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Review

Nanoparticles applied to cancer immunoregulation☆

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ABSTRACT

Aim: In recent years, we have seen a considerable increase in the relevance of nanostructures for the safe delivery of therapeutic agents and their capacity as an immunomodulatory tool.
Materials and methods: Potential clinical applications related to their unique structural properties have been described in the evolving landscape of immunotherapy.
Results: This review briefly summarizes the evidence for the role of nanoparticles in regulating the immune response.
Conclusions: Their main features to highlight how to provide an innovative means of biomedical application to oncology research.

1. Background

Despite the great progress made over the past years with advances in surgical resection, radiotherapy and chemotherapy, cancer is still the second leading cause of death in Europe and the United States.[1] Many forms of the disease remain fundamentally difficult to manage mainly due to the wide heterogeneity of tumors.[2] Nowadays, in the post-genomic era, the tendency is to find more personalized treatments with the use of targeted therapies, immunotherapies or many combinatorial approaches changing the way that physicians attack neoplasms.[3] In particular, immunotherapy has emerged as a powerful new strategy and holds tremendous promise for improving cancer treatments but, unfortunately, response rates for this strategy remain generally low, and it has become quite clear that there is no "cure-all wonder drug" to be discovered.[4]

For this reason, in these coming years there will remain three urgent unmet medical needs: to identify novel methods to enhance the treatment response to immunotherapy, to improve the efficacy of the "traditional" treatments, and also to reduce the side effects of these treatments in many instances. A fascinating approach which has recently been demonstrated to potentially include most of these hallmarks, is the medical application of nanotechnology, summarized as nanoparticles (NPs).

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NPs, defined as synthetic particles with a diameter of less than 100 nm[5] and generally derived from polymers, lipids, or metals, such as gold, have been found to be highly useful in several medical applications, from diagnostics to cancer therapy. The size of these NPs are very similar to the majority of biological structures and molecules; thus conferring functional properties for both in vitro and in vivo cancer research.[6] Such NPs, if accompanied by biodegradable carriers, can be safely loaded with therapeutic compounds, to achieve concentrated local drug delivery with potential for sustained release.[7] Thanks to this, they can enter into the body cavities and the blood circulation for treatment with minimal invasion and improved bioavailability.[8]

In addition, NPs have a larger surface-to-volume ratio than that of micro and macro sized particles, which enables them to be covered with various ligands at once (leading to superior drug loading) and can facilitate interaction with a number of molecules, such as receptors present on the surface of target cells.[9]

Immunogenicity is the ability of different substances to trigger an adaptive immune response of cellular and humoral type that in the long term constitutes immunological memory. Immunotoxicity is damage to the immune system caused by exposure to chemicals. The analysis of immunotoxicity is a standard part of the development of substances as possible new drugs.

Their applications as nanocarriers have grown immensely over the last ten years, now we can find several publications describing their many functions, namely: (1) to concentrate anti-cancer drugs in the tumor microenvironment with a superior therapeutic efficacy[10]; (2) to deliver cancer antigens to immune cells, or to directly stimulate T cells as an artificial antigen presenting cell (APC)[11]; (3) and also to induce and enhance the abscopal effect (a phenomenon in which local tumor treatments produce a systemic regression of distant lesions) by capturing the tumor-derived protein antigens (TDPAs) released by radiation therapy.[12]

Cytotoxicity of NPs can be affected by size, concentration and surface functionalization. Even though NPs are inert and biocompatible, conflicting results have been reported regarding their toxicity to cells (Table 1). NPs' cytotoxicity may be due to the tiny size which makes them have a larger reactive surface area relative to the volume ratio for extracellular or intracellular interactions[25] involved in oxidative stress production.[26] On the other hand, studies (Table 1) have shown a very low cytotoxicity for different sizes of NPs on T cells and DCs, regardless of surface functionalization and concentrations,[27] which is important for their application in immunotherapy development.

2. Delivery systems used in cancer research

Recent advances in chemistry and synthetic biology have encouraged the development of nanoscale devices with controllable structures. Despite this, the large-scale manufacturing of these materials remains challenging because of the difficulty to produce structurally homogeneous populations of particles.[28]

On the other hand, nanomaterials based on viruses allow production on living cells of millions of identical nanoparticles. Viruses are an excellent starting point because they have evolved to interact with specific cellular proteins, delivering their nucleic acid cargo and taking charge of the intracellular machinery to develop the components of progeny. Therefore, they can be programmed for the delivery of other subsets of molecules, such as drugs.

3. Viral delivery systems

Virus-based nanoparticles (VNPs) own regular layers of coat proteins with a highly defined three-dimensional structure, providing an engineering scaffold which is superior to that of synthetic particles. The structural characteristics of viruses allow for the resistance to physical and chemical degradation and the protection of viral genome from nucleases. It is an advantage for the development of VNPs because it means that they have a long lifespan and can withstand the chemical treatments necessary to conjugate them with ligands or to load with payloads.[29] The structure of VNPs can be changed by modifying the genetic information that codes for viral proteins prior to synthesis. VNPs can also be altered by chemical modification adding conjugates to specific side chains of amino acids. These characteristics have led to the production of VNPs based on mammalian viruses for their use in gene therapy; nevertheless, it is difficult to dismiss pathogenic effects resulting from natural virus–host interactions.[30,31] However, VNPs based on bacteriophages and plant viruses have shown to be safe, because they cannot infect humans.

VNPs can be crafted to target particular cells, including specific cells of the immune system or cancer cells. Targeting can be achieved by the genetic or chemical addition of ligands which bind to overexpressed receptors on distinctive cells. For example, VNPs have been targeted through the use of folic acid,[32] epidermal growth factor[33] or transferrin.[34] Tissue specificity can also be swayed by the size, shape and aspect ratio of the VNPs. Therefore, these are properties that can be considered at the design state. As an example, filamentous or tubular VNPs can have better properties in vivo than spherical VNPs, like enhanced flow and margination towards vessel walls, and reduce the clearance by the mononuclear phagocytic system, which means increased tumor homing.[35]

4. Gold nanoparticles

NPs can be developed using various types of materials, such as metal oxides (e.g. iron oxide, titanium oxide), organic biodegradable (e.g. polysaccharide, lipid, polymeric matrix), inorganic biocompatible polymers (e.g. polystyrene), metalloids (e.g. crystalline or amorphous silica) or inorganic metals (e.g. gold, silver and carbon).[36] Gold NPs are adaptable in their shape, surface, functionality and size, which provides advantageous properties for immunotherapies. It is therefore necessary to determine a suitable and appropriate method for gold NP synthesis. The most popular mechanism of synthesizing gold NPs is through citrate reduction of hydrogen

Table 1 – Examples of common therapeutic nanoparticles conjugated with different types of drugs in pre-clinical models.

Nanoparticle category	Diameter (nm)	Binding molecule	In favor	In detriment	Indication
Gold	12	TKIs and FLT3 Inhibitors	Inhibition of BCR-ABL and FLT3 pathways	Resistance to chemotherapy, ↑ risk for relapse	AML[10]
	50	Doxorubicin	↑ Cellular uptake, ↑ cytotoxicity vs multi-drug resistance, blood-brain barrier pass	Cardiotoxicity, haematological toxicity	Breast cancer[13]
	50	Oxaliplatin	↑ Cytotoxicity and uptake	Undiscriminated cytotoxicity	Colorectal cancer[14]
	50	Cisplatin	↑ cytotoxicity, free active form of the drug	Renal toxicity and irreversible neuropathy	Gynaecological cancer[15]
Liposomes	80–100	Doxorubicin, EGFR, Epirubicin, Vinorelbine	↑ antitumor effect	↓ tumor internalization rate	EGFR+ tumors[16]
	90–100	Anti-HER2 fragments, Doxorubicin	↑ antitumor effect and drug accumulation in tumor cells	Toxicity profile and efficacy to be determined	Breast cancer[17]
	≈100	Folate, Doxorubicin	↑ citotoxicity	Folate efficacy was inversely proportional with liposome uptake	Lung cancer[18]
	<200	Thiolated Anti-HER2, Paclitaxel	↑cellular uptake and antitumor effects	↑ adverse events	Breast cancer[19]
Dendrimers	17	Paclitaxel	Stop mitosis in a dividing cell	↑ side effects	Ovarian cancer[20]
	≈30	Trastuzumab + Docetaxel	↑ apoptosis and cellular uptake	↑ hemolysis	Breast cancer[21]
	50–100	Doxorubicin	↑ anticancer activity and tumor site infiltration	↑ adverse events and out of target accumulation of dendrimers	ALL[22]
Carbon nanotubes	2–3	Doxorubicin	↑ blood circulation, ↓ toxicity and ↑ tumor uptake	↑ side effect of free Doxorubicin and out of target accumulation	Non-Hodgkin lymphoma[23]
	200	Paclitaxel	↑cell penetration and cytotoxic effect	Accumulation in internal organs and ↑ side effects	Not a specific tumor line[24]

nm: nanometers; TKIs: tyrosine-kinase inhibitors; AML: acute myeloid leukemia; siRNA: short interfering RNA; EGFR: epidermal growth factor receptor; ALL: acute lymphoblastic leukemia.

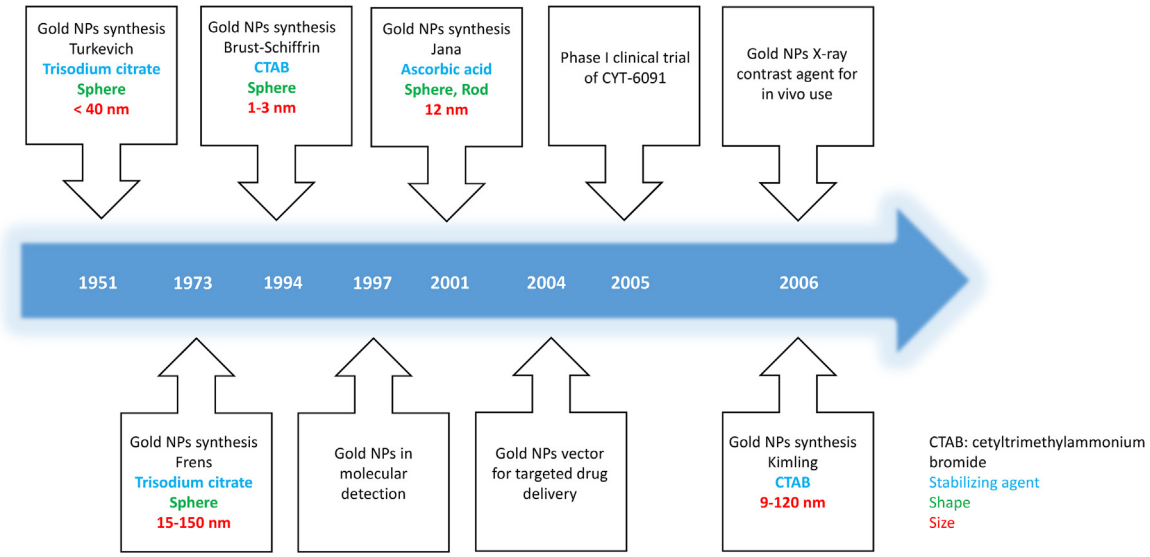

Fig. 1 – Timeline of Gold NPs development and methods of synthesis.

tetrachloroaurate in boiling water. This route was first proposed in the early 1950s by Turkevich et al.,[37] and later modified by Frens in 1973, improving the regulation of particle size resulting in gold NPs with diameters ranging from 15 to 150 nm.[38] The Turkevich and Frens methods were used for about three decades until they were further improved by Kimling et al.[39] who reported that the final particle size is independent of the absolute concentration of gold precursor. Many years of research have left us with several gold NPs techniques that have been explored and studied to produce well defined gold NPs. Currently, the most applied method is called the seed growth method, developed by Richard Zsigmondy, that synthesizes gold NPs in two separate nucleation processes followed by successive growth steps.[40] The initial production of small-sized gold NPs called seeds, were enlarged with the addition of growth solution, thus reducing and stabilizing agents in the second step. This technique achieves large size gold NPs with a more defined shape and more varied forms due to the first nucleation process. The current trend in gold NPs synthesis method is the use of biological compounds (e.g. star anise, pomegranate) rather than chemicals (e.g. citrate) that have been shown to be toxic at the cellular level. Therefore, this new trend is more environmentally friendly and also reduces the cellular toxicity of gold NPs which is crucial for future implementation[41] (Fig. 1).

As stated above, the customizable shape, size and properties of gold NPs have an important role in their interaction with APCs or other targeted cells (e.g. dendritic cells), and interaction capacity with these cells, such as the internalization process. The role of gold NPs for immunomodulation has been harnessed to deliver therapeutic compounds in diverse diseases, but the best effort is focused on cancer nanomedicine. Targeted delivery in immunotherapies for cancer and other immune diseases (e.g. rheumatoid arthritis) is important in terms of reducing systemic toxicity and achieving optimal therapeutic effects. The characteristics of the above mentioned gold NPs for surface association with drugs or other molecules to target immune cells turn these NPs into a promising therapeutic tool.[42,43]

This targeted method could be a different option to the conventional chemotherapy, with a reduction of related side effects. As an example, a new cancer chemotherapy strategy focuses on the association of gold NPs with chemotherapy drugs, showing a significantly lower cellular proliferation of hepatocellular carcinoma-derived cells than those exposed to chemotherapy alone.[44]

Similarly, several approaches have been adopted to develop cancer immunotherapies using gold NPs, and some of them are in their clinical trial phase (see Table 2). One of them is CYT-6091, a gold NP conjugated with PEG and recombinant human tumor necrosis factor (rhTNF), which was clinically tested in patients with advanced-stage of solid tumors. Results obtained from this study showed that the combination of rhTNF with gold NPs allowed to reach concentrated doses in tumor cells that had previously been toxic.[45]

Due to the ability and main roles of NPs in antigen presentation and T cell activation, there is great interest in manipulation of DC functions helped by gold NPs for induction of immune responses in immunotherapy development. This strategy is conducted to manage several challenges of immunotherapies, such as the lack of vaccine effectiveness,[46] persistent viral infection, immune escape in cancer and the adverse effects of therapies on healthy cells.[47]

One of the current strategies in DCs immunotherapies is to use materials to trigger immune specific response and also modulate immune-cell functions. As previously mentioned, gold NPs have plenty of characteristics that make them a potential tool to target DCs. To modulate the immune function of DCs, gold NPs are an instrument utilized to resolve several points in DCs immunomodulation such as to penetrate the mucosal barriers and tissues, enhance antigen uptake and to improve intracellular targeting.[48] Conducting studies on interactions and effects of gold NPs and human DCs is crucial due to the main roles of DCs in the activation of immune response,

Table 2 – Examples of on-going clinical trial with novel therapeutic nanoparticles conjugated with different types of drugs.

Nanoparticle Category	NCT	Status	Clinical phase	Binding molecule	Challenges	Questions
Gold	NCT03020017	Recruiting	Early Phase I	siRNA (Spherical Nucleic Acid)	Targeting BCL2L12 gene in glioblastoma	Safety to be determined
	NCT01420588	Recruiting	n/a	Na-Nose (chemical nanosensors)	Discrimination between gastric lesions	Effectiveness to be demonstrated
Liposomal	NCT01455389	Recruiting	Phase I–II	FUS-1 and Erlotinib	Transfer FUS-1 gene into lung cancer cells	Efficacy and dosing to be determined
	NCT00734682	Completed	Phase I	Liposomal Irinotecan	To treat patients with recurrent high-grade gliomas	Safety and dosing to be defined
(SMARTICLES©)	NCT02716012	Recruiting	Phase I	MTL-CEBPA (double stranded RNA)	To activate the CEBPA gene in advanced liver cancer	Toxicity profile and dose for phase II to be determined
	NCT02631733	Recruiting	Phase I	Liposomal Irinotecan plus Veliparib	To test this combination in solid tumors	Safety and dosing to be determined
Dendrimers	NCT03255343	Recruiting	Phase I	ImDendrim o [188Re] rhenium complex	To evaluate responder in Stage IV liver cancer	Non-removal nanodevice
Carbon nanotubes	NCT02123407	Unknown	Phase III	Harvesting lymph nodes	Guidance to lymph node dissection in gastric cancer surgery	Insufficient evidence to date
	NCT03350945	Not yet recruiting	n/a	None	To better localize tumor and lymph nodes mapping in the colorectal surgery	Safety, efficacy and economy efficiency To be determined
Other NPs categories						
Sugar molecule (CRLX101)	NCT02769962	Recruiting	Phase I–II	Camptothecin plus olaparib	To test in small cell lung cancer	Toxicity profile to be determined
Magnetic NPs	NCT02033447	Completed	Early Phase I	None	To treat prostate cancer with thermoablation	Retention and/or maintenance in the prostate
Polysiloxane Gd-Chelates	NCT03308604	Not yet recruiting	Phase I	With chemo-radiation and brachytherapy	To escalate dose in cervical cancer	Dosing to be determined
Radio-enhancer (NBTXR3)	NCT02805894	Recruiting	Phase I–II	Activated by radiotherapy	To treat locally-advanced prostate cancer	Efficacy to be demonstrated

NCT: number of clinical trial (reference of clinicaltrials.gov); siRNA: short interfering RNA; n/a: not applicable; NPs: nanoparticles.

either adaptive or innate. Studies and applications of gold NPs often require that nanoparticles be introduced into biological systems, such as into the bloodstream of an organism, cell of interest or growth medium. Thus, when exposed to biological systems, consisting of a complex mixture containing proteins, nutrients and metabolites, gold NPs would interact with them and change their physicochemical composition in terms of surface charge, surface chemistry, aggregation and size. Gold NPs would embed proteins of the medium, building a protein shell named "protein corona" which alters the effective surface charge of gold NPs.[49] On this basis, altered surface charge would later mediate the cellular uptake of gold NPs throughout several mechanisms including receptor-mediated endocytosis, phagocytosis and macropynocitosis, depending on their physiochemical properties of sizes and surface modifications.

In terms of size, some studies have shown a better cellular uptake of smaller size gold NPs (1–2 nm) by DCs rather than larger gold NPs (12 nm). These results were attributed to a higher diffusion capacity of smaller particles compared to a larger equivalent, in addition to a greater cellular uptake interaction.[27] These results were contradictory to a previous study in which 50 nm gold NPs were more efficiently internalized by DCs than 10 nm gold NPs.[50] This difference may be attributed to the minimum wrapping time required for 50 nm gold NPs, compared to smaller counterparts, which results in a faster and higher accumulation into DCs. Wrapping time is the ratio between the free energy required to introduce particles in a cell and the diffusion kinetics of the receptor, and depends on the size of the particles.[51]

Surface chemistry used to functionalize gold NPs is another critical factor that allows cellular uptake and internalization into DCs. In addition, surface modification of gold NPs protects them from aggregation and protein surface coating formation that leads to reduced cellular uptake in different lines.[49,52]

Cellular uptake and antigen internalization by DCs would activate their maturation and starts a T cell response based on antigen presented. Nevertheless, internalization and cellular uptake of gold NPs does not always result in enhanced DCs maturation and T cell response activation. Several factors such as size and type of ligands on the surface of gold NPs would affect DCs maturation, T cell responses and cytokines secretion in many ways.[27] Therefore, careful selection and further experimentation with the size of gold NPs and their protein corona will be critical to achieve the effects obtained when future research is done.

5. Dendrimers

Dendrimers are well-defined polymeric systems, which have the ability to interact with various forms of nucleic acids to form complexes that protect the nucleic acid from degradation. They are the key focus of biomedical research due to their precise molecular weight, biocompatibility, high water solubility and polyvalence.

The extremely branched structure of dendrimers allows them to integrate an extensive diversity of therapeutic (hydrophilic or hydrophobic) molecules. We can appreciate differences in the structure of the dendrimers with respect to other polymeric delivery systems that allow them to obtain more favorable physical–chemical properties and higher biocompatibility.[53] Their structure consists of a core, dendrons, and surface active groups. The electrostatic interaction between the dendrimers and the nucleic acids, where the cationic dendrimer condenses the anionic nucleic acids, the selection of a core and the surface functional groups, determines their cytotoxicity and biological activity. For example, the presence of several surface functional groups enables a concurrent interaction with numerous receptors. The drug properties are a fundamental factor in choosing the immobilization method. Encapsulation is chosen when the active compounds are virtually insoluble, labile or toxic, whereas chemical attachment offers the possibility to control the number of drugs on the dendrimer surface by modifying the number of covalent bonds.[54]

Commercially available dendrimers, such as Poly (amido amide) (PAMAM), are frequently used in biomedical applications and have been intensively investigated (Table 2), their structure is composed of a diamine core reacted with methyl acrylate, followed by another diamine, which results in regular radially concentric layers or 'generations' that give rise to successively larger dendrimers.[55]

One of the most helpful features of dendrimers is that they can modulate the release of cytokines and chemokines; in contrast, they can also produce severe secondary effects.[56] The PAMAM creation of 3,5-glucosamine dendrimers stimulates an important immunomodulatory effect generating the production of pro-inflammatory chemokines, such as MIP-1a, IL-8, MIP-1 beta, and cytokines TNF-α, IL-6 IL-1beta in human dendritic cells and macrophages. Chauhan et al. studied in vivo a PAMAM dendrimer toxicity profile in mice, elaborating a study of the dendrimer surface chemistry and different dosage levels and concluding that dendrimer toxicity is essentially a function of its outer surface layer, and can be controlled judiciously by pacifying the surface layer. The researchers assessed that PAMAM dendrimers can be used safely by correct choice of concentrations, surface groups and additives.[57]

Dendrimer glucosamine inhibited Toll-like Receptor 4-mediated inflammatory responses, allowing upregulation of the costimulatory molecules CD25, CD80, CD83 and CD86.[58] The dynamic behavior and the structural characteristics of this moderately glycosylated dendrimer revealed that its flexibility and polarity modified with the addition of glucosamine molecules. Remarkably, these surface glucosamine molecules continued to be available for interaction with the biological target.[59] Using this approach, it could be useful to design additional macromolecular dendrimers as novel antagonists of biologically important Toll like receptor–ligand interactions for treating a variety of malignant and inflammatory diseases.

A complete loss of TLR8-stimulatory activity with selective retention of the TLR7-agonistic activity have also been studied using a dendrimer synthesized from the TLR7 and TLR8 agonist imidazoquinoline.[60] Even though the mechanisms by which Toll-like receptors and dendrimers connect innate immune responses with adaptive immunity are not clear, it offers an opportunity that can be exploited to modulate the innate immune response for therapeutic use.

6. Solid lipids nanoparticles, liposomes and micelles

There are certain differences between these compounds, although all can be included within the category of Lipid-based nanotechnology. Solid lipids nanoparticles are characterized by being solid at room temperature, while micelles and liposomes are liquid at these temperatures.

Liposomes were the first colloidal drug carriers investigated in gene therapy and have been extensively studied in various drug delivery applications. They consist of spherical lipid vesicles with phospholipid bilayers with a hydrophilic centre enclosing an aqueous core in the size range of 80–300 nm.[61] Because of their high versatility of surface chemical modication, specific targeting using selected ligands[62] and potential of encapsulating both the hydrophilic and hydrophobic drugs, liposomes are attractive for drug designing, enable ease of surface manipulation and have been extensively studied in multiple clinical trials increasing the solubility of drugs, improving their pharmacokinetic properties and diminishing the side effects of chemotherapy or antimicrobial therapies.[63,64]

The release of a drug from liposomes depends on the liposome structure, biocompatibility, and the surrounding environment,[65] among others. However, liposomes have several unresolved problems, such as their accumulation in cells (liver macrophages) outside the target tissues, low encapsulation efficiency and short release time.[66]

Data on the use of liposomes and micelles as carriers to achieve immunosuppression are available, including the use of bisphosphonates for liposomes[67] and camptothecin for micelles.[68] The ability of liposomes to target specific cells is exemplified by the selective delivery of small interfering RNAs (siRNAs) to DCs using CD40 siRNAs containing liposomes with a monoclonal antibody that is specific to DCs[69] (Table 2).

7. Carbon nanocarriers

Among the most widely studied nanomaterials are carbon nanocarriers, differentiated into nanotubes (CNTs) and nanohorns (CNH). CNTs belong to the fullerene family of carbon allotropes and are formed by rolling of single (SWNCTs – single walled carbon nanotubes) or multi (MWCNTs – multi walled carbon nanotubes) layers of graphene sheets with a cylindrical nanostructure and an excellent electronic and thermal conductivity.[70] CNH exhibit similar properties to CNTs.[71]

The physicochemical and biological characteristics of CNTs make them a promising carrier for drug delivery, these nanoparticles are able to immobilize therapeutic agents, diminishing the cytotoxicity for healthy tissues. On the other hand, CNTs have several disadvantages, such as poor water solubility, low degradation rate and toxicity[72]; however, they have the ability to be surface functionalized using various methods such as absorption and electrostatic and covalent interaction, so that the release of drugs from carbon nanotubes can be electrically or chemically controlled.

8. Conclusions

Nanotechnology is a field that is currently being used to engineer specific immune responses for prophylactic and therapeutic effects. In the near future, the application of nanoparticles that have distinctive immunological properties determined by the type of polymer, its charge, size and the route of administration could offer tremendous potential to produce an appropriate immune response for elimination and prevention of future recurrence of cancer. Nanoparticle engineering for this purpose will remain an exciting opportunity for ongoing and future studies and an increasing focus of clinical trials and cancer treatments.

Conflict of interest

None declared.

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