Role of nanostructures in allergy: adverse effects, diagnostics, and treatment

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Abstract

Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers. It has led to the development of nanomaterials, which behave very differently compared with materials with larger scales and can be applied in a wide range of applications in biomedicine. The physical and chemical properties of materials of such small compounds depend mainly on the size, shape, composition, and functionalisation of the system. Nanoparticles, carbon nanotubes, liposomes, polymers, dendrimers, nanogels, among others, can be nanoengineeried for controlling all parameters, including their functionalisation with ligands, which provide the desired interaction with the immunological system. However, undesired issues related to their toxicity and hypersensitivity responses have impeded more rapid health applications. Through interactions with the immune system, some of these nanostructures show promising applications as vaccines and diagnostics tools. Dendrimeric Antigens, Nanoallergens, and nanoparticles are potential tools for the in vitro diagnosis of allergic reactions. Glycodendrimers, liposomes, polymers, and nanoparticles have shown interesting applications in immunotherapy. There are wide panels of structures accessible, and controlling their physico-chemical properties would allow the obtainment of safer and more efficient compounds for clinical applications goals, either in diagnosis or treatment.

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Introduction

Allergic diseases, including respiratory (allergic rhinitis and asthma) and food allergy (FA), and drug hypersensitivity reactions (DHRs), have shown an important increase in their prevalence over the last decades.[1-4]

Severe rhinitis and asthma, and potentially fatal anaphylaxis due to food and drug intake are a burden because of their high impact in life-quality and cost for the health system.[5-7] Moreover, there is a great heterogeneity of clinical symptoms, mechanisms, and specific gaps regarding comorbidities, making diagnosis complex, with low sensitivity or specificity, and treatments with low efficacy or no achievement of disease control.[8-11]

Regarding the diagnostic approaches, the main goal is improving sensitivity and specificity to diminish the false negative results, which can be critical in severe reactions, and the false allergic labelling of the patients, which is a main problem in DHRs.[12-16] A precise diagnosis will improve the management of the patients by applying accurate treatments. There is a need for new validated *in vitro* tests since, despite the existence of several approaches, they pose limitations regarding the real clinical relevance of positive results in cases of rhinitis, asthma, and FA, and their low sensitivity in DHRs.[17-23]

Concerning treatment, although the first line is the allergen/drug avoidance, this is not always possible, especially FA, in which accidental ingestion could happen due to the ubiquity of allergens and hidden sources. Therefore, other managements that influence the aetiology of the disease, as allergen specific immunotherapy (AIT), must be applied. Although different formulas have been commercialised with beneficial results in inducing tolerance to the patients[24-27], AIT does not completely reduce the risk of severe reactions and shows a lack of homogeneity between batches and difficulties on the obtainment of the natural allergenic extract.[28,29] Thus, it has been suggested the need of improving the efficacy of AIT using different approaches.[30]

During the last years, applications of nanotechnology for diagnosis and treatment in the field of immunology and allergy have increased and are being referred as nanomedicine.[31] We aim to present an overview of different nanostructures used in biomedicine and their potential suitability for *in vitro* diagnostic tests as well as for their role in novel immunotherapy.

Diagnostic *in vitro* tests. Immunoassays and cellular tests

The appropriate test for diagnosing allergic diseases depends on the suspected mechanism involved: specific immunoglobulin E (sIgE)-mediated or T cell-mediated, especially in DHRs.[22,32-36]

IgE-mediated allergic reactions can be induced by aeroallergens, food allergens, and drugs.[32,37] Additionally, for the latter, the drug structure coupled to a carrier protein of sufficient size may be involved in the sIgE recognition.[38,39] The major issue is the low blood concentration of sIgE, which is approximately 25% of total IgE for aeroallergens; and even lower for drug-sIgE (0.2% for betalactam).[40] Thus, extremely high sensitive methods are required.

The best validated and used *in vitro* approaches are based on the quantification of sIgE, either in serum by immunoassays (radioimmunoassay, enzyme-linked immunosorbent assay, or fluorescent enzyme immunoassay) or on basophil surface by functional basophil activation test (BAT).[41-44] The latter is quite specific, but complex to perform, and therefore limited to research laboratories.

Serum sIgE assays against allergen sources/molecules are the most commonly used and can be performed by singleplexed (which use single allergens) or multiplexed strategy.[37] In general, they are sensitive but show low specificity due to potential antigenic competition and isotype (IgG) inhibition.[45] There are several market leaders of singleplexed assays, whose main advantages are the automation, with increased precision and shorter turnaround times; the miniaturisation chip technology that reduces serum volumes, and the adaptability for use with purified native and recombinant allergens.[45] Multiplexed arrays offer the advantage of providing information on the sensitisation pattern of a patient for a large number of molecules with a small amount of serum. However, it can be difficult to differentiate clinically relevant from irrelevant sensitisations.[46] Moreover, allergen specificities on multiallergen screen are not defined and differ among various manufacturers.[45]

Currently, the available multiplex platforms can provide up to 112 allergens (allergen sources and protein groups).[46] However, the clinical relevance of many of these epitopes is not known and there is a higher degree of variability in low IgE levels,[47,48] cases in which singleplex platforms may be more sensitive.[37]

In the case of DHRs, solid-phase immunoassays have to include drug-carrier conjugates to detect serum-drug-sIgE.[42,49] Due to the extremely low levels of drug-sIgE, they generally have low sensitivity, although this depends on the clinical manifestations, the drug involved, and the time interval between reaction and diagnostic assay.[50,51] The carrier molecule can also affect the sensitivity, poly-L-lysine is the most used artificial carrier due to its multivalency, which allows a high hapten density,[52] although its polydispersity impedes adequate characterisation, reproducibility, and conjugate control.[53] Moreover nonspecific interactions and immobilisation on solid-phase can reduce immunological capture. Both commercial and in-house radioimmunoassays are used, although enhanced sensitivity is needed.[54]

The use of BAT has increased in the last years, being seen in the overall context of molecular diagnostics in food and aeroallergen allergy.[37,43,55] A major issue is the allergen source,[37] since results differ according to the variety employed.

In the case of DHRs, BAT has overcome the immunoassay limitations of amount of drugs available and has been mainly studied for neuromuscular blocking agents, betalactams, and iodinated contrast media, with a sensitivity ranging between 50% and 60%, and a specificity of 80%.[32] Moreover, BAT has a complementary role for skin test for many drugs to which no other approaches are available.[39,56-60] Both commercial BAT and in-house protocols are rarely thoroughly validated and require additional investigation before they can enter mainstream application.[37,41]

Nanotechnology in biomedicine

The famous conference of Richard Feynman in 1959 and the mythical phrase "there is plenty of room at the bottom" is considered as the starting point of nanotechnology. Nanotechnology is the development of materials with nanometric size for searching new properties at this scale, which could be used for different applications. Some of these materials have been considered as ideal platforms for their functionalisation with ligands with applications in biomedicine.[61]

Metallic and non-metallic nanoparticles (NPs), carbon nanoforms such as single- or multiple-walls carbon nanotubes (CNTs) and graphene, liposomes, polymers, dendrimers, nanogels, etc. are popular representatives (Figure 1). They show different physical and chemical properties that depend mainly on the size, shape, composition, and functionalisation of the system. Nanomaterial engineering provides the tools to control all these parameters and to achieve the desired requirements for health applications.[62] In fact, the functionalisation of these scaffolds is the way to modulate their physical, chemical, and biological properties at will. Their applications in biomedicine include therapeutics, diagnostics, and theranostics [63,64] for drug delivery,[65] bioimaging and biosensing,[66] as implants,[67] cancer immunotherapy,[68] gene therapy,[69]etc. Besides their potential and the expectative in the biomedical area, nowadays not many nanodrugs are approved for medical use, although some promising compounds are still under clinical trials.[70]Issues related to their nanotoxicity, reproducibility, and homogeneity have impeded a rapid development of this field in health applications.[71]

There is a plethora of examples, in which these nanomaterials have demonstrated very promising and interesting properties at *in vitro* level, for which they were decorated with ligands for specific receptors expressed or over-expressed in target cells or tissues. On one hand, these nanosystems behave as selective drugs carriers reducing the toxicity, being selective for specific targets and decreasing side effects. On the other hand, nanostructures can provide the means to protect their cargo, improving their stability against degradation, their solubility, and their drug availability.

Adverse effects of nanoparticles. Promoters of allergic diseases

The existence of numerous applications of different nanomaterials in biomedicine has arisen the need of evaluating the possible adverse effects, either toxicity or hypersensitivity responses.

One of the main concerns about nanostructures is their toxicity, which made nanotoxicology discipline emerge.[72] In general, physico-chemical properties including size, surface charge or area, solubility, morphology or reactivity, redox-active properties, and aggregation capacity will contribute to the toxicity of the compounds.[73,74] *In vitro* studies have shown a correlation between lower size and higher toxicity, probably because small nanostructures are better uptaken by cells.[75] Nanostructures can also generate reactive oxygen species and oxidative stress inducing DNA damage or apoptosis, as observed in keratinocytes, fibroblasts, and macrophages.[76] The route of administration can also affect their toxicity, being it higher intravenously administered, since body distribution increases. Nevertheless, although there are no definitive rules, toxicity can be modified by changing nanostructures properties as reducing surface charges or including low cytotoxic groups such as zwitterionic segments.[77]

Nanostructures can be recognised as foreign compounds by immune cells inducing dual effects as an allergen or sensitiser, or as booster or adjuvants even acting as immunomodulators.[78,79] The mechanism by which nanomaterials can immunomodulate is related to their ability to interact with antigen presenting cells (APCs) as dendritic cells (DCs), modifying their activation and maturation, and thereby leading to T-lymphocytes activation.[78] The physico-chemical properties of NPs have demonstrated to strongly affect DCs responses.[80]Small nanomaterials (<200nm) favour the uptake and migration of DCs and macrophages towards draining lymph nodes,[81,82] improving the induction of immune response. Moreover, the type of response, Th1 or Th2 could depend on the nanostructure redox potential, with oxidant titanium dioxide NPs (TiO_2NPs)[83] inducing a Th1 response, whereas antioxidant cerium oxide NPs inducing a Th2 phenotype with IL-10 production.[84]

Nanomaterials have been developed to interact with DCs through C-type lectins and Toll-like receptors (TLRs) for modulating immune responses.[85] Different nanostructures have been applied as vaccines in cancer and viral and bacterial infections, etc.[86] In allergy, the application of nanotechnology is especially interesting for immunotherapy since NPs can present a dual action, being an adjuvant and protecting allergen from degradation.[31,87] At the same time, they could be used as co-delivering immunostimulatory agents. In this sense, dendrimers, functionalised with sugars (glycodendrimers) have been used for targeting DCs through the DC-SIGN or mannose receptors[88] influencing the internalisation process and presentation through major histocompatibility complexes to T-cells. This has been applied to develop compounds than can be used in Flu viral infection immunotherapy[89] and as adjuvants to treat allergic diseases. In fact, NPs have shown efficacy in oral immunotherapy for FA.[90-92] See structures in Table 1.

Nanostructures can be internalised in cells by phagocytosis, macropinocytosis, as well as clathrin-, caveolae-, and scavenger receptor-mediated endocytosis, which will deeply depend on nanomaterial properties, again dependent on the NP size.[93] Several studies support evidences of active mechanisms such as endocytosis, with NPs present in both endosomes and lysosomes of DCs.[80,93] The functionalisation with multivalent mannose ligands that interact with C-lectin receptors can facilitate the internalisation on DCs and major histocompatibility complexes class presentation to T-cells inducing preferentially a Th1 response.[89,93] Other chemical groups decorating the NPs have also showed to impact the modulation: oxidised or hydrocarbonised porous silicon induce immunoactivation, whereas zwitterionic-stabilised gold nanoclusters strongly immunosuppress the response.[94]

Allergic responses.

Nanomaterials can produce adverse effects on respiratory systems, producing asthma exacerbation and also altering the response to allergens.[78] Moreover, they can enhance the sensitisation to an allergen by a depot capacity that increases the local antigen level, persistence, and prolonged release as demonstrated with TiO_2NPs.[95] This effect has been observed even though the allergen–nanomaterial compounds do not penetrate the epidermis.[96]

CNTs, TiO_2NPs, gold (AuNPs), silver (AgNPs), silica (SiNPs), and zinc oxide (ZnONPs) NPs have demonstrated exacerbation of Th2 allergic models.[97] The pulmonary exposure to NPs can induce the lung expression of inflammatory mediators, TARC, MIP-1a, GM-CSF even in the absence of allergen, although with an increase of this effect in its presence.[98,99] Although these results suggest that small NPs could potentiate allergic lung inflammation,[100] others indicate that they can attenuate these responses,[101,102] indicating the complexity of the NPs interaction with the immune system and the need for further research.

In general, nanomaterials can induce hypersensitivity reactions by interacting with both innate and adaptive immune systems at different levels: antigen presenting cells, mainly DCs affecting their antigen processing and presentation to T-cells inducing effector cells, as mast cells, basophils, and eosinophils; or complement system activation and pattern recognition receptors and/or release of alarmin molecules producing inflammasome activation.[97]

Metal-based nanomaterials can present an additional concern in allergy because they include metals known to cause allergic contact dermatitis, asthma, and allergy adjuvancy.[72,96]TiO_2NP and ZnONP have been extensively incorporated in sunscreens and cosmetics for their ultraviolet radiation protective effects, AgNP due to their antimicrobial properties, and SiNP in cosmetics and to alter the properties of other materials. For these extensive uses and their potential capacity to penetrate the skin, they could induce sensitisation.[72] Small size has shown to cause greater inflammatory response mainly because they can deeply penetrate the tissues and have a larger surface area.[96,98] In cases of skin barrier dysfunction, TiO_2NP can exacerbate atopic dermatitis symptoms[103] and polystyrene NPs are able to stimulate skin inflammation even without the allergen by overexpressing CC-chemokines.[103]

Pseudoallergy or idiosyncratic reactions that are non-IgE-mediated hypersensitivity have been associated to a wide range of NPs such as AuNPs, AgNPs, copper oxide, SiO_2NPs, TiO_2NPs, and CNT.[97] One possible mechanism could be the complement activation leading to anaphylatoxin (C5a and C3a) secretion and subsequent activation of mast cells, basophils, and possibly other inflammatory cells in blood.[104,105] Moreover some reports demonstrated that NPs activate the NLRP3 inflammasome,[106] which is one of the pattern recognition receptors expressed intracellularly promoting IL-1? and IL-18 production.[107]

Besides the immunological mechanisms described above, NPs can also produce allergy and asthma by damaging the epithelial barriers (pulmonary and intestinal mucosa, skin, etc.), inducing not only an innate immune response but also promoting the entrance of allergenic proteins.[97,108]

The identification of possible side effects should be done to assess the safety and efficacy of these nanomaterials before product commercialisation. These effects cannot be generalised, since the immune effects are highly dependent on the physico-chemical structure and properties of each type of nanomaterial and, even with the same material, on the administration conditions. Thus their potential risks should be identified in each particular case by preclinical studies.[109,110]

Nanotechnology in allergic diseases

1. How nanostructures can improve the current in vitro approaches

Boosting the interactions and response of nanomaterials with the immune system is essential to design new systems for in *vitro/in vivo*applications. Due to the physico-chemical properties, precise control, and tuneability for designing nanostructured materials, their use can drive the improvement of *in vitro*diagnosis.[54,111]

Different nanomaterials have been used to develop nanotechnology-based diagnostic tests, such as metallic NPs, quantum dots (QDs), SiNPs, carbon-based nanostructures, dendrimers, and liposomes (Figure 1).[54,111] Most of them focus on sIgE determination to drugs and allergens, whereas only few applications are based on cell assays.

Approaches involving nanotechnology have been applied in sIgE testing. Nanomaterials are used either as a solid support to capture antibodies or allergens, or as a detection tool to enhance the measurement signal.[46,112] Nanofluidics allow to minimise assay time by enhancing molecular interaction.[113] Besides, in DHRs, dendrimers have been used for emulating carrier proteins and sIgE recognition after drug haptenation has been proven.[54,111]

Based on dendrimer ability for mimicking proteins, Dendrimeric Antigens (DeAns) have been designed consisting of dendrimers peripherally decorated with multiple units of the drug (hapten) (Figure 2A, bottom).[114] These DeAns, presenting penicilloyl units in the periphery, are recognised by sIgE from penicillin-allergic patients, with increasing recognition extent for higher hapten density (Table 1).[114] Moreover, DeAns are valuable for understanding interactions between immunoglobulins and haptens: proving the relevance of antigen tridimensional structure, showing differences between epitopes of betalactam conformation (Figure 2C, bottom).[53] The inclusion of these two different drugs on the same DeAn has enabled the detection of sIgE from selective and cross-reactive patients (Table 1). These findings indicate that including appropriate bi-epitope-DeAns could represent the basis of a method for screening a major proportion patients with a single test.[53]

Further studies have focused on immobilising these DeAns on different solid supports for direct diagnosis application through RadioAllergoSorbent Test (RAST). Using DeAns facilitates controlling reproducibility, reduces nonspecific interactions, and enhances accessibility to sIgE in whatever solid supports (Figure 2B, bottom).[54,115]

Cellulose materials have been hybridised with penicilloyl DeAns of different generations, showing the effects of hapten density, and size scaling on penicillin-sIgE recognition.[115] Further development of hybrid DeAns-cellulose materials, using haloalkanoyl halides or hydrophilic spacer linkers to anchor DeAns to surfaces, leads to higher RAST sensitivity.[116,117]

Recent progress on nanomaterials for biosensors has been reported on the use of other solid phases such as zeolites,[117] and SiNPs,[118] which permits easier handling protocols in RAST, whereas larger surface area permits efficient functionalisation and effective quantification of amoxicilloyl-sIgE.[118]A different approach used dendrimeric gold nanodisks as a solid phase for quantifying amoxicilloyl-sIgE, showing a good correlation with ImmunoCAP.[119] The nanoplasmonic biosensor device uses label-free anti-IgE, requiring short analysis time. This represents a potential new assay for the diagnosis of betalactam allergy.[119]

NPs functionalised with allergens have been also used as a solid supports to capture IgE related to FA.[46] For instance, iron-oxide magnetic NPs coated with peanut extract were used in an immunoassay in which an external magnetic field shows high sensitivity. Peanut-sIgE was detected in concentrations close to the minimum detection range of CAP assay.[120] Another approach used magnetic core-shell NPs coated with alpha-lactalbumin in a microfluidic assay, detecting low concentrations of sIgE in serum. This is a potential quick diagnostic tool which still needs more evaluations for performance.[46]

Besides, NPs can be chemically modified to allow coupling of detecting molecules, such as antibodies, ap-

tamer, or enzymes, to amplify the signal for reaching improved sensitivities.[46] AuNPs coated with oligonucleotide aptamers with high specificity for human IgE have been used in several systems to measure total IgE level. In a system relying on surface plasmon resonance (SPR), signal amplification was clearly achieved by adding IgE aptamer-coated AuNPs.[121] However, such system needs further evaluations with human blood specimen, as matrix effects may influence test performances. Recently, to overcome these issues, an antifouling sensing interface for electrochemical biosensor was fabricated through the self-assembly of a zwitterionic peptide and the IgE aptamer onto a macroporous Au substrate. The zwitterionic peptide reduces the non-specific adsorption and fouling effect, whereas the high surface area arising from porous morphology and the high specificity of aptamer permit it exhibit ultrahigh sensitivity and selectivity towards IgE, capable of sensitively assaying IgE in biological samples.[122]

QDs technology has shown potential for IgE detection but has not been integrated into functional devices for clinical use yet.[123] For instance, IgE interaction with casein immobilised onto a sensor chip has been detected using dual polarization interferometry with signal enhancement using streptavidin-conjugated QDs. This QDs assay for casein-sIgE had comparable sensitivity to ImmunoCAP.[124]

New methods are required for the detection of trace concentrations of allergens in complex food matrices.[125] In this sense, NPs use for enhancing signal detection in biosensors for applications in food analysis is a challenging area of growing interest (Figure 2, top).[126,127] A multiplex competitive microimmunoassay for the simultaneous detection of four food allergens (gliadin, casein, β-lactoglobulin, and ovalbumin) uses Digital Versatile Discs as sensing platforms. The immunointeraction is detected using a mixture of specific gold-labelled antibodies and the signal is amplified with the silver enhancement method. Limit of detection below the accepted levels of the international legislations were obtained in real food samples, which allows promotion of food safety and quality.[128]

Regarding cellular tests, only few using nanomaterials have been addressed to allergy diagnosis, mainly to DHRs. Nanoallergens have been used for detection of platin drug allergies in oncologic patients.[129] These nanoallergens consist of liposomes as platforms for drug (oxaliplatin and carboplatin) metabolites displayed in a highly multivalent fashion. These systems trigger significant degranulation responses from mast cell–like cells (RBL-SX38) primed with serum IgE from patients with platin allergy. Interestingly, the nanoallergen concentration that triggered significant degranulation responses *in vitro* depended on the clinical entity.[129] In another study, the ability of penicilloyl DeAns to stimulate basophils was demonstrated in patients with betalactam allergy (Figure 2D bottom). Those nanoconjugates of bigger size and displaying higher valency of haptens, 4^{th} generation compared with 2^{nd}generation, were more effective in inducing activation.[130] Further studies are needed to evaluate the potential improvement of BAT with DeAns compared with the test using the free drug for evaluating penicillin allergy.

2. How nanostructures can improve the treatment approaches

Nanomaterials can interact directly with the immune system triggering immune responses, consequently they have been considered as potential adjuvants.[97] Their functionalisation with ligands produces a particular effect in a more controlled way. NPs have been considered as adequate platforms to conjugate ligands that can tune the physico-chemical properties of these nanostructures, but also to facilitate the interaction with target cells for a selective drug delivery. In addition, they can encapsulate allergens to be delivered selectively in cells of the immune system.[131]

CpG oligodeoxynucleotides (CpG-ODNs), a conserved sequence present in bacteria and viruses, are recognised by TLR9 receptors, inducing a Th1 response. CpG-ODNs have been used as potent and efficient adjuvants in allergic models. Mesoporous silica NPs, boron nitride particles, AuNPs, liposomes, CNTs, polymers, among other platforms have been extensively used to deliver CpG-ODNs into cells. The NPs not only prevent the degradation of the ODNs but also increase the cell uptake, facilitating the internalisation, and inducing the enhancement of the immunostimulation, and the release of pro-inflammatory cytokines.[132]

NPs have been also used for topical administration of drugs such as betamethasone, hydrocortisone, hydroxytyrosol, tacrolimus, anti-histamine drugs, etc. with the aim to treat atopic dermatitis. NPs improve the solubility of these drugs, their stability against degradation and facilitate the skin penetration capabilities, reducing side-effects.[133]

The use of nanomaterials has been also extensively applied to the treatment of asthma.[134] Different types, as PAMAM and Polyethyleneimine dendrimers, polyethylenglycol (PEG) NPs, and liposomes have been used as carriers of CpG, DNA plasmids, beclomethasone dipropionate, dexamethasone, salbutamol, etc.

NPs based on chitosane, poly(D,L-lactic-co-glycolic) acid (PLGA), ceramic, plygammaglutamic acid, etc. have been used as mucosa adjuvants encapsulating antigens for oral vaccination. The properties of the polymers are fundamental for an adequate allergen release. PEG functionalisation can retain the NPs in the mucosa, reducing the treatment dose. Microbial adhesins functionalisation enhances NPs bioadhesive properties to colonise the gut.[135]Besides, a water soluble self-assembled micellar formulation, using PEG400, propylene glycerol and ethanol, has been used to encapsulate Cannflavin A, a flavone derivative with anti-inflammatory activity and poorly soluble in water. This composition was tested in an experimental asthma/chronic obstructive pulmonary disease in rats enhancing the anti-inflammatory effects *in vivo* .[136]

Protein-cage NPs using ferritin of 12 nm of diameter have been functionalised on their surface with 24 units of a small peptide (AP-1), a ligand for the receptor IL-R4 which abolishes the IgE response and has been tested in a murine model of allergic asthma. The treatment clearly decreased the severity of the symptoms.[137]

NPs combined with allergens have been applied for treating allergy. PLGA nanospheres particles loaded with Bet v 1, the major allergen of birch pollen, prevent the production of IgE and modify the Th2 response producing IFN-γ and IL-10.[138] This can be considered the first example of that an allergen entrapped in PLGA polymer for allergy treatment reduces the predominance of the Th2 response.[92] Poly(anhydride) NPs containing cashew nut proteins can induce a strong Th1 and Treg immune response after oral administration.[92,139] This can be considered the first example of how an allergen entrapped in PLGA polymer for allergy treatment reduces the predominance of the Th2 response.

As result of the previous research [87,90], the first peanut oral immunotherapy clinical trial has started. It is a multicentre, double-blind, randomised, placebo-controlled phase I/II study (ClinicalTrials.gov Identifier: NCT04163562).

A copolymer based on poly(hydroxyethyl)aspartamide (PHEA) functionalised in the side chains with butyryl and succinyl moieties has been used to form nanoaggregates of 90 nm of diameter with the allergen lipid transfer protein Par j 1 and 2 (from Parietaria judaica pollen).[140]

After that, several examples demonstrate the use of NPs for allergy treatment in animal models. PLGA NPs have been used to load rChe a 3 (the most abundant allergen in Chenopodium album pollen) with the aim to modulate a Th2 immune responses by sublingual immunotherapy in a mouse model of allergic rhinitis.[141] The Polyethyleneimine polymer loaded with Bet v 1 plasmid has demonstrated to induce a reduction of IgE and an increase of Th1 response in mice against birch pollen.[142] Der f 1 plasmid complexed with a copolymer of propylene oxide and ethylene oxide has been used to reduce inflammation in asthmatic mice.[143] Ara h 2 (peanut allergen) together with the adjuvant CpG was combined with protamine (arginine rich protein) NPs to induce a Th1 response.[144] Natural polysaccharides have also been used as adjuvant together with allergens to decrease allergic responses. Alginate, extracted from algae, in combination with grass pollen extracts, induces a reduction of IgE in mice.[145]Studies using chitin as adjuvant produce a reduction of IgE and Th2 cytokines in allergic mice.[146] Many other examples using biodegradable and non-biodegradable NPs have been revised recently,[31] indicating that these platforms provide interesting approaches for the treatment of allergy.

Dendrosomes (hyperbranched dendritic spheroidal particles) have been used as adjuvants with plasmid for Bet v 1 in DNA vaccination in birch pollen allergy. In mice, footpath administration of these dendrosomes produces the inhibition of the allergic reaction, inhibiting the production of IgE, and maintaining the

Th1/Th2 balance.[142]

Small CdSe/ZnS core/shell QDs NPs with a negative surface (using glutathione) show immunosuppressive effects when tested in skin allergy and used topically in combination with the allergen 1-fluoro-2,4-dinitrobenzene. The NPs penetrate the skin, facilitating their effect.[147]

Besides NPs, dendrimers have also been used to treat allergic diseases (Figure 3). Glycodendrimers containing mannoses (Table 1) have been conjugated with a peptide corresponding with a T-cell epitope of the major allergen of olive tree pollen (Ole e 1). This compound has demonstrated to induce Treg proliferation *in vitro* , in particular in cells from allergic donors.[148] The same glycodendrimer conjugated with an epitope for the Pru p 3 lipid transfer protein induces long-term tolerance in a peach anaphylactic mice model when administered sublingually.[149] These promising results indicate that glycodendrimers can be considered as promising adjuvants to be applied in allergy.

Conclusions

Nanotechnology is a fascinating area of development providing remarkable solutions in many fields including allergy, where it represents a promising tool for improving diagnosis and immunotherapy. The specific properties that nanostructures can offer, which are tuneable at will, provide the chance to design adequate systems to be applied in this field. There are wide panels of structures accessible, and controlling their physico-chemical properties would allow the obtainment of safer and more efficient compounds for our goals, either in diagnosis or treatment. Interesting tools for detection and diagnosis are available for clinicians and researchers in this field. In addition, relevant advances have been described for application of nanostructures in immunotherapy. Besides these promising facts found in the literature, the application of nanotechnology to allergy is still in its infancy and have to face new important challenges in the next years to achieve important goals.

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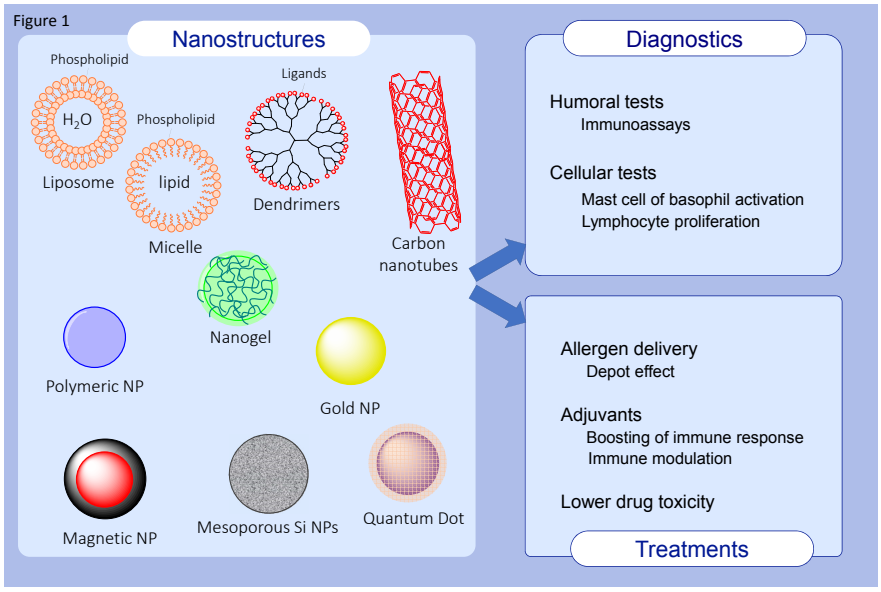


Figure Caption

Figure 1. Different types of nanostructrures and their possible uses in allergy

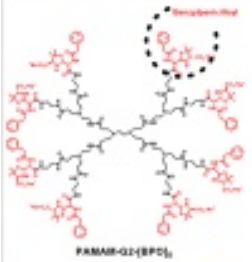
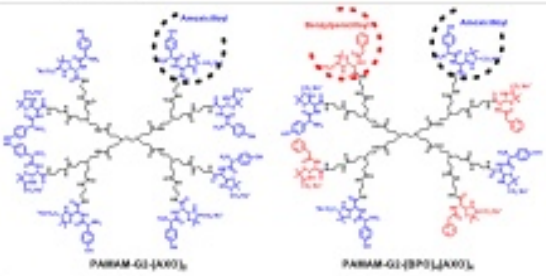
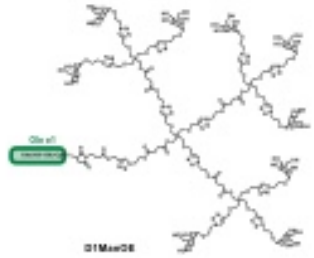
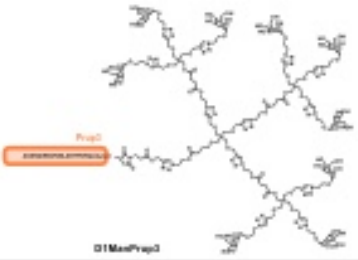
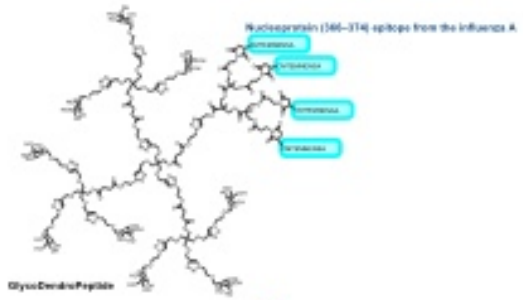
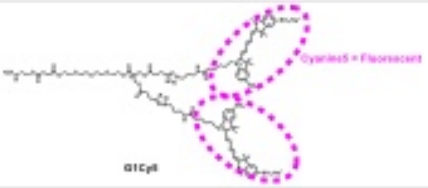
Figure 2. Nanostructures in *in vitro* diagnostics.

Top: Examples of nanomaterials amplifying the signal detection in immunoassays. A) Scheme of a competitive microimmunoassay on DVD. The allergens are immobilised on the Digital Versatile Discs (DVD) surface, to which AuNPs-labelled specific antibodies bind. The antibody-antigen interaction is exhibited as a black precipitate after antigen enhancement step. B) Fibre optic surface plasmon resonance (SPR) probe for detection of allergens in food. Detection step uses antibody linked nanobeads.

Bottom: Dendrimeric Antigens as emulators of drug-protein conjugates in the molecular recognition of in vitro tests. A) Schematic structure of multivalent dendrimeric scaffold; B) Direct in vitro immunoassay, in which Dendrimeric Antigens are covalently coupled to the solid phase; C) Competitive immunossay in which poly-L-Lysine conjugated to drug in solid phase competes for immunological recognition with Dendrimeric Antigens in fluid phase. D) Basophil activation test using Dendrimeric Antigens.

Figure 3. Schematic graph of immunological mechanism in immunotherapy with nanostructures. Interaction with dendritic cells that present the antigen to T lymphocytes, increasing Th1 regulatory response and decreasing Th2 effector cells.

Table 1. Chemical structures of dendrimers with different applications in allergy.

Dendrimer structure	Application	Reference
	In vitro drug allergy diagnostic test	Bioconjug Chem. 2002; 13(3):647-653.
	In vitro drug allergy diagnostic test	Nanomed-Nanotechnol Biol Med. 2015;11(3):579-588.
	Immunotherapy	Sci Rep 2019;9(1):4043.
	Immunotherapy	Mol Pharm. 2020; 17(3):827-836.
	Immunotherapy	MedChemComm 2015; 6:1755-1760.
	Detection in vitro diagnostic tests	Polym Sci, Part A: Polym Chem. 2018;56(15):1609-1616.