrity of the frustules is usually sought, and thus, high pressure or temperature should be avoided. Fig. 1 represents some of the methods used for diatom immobilisation, which are described below.

Most of the immobilisation methods used are based on the modification of either cleaned diatom frustules or immobilisation solid support for the subsequent assembling. As an exception, Umemura et al. [16] first cultured the diatoms on a mica surface treated with 3-aminopropyltriethoxysilane (APTES) and then baked the surface to remove the organic components of the diatom cells adhered to the surface.

Wang et al. [17] modified a glass substrate with a polyelectrolyte multilayer by inkjet printing for the subsequent immobilisation of diatom frustules by electrostatic attraction between the final positively charged layer and the silanol groups of the frustules (Fig. 1A). Two centric diatoms were immobilised, *Coscinodiscus wailesii* and *Cyclotella* sp., being 200 μm and 10 μm in diameter, respectively. Tailoring the diameter of the positively charged multilayer dots to 200 μm, deposition of only one diatom frustule per dot was achieved. Several hundreds of the small *Cyclotella* frustules were deposited as a monolayer onto dots of equal size.

Lin et al. [18] used a more sophisticated technique for the site-directed immobilisation of only one diatom frustule onto a specific spot. After coating the gold microelectrode chip with polylysine, an individual *Coscinodiscus wailesii* frustule was micro-manipulated by a positioner and attached onto the chip. Since the *Coscinodiscus* frustule was rather large, it completely covered the gold sensing site, comprised of a 25-μm diameter working electrode and a 125-μm diameter counter electrode. It is important to note that by covering the whole sensing area the nanoscale pores of the frustule structure mimicked arrays of nanowells, which were used as biosensor platforms for the electrochemical detection of cardiovascular biomarker proteins, as explained in detail in section 4.

Conventional Si-O-Si bonding techniques have also been explored to immobilise diatoms onto Si-based substrates, either directly [19,20] or through polymers such as poly(dimethylsiloxane) (PDMS) [21,22] and ethylene-vinyl acetate (EVA) [22,23] (Fig. 1B). The combined use of these polymers with photoresists and photolithography techniques provides microarrays of defined activated patterns where diatoms are specifically immobilised. The process usually requires energy supply in the form of pressure [19,20], ultraviolet light [21] or heat [22,23]. The incorporation of a catalyst like HCl allows the bonding of diatoms under more gentle conditions [19,20]. Once bonded, diatom frustules retain their physical and chemical properties, such as their photoluminescence that allowed their use as gas-sensors [19], or their three-dimensional nanoporous structure that was used as a mask to obtain nanogold pillar arrays for surface-enhanced Raman scattering (SERS) detection [20]. Combining photolithography and frustule flotation, multi-layer diatom arrays have been formed packaging *Nitzschia soratensis* into *Coscinodiscus argus* frustules. These arrays enhanced the fluorescent intensity from a fluorescein isothiocyanate-labelled anti-IgG antibody after binding to the immobilised IgG, as explained in section 4 [23].

Recently, Chandrasekaran et al. [24] reduced diatom frustules from SiO_2 to Si by magnesiothermic conversion to allow their functionalisation via hydrosilylation with allyl mercaptan. Thiolated diatom frustules were subsequently self assembled on gold-plated glass slides (Fig. 1C). To achieve high diatom immobilisation yields, five consecutive immobilisation cycles were performed. These nanostructured surfaces worked as photoelectrodes for current generation from solar energy.

So far, only a few immobilisation techniques, tailored to the unique properties of diatoms, have demonstrated to be effective for diatom-based device manufacturing. Furthermore, they usually require sophisticated instrumentation, multi-step processes or drastic experimental conditions that may affect diatoms 3D structure. Simple, fast and efficient methods for stable diatom immobilisation onto several supports are highly desired for the development of biosensors.

3. Diatom modification

The silica shell of diatoms provides them a perfectly ordered, symmetrical and 3D structure, with thermal and mechanical stability, biocompatibility and unique optical properties. However, their poor electrical properties make difficult their use in electrochemical biosensors. SiO_2-based diatom frustules can be converted into new materials with additional attractive features, such as high electrical conductivity, catalytic activity and/or optical properties that can be exploited in the development of electrochemical as well as optical biosensors.

Several approaches have been used to change the composition of diatom frustules while preserving their 3D morphologies. These processes, referred collectively as BaSIC (Bioclastic and Shape-preserving Inorganic Conversion), merge the attractive characteristics of nature with the chemical versatility of synthetic processes, providing a promising route towards mass production of nanostructured devices with complex 3D shapes and tailored chemistries. Changes in diatom composition include the direct processing of frustules, where frustules made of a new material are directly prepared by means of displacement reactions, or the use of frustules as templates, where coating methods are performed to produce composite frustules. If the coating is continuous and rigid, but does not fill or coat the nanopores, frustules can be selectively removed (e.g. by selective dissolution) to yield a replica with the desired composition. This replica retains the 3D features of the starting diatom template and features new material properties. In other cases, coating methods are used to replicate the pore patterns of the diatom frustules and obtain inverse replicas with the same multi-level pore and nanoscale precision as the original frustules.