Hierarchical Nanobiosensors

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Nanostructures have played a key role in the development of different techniques to attack severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Some applications include masks, vaccines, and biosensors. The latter are of great interest for detecting diseases since some of their features allowed us to find specific markers in secretion samples such as saliva, blood, and even tears.

SARS-CoV-2 hierarchical nanostructures nanoparticles

1. Introduction

Sensors based on hierarchical nanostructures in the area of nanomedicine have been meticulously investigated in order to identify different enzymes and organisms such as bacteria or viruses. Biosensors are fascinating instruments that basically serve to detect biological or chemical parameters such as those related to molecules in tissues, microorganism cultures, and nucleic or acid chains [1]. The characteristics related to biodetection like selectivity, response speed, and stability depend on the morphology and structure of the sensing materials [2].

The main types of sensors used in biodetection are electrochemical [3], thermometric [4], piezoelectric [5], magnetic [6], and optical sensors (plasmonic [7], UV-Vis/infrared spectroscopy [8], Raman and SERS [9], or attenuated total reflection [10]). Biosensors that are developed using hierarchical nanostructures can be manufactured with different nanomaterials. For example, nanohybrids can be integrated into diverse materials such as noble metals [11], graphene [12], copper, titanium [13], zinc oxide [14], and bimetallic oxide [15], among others. The biosensors can be classified into three groups according to their mechanisms: the biocatalytic group that uses enzymes, bioaffinity group that involves antibodies and nucleic acids, and microorganism group that uses microbes [16].

A strong selective control of the manufacturing parameters of noble metals is possible [17], allowing their structure to be modified [18] to improve their physicochemical properties and adjust their shape [19]. There are multiple techniques for designing nanobiosensors, but the most common ones are based on electrochemical deposition [20], electroless deposition [21], electrocatalysts [22], and physicochemical methods [23].

Besides different processing routes that have been extensively explored to improve biosensing effects, the use of the LSPR phenomenon is very attractive [24], and the development of hierarchical nanostructured biosensors can promote exceptional optical, electrical, and chemical properties based on LSPR. Some of the special characteristics exhibited by hierarchical nanostructures are derived from their ultra-high specific surface area, high flexibility, light weight, high electrical conductivity, and bio-compatibility [25][26][27][28][29][30].

The hierarchical nanostructures are replacing conventional random hybrids in counterparts thanks to their physical characteristics, stability, and efficient transfer of electronic and ionic charges [31][32]. For example, their morphologies show a high surface area with adjustable porosity or packing density. Some hierarchical assemblies serve as programmable scaffolds that provide molecule-level control over the distribution of fluorophores and nanometer-scale control over their distance. Several strategies can be used to study imperfections and to stabilize various types of nanostructures, such as hollow ones [33] or cage frames to obtain a better performance [34].

It is worth noting that hierarchical metamaterials have been reported for the development of virus-based light learning systems, in plasmonic structures for application in high-performance metamaterials, and in binary nanoparticle networks and liquid crystal arrays for sensing technologies and imaging [35]. With these procedures, diverse techniques have been demonstrated strong fluorescence intensity and mild levels of enhancement, which allows them to manipulate photonic excitation and photoemission [36].

Hierarchical nanostructures represent a potential key to the next generation of new nanomaterials. For example, a controlled structure in the agglomeration between nanoparticles can increase plasmonic effects while the stacking distance between other nanoparticles decreases; all of this can be used to develop new and effective detection methods. Some of the representative hierarchically structured shapes are nanopillars [37], nanocones [38], nanoholes [39], and gecko pillars [40], among others.

Hierarchical nanostructures can be fabricated using techniques such as nanosphere lithography [41] with multiple patterns [42], electron beam lithography [43], pattern transfer [44], and focused ionization [45].

The characterization of the morphology, structure, and stability of hierarchical nanostructures can be explored by different methods. The typical characterization techniques for hierarchical nanostructures are X-ray diffraction [46], electrical effects [47], TEM [48], energy dispersive spectroscopy (EDX) [49], AFM [50], optical interactions [51], PL [52], Brunauer–Emmett–Teller surface area analysis [53], UV–visible absorption spectroscopy [54], photovoltaic performance [55], photocatalytic processes [56], Raman spectroscopy [57], and magnetic phenomena [58].

A hierarchy in nanostructures can be developed through in situ plasmon-driven syntheses [59] or through amino acids [60] to easily detect analytes at trace levels, such as pesticides, heavy metals, explosives, proteins, pathogens, and other chemical and biological contaminants [61]. It is clear that nanomaterial sciences are essential for developing biosensors with high reliability and speed using innovative technology [62][63][64][65].

In the last two years, diverse experiments have been carried out in the development of biosensors using different hierarchical nanostructures. It is worth highlighting some examples that have been very useful in the commitment to developing biosensors with better properties.

It has been pointed out that biosensors can be used to see the effectiveness of the vaccines in healthy, convalescent, or vaccinated people [66]. They can be used to monitor diseases, observe how many antibodies exist in people's fluids, as well as determine whether the vaccines are effective for the test subjects [67]. In the faster biosensors, it takes approximately 20 min to obtain the result. The research has sought to develop biosensors with these nanomaterials to achieve a relatively rapid response, achieving a response time of 15 min.

It has been observed that current biosensors also have some disadvantages such as not being capable of detecting analytes in samples when there are external stimuli. This has to be addressed with the development of different biosensors with the properties of nanomaterials, such as different probes, including plasmonic [68] and incorporated ones [69]. Biosensors capable of detecting pathogens with very little genetic material compared to other assays have also been developed [70]. Additionally, calorimetric strips for smartphones aimed at antibodies or antigens to combat the rapid spread of these diseases have been considered since wearable biosensors can constantly monitor patients [71].

With this motivation, different aspects of the cutting-edge biosensors in the detection of SARS-CoV-2, focusing in those based on hierarchical and hybrid nanoparticles. **Figure 1** shows the main characteristics.

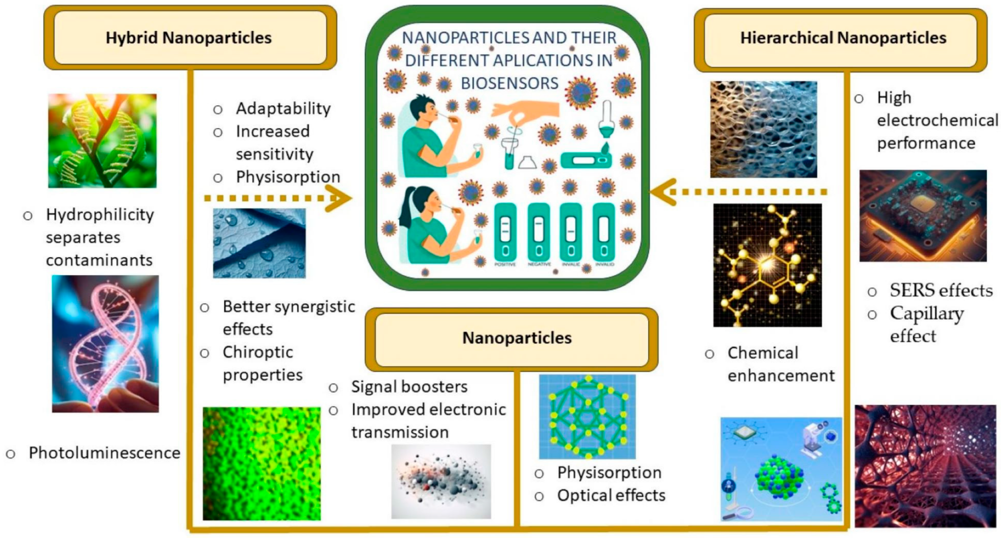

Figure 1. Representative characteristics exhibited by different nanostructures in biosensing applications.

2. Synthesis of Hierarchical Nanostructures and Multicomponent Assemblies for Biosensors

Materials with hierarchical nanostructures have excellent mechanical properties due to the functional adaptation of their structures into different hierarchical levels. Hierarchical structures can be observed in nature, such as in bones, wood, cork, and plant stems, or in glass sponges [72]. Hierarchical nanomaterials show different architectural designs that are ordered at multiple length scales. They are grouped according to their main characteristics; in the case of porous materials, they contain interconnected pores with at least two levels of pore hierarchy from molecular (1–100 A), nano (10–100 nm), and meso (1–100 μm), to macropores [73]. It should be noted that the construction of hierarchical nanostructures requires knowledge of particular principles to avoid limitations on their properties [74]. Hierarchical materials can mimic the mechanical properties of their biological counterparts. Smart hierarchical materials can exhibit specific stimulus-response properties [75], such as selfhealing and self-regeneration [76] in order to improve fracture resistance and increase strength [77]. Arrays can be constructed using proteins and microscale mechanical constraints can be used to form ordered networks within macroscopic structures [78]. The synthesis at different orders of magnitude from nanoscale to macroscale can be used to acquire outstanding characteristics through interacting with different analytes of different sizes [79], from small proteins to living cells. Different networks can be designed according to the geometry of the templates used [80]. The nanoclusters can be protected by ligands that can be prepared with atomic precision, exhibiting well-defined structures and resulting in versatile building blocks to manufacture excellent structures capable of performing certain functions [81]. For instance, nanofibers are used to construct multifunctional walkways with up to five levels of organization (depending on the method used). In the first level, there is a composite nanofiber; in the second level, a layer of composite material coated on the composite nanofiber that will result in the third level. The fourth level organizes the nanofibers to form an assembly and finally, in the last level, an assembly of nanofibers can be encapsulated within a matrix to form a massive structure by default [82]. Nanotubes are commonly used for the manufacture of hierarchical materials since they consist of molecular blocks, whose characteristics can be related to an anisotropic supramolecular self-assembly behavior at a personalized nanoscale, which allows for the creation of a percolation network at the mesoscale. They are regulated by dynamic self-assembly into four hierarchical levels of self-organization [83]. Nanosheets are composed of 2D building blocks, which have atomic or molecular thicknesses and they are considered the thinnest functional nanomaterials. They can be organized into various nanostructures or combined with a variety of materials at the nanoscale. Thanks to this, wide-range assemblies such as organic molecules, polymer gels, and inorganic nanoparticles can be designed [84].

Although hierarchical nanomaterials can be considered hybrid materials [85], nanohybrids are composed in a different way. Hybrid materials can have a variety of complex architectures with or without hierarchy. Their size varies from nanometers to several micrometers and several millimeters. Hybrid nanomaterials are combined through the synergistic mixture of two or more nanomaterials, which can be either inorganic or organic [86], that create a single material with properties that go beyond their properties as individual elements. They consist of groups of blocks with similar properties and structures with groups that cross-link the polymer into chains [87]. Their properties are determined by a combination of structure and composition at each length scale [88]. As a result, their properties are expressed in molecular length scale structures [89]. This indicates that the new mixture has superior properties compared to the original mixture. The properties to look at are the advantages derived from nanomaterials at a macroscopic level, such as energy absorption performance. The lightweight structure maximizes its functionality and improves the efficiency of the material [90][91].

There are different forms of nanohybrids such as sandwich structures, foams, reticular structures, segmented structures, zero expansion [92], and meso-structured thin films [93]. These structures serve different purposes, especially in integrated refractive and diffractive optical devices. Since these nanomaterials have a large thermal stability and better compatibility, they are typically used in the production of semiconductive devices [94]. In order to characterize organic–inorganic materials, techniques such as FTIR, Raman spectroscopy, LSPR, and various techniques based on MS are used [95].

Hybrid nanomaterials are good candidates for developing nanomaterials in the fight against bacteria and viruses thanks to their high sensitivity, good stability, and selectivity. In particular, they can detect antigens in plasma since their good electrochemical activity helps in the immobilization of the chains of different aptamers [96].

Nanomedicine, based on hybrid and hierarchical nanomaterials, has achieved great progress in the field of biosensors for the diagnosis, prevention, detection [97], and treatment of diseases in the post-pandemic period [98]. Compared to bulk materials, nanostructures are more precise, more reliable, less invasive, and easier to carry according to their chemical elements [99]. The effectiveness of nanomaterials has advanced to detect diseases at a very early stage using new technologies based on nanobiosensors [100], whose physical principles at the nanoscale level allow the biological receptors to be highly sensitive [101]. Nanobiosensors can be tailored by using different types of nanomaterials and structures [102].

Depending on their interactions, nanobiosensors can be classified into two different groups called biocatalytic or biophilic. These two groups can be classified according to recognition factors, for example, cells, organelles, tissues, enzymes, receptors, antibodies, nucleic acids, MIPs, PNAs, or aptamers.

Nanostructures are capable of obtaining information through molecular interactions in real time, and in normal and pathological biological states which provides an effective and relatively fast result. For example, in a drop of blood, an enzyme such as glucose oxidase, glucose dehydrogenase, or hexokinase can cause a reaction, which can be measure by a low-dimensional detector in a glucometer (biosensor) [103].

Because the manufacturing of biosensors has several drawbacks, efforts have been made to develop improvements in manufacturing [104][105][106][107][108]. Characteristics like adhesion ability, strong adsorption capacity, chemical catalytic efficiency, and corrosion and oxidation resistance facilitate the fabrication [109], chemical stability, and electron transfer kinetics [110]. The challenges for optimizing highly selectivity binding properties are continuously being overcome to analyze nanoscale elements of biomolecules [111].

High crystallinity with insignificant structural defects can be relevant to detecting different samples such as glucose, proteins, and nucleic acids [112]. The other main advantages of nanohybrid materials are the specific binding sites that generate a selective sensor signal, which also improves its magnitude and composition. The high surface-to-volume ratio of nanofibers can also improve the capture efficiency and it provides some surface area-related phenomena, including ion exchange and catalysis [113][114].

Heterounions in hierarchical nanomaterials can promote the selective formation of specialized structures and sensitive responses not found in other sensors [115][116][117][118]. Nanomaterials produced through molecular printing can create selectivity for specific enzymes. This method can be worked with 3D nanostructures and it is used to manufacture versatile materials for the construction of sensors to detect various analytes [119]. It has been demonstrated that these nanostructures can eliminate pathogens and better detect enzymes compared to other nanomaterials [120].

The existing improvements found when assembling nanostructures are versatile, and they open new methods for different technologies to control their structure and combine physicochemical properties [121][122][123][124][125][126][127][128][129][130][131]. On the other hand, some nanostructured systems based on organic polymers have been proposed [132], and applications for spectrochemical biosensing have been demonstrated. Biosensors based on RNA hybridization can be considered for several biological reactions and for generating analytical signals that are easily detected by different electrochemical aptasensors [133], electrochemical luminescence sensors [134], and optical transducers, among others [135]. It has been pointed out that RT-PCR [136] can be used to amplify cDNA from virus RNA [137]. This is of great interest in the studies that have to do with inhibitors that target the enzyme helicase since it is known to participate in the processes of duplication and cell reproduction [138].

Photonic nanobiosensors have been also highlighted with respect to their potential use against SARS-CoV-2. In addition to monoclonal antibody pairs, which are rapid antigen tests [139], it is important to look for more efficient ways to detect pathogens [140]. In this direction, biosensors using some promising plasmonic nanoparticles are the most powerful tools employed for the detection of viruses [141]. Moreover, polystyrene nanoparticles, graphene, and carbon nanotubes [142] present different advantages such as selectivity towards particular molecular expressions corresponding to an important challenge that requires high specificity, sensitivity, and a multiplex detection capability to offer good virus detection. The design of POC testing arrangements [143], such as LFIAs, should be mentioned as they offer fast and easy-to-use methods, as well as reliability. Each synthesis procedure can be functional, but inherent limitations in the quantitative analysis of the virus in the application of biosensors should be noted [144].

In summary, hierarchical nanostructures are formed by hybrid nanoparticles, which are good candidates for the development of nanobiosensors for pathogen detection. These nanoparticles are prepared through different synthesis methods, as it is illustrated in **Figure 2**.

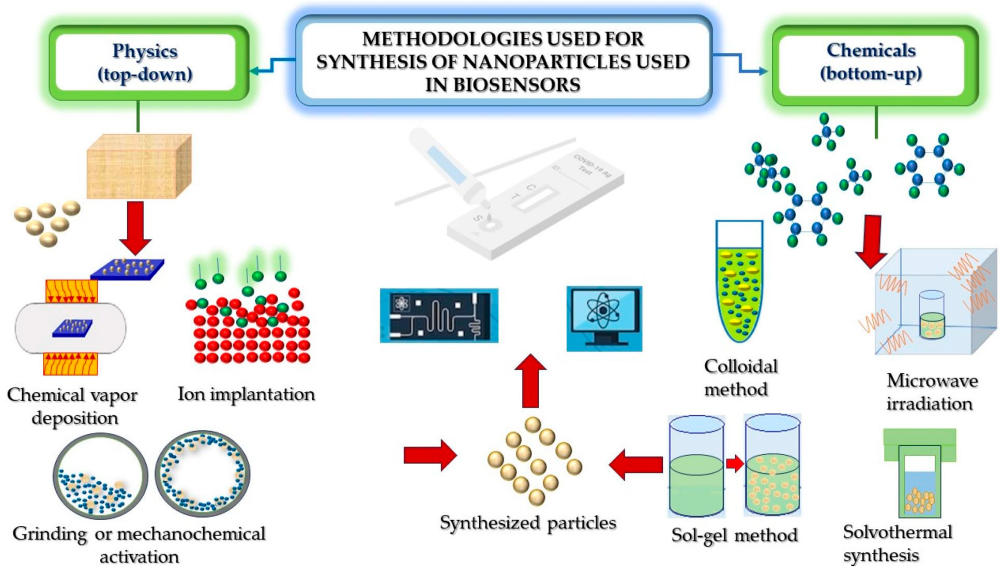

Figure 2. Representative processing routes for the synthesis of hierarchical nanostructures used in the development of biosensors.

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