Review

Biofunctionalized Nanomaterials: Alternative for Encapsulation Process Enhancement

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Abstract: In recent years, interest in the development of nanometric materials with specific characteristics has grown; however, there are few scientific contributions that associate encapsulation methodologies and matrices with the particle objective (metabolic directions, type of administration, biological impact, and biocompatibility). This review focuses on describing the benefits and disadvantages of different techniques for designing custom particles and alternatives for the biofunctionalization nanomaterials regarding the biological impact of a nanomaterial with potential use in foods known as nutraceuticals. The study of optical properties, physicochemical factors, and characteristics such as rheological can predict its stability in the application matrix; however, not only should the characterization of a nanocomposite with applications in food be considered, but also the biological impact that it may present.

Keywords: nanomaterials synthesis; encapsulation process; biological impact; biofunctionalization

1. Introduction

In recent years, the impact of nanoscience in research has contributed to the design of nanometric structures of advanced high-performance materials. Given their unique optical, electro-negative, and catalytic properties, some nanoparticles, such as inorganic ones, are attractive as building parts [1], and these characteristics can be modulated by altering the surface state, functionality, and even nanoparticle shape. Other types of structures are made with different polymeric matrices such as polyvinyl alcohol, poly(lactide-co-glycolide), alginic acid, chitosan, etc., which generally have a larger size but have been considered to have the greatest biocompatibility to processes of digestion [2,3]. For the development of nanostructures, simple encapsulation processes are required, such as emulsion and solvent evaporation, or more thorough synthesis processes, for example, sol-gel [4], nanoprecipitation [5], thermal evaporation [6], or those assisted by specific technologies such as spray drying, spray freeze drying [7], solvothermal [8], microwave [9], etc.

An important aspect in the synthesis of new materials is the biofunctionalization, which involves modification of the physicochemical properties of the surface of any material, which allows a wide variety of applications, with a particular biological interest (as an implant, a prosthesis, or any additive administered topically or orally in a living being) to improve the biological response of the organism to the material that has been manufactured [10]. This new concept allows the improvement of surface properties to obtain beneficial effects such as better digestibility and bioaccessibility of encapsulated

compounds, as well as faster response time for the biological effect, among other benefits. Therefore, there are alternatives in silico that allow for predicting the compatibility or percentage of charges of different materials for particle formation [11,12].

In this sense, nanoparticles, due to a wide field of application, have been implemented in various industries, such as pharmaceutical, food, and chemical, among others, for the encapsulation of compounds or even microorganisms. However, the analysis of polymer methodologies or matrices is analyzed with respect to the objective of the nanostructure that is intended to be designed [13]. Conversely, it is compelling to characterize the material with certain methods such as dynamic light scattering (DLS), from which the Zeta potential (ZP), polydispersibility index (PdI), and particle size can be measured [14]. DLS can predict the stability in a matrix in different systems, such as a colloid. Fourier-transform infrared spectroscopy (FTIR), Ultraviolet–visible spectroscopy (UV-vis), and Raman spectroscopy provide structure-related information [15], scanning electron microscopy (SEM) shows the morphology of the materials, and with simple uranium staining techniques, the material encapsulated is demonstrated with transmission electron microscopy (TEM) [16].

A relevant factor to evaluate is biocompatibility, for which there are in vitro assays to evaluate the possible damage of nanomaterials in tissues. Among the possible effects that depend on the uptake and internalization of nanoparticles in the cells are short-term cytotoxicity, which includes the affectation of cellular metabolism, alterations in the integrity of the plasma or lysosomal membrane, alterations in the mitochondrial metabolism, reactive oxygen species (ROS) generation, or genotoxicity [17,18]. Nanoparticles could also generate other long-term effects in cells, such as changes in the structure of the cytoskeleton, which alter cell morphology and reduce the proliferative capacity and defects in cell migration or differentiation [17].

This review considers the most important characteristics of the development of biofunctionalized materials, the tools for their characterization, and the biological impact (Figure 1).
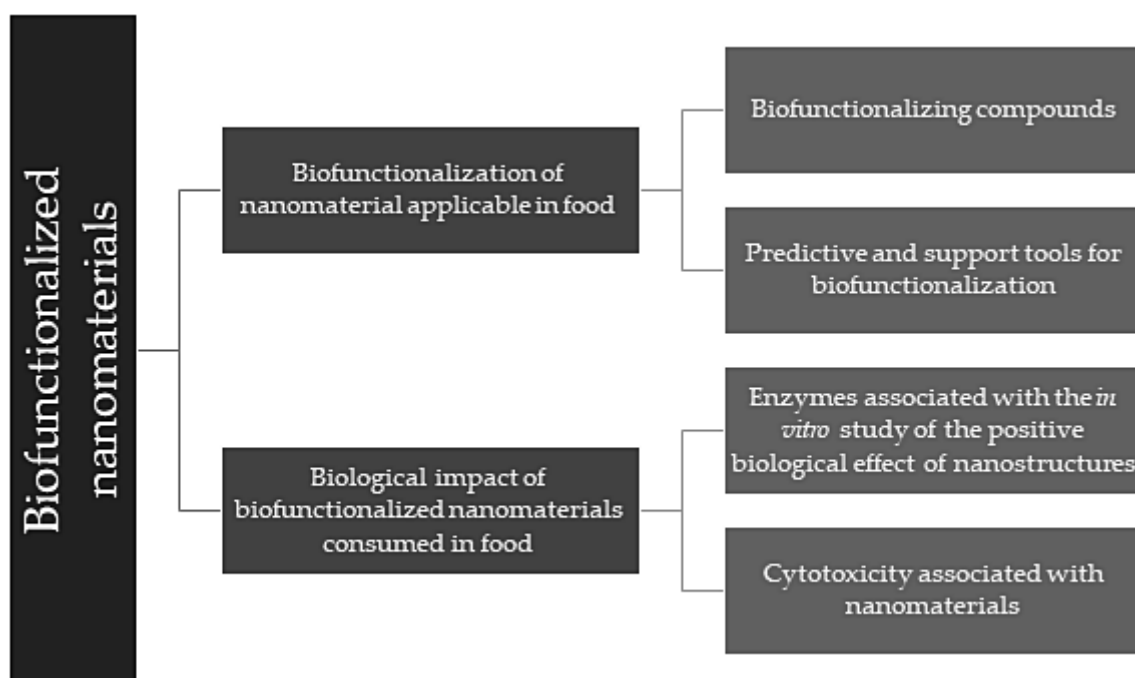
Figure 1. Schematic content.

2. Biofunctionalization of Nanomaterials Applicable in Food

The implementation of nanoscience methodologies has generated new sources of scientific interest [19]. Specifically, the most important synthesis parameters are the chain length, degree of branching, and crystallinity, particularly in polymers, since these param-

eters influence the physical, chemical, and transformation behavior. The longer it takes for the polymer chains to be obtained in the synthesis or the higher the degree of branching, the less crystallinity the polymers have, which improves the mechanical resistance, stability, and chemical resistance. Nevertheless, bioactive compounds' inclusion through different novel methods is involved in the development of biofunctionalized polymeric materials [20]. An example of application is in the development of biopolymers for food packaging. Among the main characteristics that they must meet are degradability, mechanical resistance, and thermal resistance. This can be achieved with the mixture of polymers known as polyvinyl alcohol, crystalline cellulose, and gelatin and allows nanocomposites to be obtained, which are structurally more resistant, with changes that are usually seen in specific FTIR regions [21].

The characteristics and properties of nanomaterials can vary with the parameters and designs used during synthesis. The materials used as the main components of the matrix can be of lipid origin (phospholipids, fatty acids), proteins (albumin, collagen), natural polymers (chitosan, dextran, alginate), semisynthetic (cellulose derivatives, and synthetic polyacrylates (polyacrylamides, polyanhydrides, and polyesters) [22]. These combinations of materials could be of higher molecular weight such as agro-industrial residues; however, the integration of polymeric molecules in the appropriate proportions, considering the structural changes and the interactions between the molecules that make it up, can allow the development of biofunctionalized materials [23].

The chemical structure of the polymer and proteins when used in a suitable encapsulation process (particle size, shape, drug release profile, etc.) can determine the behavior in terms of encapsulation, degradation, and the release of molecules [24]. Due to the wide range of possible combinations of materials, nanoparticles with covers have been classified based on the material from which the core is manufactured and the cover/envelope. Nevertheless, advances in science have implemented other trends to prepare complex materials to mimic the supramolecular architecture of natural structures, with sophisticated and/or multifunctional added capacities (Figure 2). Previous research [25] has shown a proposal of the possible structural conformations for the functionalization of material by incorporating a polymer, a bioactive molecule, and a functionalizing structure, which aims to wrap the structures to confer stability of the particle.
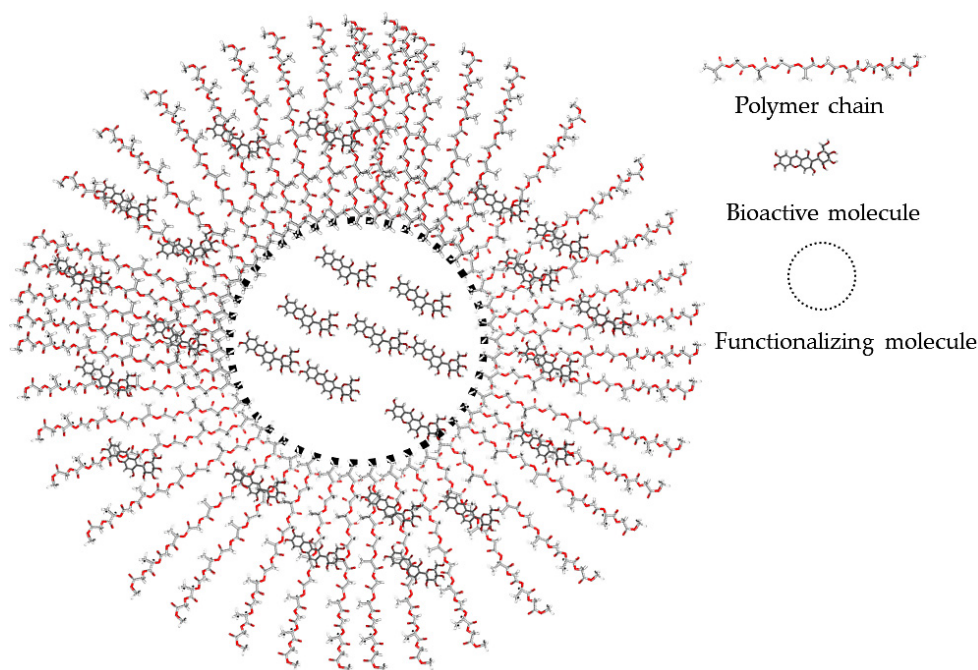

Figure 2. Hypothetical structure of nanoparticles [25].

2.1. Bio-Functionalizing Compounds

Bio-functionalization is possible by using compounds of different chemical natures with relevant biological activity, i.e., combining the biological attributes of two or more compounds by enhancing the effect of one of these compounds [26]. One strategy for bio-functionalization involves mixing a compound of low water solubility with one of high water solubility in stoichiometric quantities to ensure the correct interaction between functional groups that can act as anchors [27]. These formulations allow the use of emerging technologies such as an electrospray, where fibers with a particular molecular arrangement can be obtained, which allows for diverse applications, from packaging materials to controlled-release systems for compounds with biological activity [28].

In addition to the above, the properties of some polymeric monomers are exploited through functional groups as anchors for the addition of biological compounds [29]. Biofunctionalizations are performed using both physical and chemical methods of both low-energy (ultrasound, homogenization, emulsification) and high-energy methods (microemulsion polymerization, interfacial polymerization, etc.), always considering the chemical nature of the compounds and their stoichiometric proportions to ensure the expected effect [30].

In biofunctionalization, it is pivotal to consider low-energy interactions such as electrostatic interactions and Van der Waals forces, since these are more likely to be obtained in the combined system. In addition to the participation of a certain surfactant that allows the stability of biofunctionalized compounds in the system of interest, Figure 3 graphically shows one of the ways to consider these energies through structural modeling, which, through a database, allows for predicting the behavior of the structure and translating this into effectiveness or feasibility of using the matrices. Once the biofunctionalization is performed, the system must be maintained with a low humidity concentration to ensure the stability of the components [31]. For this, lyophilization, spray drying, or other physical methods are performed to ensure its subsequent use in vitro and in vitro assays in which the compounds have been considered active [32].
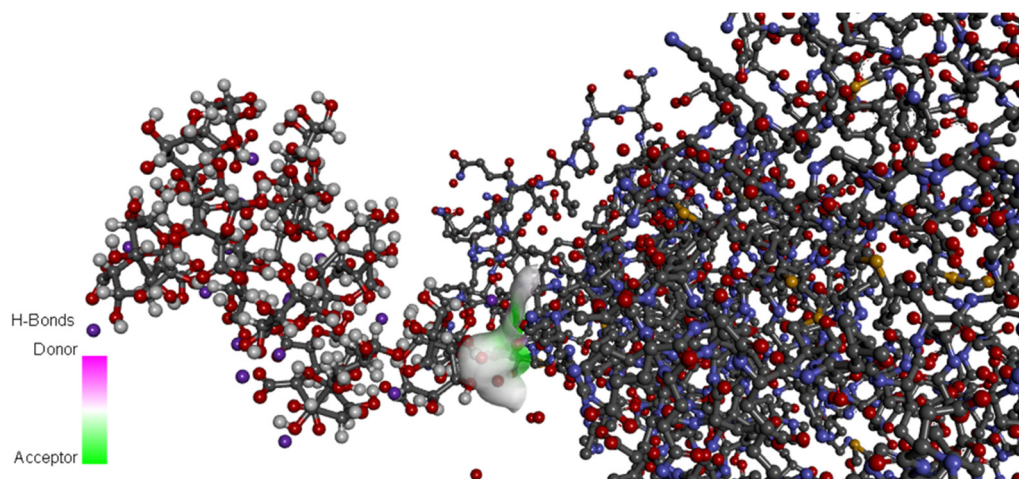
Figure 3. Example of in silico visualization of the biofunctionalized polymer [31].

Some biofunctional materials with the structural characteristics and information are provided in Table 1, which are considered important in nanomaterials design since they allow one to predict their behavior. We thereby consider applications together through an analysis of the information collected, which concern parameters, such as solubility and particle size dispersion, among others, that can guide the use of a vehicle such as a patch, drink, or food solid, among others, considering the matrix in which this functionalized material is reported and loaded with a compound that has beneficial characteristics for health [33,34]. The information provided in Table 1 describes the characteristics of

the size and composition of the nanoparticles, although the authors do not mention the cytotoxic evaluation.

An important part of biofunctionalization is the risk assessment to obtain these measurements and extract patterns of nanomaterial behavior that can be predicted based on these physicochemical parameters. The toxicity of a specific nanomaterial is correlated with the size of its particles, which can predict the toxicity of a nanomaterial based on a specific parameter in its characterization as FTIR. Through the formation of specific signals in FTIR spectra, some bonds can be identified that can be attributed to possibly toxic substances generated during the synthesis that, through deep analysis, can show a lethal effect on cell lines, but analysis through in vitro tests is also necessary [34].

Table 1. Biofunctionalized materials and relevant information on characterization techniques.

| Material	Load	Generalities	Characterization	Results	Ref
PLGA	Donepezil	89.67 ± 6.43 nm Nanoparticles	SEM	Spherical nanoparticles	[35]
			FTIR	At 3008 cm^{-1} the characteristic peak of aromatic CH group stretch appeared and at 2924 cm^{-1} the characteristic peak for aliphatic CH$_2$ stretch appeared. Peaks appeared at 1690 cm^{-1} and 1589 cm^{-1} corresponding to the C = O carbonyl stretching and aromatic C = C stretches, respectively	
			XRD	PLGA: 20–20° characteristic signal Load: signals were shown at 5°, 15° y 20°	
Chitosan	Kaempferol	137.51–272.91 nm PdI = 0.25 Nanoparticles	ZP	Positive zeta-potential values from + 18.5 to + 38.1 mV.	[36]
			SEM	Nanoparticles are spherical in shape, and uniform formation.	
			FTIR	Characteristic absorption bands: 3324 cm^{-1} (O–H stretch), 1661 cm^{-1} (C = O), 1604 cm^{-1} (C = C), 1378 cm^{-1} (C–OH), 1257 cm^{-1}(C–O–C) of chitosan and kaempferol shifted to the 1653 cm^{-1} and the OH stretch of kaempferol completely disappeared at 3363 cm^{-1}.	
			XRD	Crystalline structure at a diffraction angle of 2°θ 10.80°, 12.50°, 15.90°, 23.95°, 24.36°, 27.46°.	
PLA	Curcumin	516 and 601 nm Fibers	FTIR	Characteristic bands corresponding to carbonyl stretching (1750, 1760 cm^{-1}) in PLA and bands corresponding to phenolic, (-OH), C = O and C = C at 1367, 958, 1613, 1505 cm^{-1}	[37]
			XRD	PLA: broad peak at 2θ = 21.84°; load: peaks at 2θ = 11.90°, 14.31°, 17.12°, 17.87°,21.03°, 23.14°, 24.34°, 25.35°, 27.15°and 28.76°.	

Table 1. *Cont.*

Material	Load	Generalities	Characterization	Results	Ref
			DSC	The glass transition of the polymer with the load is increased	
PLC	Quercetin	Spheres	ZP	Potential ranged from -120 to 120 mV.	[38]
			FTIR	Characteristic bans of quercetin as aromatic bending and stretching (1093–1615 cm^{-1}), –OH phenolic bending (1211–1435 cm^{-1}), and –CO stretching (1654 cm^{-1}) and broad phenolic –OH around 3392 cm^{-1}, polymer –CH stretching (2868–2947 cm^{-1}) and –CO stretching (1724 cm^{-1}).	
			XRD	Quercetin: 2θ of $10.80°$, $14.16°$, $17.23°$, $24.33°$ and $26.97°$, but with polymer $21.08°$, $23.47°$ and $26.80°$.	
Gelatin + ZnO	Cefazolin	Diameter 47.5 nm PdI: 0.325 Fibers	ZP	Zeta potential negative -22.67 mV indicated the stable colloidal.	[39]
			XRD	2θ values of $32.42°$, $34.51°$, $36.78°$, $48.1°$ confirmed the crystalline nature.	
			FTIR	Characteristic peaks of cefazolin (1761, 1388, 1285 and 1183 cm^{-1}).	
PEG	Naproxen	Particles Up to 20 μm	SEM	Exhibited irregularly shaped particles alongside a few spherical microspheres with a smooth surface	[40]
			FTIR	Absorption peaks of 1724 cm^{-1} and 1681 cm^{-1} are related to the carboxylic acid bond and benzene ring and peaks of 1155 cm-1 and 1174 cm^{-1} are correlated to etheric bond.	

Ref: Reference; SEM: Scanning electron microscopy; XRD: X-ray diffraction; DSC: Differential scanning calorimetry; ZP: Zeta potential; FTIR: Fourier-transform infrared spectroscopy; PLGA: Poly (lactide-co-glycolide, PLA: Polylactic acid; ZnO: Zinc oxide; PLC: Poly (caprolactone); PEG: Polyethylene glycol; Millivolts: (mV).

2.2. Predictive and Support Tools for Biofunctionalization

Usually, for functionalization, the combination of the compounds is performed via stoichiometric and chemical reactions [31]. However, at present, the implementation of software, specifically in silico methods, allows the establishment of parameters, concentrations, and potential intramolecular interactions for functionalized biomaterial design. For this, it is necessary to carry out the design of the molecules using one of the free or paid platforms, which subsequently requires minimization of energy using an algorithm such as harder fork, beck tree approximations, etc., Subsequently, calculations are carried out to identify the Highest Occupied Molecular Orbital (HOMO)/Lowest Unoccupied Molecular Orbital (LUMO)/Single Occupied Molecular Orbital (SOMO) in addition to the polar and apolar zones of the designed molecules, with which the most stable configuration can be determined in addition to elucidating whether the stability of the compounds or interactions will be strong or not, depending on the distances and the Gibbs-free energy required for its development [41]. Figure 4 shows the interaction between lupeol and

mangiferin using the Spartan Student version 8 software as a preliminary stage for the design of nanoparticles obtained in the comprehensive food research laboratory of the Technological Institute of Tepic, to obtain the adequate proportions of both bioactives to be encapsulated with polylactide-co-glycolide (PLGA) (original figure).
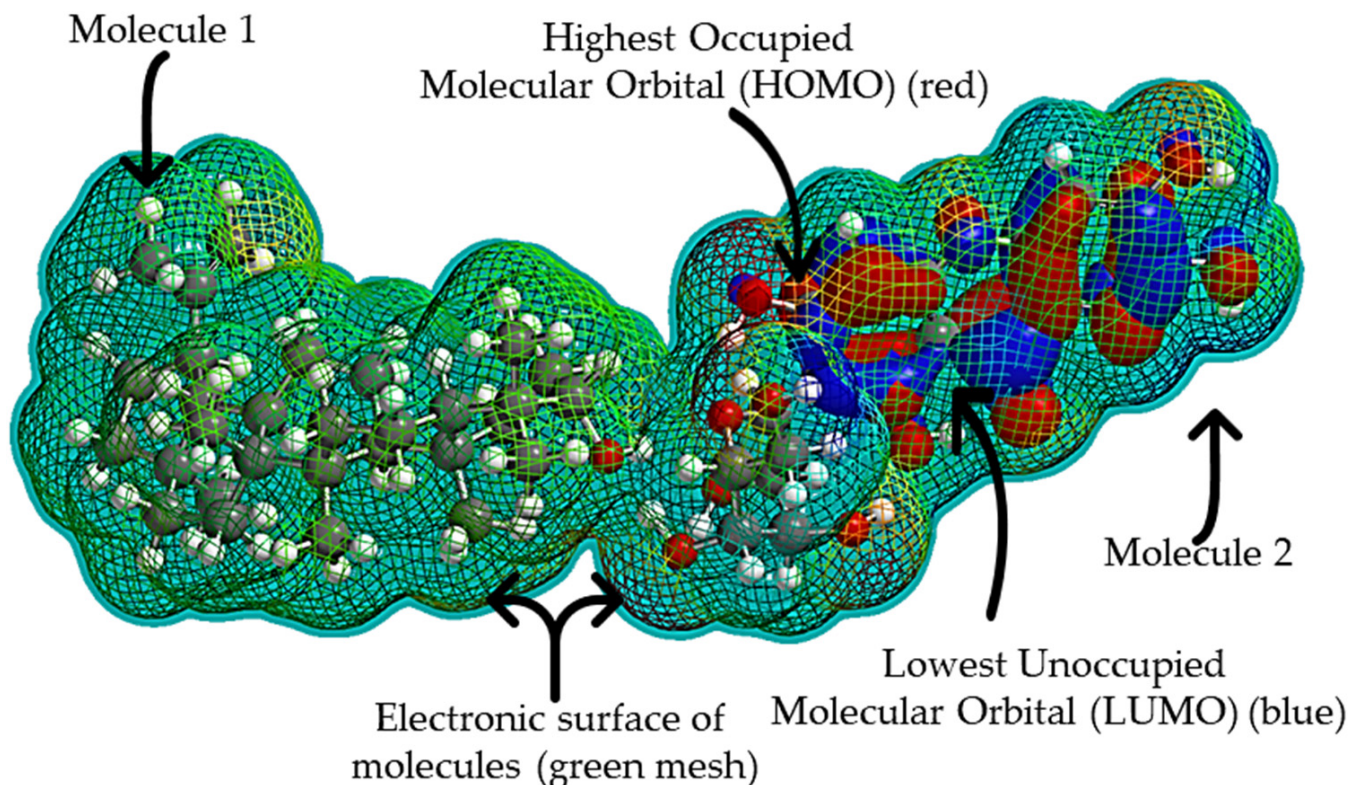
Figure 4. Example of HOMO and LUMO surface in the interaction of two molecules from different chemical characteristics.

The molecules obtained can be used to estimate molecular proportions (stoichiometric quantities). Finally, the appropriate proportions have been established, and functionalization can be carried out with the concentrations closest to reality and with not-so-complex experimental designs (Taguchi, fractional factorial, mix design). The development of biofunctionalized materials is validated with traditional characterization tests, and together with predictions, more satisfactory results can be obtained [42].

Some of the methodologies most associated with functionalization applied in food are those involving the use of polymers due to their effectiveness in encapsulating and biocompatibility, as shown in Table 2. The emulsion and evaporation of solvents, as well as electrospinning, have been some of the most used methodologies in recent years for biofunctionalization since, regularly, they do not form residues that may affect health. However, the stability of the product obtained must be detailed and controlled, so characterization helps to predict its stability in the desired application. It has been observed that zeta potential inferences at 10 mV in aqueous shades can precipitate, and on the other hand, it is important to evaluate the biological activities that are not affected by these encapsulation methods.

Table 2. Biofunctionalized compounds by solvent evaporation method and electrospinning.

Biofunctionalization Nanomaterials	Synthesis Type	Size (nm)	Results	Ref
PLA + quercetin	Solvent evaporation method	130	Enhancing solubility and stability (ZP 15 mV), spheres by SEM.	[43]
PLGA + curcumin		150–200	Enhancing solubility and increase of antioxidant activity (ABTS).	[44]
PLGA + cucurbitacin		399 ± 66	Promote the encapsulation efficiency until 45%	[45]
Cyclodextrin + voriconazole	Electrospinning	<800	drug delivery is a dynamic and complex process and can be adapted to meet the needs of the targeted application	[46]
Polycarpone + plants phytochemicals		<700	Non-toxic formulation of phytochemicals for tissue regeneration and repair.	[47]
Lecitin +		354 ± 12	Improvement of drug loading in the lipid nanoparticles with lecitin.	[48]

PLGA: Poly (lactide-co-glycolide, PLA: Polylactic acid, ZP: Zeta potential; mV: Millivolts.

In fact, no inorganic materials have been reported for foods and the biofunctionalization of materials since they are toxic; however, some studies have shown that modifying the synthesis processes with less harmful reagents improves this impact [49].

3. Biological Impact of Biofunctionalized Nanomaterials Consumed in Food

The evolution of nanoparticle preparation methods has been characterized by the need to synthesize non-toxic and viable compounds, simplify procedures, and optimally improve performance and efficiency in encapsulation. Different techniques that are simple and safe for the encapsulation of drugs have been mentioned above, such as nanocapsules, nanospheres, nanofibers, etc. A biofunctionalized surface allows a more controlled and directed release. Therefore, nanoparticle applications are widespread, and their development is very promising [50]. Some of the applications associated with nanoparticle shape are identified in Table 3. For human use, the particle size must be greater than 100 nm and frequently implements the use of biocompatible polymers; therefore, the size and shape of the nanomaterial are important parameters to consider in biofunctionalization.

Table 3. Shape and size associated with applications.

Shape	Synthesis	Generalities	Application	Ref
Nanocapsules/nanospheres	emulsion and solvent evaporation	Size: 200–300 nm.	Cutaneous	[51]
	Hydrothermal carbonization (HTC)	Size: 20–50 nm. monodispersed and mesopores	Electrocatalytic	[52]
	Nanoprecipitation method	Size: 380.80 ± 37.97 PdI: 0.15 ± 0.06	Nutraceutical	[53]
Nanofibers	Electrospinning	Diameter: 400–600 nm.	Active and intelligent food packaging products	[54]
	Electrospinning	Diameter: 139.0 ± 25.6 nm.	Nutraceutical patch	[55]
	Coprecipitation	Diameter 100–200 nm.	Immobilize enzymes, bioelectroanalysis, and bioelectrocatalysis	[56]

3.1. Enzymes Associated with the In Vitro Study of the Positive Biological Effect of Nanostructures

The areas covered by research on nanoparticles are numerous as they offer better therapeutic options for disorders of public health importance [57]. Some of them have even been used to treat diseases such as cancer. Biomaterials composed of polymers have been widely studied, such as alginate, chitosan, gelatin, hyaluronic acid, polylactic-co-glycolic acid (PLGA), polylactide (PLA), polycaprolactone (PCL), and polyanionic cellulose (PAC). The most commonly used correspond to PLA and PCL [58] due to the bioavailability in the digestion system, improved encapsulation, controlled release, and cytocompatibility and biocompatibility properties, which improve the therapeutic value of the agents encapsulated in colloidal nanoparticles [59]. Studies on the production, characterization, and application of colloidal nanomaterials are rapidly evolving. For nanostructures applied in the oral cavity, it is necessary to evaluate the behavior under different conditions, including factors such as pH, saliva buffer capacity, contact with the mucosa, and dissemination in dental tissues [60].

- Cyclooxygenase (COX): COX is related to the inflammatory phenomena, and there are currently immunoassay kits that allow the enzyme inhibition to be quantified from a product associated with inflammation (thromboxanes). Therefore, by reducing the activity of the enzyme, the inflammation can be stopped. There are three types of cyclooxygenase: Cyclooxygenase-1 (COX-1), whose function is to regulate the proliferation of normal or neoplastically transformed cells and is found in all tissues, especially in the kidney and gastrointestinal tract, and participates in the production of prostaglandins involved in normal physiological processes such as the protection of the gastric epithelium, maintenance of renal flow, and platelet aggregation. Cyclooxygenase-2 (COX-2) modulates inflammation and prostanoids pathways; therefore, the objective of the design of nanomaterials with anti-inflammatory properties seeks the selective inhibition of COX-2, and current existing immunoassays allow for evaluating this type of effect [59,60].

- Topoisomerase (TOP): The exacerbated replication of cells, such as the development of carcinoma, is also associated with enzymatic dysregulation, such as TOP, which is the enzyme responsible for maintaining the tertiary DNA structure throughout the cell cycle, being the one in charge of the winding and unwinding of DNA strands during synthesis, replication, condensation, and recondensation. Three types of topoisomerase DNA have been characterized according to their catalytic properties, energy expenditure, and protein structure: Topoisomerase type I DNA (TOP-I) is involved in the opening of the DNA so that the copy of the material is carried out

genetically, and the enzyme known as TOP-Ib or TOP-III isogenic of TOP-I is not found in all eukaryotic cells and is responsible for causing a break in one of the chains and forms a phosphodiester bond between the 5 'phosphate and the OH of tyrosine. The type II topoisomerase DNAs (TOP-II) are associated with the closure of the same post-process structure and act together to maintain the appropriate level of supercoiling so that biological processes such as cell development are carried out. It is interesting to inhibit these enzymes in processes associated with cancerous processes due to the overexpression of the TOP [61,62].

3.2. Cytotoxicity Associated with Nanomaterials

One of the most used tools for the study of toxicity associated with nanoparticle-based treatments, before their application in vitro, is cell cultures. These have several advantages over in vitro studies: Minor complexity, costs, and ethical issues. Additionally, in vitro assays allow one to control the cellular environment and homogeneity both morphologically and compositionally, allowing a deeper understanding of the biological and biochemicals processes that take place during treatments [63]. The internalization of large particles is favored through phagocytosis processes. Non-specific internalization of particles of ~ 1μm enter via mechanisms of pinocytosis, and particles <100 nm enter the cell via endocytosis, mediated by clathrins or caveolins [64]. Table 4 shows the cell viability of the polymeric nanostructure sizes. It is shown that one of the characteristics of biofunctionalized nanoparticles is that they do not generate an invasive effect or cytotoxicity since these materials lack structures with unpaired electrons or molecules that can break the osmotic balance of a cell such as sodium and zinc, even at sizes below 100 nm.

Table 4. Cell viability associated with the size of the polymeric nanostructures.

Cell Line	Size	Essays	Results	Ref
Fibroblasts humans Normal	450 ± 20	WST	Low effect toxic.	[65]
Macrophages murine RAW264.7	80 ± 18 nm	MTT	No significant alterations.	[66]
HeLa	30 to 70 nm	MTT	No significant alterations after 24 h.	[67]
Fibroblasts L929	49 nm	MTT	No toxic seven days after.	[68]
Crops primary of Astrocytes	60 nm	Action enzymatic of LDH	No significant alterations.	[69]
Cells oligodendroglia's OLN-93	60 nm	Action enzymatic of LDH	No significant alterations.	[70]
Stem cells derivate of adipocyte	20 nm	Kit-8 for counting mobil	No significant alterations.	[71]

The cytotoxic effects of a nanoparticle-based treatment may be independent of the cell–particle interaction or can be associated with the process of the internalization and transformation of intracellular nanoparticles. In the first case, this type of cytotoxicity is given by nanoparticles' direct interaction with the culture medium [72]. Due to this, adding particles to the culture medium can alter pH or osmotic pressure and indirectly cause cell death. To avoid confusion, these possible cytotoxicity results not associated with the cell–nanoparticle interaction were suggested, including in the working protocol the previous incubation of the nanoparticles, its decantation, and resuspension in a fresh culture medium. Besides, it is also recommended to conduct cytotoxicity assays using inhibitors of the endocytosis process or with cell types with very low endocytic capacity

(for example, erythrocytes) to evaluate the indirect toxicity induced by nanoparticles in the culture medium [73,74].

Hematoxicity: Hemostasis is the set of mechanisms suitable to make the blood circulate in a liquid state in the blood vessels, while also regulating the process whereby when one of these structures is damaged, the mechanisms of clot formation activate to stop bleeding, as well as the dissolution processes of thrombi (fibrinolysis) [75]. Bearing in mind that the preparation of these nanoparticles has a biomedical end, when administered, they will meet the different components of the blood [76]. Therefore, Table 5 shows some studies that demonstrate the hemotoxic effect of nanomaterials associated with the size. One of the most important barriers to the absorption of nanoparticles in food is blood. In Table 5, all the inorganic nanoparticles show different cellular alterations, given by the energetic composition of the materials since this type of charge affects the erythrocyte membrane, leading to anomalies in health. However, at low concentrations, it is unlikely to show this type of effect [77].

Table 5. Hematoxicity associated with inorganic nanoparticles.

Material	Size	Effect	Cite
Gold	40 nm	Promotes erythrocyte scavenging.	[78]
Selenium	30 to 100 nm	Hematological alterations in female mice.	[79]
Selenium and zinc oxide	36–40 nm	Ameliorative effect on hematology and antioxidant systems.	[80]
Silver	17–22 nm	Alterations in the production or synthesis of hematological contents.	[81]
Zinc oxide	30–50 nm	Induced changes in hematological parameters.	[82]

4. Conclusions

The biofunctionalization of nanoparticles is a strategy that has made it possible to enhance the biological activity of different bioactive compounds; however, the physical-chemical characteristics acquired by the nanomaterial during the biofunctionalization process can compromise the biological effect, with the shape, size, and origin of the encapsulating material having the greatest effect on its toxicity. These "toxic attributes" can be identified during characterization and verified by in vitro testing. Undoubtedly, the process of synthesis or development of nanoparticles can lead to the formation of undesirable molecules, therefore evidencing the importance of using biofunctionalizing matrices approved as biocompatible. The challenge for the scientific area is to develop safe nanoparticles, with optimal concentrations of a phytochemical or extract considering characteristics such as the size and shape of the designed material. Additionally, the biological activity of the bioactive compound (autoinflammatory, antioxidant, antiproliferative, etc.) and its route of administration, metabolism, and excretion must be considered since this is an essential point that the physicochemical characteristics can influence.

It is important to carry out the characterization of the biofunctionalized materials before the in vitro biological evaluation since the potential toxicity that it may present can be deduced, through which biomaterials that are less toxic, more biocompatible, and metabolically viable for their degradation could be obtained.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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