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Overview of Nanomaterials and Cellular Interactions

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Abstract: In the last three decades, there has been wide progress in nanomaterials development, and several studies are being performed to show its biological effects and cellular interaction for biomedical applications. Due to the exponential increase in nanomaterial diversity, production, and possibilities of applications in different areas, there is an important concern about its toxicity for humans, animals, and ecosystems. There is a great effort to minimize experimental assays in animals, and this is a commendable initiative. Several alternatives *in vitro* assays are available; however, several new protocols have been introduced to elucidate the mechanisms of cell-nanomaterial interaction. Wide and fast progress in nanotechnology has been observed. Nonetheless, the nanomaterial interaction with cells or biological systems is still not totally described. In this aspect, this paper is a brief overview of nanomaterials and cellular interactions (nano-bio interaction).

Keywords: nanomaterial; biological systems; nano-bio interaction.

1. Introduction

Despite the evident progress of biology, in the 1960s, there were microscopic limitations, mainly due to the computer technology that did not offer efficient tools for processing samples and data analysis in biology. Images of biological molecules such as deoxyribose nucleic acid (DNA) [1] and the outer sheath of the cell wall of the archaebacterium *Methanospirillumhungatei* [2] were reported only in 1988, allowing advances in the field of DNA technology.

The first presentation of public knowledge regarding nanotechnology was made in 1959 by Richard P. Feynman, that postulated the possibility of carrying a huge amount of information in an extremely small space such as a cell, which, at that time, was already known to be able to store information, and to work as a small manufacturer, in a very small scale. However, a high level of organization maintains the biochemical processes and the performance of complex structures, such as those from the human body [3]. Assertively, it was postulated the possibility that, on a small scale, materials would increase their properties in a wider range, representing new opportunities for conceiving and developing new nanomaterials in different areas.

It is possible to say that the high performance of the biological phenomena was considered a kind of model for developing and improving computer technology to a high level of efficiency and speed for processing data on a smaller scale. Although smaller, the technology would be precise through a correct operational process.

2. Current Numbers in Nanotechnology

Although there is no difference in its meaning, it is interesting to register that the Web of Science shows that the first study referring to "nano material" was published in 1963, and to "nanomaterial", only in 1995.

Figure 1 shows the huge increase in publication numbers related to the nanomaterials area since 1963. According to the Web of Science, the first three studies considering the interaction of "nanomaterial" and "cellular" systems were published only in 2005. Probably, there are others studies; however, they were not reported in these scientific databases before 2005. As can be observed, currently, there is a high increase in publications in this area.

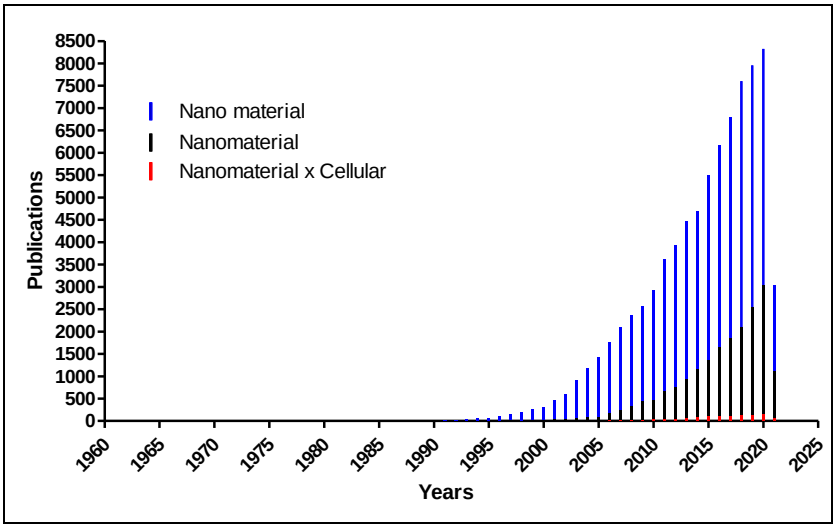

Figure 1. Overview of publication profile containing in their topic the terms: "nano material", "nanomaterial", and nanomaterial x cellular", from 1960 to 2021.

Nanomaterials are the result of studies and manipulation of materials on the nanoscale in different areas, such as biology, chemistry, physics, engineering, etc. Although introduced a few decades ago, the types of nanomaterials have exponentially increased, as well as the diversity of their applications.

3. Strategies for Nanomaterials Development and Physicochemical Characterization

Nanomaterials can be obtained through top-down or bottom-up approaches; among them, chemical, physical or biological methods are proposed as routes. All these methods are effective and have advantages, drawbacks, and shortcomings that should be balanced considering the cost, energy consumption, high temperature and pressure for the process, use of toxic chemicals and generation of toxic residues, and finally, the ecotoxicity that has been the ultimate counterbalance for the choice.

Nanoparticles have sizes in the range of 1-100 nm, and with the progress of nanotechnology, they are being developed in different shapes and sizes. Furthermore, the association of different nanostructures, forming even hybrid nanostructures, is being reported. The properties of nanomaterials, such as physical, mechanical, catalytic, optical, and biological, are different from those of their larger counterparts and are related to the method applied to obtain them [4].

Although there are challenges to overcome, green nanotechnology is a versatile pathway that has been successfully and sustainably developed. The green synthesis of

nanomaterials has been performed in a few steps by applying plant extracts, algae, bacteria, yeasts, and mainly fungi species, which work as biological nanofactories [4].

The size, surface area-to-volume ratio, shape, morphology, coat, dispersity, reactivity, and compatibility are extremely relevant for the interaction of nanomaterials with other structures, such as the cells and their functionality. In this regard, several studies describe the presence of capping agents in metallic nanoparticles (silver, gold) formed by green synthesis through biological systems, which are more stable and biocompatible [4,5].

For physicochemical characterization of nanomaterials regarding their morphology, size, chemical composition, forces, and surface charge, the most useful and applied tools are UV-Visible Spectroscopy (UV-Vis), Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), High-Resolution Transmission Electron Microscopy (HR-TEM), Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and Energy Dispersive X-Ray (EDX) [6]. Nonetheless, fortunately, several other important techniques are available and can be performed to complement the characterization according to the required specification. Among them are Nanoparticle Tracking Analysis (NTA), Atomic Force Microscopy (AFM), High-Resolution Transmission Electron Microscopy (HRTEM), Energy Dispersive Spectroscopy (EDS), Selected Area Electron Diffraction (SAED), X-Ray Photo-Electron Spectroscopy (XPS), Diffuse Reflectance Spectrometer (DRS), inductively coupled plasma optical emission spectrometry (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Raman Spectroscopy (IRS), Scanning Tunneling Microscopy (STM), Spectroscopic Ellipsometry (SE) and Brunauer - Emmett – Teller (BET) [6].

The characterization is important to understand the interaction at the nano-bio interface that is being discussed in the next topics.

4. Interaction of Nanomaterial with Biological Systems - Nano-Bio Interaction

For further possibilities of application in very distinct areas such as agriculture or spacial area, several different types of nanomaterials have been designed and studied as promising diagnostic tools or pharmacological alternatives to improve the quality of human life. Studies have been intensively performed on nanomaterials as antimicrobial, antitumor, inflammatory, immune system modulation, antidiabetic, antioxidant, membrane permeation, and biocompatibility [7]. Alternatives to conventional treatments are, though, and despite the efforts, the mechanisms for the interaction of nanomaterials with biological systems (nano-bio interaction) are not totally described yet and are a limiting factor for its application [8]. The effects of nanomaterials are not the same as that of their larger bulk counterparts, and therefore, it is important to study and understand their interaction with biological systems. In the nano-size range, the interactions with biological systems are not totally predictable [9]. Furthermore, the interactions can be modified after engineering processes, which can change the nanomaterial properties.

5. Phytochemistry Modulation by Nanomaterials

A recent and new area of interest for the physiologist is the modulation of secondary metabolite biosynthesis and accumulation in plants through the application of nanoparticles, which has a lower cost than phytohormones. Flaxseed (*Linum usitatissimum*), an oil seed used in industrial and natural health products, responded positively to the application of ZnO,

titanium dioxide (TiO_2), silicon dioxide (SiO_2), and ferric oxide (Fe_2O_3) nanoparticles, which improved the growth, carbon and nutrient assimilation, and salt tolerance in the plant [10]. On the other hand, on rice seeds (*Oryza sativa*), AgNPs displayed a dose-dependent inhibitory effect on germination and their subsequent growth and development [11]. Also, it was shown that single-wall carbon nanotubes (SWCNTs) influence the morphological, physiological, and biochemical properties of *Thyme callus* (*Thymus daenensis*) culture, which is extensively used in folk medicine [12]. Various other studies also reported Benefic effects for plants, revealing the potential of nanomaterials in agriculture [13-16].

These data confirm the relevance of a careful and detailed analysis for selecting the most appropriate nanomaterials for phytochemistry purposes. Otherwise, unwanted and negative impacts could be unleashed.

6. Interaction of Nanomaterials with Microorganisms

Several nanoparticles present antiviral activity, such as those containing zinc oxide (ZnO), used as a disinfectant nano-spray against coronavirus (SARS-Cov-2) [17], or acting as an intracellular antimicrobial for the treatment of *Salmonella typhimurium* without causing any deleterious effect on the macrophages host cell [18].

Bacterial cells in starvation conditions commonly shut down metabolic activity; however, in starvation conditions, graphene oxide (GO) was able to protect *Staphylococcus aureus* bacterial cells from death, even improving cell viability [19]. There was a growth in the production of specific enzymes from the glyoxalase detoxification pathway along with repressed autolysis. The presence of oxygen-containing functional groups of GO mimics the molecular structure of methylglyoxal usually produced by bacteria to keep the nutrient imbalances. GO is perceived as a methylglyoxal-mimicking nanomaterial able to reshuffle cellular metabolism and defenses to survival.

Nanoparticles can interact with microorganisms (bacteria, fungi) cell walls or membranes by electrostatic attraction or transpose the cell wall or membrane. Once in the cells, it can act by free-radical and ROS generation or by interaction with intracellular structures or biomolecules. The nanomaterial can induce cell permeability through membrane damage and lysis [20], resulting in leakage or loss of intracellular contents such as ions, proteins, and ATP [21]. The cellular death mechanism can also occur by the nanomaterial bound to membrane enzymes [22,23], reducing DNA replication through the inactivation of the ribosomal subunit and finally inhibition of protein synthesis [24].

When exposed to a nanoparticle coated with a protein surface, the death of promastigote and amastigote forms of protozoal occur by apoptosis-like events, triggered by damage in their membrane, increase in the generation of ROS, loss of mitochondrial integrity and phosphatodylserine exposure [4,25,26]. Immunomodulation of infected macrophages was also reported [25,26].

7. Interaction of Nanomaterials and Mammalian Cells

Simultaneously to the increasing diversity and design strategy, there is a concern to optimizing the properties of nanomaterials to allow their internalization or uptake by mammalian cells to induce the biochemical or pharmacological effect.

Intrinsic physicochemical properties such as the size, morphology, Van der Waals forces, hydrophobic interactions, and hydrogen bonding are relevant properties for the nano-

bio interaction. They can still be associated with and guided by the surface topography and energy of the nanostructure.

In addition to the unique nanomaterial properties, characteristics of the cells, such as the membrane tension, stiffness, and curvature (spherical, discoidal, concave), are also important parameters that can interfere with the cell-particle interaction [27], and there is an increasing concern regarding the design and synthesis of nanoprobes for targeting cells. At large, particles are internalized through phagocytosis, pinocytosis/macropinocytosis, and clathrin or caveolin-mediated endocytic pathways uptake [28,29].

Nanomaterials can reach the human body by transposing routes like the epidermis, gastrointestinal and respiratory systems [30]. Tissue and cell barriers are challenges that the nanomaterial could also transpose, and the efficiency on the specific site of action depends on its circulation, biodistribution, and biodisponibility. Once in the biological fluids, the interaction of the nanomaterials with surrounding biomolecules through adsorption on its surface can form protein corona and induce modifications of the functional proteins or redox reactions [8]. The function and toxicity of nanomaterials are also regulated by the redox reaction. ROS-related diseases, such as Alzheimer's, hepatitis, and radiation damage, ROS overexpression exists. Nanomaterials with enzyme-like catalytic activity have been studied as a therapeutic approach for ROS scavenging. Metal-based nanomaterials are promising and can be designed especially for this application [31].

The cellular function of the nanomaterial depends on its intracellular release and interaction with the biological system, such as with endothelial cells or fibroblasts exposed to biogenic silver nanoparticles (AgNPs) surrounded by a corona protein, as previously reported by our group [32,33]. The rate of metallic nanoparticle release is related to its chemical composition, structure, metalcore, surface coating, and environmental conditions such as pH and ionic strength.

In general, independently of the method applied for obtaining, new nanomaterials are commonly submitted for modifications to improve their solubility, reduce toxicity and side effects on normal cells, or even promote a controlled drug release [34].

Tumor cells' exposure to nanoparticles coated with a surface protein can undergo sub-G1 arrest, resulting in an apoptotic process [35]. As in normal cells, the activation of apoptosis in tumor cells can be due to an increase in ROS generation, influence in the signal transduction pathway, activation of the enzyme lactate dehydrogenase (LDH), upregulation of p53 protein, nuclear fragmentation, loss of cell membrane integrity, oxidative stress and expression of cleaved/active caspase-3 [36-38]. These events were already reported in tumor cells such as MDA-MB-231 human breast cancer cells, MCF-7 breast cancer, B16F10, Caco-2, and gastric cancer cell line (AGS).

In the cancer context, immunotherapy agents can act through the activation of the host immune system. However, the effect is not totally guided or controlled due to the difficulty of stimulating only tumor-specific immune cells. Consequently, unwished pathways can be activated, causing side effects for the patients. For guiding the immune response triggered by nanomaterial, many concerns have to be evaluated and considered [39]. As a safe and effective solution, immunomodulation can be centered on the engineering of immunotherapeutics through the formulation of nanoparticles with physicochemical properties able to promote the uptake by tumor cells and accumulation in tumor tissues. Polymers, lipids, metals, and inorganic materials have been used in the formulation of immunotherapeutics by the materials science in the nanoscale using chemical conjugation, encapsulation, or physical incorporation

of biological or small-molecule drugs. The formulation can carry only one immunotherapeutic or multiple drugs for co-delivery or to promote intensification of the effect by synergism [40].

The different physicochemical properties of the nanomaterial will allow new mechanisms of action and can induce the accumulation of immunotherapeutics in tumor sites, improving pharmacokinetics and reducing toxicity. Particles in the range of 10-100 nm are accumulated within tumors due to defective lymphatics that prevent their clearing from the tissue due to enhanced permeation and retention (EPR) [41]. The immune system acts by a dynamic biochemical process triggered by an effective cascade of cell-to-cell communication. Therefore, effective stimulation of a small number of leukocytes in tumors or lymphoid organs can significantly change the microenvironment [40].

Nanomaterials can be morphologically, optically, or chemically modified and/or functionalized with ligands on their surface to modulate the affinity for biomarkers (proteins, phospholipids, membranes, DNA, free radicals), cross biological barriers, regulate intracellular delivery of compounds and their time of release and/or activation, targeting specific tissues, organs, or cells [42,43]. In addition, nanomaterials can be designed with ligands to interact with external energy sources directly, promoting intensification of the immunogenic cell death (ICD) induced by radiotherapy and magnetic hyperthermia [44].

Several nanomedicine-based-treatment strategies for cancer immunotherapy have been discussed [40]. As an example, the fluorescent and stable fullerene nanoparticles are a sensitive and specific probe for folic acid detection and quantification that works as a biomarker for targeting cancer cell imaging [45].

Carbon dots (CDs), owing to excitation-independent long-wavelength emission, are rarely reported but are highly desirable for biomedical applications. Bright-orange-emissive, polyethyleneimine-modified carbon dots (PEI-CDs) were obtained and can be bound to small interfering RNA (siRNA), showing excitation-independent emission and a promising siRNA delivery system [46]. The PEI-CDs siRNA complexes can be an effective strategy for down-regulating the expression of hepatoma-derived growth factor (HDGF) in glioblastoma cells, potential targets for glioblastoma treatment [46].

For the development of efficient bionanoprobes, it is crucial to understand the uptake efficiency, nanobiointeraction, and the pathway of luminescent nanomaterials. In this regard, recently synthesized orange-emitting activator Mn^{2+} ion doped ZnSe quantum dots (QDs) with ultrasmall zinc-blend cubic crystal structure and an average diameter of 4 nm [47]. In this study, the QDs were internalized predominantly via clathrin- and caveolae-mediated pathways and were found as aggregates inside the vesicles in the cytoplasm. These QDs showed elliptical shape nanocrystals and a high intensity of orange luminescence, photochemically stable in the intracellular environment of RAW 264.7 macrophages. These data show that QDs are promising tools for the development of bionanoprobes.

As another example, polymers-lipid hybrid nanoparticles can be modified to form new nanocarriers to overcome multiple transport barriers and facilitate tumor penetration, cellular uptake, and intracellular targeting of drugs like anticancer [48]. In this regard, amino groups have been applied to decorate composite nanoparticles for a greater cellular uptake [49]. Previously, it was shown that PEGylation of AgNPs difficulted the cellular uptake by endothelial and murine C17.2 cells, reducing the interaction of nanoparticles with the cell membrane [28]. The PEGylation induced higher levels of ROS and autophagy than on AgNPs coated with either mercaptoundecanoic acid (MUA) or dodecylamine-modified

poly(isobutylene-alt-maleic anhydride) (PMA), and also protected the cells from any cytoskeletal deformation [50].

It is known that the chemical modifications on nanomaterials are an efficient strategy to improve their properties and stimulate cellular uptake. Alternatively, for the co-administration of nanoparticles and a transportation peptide, an amphiphilic cell-penetrating peptide (CPP) was reported as a simple procedure by which the cells were able to engulf a variety of nanoparticles mediated by a receptor-dependent micropinocytosis mechanism [46,51,52]. This strategy bypassed the requirement of structural modification and opened up a possibility for applying this peptide to improve nanomaterial delivery [51].

Nanomaterials have been designed to regulate cellular gene expression, and several studies have focused on applying gold nanoparticles (AuNPs) and CNTs for this purpose [39]. To overcome non-specific toxicity to normal cells and genes, the surface and size variation are attempts that have been applied to improve the physicochemical characteristics of nanomaterials. AuNPs and CNTs are nanomaterials commonly used, but random methylation and damage to target cells and genes have been reported [53,54].

As it is known, surface coating is related to the stability and biocompatibility of nanoparticles. Recently, an innovative nanosystem containing an inorganic material coated with chitosan and alginate and tagged with pCRISPR was effective in drug and gene delivery [55]. This nanosystem can be improved for different drugs and gene delivery.

In normal cells, gene regulation is activated to suppress the expression of abnormal genes, which can trigger conditions such as autoimmune disease, inflammation, and obesity [39]. In case of a shortcoming of this regulatory mechanism, exogenous factors can be introduced to emulate the normal physiological and metabolic regulatory system, preventing diseases such as those mentioned. For this purpose, various nanomaterials have been developed, including general nanoparticles, carbon-based materials, and polymer structures that are not toxic, biocompatible, and biodegradable. In functionalized nanoparticles, functional and specific components on their surface are able to recognize target cells and trespass the membrane for intracellular entry and the proposed effect, avoiding an immune response of the body [56].

The regulation of cellular metabolism as gene expression and cellular growth and death can be achieved through specific nanomaterials designed and developed for this purpose. Additional alternatives for gene regulation in cells are drug delivery by magnetic nanoparticles (MNPs) (iron, nickel, or cobalt) and application of graphene [57,58], QDs, single and multiwall carbon nanotubes (CNT) [59], and silica nanoparticles by siRNA or antisense RNA interference [39]. These nanomaterials are capable of high transfection efficiency, transferring genes into targeted cells. However, they may cause toxicity to DNA and targeted cells and genes [53]. To overcome this critical drawback and move forward, an alternative is to develop engineered nanomaterials by modifying the surface, size, shape, or association with other materials that prepare a nanomaterial with new properties.

Gold nanoparticles (AuNPs) have unique optical properties and have been studied for applications in therapeutic and diagnostic purposes. Gold nanorods (GNRs) were used in photothermal therapy (PTT) developed for gene regulation by silencing the *BAG3* gene (using SiRNA), which is related to an increase in heat shock response of cancer cells, mainly when combined with polymers, reducing the demand for laser power [60].

The PEGnylation of AgNPs avoids significant changes in genes in murine C17.2 cells; however, for AgNPs coated with MUA and PMA genes related to immunotoxicity (Ccl12, Il1a,

and II1b), DNA damage response (Gadd45a and gadd45g), hypoxia-like toxicity (Adm, Hmox1, and Serpine) were upregulated [50].

8. Nano-bio Interaction by *in silico* and/or *in vitro* Analysis

The incorporation of nanomaterials in different types of products is increasing day by day, so it is important to think about and evaluate its interaction with biosystems such as the cells and organs of the human body. This approach is relevant for decisions regarding the risk of their use. Products containing AgNPs have been explored mainly as antimicrobials, such as clothes and dressings. There is a huge amount of data exploring the toxicity of these nanoparticles for humans [9] and the environment. Otherwise, there is no clear definition of public policies or rules to guide their use.

Currently, there is a high tendency and demand to minimize the use of animals in experimental procedures. An alternative and effective purpose of achieving this goal in the nanotechnology area is to evaluate the nano-bio interaction by *in silico* and/or *in vitro* analysis.

In silico analysis using computational models is a theoretical method and an alternative to predicting the physicochemical properties of molecules or nanomaterials. Although *in vitro* assays are more widely applied, their association with *in silico* analysis is common in describing the nano-bio interaction. High-content screening strategies can generate a data set that can be useful for bioinformatic study to predict models for nanomaterials design and development [28,50].

Several studies contributed to elucidating the mechanisms of nano-bio interaction of classically engineered nanomaterials, such as fullerenes [61] and CNTs by genome expression array analysis [62]. The interaction of single-wall CNT (SWCNT) with abundant blood proteins is by hydrophobic interaction [52], and the adsorption of protein by two-dimensional graphene oxide (GO) nanosheets is more intense than that of one-dimensional SWCNT [63].

Recently, a highly efficient coarse-grained model was proposed through the endocytosis of nanorods incorporated with polymers and the entry of multiple nanorods into a lipid membrane [45]. Through this model, the effect of ligand-receptor binding can be captured, and the cooperative or separated cell entry of multiple nanorods can be characterized. According to the authors, the model will afford an understanding of the interactions between cells and nanomaterial in combination with molecular dynamics simulations.

In an effort to optimize nanomedicine application, a nanoplatform coated with AuNPs was designed [64]. The platform operation is based on a tool able to estimate enough number of nanoparticles to interact with cells promoting the desired physical or biological effect. The system can be expanded to several other nanoparticles, coating, and cell types.

In vitro assays are efficient and valuable tools for studying the nano-bio interaction and estimate the toxicity of nanomaterials by evaluating cellular viability, proliferation, metabolism, apoptosis, DNA damage, genotoxicity, and mutagenicity [30]. Several different cell lines can be used in a considerable number of cost-efficient procedures. However, the diversity of nanomaterials and different *in vitro* assays are very wide, and consequently, it is not simple to compare the effects of nanomaterials, including their mechanisms of interactions with cells.

When in contact with biological fluids, the nanomaterials will interact rapidly with biomolecules, mainly free proteins, forming the "biomolecular corona" [5,65-68]. This biomolecular coating, associated with the nanomaterial surface, has been shown to have critical influences on cellular interactions such as internalization, biodistribution, toxicity, and even

immune system activation [69-71]. The intensity of the interaction can be soft or hard, according to the affinity of the biomolecules with the nanomaterial surface.

It is essential, therefore, to evaluate the physiological effect of the biomolecular coronas formation at nanomaterial-cellular interfaces to understand the links between *in vitro* and *in vivo* effects under different physiological or biochemical conditions through nanoinformatics approaches for predicting and modeling complex biological/toxicological outcomes [72,73].

The protein corona presence can affect the cellular uptake of nanomaterials, limiting the penetration into the cell membrane mainly due to a modification and decrease in the available surface area and/or lipid bilayer damage [8]. An example of the biomolecular corona effect was shown by quantifying the uptake of AuNPs coated with bovine serum albumin (BSA), or citrate-stabilized. The *in vitro* uptake by Lewis lung carcinoma (LLC) cells or *in vivo* biodistribution was estimated using high-resolution computed tomography (HRCT) and ICP-OES [74].

In the cell and nanomaterials interaction, the nanoparticles uptake and adsorption on the cell surface can usually be observed, as well as changes in nuclei/cell phenotype and chemistry, which can outcome in oxidative stress, genotoxicity, and carcinogenicity. These effects are determined by the physicochemical properties of the nanomaterial (such as shape, size, concentration, zeta potential, diffusion coefficients, and polydispersity) [75] that, on the same scale as biological entities, can easily cross the blood-tissue barriers. In this regard, innovation is the machine-learning-based approach used to decode the interaction of nanomaterial with cells [76].

Although the nanotoxicity of several different types of nanomaterials, including those with "biomolecular corona", has been widely studied, details regarding the cell shape and nuclear area factors (NAF) are not well-explored descriptors of the type of nanomaterials. To create a set of nanodescriptors, which can contribute to the cell-nanomaterial interaction through phenotype adjustments, intrinsic and extrinsic physicochemical characteristics of representative nanomaterials (PEGylated gold nanosphere, CTAB-gold (cetrimonium bromide capped spherical AuNP), dendrimers, nanocarbon and PEGylated gold nanorods (GNRs)) such as dynamic light scattering, nanoparticle tracking analysis were measured by optical methods [76]. These nanomaterials' cell and nuclei form and polarity functions were predicted using a correlation function as the machine-learning algorithm. The authors recommended the application of the cell shape index (CSI) and NAF nanodescriptors for cell phenotypic parameters to determine the safety of nanomaterials available in commercial products and nanomedicine.

Recently, [27] critically analyzed various properties that affect cell-nanomaterials interactions. Considering their small size, nanomaterials should not interfere with the cell's functionality as their motility, metabolic activity, or responsiveness to chemical cues, and they would be able to evade immune clearance [27]. As described above, along the interaction, in addition to the cellular uptake, there is surface adsorption and changes in nuclei/cell phenotype and chemistry. In regard to it, calcium pectinate/hyaluronic acid/rhein-nanoparticles significantly alleviated inflammation in ulcerative colitis. It accelerated colonic healing by enhancing the uptake rate through lactoferrin and hyaluronic acid ligands [77].

9. Considerations about *in vivo* Analysis of Nanomaterials

Although there are several different possibilities for *in vitro* analysis of nanomaterials, the *in vivo* environment is more complex and can be considered necessary. It is relevant to

remember that in a tissue or organ, there is more than one cell type, and *in vitro* assays are usually performed with only one cell type. Furthermore, usually on *in vitro* assays, cell-cell communication is not considered, and consequently, the result is not totally representative of the *in vivo* phenomena.

Alternative models for nanomaterial assessment are being used to contribute to the 3Rs proposal to reduce, replace and refine assays with animals. Among them, embryonic zebrafish cells (ZF4) are a promising early-stage aquatic model that evaluates the molecular and cell death mechanisms related to nanomaterial toxicity [78]. Another advantageous model that has been successfully applied is the larvae *Galleria mellonella* model, which successfully determines drug candidates and nanomaterials toxicity for several different types of samples [11,32].

An overview of the state of art on nanotoxicity, including a description of available assays for toxicity and recent advances *in vitro* and *in vivo* toxicity studies of nanomaterials, was recently made and very well discussed [29]. Due to several different types of nanomaterials and bioassays available, a wider perspective and establishment of a systematic standardization for evaluating the cellular-nanomaterial interaction, biodistribution, toxic kinetics, or genotoxicity, allowing a trustful and accurate comparison between studies is a big challenge and would be very useful for biomedical applications of nanomaterials. This point of view is a consensus pointed out by several authors [28].

10. Conclusions

Consistent with the innovative and wide progress in nanomaterial science, remarkable efforts are being applied to transpose nanotechnology knowledge from the bench to the clinic or for several other applications. Despite the efforts, the interactions of nanomaterial-cell (or biological systems) have not been totally described. Furthermore, the diversity of nanomaterials and their environmental impact is still an open question requesting a wide and crucial discussion to ensure safe use for both nature and humans.

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Conflicts of Interest

The author declares no conflict of interest.

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