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ARTICLE

Interactions of carbon nanotubes and fullerenes with the immune system of the skin and the possible implications related to cutaneous nanotoxicity

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ABSTRACT

The understanding of the interaction of carbon nanotubes and fullerenes with the constituents of the skin, especially the skin immune unit, is relevant to the determination of toxicological endpoints. A systematic review was done focused on such aspects. Considerable part of the found references concentrated in cytotoxicity and skin permeation. On a smaller scale, there are articles on immunomodulation and activation of immune cells and other elements. Few of the found studies deal specifically with cutaneous immune response, limiting the related knowledge. The findings suggest that nanomaterials studied may be involved in skin problems such irritant contact dermatitis, anaphylactoid reactions, urticaria, angioedema, and raised the need for performing additional studies to confirm the findings. The standardization of the description and testing of nanomaterials characteristics used in experiments can facilitate comparison of results.

KEYWORDS: Fullerenes; Carbon Nanotubes; Allergy and Immunology; Skin Diseases

Introduction

Nanoscience has been developing at a fast pace, and nanomaterials can now be found in a wide range of products such as electronics, food, pharmaceutical products, and industrial and domestic utensils.

For a material to be considered as a product of nanotechnology, at least one of its dimensions must be between 1 and 100 nm[1]. This nanometric size means that most atoms in the molecule are on its surface, which explains the high reactivity of nanomaterials and their various applications[2].

Among nanomaterials, the production of carbon allotropes such as fullerenes and carbon nanotubes (CNTs) is the highest, and they have the most applications[3]. Therefore, there is an increasing concern about risks associated with the environment and human health because of occupational exposure to these nanomaterials, in particular via the skin and airways[4,5].

The skin is a major barrier between man and the environment, and it plays an important role in the protection against physical, chemical, and biological aggressions[6,7]. Exposure of the skin to nanomaterials can occur via direct application of medications that contain nanocarriers, through tissues treated with nanoparticles, or involuntarily via contact with raw materials or residues from industrial production processes[8].

There are several issues regarding the interaction of nanomaterials with the skin. These include penetration through intact and damaged physiological barriers, cytotoxicity, induction of oxidative stress and inflammation, and mutagenicity[9].

Similar to any new chemical substance, nanomaterials pose potential risks of allergic or irritant contact dermatitis because these structures can represent new allergens, haptens, and cross-reaction agents and cause unknown damages[10]. Thus, many of the predicted risks associated with the interaction of nanomaterials with the skin involve the skin's immune response elements. It is important to understand each phase of this process to acquire knowledge about skin nanotoxicity.

Carbon allotropes

At present, carbon allotropes are one of the most investigated materials for their wide applicability in medicine and technology. The specific hybridization of carbon, bonds between atoms, and conformation will determine the type of the allotrope, namely CNT, fullerene, graphene, graphite, or diamond[11].

Fullerenes have the form of a hollow sphere[12]; all carbon atoms are organized in polycyclic rings[13]. Different types of fullerenes can be created depending on the radical present on the surface, the synthesis process, and the number of carbon atoms, e.g., C_{60} and C_{80}[5].

At present, fullerenes are used in the synthesis of systems that carry drugs, cosmetics, lubricants, catalysts, modified polymers, and sport articles[5]. Owing to their ability to react with various chemical species, in particular, free radicals such as superoxides, hydroxyl groups, and lipid radicals[14,15], fullerenes have been used in cosmetics as antioxidants. This effect is further enhanced by the inhibitory action of this nanomaterial on the monooxygenase-dependent cytochrome P450[16]. In addition, owing to their antioxidant effect, the topical use of fullerenes has been suggested for treating acne because of the possible beneficial action in reducing sebum and neutrophil infiltration[17]. Moreover, the ability of fullerenes to reduce the synthesis of free radicals induced by exposure to UVA and UVB radiations favors the use of these nanomaterials in sunscreens[18,19]. Another characteristic is their ability to inhibit melanogenesis induced by UVA radiation in human melanocytes; thus, they can be used as depigmentation agents[20].

CNTs are composed of one or more curved walls of graphene[21,22]. There are three types of CNTs, depending on the number of walls: single-wall CNTs (SWCNTs), double-wall CNTs (DWCNTs), and multiple-wall CNTs (MWCNTs), which also differ in terms of the diameter[23].

During CNT synthesis, significant amounts of iron[24], nickel[25], and cobalt often remain in their interior[26] because they are used as catalysts in the synthesis process[24,26]. These contaminating metals can have a biological effect by acting as catalysts in oxidative stress reactions[24] and can induce the formation of free radicals, accumulation of peroxide, and depletion of antioxidants[27] in addition to playing a role in acute inflammatory responses[28].

Due to their mechanical, structural, and differentiated transport properties, CNTs have been used in the synthesis of nanocarriers for drugs, biosensors, resistant plastics, electromagnetic shields, scanning probe microscopes, and resistant fibers in addition to other uses within the medical, aerospace, computation, and electronic fields[13,25,29,30,31].

Furthermore, CNTs have been applied as transport vectors for therapeutic molecules[32], thereby allowing the development of more immunogenic vaccines[33] and drugs with site-specific action and reducing side effects[34].

The surfaces of CNTs and fullerenes are chemically inert, which hinders their interaction with biological structures[23]. Functionalization consists of the bonding of functional groups and compounds to surfaces. It occurs through covalent or noncovalent bonds, such as those of amino acids or hydroxyl groups, on the surface of nanomaterials[35], and it determines new physical and chemical properties[27]. This process leads to an increase in the solubility of CNTs and fullerenes in water (thereby enhancing their interaction with biological elements), the mobility of these structures between different compartments in organisms[23,36], and the release of elements of the immune system, thereby increasing the bioavailability of carried substances[37].

The interaction between carbon allotropes and elements of the immune system is already known, particularly because these structures have been used in vaccines, including those with intradermal and intramucosal administration[38]. The aim of describing the interaction with the skin immune system (SIS)

is not only to search for new applications of these nanomaterials but also to predict possible complications related to a prolonged stimulus of the immune response.

SIS

The skin is constantly exposed to numerous antigens and irritants, leading to the need for an effective and differentiated immune system. The diversity of the skin's immune response has led to the hypothesis, in the 20th century, about the existence of a specific lymphoid tissue associated with the skin, the skin-associated lymphoid tissue (SALT)[6], which was later termed SIS[39].

With regard to the type of immune response, the immune system of the skin includes elements of innate and adaptive immune response in addition to elements that mediate the two types of responses.

The skin physical barrier

The skin is composed of three layers, namely the epidermis, dermis, and hypodermis, which differ structurally and functionally [40] and together represent a physical barrier.

The cornea layer is composed of corneocytes[41] that are connected to each other through corneodesmosomes and are surrounded by an extracellular matrix rich in nonpolar lipids, organized in a double layer. In the epidermis, keratinocytes are covered by the cornea and are organized in a manner that prevents the entry of microorganisms and potentially harmful chemical substances[42]. They are the predominant cells organized in four layers (basal, spinosum, granulosum, and stratum corneum) and are connected to each other through desmosomes and to the basal layer through hemidesmosomes. These cells are not inert and participate in the immune response by secreting cytokines, chemokines, antimicrobial peptides, components of the complement system, and arachidonic acid metabolites[43,44,45]. In addition, they are involved in the phagocytosis of bacteria and fungi[44]. Langerhans cells (LCs) and Merckel cells and a fluctuating population of inflammatory cells lie between keratinocytes and melanocytes[40].

Underneath the epidermis is the basal membrane, followed by the dermis, with a diversity of cells such as fibroblasts, mastocytes, macrophages, and transitory inflammatory cells as well as the skin appendixes and the vascular and nervous plexus[40,45]. The skin annexes consist of the pilosebaceous unit and the sweat glands.

Transcutaneous penetration of chemical products occurs via passive diffusion according to different concentration gradients[46]; usually, only particles that weigh up to 500 Da can penetrate the skin[47]. In general, structures with a size of up to 1000 nm penetrate the intact skin in flexural areas; in other areas, if the skin is not intact, particles of up to 7,000 nm can reach the deeper skin strata[10]. This process presents physical obstacles such as the corneal layer, an essentially lipophilic structure[48].

Sweat glands and hair follicles represent points that exhibit lower resistance to the penetration of substances into the skin because they have direct openings on the skin surface[48]. In general, it has been reported that particles smaller than the diameter of the follicular opening, between 10 and 210 µm, can penetrate the skin through that route[48].

Cells involved in the skin's innate response

Neutrophils are the major components of the innate immune response, although they are not naturally present in the skin[48]. Monocytes leave the blood circulation and reach the skin, where they transform into macrophages[49]; they exist in large numbers, particularly in the dermis, near the basal membrane[50,51].

Antigen-presenting cells are responsible for the internalization, processing, and presenting of antigens to T cells in lymph nodes, thereby contributing to acquired immunity[52]. In the skin, two populations of these cells are observed: LCs and dermal dendritic cells[53].

LCs are a subtype of immature dendritic cells present in the epidermis[54], in the basal and suprabasal layers, whose extensions cover an area of approximately 20% of the epidermis, thereby facilitating antigen capture[55]. Dendritic cells are predominantly found in the dermis and perform functions to those of LCs[53].

Mastocytes present in the skin are located around capillaries, arterioles, and vennules of the dermis and hypodermis[50]. Basophils are present in lesions in some skin disorders such as atopic dermatitis, hives, and itching, and they are structurally and functionally similar to mastocytes[56].

The distribution pattern of eosinophils in the skin is similar to that in other tissues; their number increases during allergic and autoimmune processes[54,57,58].

Soluble elements of the skin innate immune response

Proteins of the complement system reach the skin via diffusion through vessels that have their permeability altered by an inflammatory stimulus[50]. Other cells such as monocytes and macrophages present in the skin or attracted to it at the start of inflammation can also be a source of these proteins. The synthesis of several elements of the complement system by keratinocytes, such as C3; C5; C7; C8; C9; factor B; regulatory factor H; factor I; complement receptors CR1, cC1qR, C5aR, and CR 2; and regulatory cytokines has been described previously[59].

Cells involved in the skin adaptive response: T and B lymphocytes

T lymphocytes represent 1% to 3% of the population of epidermal cells[39,50,60], whereas lymphocytes B are not found in the intact skin or oral mucosa[39]. These T cells are memory CD45R0+ lymphocytes that express adhesion molecules on their surface, such as the cutaneous lymphocyte antigen (CLA), in addition to chemokine receptors and enzymes that contribute to migration to the skin[61].

Under homeostasis conditions, T lymphocytes are present in the epidermis near the basal membrane and LCs and in the dermoepidermal junction around the postcapillary vennules of the superficial and deep plexus[50,39].

Method

A bibliographic review of scientific articles was conducted, including clinical and laboratory studies, case reports, reviews, editorials, letters, and websites on the topic. The bibliographic search focused on the immune system, anatomy, and specificities of skin immunology, carbon allotropes, and their interactions. We considered articles and documents (printed or online) published until November 30, 2011.

The electronic databases Academic Search Premier (EBSCO), Cross Search (Isi Web Services WOK), Medline, Ovid MEDLINE, Scielo, and Scopus were used for the bibliographic search. Bibliographies of the selected articles were also reviewed to search for unselected sources. The search was limited to documents written in English, French, Spanish, and Portuguese.

A list of the relevant applications and physical and chemical characteristics of the nanomaterials under study was prepared in addition to a brief description of the cutaneous immunological structure. Moreover, a report of the possible interactions of nanomaterials with each element of the skin immune response was prepared.

Interaction between carbon allotropes and elements of the innate immune response

Interaction with the elements of the physical barrier and skin penetration

The penetration of CNTs and fullerenes into keratinocytes and other skin cells has been studied not only to assess the toxic effects but also to develop matrices that carry pharmaceutical compounds. Monteiro-Riviere et al.[62] demonstrated that MWCNTs can penetrate cultured keratinocytes after observing them in cytoplasmic vacuoles via electron transmission microscopy (ETM). Bullard-Dillard et al.[63] described the ability of fullerene C_{60} and of a more soluble version of the nanomaterial, fullerene C_{60}, derived from quaternary ammonia salts, to be taken up by immortalized human keratinocytes. Both the fullerene forms were internalized by the keratinocytes, although the substance derived from ammonia salts accumulated more slowly. The precise mechanism of the penetration of carbon allotropes into keratinocytes has not been described, although this internalization probably occurs through endocytosis or phagocytosis[64].

Analysis of cytotoxic effects of nanomaterials on cells of the cutaneous structure is relevant because their destruction can lead to alterations in the physical barrier as well as the production of cytokines that trigger the inflammatory process. Shvedova et al.[25] assessed the effect of SWCNTs in cultured HaCat keratinocytes and observed the induction of free radicals synthesis, accumulation of peroxides derivatives, reduction of antioxidants, and subsequent toxic effects on keratinocytes. Moreover, studies have suggested that regardless of the concentration of the nanomaterial in the medium, there is a difference in cytotoxicity among CNTs in the following order: MWCNO < MWCNT < SWCNT[65,66]. This difference may be explained by the diameter: smaller structures may exhibit a higher cytotoxic activity. The cytotoxic effect of fullerenes on keratinocytes depends on the nature of the radical used in the functionalization of the nanomaterial and its concentration[24,67].

Metal contaminants, present as residues of the process of nanomaterial synthesis, play a relevant role in cytotoxicity because they catalyze reactions that produce free radicals[25]. Murray et al.[26] exposed artificial human skin (EpidermFT) and murine epidermal cells JB6 P+ to partially purified and nonpurified SWCNTs containing iron. The JB6 P+ cells produced OH radicals when exposed to SWCNT; the process was inhibited when deferoxamine, a metal chelator, was added to the medium. The artificial skin, with human cells in the epidermis and dermis, after exposure to 75 µg of SWCNTs, exhibited hyperkeratosis; parakeratosis; an increase in basal cells in the epidermis and in fibroblasts in the dermis; and an increase in collagen fibers. Moreover, there was an increase in the synthesis of inflammatory cytokines after exposure to nonpurified SWCNT, particularly IL-6, IL-12, and IFN-γ. The association between the destruction of keratinocytes via the cytotoxic activity of the released cytokines and histological changes in the skin indicates a possible irritant contact dermatitis triggered by this class of nanomaterials[68].

The interaction of nanomaterials with the immune system necessarily depends on the ability of these structures to penetrate the intact skin or skin damaged by trauma or inflammatory processes. This internalization can occur via hair follicles and sweat glands, between cells or through cells; in this case, it occurs via processes such as endocytosis, direct penetration of the cell membrane, or transport through transmembrane channels when the nanoparticle is smaller than 5 nm[50].

Several characteristics intrinsic of nanomaterials appear to be relevant for analysis of skin penetration, such as size, hydrodynamic diameter, surface charge, shape, composition, zeta potential, aggregation, metabolism, and deformity[50].

Furthermore, at present, there is a great concern regarding carbon allotropes and occupational exposure. Previous studies have simulated work conditions susceptible of altering this internationalization, such as performing repetitive movements. Rouse et al.[69] have demonstrated the occurrence of passive diffusion of functionalized fullerenes [Baa-Lys (FITC)-NLS] in pig skin subjected to prolonged repetitive flections. ETM revealed that the penetration of these fullerenes predominantly occurred through the intercellular space and that after 24 h, they were located in the granular layer. In an attempt to simulate occupational exposure, Xia et al.[70] assessed the effect of different solvents on the penetration of fullerene C60-pristine in the skin of Yorkshire pigs. They observed that only fullerenes diluted in toluene, cyclohexane, and chloroform were present in the deep corneal layer. Although caution should be taken regarding the amounts and concentration of nanomaterials used in these experiments and their real concentration in the environment, one should be careful regarding the substances present in the workplace that may enhance the skin absorption and toxicity of nanomaterials.

Interaction with neutrophils

Most studies on the interaction of carbon allotropes with neutrophils have focused on the airways. This is because exposure to CNTs and fullerenes mostly occurs via the airways in occupational situations[71] (Table 1).

Neutrophils internalize functionalized and nonfunctionalized carbon allotropes and accumulate them in intracytoplasmic vesicles. The surface charge of these structures appears to influence the action of neutrophilic enzymes, and SWCNTs with a negative surface charge can be degraded by myeloperoxidases present in cytoplasmic vesicles[72]. This fact is important because it suggests that changes in the surface of nanomaterials can alter the biopersistent nature of these structures, thereby increasing biocompatibility and reducing toxic effects when they are used, for example, as drug carriers. Kagan et al.[72] produced CNTs after modeling sites active after the action of neutrophil myeloperoxidase. The results revealed that in contrast to compounds without the active sites under study, these compounds were susceptible to destruction by myeloperoxidase of laboratory animals, without the appearance of granulomatous lung lesions.

Once inside the cell, nanomaterials can affect the mechanisms of action of neutrophils. Thus, it is possible that nanomaterials such as fullerenes, which have antioxidant action, neutralize the reactive oxygen species (ROS) of phagolysosomes. Because these oxygen species are important for the degranulation of neutrophils, the function may be reduced by the effect of the immune response to infectious agents. Moreover, this neutralization of ROS can be useful in treating inflammatory dermatosis such as acne[17].

However, this depressor effect on the neutrophilic action was not observed in the study by Tsao et al.[73], where it was suggested that the carboxyfullerene increased the viability and bactericide power of these cells against gram-positive bacteria. Moreover, other studies have demonstrated the pro-inflammatory action of carbon allotropes, particularly those associated with lung instillation of nanomaterials with increased neutrophilic infiltrate. Kagan et al.[72] have suggested that the anti-inflammatory effects observed for the allotropes occurred when the structures used in the experiments were not functionalized. Additional studies need to be conducted for assessing the factors involved in these diverging responses.

A study with guinea pigs has suggested that skin contact with SWCNTs promotes the influx of neutrophils to the site only at high doses such as 160 µg[26]. The preferential location of the cell infiltrate around the follicles raises the question about a possible penetration via these structures. A study using CNTs instilled in lungs has shown that the neutrophilic infiltrate only occurs in lung tissue if the nanomaterial is not aggregated[74].

Interaction with macrophages

Phagocytosis of nanoparticles by macrophages depends on the physical and chemical properties of these structures, such as size, solubility, surface charge, and functionalization [75]. Thus, larger nanoparticles with a surface charge would have a greater chance of being internalized via this process.

Opsonization of nanoparticles, with the adhesion of antibodies and fractions of the complement system to their surface, instantaneously occurs when they are administered parenterally, thereby boosting their phagocytosis[76]. This observation can be transposed to nanoparticles capable of entering the skin, which would be more easily internalized by macrophages once opsonized by antibodies and complement factors present in the epidermis and dermis.

Internalization of carbon allotropes by macrophages can occur via phagocytosis, particularly when they are functionalized and aggregated[72,77]. Although most reports did not specify the term "pinocytosis," smaller and nonaggregated allotropes possibly penetrate the cell via this process.

Research on the cytotoxicity of carbon allotropes in macrophages is divergent. Although the reduction in cellular viability has not been demonstrated[77], it has been reported that the toxicity of these nanomaterials is dependent on their concentration[67], the presence of metal contaminants[24], and their solubility[78]. The fact that different concentrations were used in the experiments may explain this difference. The study in which cytotoxicity was not observed used nanoparticles at a concentration of 10 μgMl^{-1}[77], whereas the other studies used concentrations ranging from 30 μgMl^{-1} to 0.5 $mgMl^{-1}$[65,67]. It has also been shown that for this cell line, there was a difference between the cytotoxic potential of SWCNTs and MWCNTs; therefore, the latter did not change cell viability even at high concentrations[67].

With regard to functional changes, one study showed that only CNTs capable of stimulating phagocytosis induced an increase in IL-6 and TNF-α, which was not observed for structures that enter the membrane of macrophages via a different process[77]. The function of macrophages with regard to usual stimuli, such as bacterial structures, can also be reduced after exposure to carbon allotropes. Dumortier et al.[77] have demonstrated that this fact depends on functionalization because only SWCNTs that contained polyethylene glycol (PEG) had an effect on the macrocytic function.

The induction of macrophage maturation by these nanomaterials, analyzed via the expression of surface molecules such as CD40 and CD86, showed divergent results. Although a lower concentration of SWCNTs did not induce the expression of these molecules[77], both macrophage maturation and activation were observed in the study in which a higher amount of nanomaterial was used[67]. Thus, similar to the cytotoxic potential, macrophage activation by CNTs and fullerenes may be dose-dependent.

The inability of macrophages to phagocytize inert materials such as nanomaterials has been observed even when they are opsonized[76]. This process may lead to the accumulation of monocytic cells around the nanomaterial, with the formation of granulomas[79] in addition to enzyme release by inflammatory cells such as neutrophils, which in turn may lead to tissue damage[76]. In a study in which the formation of skin gra-

nulomas was analyzed, SWCNTs and MWCNTs of 20 and 80 nm, respectively, were implanted under the skin of guinea pigs[80]. The formation of granulomas was observed after 2 weeks; at a late stage, SWCNTs induced higher macrophage infiltration and the granulomas had thicker walls. The use of highly purified nanomaterials enabled the observation that these structures alone, without metal contaminants, can induce the formation of granulomas (Table 1).

Interaction with dendritic cells

Analysis of the interaction of nanomaterials with dendritic cells, the most effective antigen-presenting cells of the human body, aims to study the possible internalization, processing, and presentation of nanomaterials, as if they were antigens.

Carbon allotropes can be internalized by dendritic cells via macropinocytosis[81] and phagocytosis[32,82]; endocytosis has not been described in the reviewed studies. Internalization seems to be dependent on the concentration of the nanomaterial and on the size of the structures because those with a larger diameter are more easily internalized[82].

The results of the cytotoxic potential of carbon allotropes are conflicting. One study has described that the toxic action of SWCNTs and MWCNTs in dendritic cells is dependent on the concentration of the nanomaterials (3, 10, 30 μgMl^{-1}). On the other hand, Wang et al.[82] have reported that carboxylic MWCNTs of different sizes and concentrations (10, 50, 100 μgMl^{-1}), with metal contaminants, do not exhibit significant cytotoxic activity on these cells. In the first study, the physical and chemical characteristics of CNTs were not described, such as functionalization, which could explain the differences that were observed. A higher concentration of the nanomaterial and the presence of varying amounts of metal contaminant would indicate a higher toxic effect, in contrast to what was observed by Wang et al.[82].

Moreover, the potential immunomodulator role of carbon allotropes in dendritic cells has been studied. CNTs can be conjugated with peptides considered as weak immunogens via unknown mechanisms and induce internalization by dendritic cells, thereby leading to the activation of CD4+ T lymphocytes, which in turn develop Th2 immunity, with the production of antibodies[81]. This characteristic may have wide applicability in the development of immunotherapy, particularly in cancer treatment; tumor antigens that have been considered as weak immunogens till date may have their immune potential amplified by conjugation with nanomaterials. On the other hand, it remains unknown whether nanomaterials can bind to autologous peptides, turning them into strong immunogens, which are then recognized by antigen-presenting cells and result in the development of autoimmune diseases.

With regard to dendritic cell activation, which is an essential process for these cells to be able to present antigens to T cells, fullerenes and CNTs exhibited distinct results. The former, according to Yang et al.[83], can induce the expression of lymphocyte co-stimulatory molecules, chemokine receptors, and inflammatory cytokines on the surface of cells, whereas CNTs the latter cannot[67,82]. The consequences of these processes should be studied. Usually, the presentation of antigens identified as potentially harmful to dendritic cells triggers their processing and cell maturation. Thus, whether fullerene can be recognized as an antigen to be presented to T cells or whether it only interferes with cell-signaling mechanisms (with unknown effects) should be studied. In addition, the fact that CNTs did not activate dendritic cells in the abovementioned experiments does not exclude the possibility that these nanomaterials act as haptens by binding peptides and becoming strong immunogens.

Although Langerhans cells are important for the presentation of skin antigens, no studies analyzed this interaction using carbon allotropes. Skin dendritic cells were also not mentioned in the studies under analysis. The importance of reproducing the findings of studies using the human skin may be reinforced by other studies involving other nanomaterials applied in skin immunization. Manolova et al.[53] have demonstrated that skin antigen-presenting cells were only relevant for polystyrene internalization and transport when they were larger than 200 nm. Smaller structures could spontaneously migrate through skin lymphatic vessels to the skin's draining lymph nodes, where they would be internalized by dendritic cells. Thus, if carbon allotropes and silver nanoparticles exhibit a similar behavior, it is possible that they only interact with skin antigen-presenting cells if they are aggregated or if they are longer than 200 nm, in case of CNTs (Table 1).

Interaction with mastocytes

Carbon allotropes can be internalized by human skin mastocytes; a study using fullerenes showed that this process occurs via endocytosis[84]. In the cell, fullerenes can be found inside organelles and in the cytoplasm[84].

Studies have indicated that carbon allotropes have distinct effects on mastocytes. Although CNTs in high concentrations are associated with the influx of mastocytes to the application site and an increase in degranulation[26], fullerenes appear to inhibit this process as well as release cytokines[85,86]. This fact may have implications in the use of these nanomaterials because CNTs may be related to nonimmunological cases of hives and angioedema in addition to anaphylactoid reactions, whereas fullerenes may be employed to prevent these reactions. The location of fullerenes in lysosomes and the mitochondria, which are sites of ROS production, may explain the inhibitory action on mastocytes[84] (Table 1).

Analysis of the effect of other factors in the activation of mastocytes, such as the concentration and functionalization of the nanomaterial, should be studied further. Even if the amount of histamine released is not sufficient to induce systemic alterations, it should be noted that the substances synthesized by mastocytes alter the vascular permeability, thereby favoring the migration of inflammatory cells to the skin and resulting in various effects.

Table 1. Summary of the interactions between carbon allotropes and innate immune response cells

	Neutrophil	Macrophage	Mastocyte	Dendritic cell
Cell permeation	Phagocytosis Pinocytosis?	Phagocytosis Pinocytosis?	Endocytosis?	Phagocytosis, Macropinocytosis- Dependent on size
Cytotoxicity	Not observed (minnow fish)	SWCNT: dependent on size and concentration in the medium (Assessed cells: Balb-c, RAW 264.7)	Not observed (Human mastocytes)	Divergent results May be dependent on concentration (Assessed cells: rat and human dendritic cells)
Functional alterations	Decreases the expression of neutrophilic enzymes Inhibits degranulation Suppresses the mechanism of NETosis	IL-6, TNF-α, TGF-β Induction Divergence of induction of surface molecules Metal contaminant: alters the redox potential Induction of skin granulomas	Increases skin influx CNT: increases degranulation Fullerene: decreases degranulation	CNT: não observadas Fulerenos – indução de citocinas e moléculas de superfície

Interaction with eosinophils

Although eosinophils participate in skin allergic processes, few studies have been found to focus on these cells and carbon allotropes.

SWCNTs, MWCNTs, and carbon black injected in the airways of mice, associated with ovoalbumine allergen (OVA), can induce an increase in eosinophils in the absence of bronchoalveolar lavage[87]. However, an increase in eosinophils has not been observed in the experiments using skin implants of carbon allotropes (Table 1).

Interaction with the complement system

Carbon allotropes can bind to some fractions of the complement system[29] and activate the classical route, alternative route[76],and lectin route[88]; however, some results are conflicting [29]. This activation of the complement by CNTs can be reduced or inhibited through changes in the surface of the nanomaterial, such as functionalizaton by covalent bonds[88].

The interaction of the proteins of the complement system with nanoparticles can increase the purification of these structures and thus reduce the bioavailability of nanoparticle drugs or induce a pseudoallergic and anaphylactoid reaction, the latter being caused by potent anaphylatoxins such as C4a, C3a, and C5a.

Despite the production of various elements of complement routes by cells of the epidermis and dermis and despite these factors reaching the skin through the bloodstream, no studies assessed the activation of the system at the site.

Interaction of carbon allotropes with elements of the adaptive immune response

Interactions with T lymphocytes

Functionalized CNTs can penetrate T lymphocytes[77], and studies on the cytotoxicity in these cells have discrepant results[77,89], which may be explained by differences in the methods used in the experiments. Functionalization can increase cytotoxicity, which also depends on the time of exposure and on the quantity of CNT[89].

With regard to lymphocyte function, CNTs appear to favor the activation of CD4+ T lymphocytes over CD8+ T cells. Koyama et al.[80] implanted BALB/c, SWCNTs (0.8 to 2.2 nm), CSNTs (cup stacked-type NTs) (50 to 150 nm), and MWCNTs (20 and 80 nm) under the skin of mice and, in most experiments, observed an increase in CD4+ T lymphocytes in the peripheral blood and reduction in CD8+ T cells in the ini tial weeks (Table 2).

Table 2. Summary of the interactions between carbon allotropes and adaptive immune response cells

Lymphocyte T	Lymphocyte B	
Cell permeation	Endocytosis?	Endocytosis?
Cytotoxicity	- Divergent results - Solubility may influence (Assessed cells: Balb-c, Jukart)	- SWCNT: not observed (Assessed cells: Balb-c) - Fullerene: dependent on concentration (Assessed cells: Raji)
Functional alterations	- Increased CD4+ T cells in skin implants - Direct activation: not observed - Activation of macrophages into Th1 (IFN-γ) - Increased response to weak antigens - May induce Th1 and Th2 responses	- Induction of immunoglobulin against weak antigens - Development of anti-C60 IgG that cross-reacts with C70

Once CD4+ T lymphocytes are activated, the developed immune response may be either of the Th1 type or of the Th2 type. Factors such as functionalization and other characteristics of nanomaterials may be involved in the type of lymphocyte response. This immunostimulating characteristic of carbon allotropes may be applied in the development of vaccines, thereby boosting the immune response against weak antigens.

The cytotoxicity observed in Jurkat T cells, commonly used in studies of T-cell viability, was not observed in human cells, which raises questions about the utility of this cell line.

Interaction with B lymphocytes and antibodies

CNTs and fullerenes appear to be able to enter B lymphocytes[78,91]. Although functionalized SWCNTs do not activate B cells and are not toxic to them[77], experiments with fullerenes have shown cytotoxicity and immunosuppression of B lymphocytes[90,91]. In this case, there should be some caution regarding variation in the concentration of the nanomaterial and the presence of functionalization.

Another interesting point in the study of B cells is their ability to recognize nanomaterials as antigens and trigger an undesired production of antibodies. Chen et al.[92] have demonstrated the development of IgG antibodies against C_{60} fullerenes, with cross-reaction with C_{70}. This finding may be important for the development of vaccines and for the assessment of risks associated with the use of carbon allotropes in pharmaceutical products. The induction of IgG directed against nanomaterials may be related to the reduction in the viability of the administered drugs as well as to inflammatory processes by the deposition of immunoglobulin. The remaining antibodies in the organism, nonspecific for carbon allotropes, have a weak interaction with these nanomaterials and are thus not relevant to the opsonization process[92] (Table 2).

Conclusion

The evolution of nanotechnology depends on the development of biocompatible nanomaterials, which involves understanding the interaction between these structures and the immune system.

In it is often difficult to distinguish between innate and adaptive immunity owing to the interconnection between these two types of immune responses. Although the isolated description of each component of immunity facilitates the assessment, it may hide the interaction between them.

The interaction between carbon allotropes and various elements of the innate and adaptive immune responses is wide and complex. Most cited studies have referred to experiments conducted in mice or *in vitro*; in the latter case, the cells of the immune system that were used were not necessarily human skin cells. Thus, caution should be taken when transposing the observed results to the human skin because immune interactions in living organisms may alter the responses that are observed in *in vitro* experiments.

Despite the importance of the physical and chemical characteristics of nanomaterials used in the experiments for assessing biological properties, no standardization was observed in their description. This fact, in addition to the use of different techniques, may hinder data interpretation.

In general, carbon allotropes are potentially involved in irritant and granulomatous skin reactions in addition to angioedema, hives, and anaphylactoid reactions. New studies are required to establish the risks and benefits resulting from the interaction between nanomaterials and SIS.

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