Trends in Immunotherapy (2023) Volume 7 Issue 1
doi:10.24294/ti.v7.i1.2039

REVIEW ARTICLE

Revolutionizing cancer treatment by boosting dendritic cell vaccine efficacy with graphene oxide

Rafeezul Mohamed[1,2,3*], Muhammad Mahyiddin Ramli[4], Ali H Reshak[5], Nurul Huda Osman[6], Mohd Yusmaidie Aziz[3,7], Mohd Syahir Mansor[3,8]

[1] *Department of Biomedical Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas 13200, Pulau Pinang, Malaysia. E-mail: rafeezul@usm.my*

[2] *Breast Cancer Translation Research Program (BCTRP), Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas 13200, Pulau Pinang, Malaysia.*

[3] *Advanced Management Liver Malignancies Research (ENRICH), Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas 13200, Pulau Pinang, Malaysia.*

[4] *Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, Kangar 01000, Perlis, Malaysia.*

[5] *Physic Department, College of Science, University of Basrah, Basra 61004, Iraq.*

[6] *Applied Electromagnetic Lab 1, Department of Physics, Faculty of Science, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia.*

[7] *Department of Toxicology, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas 13200, Pulau Pinang, Malaysia.*

[8] *Department of Biomedical Imaging, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas 13200, Pulau Pinang, Malaysia.*

ABSTRACT

Dendritic cells (DCs) are potent antigen presenting cells that play a crucial role in stimulating T cell responses against cancer. DC vaccines have been utilized as an immunotherapy approach for cancer treatment, but their effectiveness is hampered by challenges in the tumor microenvironment. Graphene oxide (GO), a cutting-edge carbon-based nanomaterial, has shown promise in modulating DC activation and function. This review highlights the recent advancements in DC vaccines and explores how GO can enhance their efficacy for cancer treatment. By leveraging the unique properties of GO, such as its biocompatibility and immunomodulatory effects, DC vaccines can potentially be optimized to overcome the limitations of the tumor microenvironment and achieve improved outcomes in cancer immunotherapy.

Keywords: Dendritic Cells; DC Vaccine; Immunotherapy; Graphene Oxide; Cancer

ARTICLE INFO

Received: 20 April, 2023
Accepted: 20 June, 2023
Available online: 17 July, 2023

1. Introduction of dendritic cells

Dendritic cells (DCs) play a crucial role in integrating the innate and adaptive immune responses and are considered the most effective professional antigen-presenting cells (APCs). The presence of DCs in lymphoid tissues and peripheral blood is crucial for antigen-specific immune responses. These cells uptake tumor-specific antigens through pattern recognition receptors (PRR), such as Toll-like receptors, and digest them into small peptides that are presented on major histocompatibility complex I (MHC-I) or MHC-II molecules expressed on the DCs surface[1]. Activated DCs then migrate into the lymphoid organ and bind with Th1 ($CD4^+$ T cells) cells through the MHC-II molecule[1]. This process stimulates the secretion of cytokine milieu such as IL-2 and IFN-γ, which are essential to activate and proliferate antigen-specific cytotoxic T lymphocytes (CTLs)/$CD8^+$ T cells (**Figure 1**)[2]. Alternatively, MHC-I molecules on DCs bind to $CD8^+$ T cells and generate

antigen-specific CTLs in the presence of Th1 cytokines[2]. Additionally, the MHC-II molecule on APCs binds with Th2 cells, stimulating the secretion of IL-4 and IL-10, as well as interaction with B cells to promote antigen-specific antibody production (**Figure 1**)[2]. T cell activation stimulates T cell differentiation into Th1, Th2, Th17, or regulatory T cells (Tregs) based on the cytokine environment[2]. Due to the effectiveness of DCs in processing and cross-presentation of antigens to both $CD4^+$ and $CD8^+$ T cells, they have been developed as vaccine platforms to elicit anti-tumor CTL $CD8^+$ T cell immune responses[3]. High penetration of blood stream-derived CTLs into tumors suppresses cancer cell growth because activated CTLs produce perforin and granzyme B that eliminate cancer cells[3].

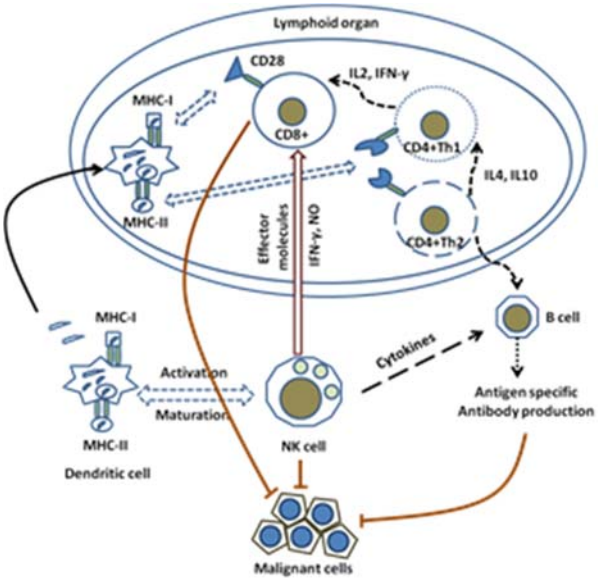

Figure 1. DCs stimulate $CD4^+$ and $CD8^+$ T cells to diminish malignant cells.

Developing effective DC vaccines is hindered by various challenges, including the limited immunogenicity of tumor-associated antigens (TAAs), low efficiency in loading antigens, and the immunosuppressive nature of the tumor microenvironment (TME). However, the emergence of nanomaterials, specifically graphene oxide (GO), offers promising avenues to enhance the efficacy of DC vaccines. GO enables the efficient loading of antigens and adjuvants through diverse mechanisms such as electrostatic and hydrophobic interactions, as well as covalent binding. Moreover, it facilitates the targeted enrichment of TAAs in lymph nodes by leveraging its unique physical and chemical properties. This comprehensive review aims to shed light on several aspects, including the crucial role of DCs in the immune system, the impact of the TME on the function of DCs, the advancements in DC vaccine production and progress within clinical settings, and the potential of GO to significantly improve the efficacy of DC vaccines for cancer immunotherapy.

1.1 Impaired function of DCs in the tumor microenvironment

The tumor microenvironment (TME) is a complex ecosystem comprising various cellular and non-cellular components. The cellular components of TME include immune cells such as lymphocytes, macrophages, neutrophils, DCs, and mast cells[4]. These immune cells interact with tumor cells and modulate their growth and spread[4]. A substantial body of evidence suggests that the TME is composed of a diverse array of immune cells that promote tumor growth and metastasis. These include tumor-associated neutrophils (TANs), tumor-associated macrophages (TAMs), Th2 cells, and Tregs. Collectively, these immune cells contribute to the establishment of an immunosuppressive environment, facilitating the survival and metastasis of tumor cells, and facilitating evasion of immune destruction[5]. However, there are still several adaptive immune cells that play an important role in the inhibition of cancer, such as CTLs, Th1 and natural killer (NK) cells[4]. In contrast, T regulatory (Treg) cells promote the growth of cancer cells because the activation of CTLs and Th1 that function in the killing of cancer cells could be inhibited by Treg cells[6]. Furthermore, tumor-associated macrophages (TAMs) secrete IL-4 and promote tumor progression as well as the penetration of cancer cells into the bloodstream[7]. In addition, tumor growth is also stimulated by myeloid-derived suppressor cells (MDSCs) since it promotes IL-4 secretion, ROS production, and arginine metabolism[8]. Pro- and anti-tumoral cells detected in TME are shown in **Figure 2**. The mode of action for each pro and anti-tumoral cells were summarised in **Table 1**.

Table 1. Mechanism of action of each pro-tumoral and anti-tumoral cells in TME

	Cells	Mechanism of action	References
Pro-tumor	Tregs	Hinders the activation and effectiveness of CTLs and Th1 cells via secretion of TGF-β and IL-10 that lead to the promotion of immune tolerance and assists the tumor in evading the immune system's attack.	[6,9]
	Th2 cells	Suppressing Th1 cell-mediated immunity and stimulate M2 macrophages via secretion of IL-4 that exacerbate tumor growth by accelerating angiogenesis.	[7,10]
	TAMs (M2 macrophage)	Exerting pro-tumoral effects by producing anti-inflammatory cytokines (e.g., interleukin-10, TGF-β) that inhibit CTLs and Th1 cells activation, secrete growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), which stimulate angiogenesis.	[7,11]
	MDSCs	Upregulate immune-inhibiting molecules such as Arginase-1 and inducible nitric oxide synthase (iNOS) that inhibit Th1 and CTLs activation, reduced antibody dependent cell mediated cytotoxicity (ADCC) function by NK cells that promote tumor progression.	[8,12]
Anti-tumor	CTLs ($CD8^+$ T cells)	Activated CTLs recognize TAA presented on MHC-I molecules and exert anti-tumoral effects by directly destroying tumor cells via perforin and granzyme B.	[12,13]
	Th1 cells	Secreting IFN-γ that promote CTLs activity, induce an anti-tumoral immune response, and hinder tumor growth.	[12]
	NK cells	Directly recognize and destroy tumor cells by secreting perforin and granzymes, inducing apoptosis in tumor cells via ADCC, produce IFN-γ that activate CTLs and Th1 immune responses and inhibit tumor growth.	[10,12]
	M1 macrophages	Producing pro-inflammatory cytokines (e.g., IFNγ, IL-1β, and TNF-α) and reactive oxygen/nitrogen species, which contribute to tumor cell death and the initiation of an immune response against the tumor.	[10,12]
	DCs	Capture TAA secreted from tumor cells, process them, and present them to CTLs cells, promoting CTLs activation that generate of an anti-tumoral immune response.	[10,12]

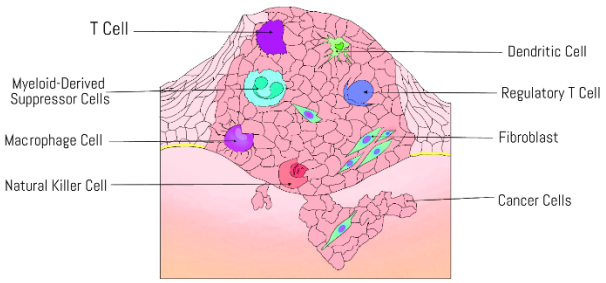

Figure 2. Tumor microenvironment.

The composition of non-cellular components of the TME are extracellular matrix (ECM), blood vessels, and wide arrays of soluble factors exist surrounding a tumor. The ECM is a complex network of various proteins namely collagen, fibronectin, glycosaminoglycans, laminin and proteoglycans[14]. ECM acts as a scaffold that support tissues structural. In TME, the ECM undergoes remodelling that alters its composition and organization. Modified ECM can stimulate tumor cell migration, invasion, and angiogenesis[14]. Blood vessel in tumor play important role in supplying oxygen and nutrients for their growth. In TME, abnormal blood vessels more prominent and tend to obstruct the delivery of oxygen and drugs, promote tumor cell intravasation, and accelerate tumor metastasis[14]. Various soluble factors such as growth factors, cytokines, chemokines, and extracellular vesicles secreted by tumor cells and surrounding stromal cells, such as fibroblasts and immune cells in TME can regulate tumor cell proliferation, survival, angiogenesis, immune responses, and tissue remodelling[14]. They create a dynamic communication network between tumor cells and the surrounding TME.

Within the TME, DCs also play a crucial role in stimulating tumour-specific CTLs, thus eliciting anti-tumor immune responses[8,15]. Human DCs can be classified as conventional DCs (cDCs) and plasmacytoid DC (pDC). cDCs can be further categorised as cDC1 ($CD141^+$ DCs) and cDC2 ($CD1c^+$ DCs) subsets[16]. Despite their crucial role in anti-cancer immunity, many evidence suggest that DCs in tumours become largely defective due

to lack of maturation markers and are no longer capable to activate CTLs to kill cancer[17]. Furthermore, they are often outnumbered by other myeloid cell subsets, such as TAMs and MDSCs that actively suppress the anti-tumor responses[18]. Recently, Lavin *et al.* reported a decrease in cDC1 accompanied by a low number of activated $CD8^+$ T cells in the tumors of early stage lung adenocarcinoma patients[19]. These observations suggest that tumor-derived factors actively suppress normal DCs function and recruitment to the TME, which, in turn, directly impacts the efficacy of DC vaccines[20,21].

1.2 DC vaccine

DC vaccines, which have emerged as a potent tool for reactivating the immune system to combat tumor cells, have undergone significant advancements in the past two decades. The typical process of preparing a DC vaccine involves inducing patient-derived monocytes with GM-CSF and IL-4 to generate DCs, exposing them to variety of tumor-associated antigen (TAA) from various sources to induce maturation, and ultimately administering the mature DCs to the recipient (**Figure 3**)[22]. DCs treatment has been shown to increase the number of these cells in the draining lymph node, where they engage with T cell to induce immune responses[23]. While it has been demonstrated that only a small percentage of injected DCs migrate to the lymph node, most of the administered DCs accumulate at the site of vaccination, triggering a strong immune response within the area due to the development of tertiary lymphoid tissues[24]. A variety of tumor antigens can be utilised, including tumor DNA, tumor RNA, tumor derived peptides, whole tumor cells, whole tumor protein and viral vector-encoding proteins[25]. In vivo DC vaccines have been investigated using various approaches such as DC-targeting delivery vectors, immunoliposomes, or moieties targeting receptors on DCs, and tumor antigens may be delivered in chimeric protein complexes[26,27]. DC vaccines exhibit minimal toxicity compared to traditional cytotoxic therapies, allowing them to be used for the clinical treatment of various cancers[22]. Food and Drug Agency (FDA) has approved DC vaccine namely Provenge to treat an asymptomatic or minimally-symptomatic castration-resistant prostate cancer[28]. Moreover, the randomized trials have been carried out in multicenter and indicated a high clinical efficacy of DCs pulsed with an immunogenic WT1 mRNA[29], whereby autologous DCs pulsed with patient-derived tumor cells has stimulated potent anti-tumoral immune response in acute myeloid leukaemia or multiple myeloma patients[30,31]. However, despite promising results in some clinical trials, most objective clinical responses have been far from satisfactory.

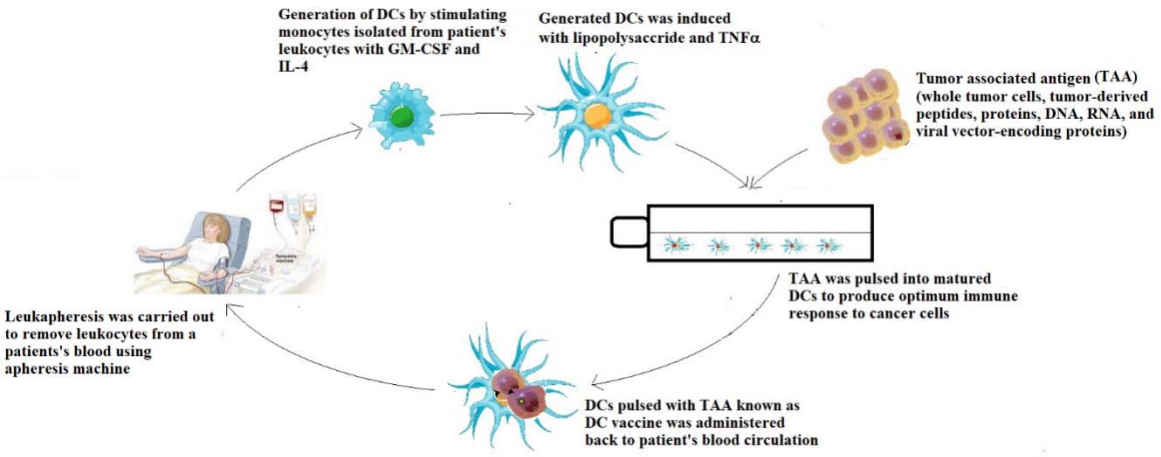

Figure 3. A typical process of DC vaccine preparation from patient's blood sample.

The lackluster performance of DC vaccines in clinical settings is largely due to tumor-induced immunosuppression. The effectiveness of these vaccines depends on a wide array of immune factors and their molecular mechanisms. The efficacy of

DC-based vaccines is often hindered by immunosuppression, immune subversion and immune penetration in the TME caused by tumor burden[32]. Poor immunogenicity, as a result of MHC molecules or tumor antigen damage, is a major contributor to immune evasion[32]. The migration and penetration of immunosuppressive cells like Tregs and MDSCs can also disrupt the anti-tumor response[20]. Immunosuppressive cytokines such as CCL22, IL-10, TGF-β and VEGF, can stimulate these cells to prevent the anti-tumor immune response[33–36]. DCs in the TME may also be phenotypically immature, leading to their reduced functionality, possibly due to their inherent plasticity[37]. Thus, novel and potent DC vaccines need to be developed to improve their anti-tumor performance.

2. Graphene oxide (GO)

Graphene oxide (GO) has gained significant attention in recent years due to its unique mechanical, chemical, and electrical properties. Structurally, GO can be described as a monolayer of carbon atoms arranged in a dense honeycomb structure, with oxygen-containing functional groups filling its basal plane, as shown in **Figure 4**[38]. It composed of an array of carboxylate groups encompassing its periphery. These groups bring about a negative charge and colloidal stability that are contingent on the pH level. Additionally, the basal plane of GO exhibits hydroxyl and epoxide groups, rendering it both polar and hydrophilic. The untouched sections of the basal plane, however, retain their hydrophobic nature[39]. These oxygen-containing groups provide opportunities for covalent and non-covalent modifications, making GO highly versatile for different application fields[39]. One of the distinctive features of GO is its increased interlayer spacing compared to pristine graphite. While the interlayer spacing of graphite is typically 0.335 nm, it increases to more than 0.625 nm in GO due to the presence of oxygen-containing functional groups[40]. This expanded interlayer spacing of GO allows for improved dispersion in solution and easy mixing with different polymers and materials, enhancing the properties of composite materials[40]. Furthermore, the high affinity of GO to water molecules makes it easier to disperse within solution, providing an advantage over graphene. This property enables efficient blending of GO with other materials, leading to enhanced properties in composite materials. Moreover, the carbon/oxygen ratio of GO can be used to evaluate the extent of oxidation during the conversion of graphene to GO, providing a means to check the potency of the oxidation process.

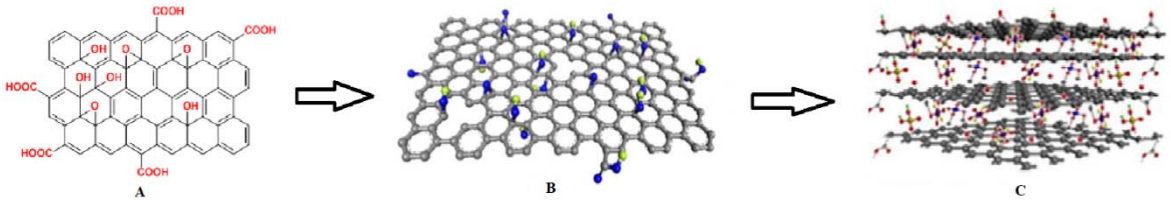

Figure 4. The structure of GO. A) Lattice structure of GO, B) Mono-layer of GO, C) Multi-layer of GO.

The importance of various GO synthesis processes lies in their ability to produce scalable quantities of GO while allowing control over its oxidation level, functional group incorporation, colloidal stability, and sheet characteristics to control the properties of GO, allowing for tailored synthesis for GO specific applications. There are several methods for synthesizing GO, including mechanical or thermal exfoliation, chemical vapor deposition (CVD), and epitaxial growth[41]. There are two main categories of GO synthesis methods: bottom-up and top-down. Bottom-up methods involve constructing pristine graphene from simple carbon molecules, while top-down methods extract graphene derivatives from a carbon source, typically graphite[42]. However, bottom-up methods such as chemical vapor deposition (CVD) and epitaxial growth are time-consuming and face scalability challenges[42]. As a result, GO synthesis has shifted more towards the top-down approach.

The earliest known synthesis of GO was presented by Brodie, who mixed graphite with HNO_3

and $KClO_3$ at 60 °C for several days[43]. Staudenmaier later improved upon this method in 1898 by incorporating chlorates such as $KClO_3$ or $NaClO_3$ and adding concentrated H_2SO_4 to enhance the solution's acidity[44]. However, both Brodie and Staudenmaier methods produced toxic ClO_2 gas and posed a risk of explosion. To address this issue, Hummer developed a modified approach in which graphite was treated with acid (H_2SO_4 and HNO_3) that did not produce hazardous ClO_2 gas, albeit requiring a longer reaction time[45]. In 1958, Hummers and Offeman discovered a further modification by reacting graphite with $KMnO_4$ and concentrated H_2SO_4 while preserving oxidation levels[45]. This process disrupted the π-π interaction on GO's surface, resulting in lower electrical conductivity. However, the hydrophilic characteristics of GO, produced from oxygen-containing functional groups, improved its dispersibility in aqueous solvents, making it more suitable for bio-applications due to its water dispersibility and biocompatibility[46].

Understanding the carbon source, pre-treatment methods, oxidizing agent, and choice of solvent with protonation is crucial for tailoring Hummer's approach to specific applications, as these factors can affect the carbon-to-oxygen (C/O) ratio of the final product. **Figure 5** provides a general overview of common approaches for GO synthesis.

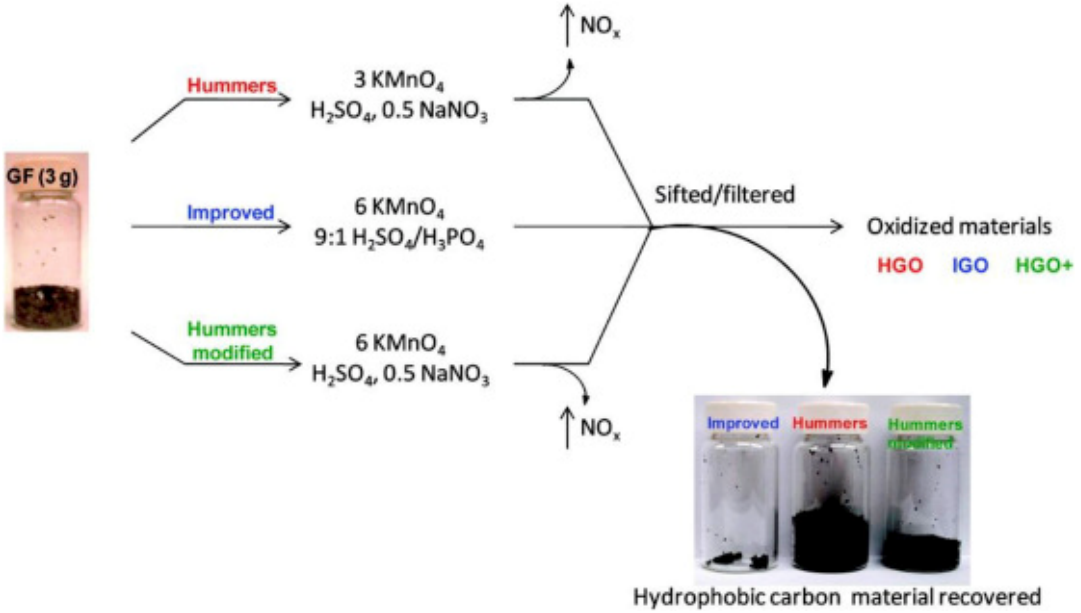

Figure 5. Schematic of most common GO synthesis methods[47].

2.1 Different size, shape of GO and antigen-coupled GO effects on DCs

GO can be synthesised into different forms depending on its size, shape and functionalization. Mono layer-GO (mono-GO) is a single layer GO sheets. These sheets typically have lateral dimensions on the micrometer scale and are often characterized by their large surface area[48]. Multi layer-GO (multi-GO) composed of several stacked layers of graphene oxide sheets. It retains some of the properties of individual GO layers but may display enhanced mechanical stability and electrical conductivity due to the interlayer interactions[49]. Small-GO (S-GO) generally refers to GO sheets with smaller lateral dimensions, typically in the range of nanometers to a few micrometers[48]. S-GO can exhibit properties such as increased flexibility, improved dispersibility, and higher reactivity due to their higher surface-to-volume ratio[48]. Large-GO (L-GO) refers to GO sheets with relatively larger lateral dimensions, typically in the range of tens to hundreds of micrometers[50]. These sheets can be useful for applications requiring large-area coverage or macroscopic assembly of GO materials[50]. Antigen-coupled GO involves the functionalization of GO sheets with specific antigens that can elicit an immune response in organisms[51]. By coupling antigens onto GO surfaces, it

becomes possible to create biofunctionalized materials for various applications, including biosensing, drug delivery, and immunotherapy[51].

DCs play a crucial role in the activation of innate and adaptive immune responses in various diseases, making them a promising target for therapeutic applications of GO[52]. Initial study has shown that pristine GO induces maturation of DCs by upregulating the expression of HLA-DR/MHC-II, CD40, CD83, CD86, and CCR6, and enhancing secretion of TNF-α and IL-1β[53]. Furthermore, GO coupled with anti-glioma antigen (GO-Ag) has been found to elicit a potent anti-glioma immune response mediated by DCs in vitro[54]. Another study by Li *et al*. revealed that GOx nanosheets coupled with ovalbumin antigen (GOx-OVA) promote DC activation and secretion of various effector cytokines that are crucial for $CD8^+$ T cell differentiation[55]. Interestingly, different effects of mono-GO and multi-GO on mouse dendritic cell line (DC2.4) have been observed. Both mono-GO and multi-GO can stimulate DC2.4 cells to produce reactive oxygen species (ROS) and TNF-α, with or without LPS stimulation[56]. However, pristine GO and mono-GO do not induce IL-6 production in DC2.4 cells under LPS influence, whereas multi-GO enhances IL-6 production in the same cells[56]. In a recent study, it was found that S-GO flakes are easily internalized by DCs without significantly affecting their viability, activation phenotype, or cytokine production[57]. On the other hand, L-GO flakes predominantly interact with the plasma membrane of DCs, with a muted impact on DC viability or activation[57]. Interestingly, delivery of OVA via S-GO flakes significantly enhances DCs' ability to induce proliferation of OVA-specific $CD4^+$ T cells in vitro[57]. Conversely, delivery of OVA via L-GO flakes augments DCs' ability to induce proliferation of OVA-specific $CD8^+$ T cells, as well as their production of IFN-γ and granzyme B, indicating distinct modulation of DCs function by different GO flakes[57]. The summary of how size and shape influence DCs activation was illustrated in **Figure 6**.

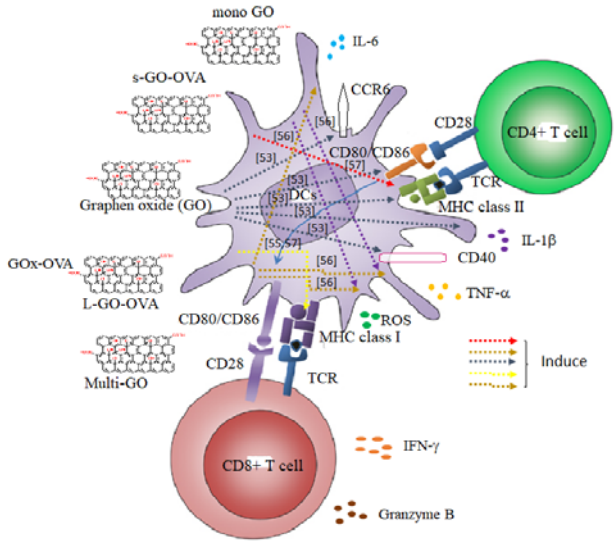

Figure 6. Different shape, size and functionalization of GO influence DCs activation. L-GO improves efficacy of DC vaccine against SARS-CoV-2.

Recent study published in the journal *Advanced Materials* by a team of researchers from Beijing has proposed a novel approach to enhance the efficacy of DC vaccines against SARS-CoV-2, the virus responsible for COVID-19[58]. The team hypothesized that by modulating the interaction between DCs and T cells, the immune response could be improved. To test this hypothesis, they developed a new platform using GO, a material known for its beneficial properties in biomedicine[58]. Previous studies have shown that ex vivo DCs, which are grown and/or modified outside of the body, require adjuvants to activate T cells. However, the currently available adjuvants have limited effectiveness, with response rates of less than 15% in cancer patients receiving standard DCs immunotherapy. Therefore, the researchers sought an alternative and turned to GO[58].

The team first investigated the effect of particle size of graphene nanosheets on their interaction with DCs using fluorescence labeling and transmission electron microscopy. Interestingly, they found that nanosheets with diameters greater than 1 μm adhered strongly to the surface of DCs, while those smaller than ~500 nm were mostly internalized by the cells[58]. Next, the researchers examined the dynamic interaction between T cells and DCs with and without the GO nanosheets. They defined meaningful interaction by determining the contact area and duration between DCs and T cells[58].

They found that the direct contact area between DCs and T cells was approximately four-fold higher in the group treated with L-GO nanosheets compared to untreated DCs, and approximately two-fold higher compared to small nanosheets[58]. The L-GO nanosheets acted like a "nanozipper", adhering to the surface of DCs and bringing together large clusters of DCs and T cells, creating a stable microenvironment for effective cell interactions and T cell activation[58]. The scientists described this phenomenon as the first evidence that L-GO nanosheets showed selective adherence to different cell membranes[58]. The high binding affinity with DCs membranes facilitated DCs-T cell clustering, while the low binding affinity with T cells prevented interference with DCs-T cell interactions[58].

These clusters of DCs and T cells induced a more than twenty-fold higher antigen-specific T cell response compared to conventional cytokine-cocktail adjuvants, and resulted in >99.7% clearance of viral RNA from lung tissues in mice inoculated with SARS-CoV-2[58]. The researchers concluded that the robust immune responses induced by DC vaccines using GO nanosheets could serve as a promising reference for developing personalized antiviral therapy against the global COVID-19 pandemic[58]. The summary of how L-GO increased DCs-T cells clustering to activate Rho-ROCK-MLC pathway and stimulate the binding of ICAM-1 and LFA-1 to fully activated $CD8^+$ T cells to diminish COVID-19 was illustrated in **Figure 7**. This approach can be implemented to increase the efficacy of DC vaccine for cancer treatment using L-GO.

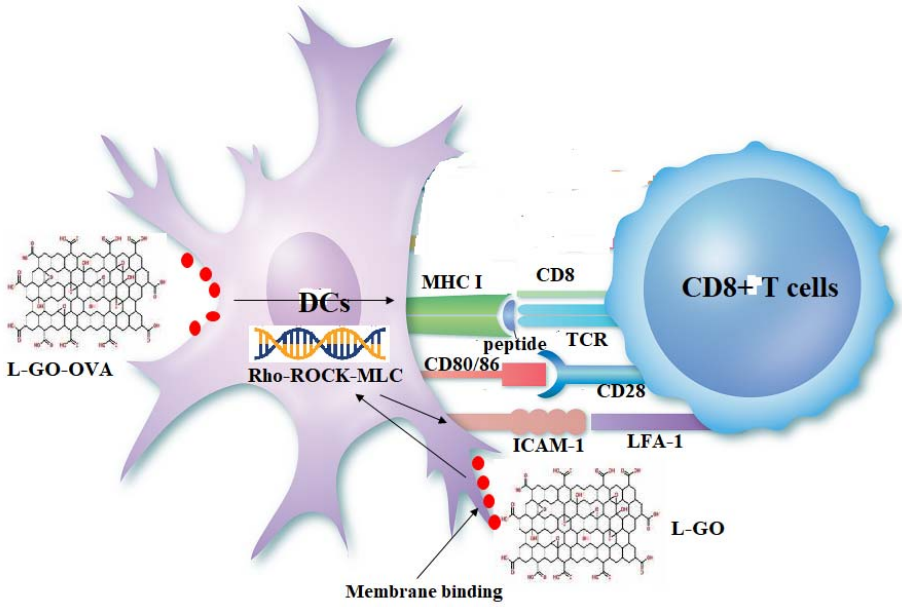

Figure 7. The intense binding capacity of L-GO to DCs membrane increase DCs-T cells receptors interaction which facilitate the full activation of $CD8^+$ T cells.

2.2 Other perspectives on how GO can enhance DC vaccine performance

Immunotherapeutic strategies for various diseases now include vaccination, with the addition of adjuvants to enhance vaccine performance and reduce the need for high-dose vaccines. In recent years, research has focused on developing adjuvants for vaccine preparations, such as alum, liposomes, and GO nanosheets. GO nanosheets have shown potential as universal adjuvants for DC vaccines, particularly in cancer treatment, as they can be tailored to different antigens. GO has been used as a delivery vehicle for vaccine antigens, effectively internalizing antigens into DCs and promoting cross-presentation to $CD8^+$ T cells, leading to anti-tumor responses[55]. However, GO's low solubility and poor stability in the human body, as well as its cytotoxicity and potential DNA damage, have

limited its application[59,60]. To overcome these limitations, functionalized GOs (FGOs) have been extensively studied as carriers and adjuvants. The surface chemistry of nanomaterials is crucial for biocompatibility, and covalent modification with oxygen-based functional groups has been a common method to fabricate FGOs with improved biocompatibility[61]. Some examples of FGOs that have been successfully synthesized through this method include GO-PEI, GO-PEG, GO-PEG-PEI, GO-PAMAM, and others. For instance, GO-PEG-PEI has a positively charged surface that can adsorb negatively charged antigens with ultra-high loading efficiency, promoting DC aggregation and antigen uptake[61,62]. GO-PEG-PEI has also been shown to induce DC maturation, upregulate co-stimulatory molecules, and enhance T cell proliferation and cytokine secretion, suggesting its potential as an adjuvant for improved immunogenicity and cellular immunity[63]. Additionally, GO can be covalently linked with alum for higher antigen loading efficiency, and OVA-loaded GO-AlO(OH) has been shown to increase antigen uptake by DCs and facilitate cytosolic delivery, indicating its potential as an effective antigen delivery system[64]. GO has also been proposed to protect proteins from proteolysis, and functionalization with chitosan has been shown to improve biocompatibility and stimulate cytokine production for enhanced cellular immune response[64]. RNA-based antigen approaches have shown promise in clinical studies, and GO-bound total RNA of tumors may elicit more potent antitumor responses in DC vaccines, as GO can enhance RNA stability and hinder RNase degradation[65].

2.3 Clinical translational challenges of using GO for DC vaccine-based immunotherapy

GO has shown tremendous results in enhanced DC vaccine-based immunotherapy, but there are several clinical translational challenges that need to be addressed. These challenges emerge from the complex interactions between GO and the immune system, safety concerns, and the need for optimized delivery strategies. GO has been displayed to have immunomodulatory effects that can modulate the immune response[52]. While this can be beneficial for eliciting the efficacy of DC vaccines, the exact mechanisms and long-term effects of GO on the immune system are not fully understood. Further research is required to enlighten the immunomodulatory effects of GO and decide the optimal dosage and treatment regimen for safe and effective immunotherapy. The biocompatibility and potential toxicity of GO are critical concerns for clinical translation[51]. While GO has demonstrated low toxicity in many in vitro and animal studies, its long-term effects and potential accumulation in vivo need to be carefully evaluated[51]. Comprehensive preclinical studies that involve toxicity assessments, biodistribution studies, and long-term safety evaluations, are necessary to ensure the safe use of GO in humans[51]. Understanding the pharmacokinetics and biodistribution of GO is essential for its clinical translation. The size, shape, surface charge, and functionalization of GO can influence its distribution, accumulation, and clearance from the body[66]. Detailed studies investigating the fate of GO after administration, including its systemic distribution and elimination pathways, are required to ensure proper dosing and minimize potential side effects[66]. Efficient delivery of GO to target cells, such as DCs, is crucial for the success of immunotherapy. Strategies for GO delivery need to be optimized to ensure effective uptake by DCs and minimal off-target effects. Various delivery systems, such as nanoparticles, liposomes, or biomaterial-based carriers, can be explored to enhance the specificity and efficiency of GO delivery to DCs[67]. Establishing standardized protocols for GO synthesis, functionalization, characterization, and quality control is essential for clinical translation[68]. Consistent and reproducible production of GO with well-defined physicochemical properties is necessary to ensure reliable and comparable results across different studies and to meet regulatory requirements.

3. Conclusion

GO has shown great promise in its ability to play a pivotal role in activating DCs and influencing various aspects of T cell effector function. This phenomenon appears to be dependent on factors

such as size, shape, and functionalization of GO. Notably, recent studies have revealed the crucial role of L-GO in promoting the assembly of the DC-T cell immune synapse, offering new insights for engineering DC vaccines based on enhancing DC-T cell communication. The potential of GO and its functionalized derivatives as adjuvants in vaccine formulations is significant, as they can enhance vaccine stability, antigen delivery, and immune response. However, interdisciplinary collaboration between researchers, clinicians, and regulatory authorities are required in addressing several aforementioned clinical translational challenges. Rigorous preclinical evaluation, including comprehensive toxicity studies and optimization of delivery strategies, is crucial to establish the safety and efficacy of GO in DC vaccine-based immunotherapy.

Author contributions

RM, MMR, MYA and MSM conceived of the scope of the review, drafted and revised the manuscript.

AHR and NHO helped in revise the manuscript and provided many valuable comments about the manuscript. All authors read and approved the final manuscript.

Funding

This research was funded by strategic research fund (ENRICH) (311/CIPPT/411988) and Research University Fund for BCTRP (1001/CIPPT/8070033) from Universiti Sains Malaysia.

Conflict of interest

The authors declare no potential conflict of interest.

References

1. Wculek SK, Cueto FJ, Mujal AM, *et al.* Dendritic cells in cancer immunology and immunotherapy. Nature Reviews Immunology 2020; 20(1): 7–24. doi: 10.1038/s41577-019-0210-z.
2. Wang Y, Xiang Y, Xin VW, *et al.* Dendritic cell biology and its role in tumor immunotherapy. Journal of Hematology & Oncology 2020; 13(1): 107. doi: 10.1186/s13045-020-00939-6.
3. Yu J, Sun H, Cao W, *et al.* Research progress on dendritic cell vaccines in cancer immunotherapy. Experimental Hematology & Oncology 2022; 11: 3. doi: 10.1186/s40164-022-00257-2.
4. Parente P, Parcesepe P, Covelli C, *et al.* Crosstalk between the tumor microenvironment and immune system in pancreatic ductal adenocarcinoma: Potential targets for new therapeutic approaches. Gastroenterology Research and Practice 2018; 2018: 7530619. doi: 10.1155/2018/7530619.
5. Wang M, Zhao J, Zhang L, *et al.* Role of tumor microenvironment in tumorigenesis. Journal of Cancer 2017; 8(5): 761–773. doi: 10.7150/jca.17648.
6. Chen BJ, Zhao JW, Zhang DH, *et al.* Immunotherapy of cancer by targeting regulatory T cells. International Immunopharmacology 2022; 104: 108469. doi: 10.1016/j.intimp.2021.108469.
7. Malekghasemi S, Majidi J, Baghbanzadeh A, *et al.* Tumor-associated macrophages: Protumoral macrophages in inflammatory tumor microenvironment. Advanced Pharmaceutical Bulletin 2020; 10(4): 556–565. doi: 10.34172/apb.2020.066.
8. Wang Y, Jia A, Bi Y, *et al.* Targeting myeloid-derived suppressor cells in cancer immunotherapy. Cancers 2020; 12(9): 1–24. doi: 10.3390/cancers12092626.
9. Verma A, Mathur R, Farooque A, *et al.* T-regulatory cells in tumor progression and therapy. Cancer Management and Research 2019; 11: 10731–10747. doi: 10.2147/CMAR.S228887.
10. Galli F, Aguilera JV, Palermo B, *et al.* Relevance of immune cell and tumor microenvironment imaging in the new era of immunotherapy. Journal of Experimental & Clinical Cancer Research 2020; 39: 89. doi: 10.1186/s13046-020-01586-y.
11. Farc O, Cristea V. An overview of the tumor microenvironment, from cells to complex networks (Review). Experimental and Therapeutic Medicine 2021; 21(1): 96. doi: 10.3892/etm.2020.9528.
12. Labani-Motlagh A, Ashja-Mahdavi M, Loskog A. The tumor microenvironment: A milieu hindering and obstructing antitumor immune responses. Frontiers in Immunology 2020; 11: 940. doi: 10.3389/fimmu.2020.00940.
13. Ostroumov D, Fekete-Drimusz N, Saborowski M, *et al.* CD4 and CD8 T lymphocyte interplay in controlling tumor growth. Cellular and Molecular Life Sciences 2018; 75: 689–713. doi: 10.1007/s00018-017-2686-7.
14. Anderson NM, Simon MC. The tumor microenvironment. Currrent Biology 2020; 30(16): R921–R925. doi: 10.1016/j.cub.2020.06.081.
15. Binnewies M, Mujal AM, Pollack JL, *et al.* Unleashing type-2 dendritic cells to drive protective antitumor $CD4^+$ T cell immunity. Cell 2019; 177: 556–571. doi: 10.1016/j.cell.2019.02.005.

16. Collin M, Bigley V. Human dendritic cell subsets: An update. Immunology 2018; 154(1): 3–20. doi: 10.1111/imm.12888.
17. Gupta YH, Khanom A, Acton SE. Control of dendritic cell function within the tumour microenvironment. Frontiers in Immunology 2022; 13: 733800. doi: 10.3389/fimmu.2022.733800.
18. Law AMK, Valdes-Mora F, Gallego-Ortega D. Myeloid-derived suppressor cells as a therapeutic target for cancer. Cells 2020; 9(3): 561. doi: 10.3390/cells9030561.
19. Lavin Y, Kobayashi S, Leader A, *et al.* Innate immune landscape in early lung adenocarcinoma by paired single-cell analyses. Cell 2017; 169(4): 750–765. doi: 10.1016/j.cell.2017.04.014.
20. Veglia F, Gabrilovich DI. Dendritic cells in cancer: The role revisited. Current Opinion in Immunology 2017; 45: 43–51. doi: 10.1016/j.coi.2017.01.002.
21. Lee JH, Choi SY, Jung NC, *et al.* The effect of the tumor microenvironment and tumor-derived metabolites on dendritic cell function. Journal of Cancer 2020; 11(4): 769–775. doi: 10.7150/jca.38785.
22. Ding J, Zheng Y, Wang G, *et al.* The performance and perspectives of dendritic cell vaccines modified by immune checkpoint inhibitors or stimulants. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer 2022; 1877(5): 188763. doi: 10.1016/j.bbcan.2022.188763.
23. Yu Z, Liu W, He Y, *et al.* HLA-A2.1-restricted ECM1-derived epitope LA through DC cross-activation priming $CD8^+$ T and NK cells: A novel therapeutic tumour vaccine. Journal of Hematology & Oncology 2021; 14(1): 71. doi: 10.1186/s13045-021-01081-7.
24. Najibi AJ, Mooney DJ. Cell and tissue engineering in lymph nodes for cancer immunotherapy. Advanced Drug Delivery Reviews 2020; 161–162: 42–62. doi: 10.1016/j.addr.2020.07.023.
25. Galati D, Zanotta S. Hematologic neoplasms: Dendritic cells vaccines in motion. Clinical Immunology 2017; 183:181–190. doi: 10.1016/j.clim.2017.08.016.
26. Badiee A, Davies N, McDonald K, *et al.* Enhanced delivery of immunoliposomes to human dendritic cells by targeting the multilectin receptor DEC-205. Vaccine 2007; 25(25): 4757–4766. doi: 10.1016/j.vaccine.2007.04.029.
27. Cauwels A, Van Lint S, Garcin G, *et al.* A safe and highly efficient tumor-targeted type I interferon immunotherapy depends on the tumor microenvironment. Oncoimmunology 2017; 7(3): e1398876. doi: 10.1080/2162402X.2017.1398876.
28. Madan RA, Antonarakis ES, Drake CG, *et al.* Putting the pieces together: Completing the mechanism of action Jigsaw for Sipuleucel-T. Journal of the National Cancer Institute 2020; 112(6): 562–573. doi: 10.1093/jnci/djaa021.
29. Anguille S, Van de Velde AL, Smits EL, *et al.* Dendritic cell vaccination as postremission treatment to prevent or delay relapse in acute myeloid leukemia. Blood 2017; 130(15): 1713–1721. doi: 10.1182/blood-2017-04-780155.
30. Amberger DC, Schmetzer HM. Dendritic cells of leukemic origin: Specialized antigen-presenting cells as potential treatment tools for patients with myeloid leukemia. Transfusion Medicine and Hemotherapy 2020; 47(6): 432–443. doi: 10.1159/000512452.
31. Rosenblatt J, Avivi I, Vasir B, *et al.* Vaccination with dendritic cell/tumor fusions following autologous stem cell transplant induces immunologic and clinical responses in multiple myeloma patients. Clinical Cancer Research 2013; 19(13): 3640–3648. doi: 10.1158/1078-0432.CCR-13-0282.
32. Garrido F, Ruiz-Cabello F, Aptsiauri N. Rejection versus escape: The tumor MHC dilemma. Cancer Immunology, Immunotherapy 2017; 66(2): 259–271. doi: 10.1007/s00262-016-1947-x.
33. Sun X, Cui Y, Feng H, *et al.* TGF-β signaling controls Foxp3 methylation and T reg cell differentiation by modulating Uhrf1 activity. Journal of Experimental Medicine 2019; 216(12): 2819–2837. doi: 10.1084/jem.20190550.
34. Chen JY, Lai YS, Chu PY, *et al.* Cancer-derived VEGF-C increases chemokine production in lymphatic endothelial cells to promote CXCR2-dependent cancer invasion and MDSC recruitment. Cancers 2019; 11(8): 1120. doi: 10.3390/cancers11081120.
35. Bah I, Kumbhare A, Nguyen L, *et al.* IL-10 induces an immune repressor pathway in sepsis by promoting S100A9 nuclear localization and MDSC development. Cellular Immunology 2018; 332: 32–38. doi: 10.1016/j.cellimm.2018.07.003.
36. Rapp M, Wintergerst MWM, Kunz WG, *et al.* CCL22 controls immunity by promoting regulatory T cell communication with dendritic cells in lymph nodes. Journal of Experimental Medicine 2019; 216(5): 1170–1181. doi: 10.1084/jem.20170277.
37. Burgdorf S, Porubsky S, Marx A, Popovic ZV. Cancer acidity and hypertonicity contribute to dysfunction of tumor-associated dendritic cells: Potential impact on antigen cross-presentation machinery. Cancers 2020; 12(9): 2403. doi: 10.3390/cancers12092403.
38. Jiříčková A, Jankovský O, Sofer Z, Sedmidubský D. Synthesis and applications of graphene oxide. Materials 2022; 15(3): 920. doi: 10.3390/ma15030920.
39. Yu W, Sisi L, Haiyan Y, Jie L. Progress in the functional modification of graphene/graphene oxide: A review. RSC Advances 2020; 10(26): 15328–15345. doi: 10.1039/d0ra01068e.
40. Shahriary L, Athawale AA. Graphene oxide synthesized by using modified Hummers approach.

International Journal of Renewable Energy and Environmental Engineering 2014; 2(1): 58–63.
41. Moosa AA, Abed MS. Graphene preparation and graphite exfoliation. Turkish Journal of Chemistry 2021; 45(3): 493–519. doi: 10.3906/kim-2101-19.
42. Chua CK, Pumera M. Chemical reduction of graphene oxide: A synthetic chemistry viewpoint. Chemical Society Reviews 2014; 43(1): 291–312. doi: 10.1039/c3cs60303b.
43. Brodie BC. On the atomic weight of graphite. Philosophical Transactions of the Royal Society of London 1859; 149(149): 249–259. doi: 10.1098/rstl.1859.0013.
44. Ojha K, Anjaneyulu O, Ganguli AK. Graphene-based hybrid materials: Synthetic approaches and properties. Current Science 2014; 107: 397–418.
45. Hummers WS, Offeman RE. Preparation of graphitic oxide. Journal of the American Chemical Society 1958; 80: 1339. doi: 10.1021/ja01539a017.
46. Lee J, Kim J, Kim S, Min DH. Biosensors based on graphene oxide and its biomedical application. Advanced Drug Delivery Reviews 2016; 105: 275–287. doi: 10.1016/j.addr.2016.06.001.
47. Marcano DC, Kosynkin DV, Berlin JM, *et al.* Improved synthesis of graphene oxide. ACS Nano 2010; 4(8): 4806–4814. doi: 10.1021/nn1006368.
48. Ma J, Liu R, Wang X, *et al.* Crucial role of lateral size for graphene oxide in activating macrophages and stimulating pro-inflammatory responses in cells and animals. ACS Nano 2015; 9(10): 10498–10515. doi: 10.1021/acsnano.5b04751.
49. Smith AT, LaChance AM, Zeng S, *et al.* Synthesis, properties, and applications of graphene oxide/reduced graphene oxide and their nanocomposites. Nano Materials Science 2019; 1(1): 31–47. doi: 10.1016/j.nanoms.2019.02.004.
50. Zhao J, Pei S, Ren W, *et al.* Efficient preparation of large-area graphene oxide sheets for transparent conductive films. ACS Nano 2010; 4(9): 5245–5252. doi: 10.1021/nn1015506.
51. Cao W, He L, Cao W, *et al.* Recent progress of graphene oxide as a potential vaccine carrier and adjuvant. Acta Biomaterialia 2020; 112: 14–28. doi: 10.1016/j.actbio.2020.06.009.
52. Yunus MA, Ramli MM, Osman NH, Mohamed R. Stimulation of innate and adaptive immune cells with graphene oxide and reduced graphene oxide affect cancer progression. Archivum Immunologiae et Therapiae Experimentalis 2021; 69(1): 20. doi: 10.1007/s00005-021-00625-6.
53. Zhi X, Fang H, Bao C, *et al.* The immunotoxicity of graphene oxides and the effect of PVP-coating. Biomaterials 2013; 34(21): 5254–5261. doi: 10.1016/j.biomaterials.2013.03.024.
54. Wang W, Li Z, Duan J, *et al.* In vitro enhancement of dendritic cell-mediated anti-glioma immune response by graphene oxide. Nanoscale Research Letters 2014; 9(1): 311. doi: 10.1186/1556-276X-9-311.
55. Li H, Fierens K, Zhang Z, *et al.* Spontaneous protein adsorption on graphene oxide nanosheets allowing efficient intracellular vaccine protein delivery. ACS Applied Materials & Interfaces 2016; 8(2): 1147–1155. doi: 10.1021/acsami.5b08963.
56. Yang Z, Pan Y, Chen T, *et al.* Cytotoxicity and immune dysfunction of dendritic cells caused by graphene oxide. Frontiers in Pharmacology 2020; 11: 1206. doi: 10.3389/fphar.2020.01206.
57. Parker H, Gravagnuolo AM, Vranic S, *et al.* Graphene oxide modulates dendritic cell ability to promote T cell activation and cytokine production. Nanoscale 2022; 14(46): 17297–17314. doi: 10.1039/d2nr02169b.
58. Zhou Q, Gu H, Sun S, *et al.* Large-sized graphene oxide nanosheets increase DC-T-cell synaptic contact and the efficacy of DC vaccines against SARS-CoV-2. Advanced Materials 2021; 33(40): e2102528. doi: 10.1002/adma.202102528.
59. Zheng T, Gao Y, Deng X, *et al.* Comparisons between graphene oxide and graphdiyne oxide in physicochemistry biology and cytotoxicity. ACS Applied Materials & Interfaces 2018; 10(39): 32946–32954. doi: 10.1021/acsami.8b06804.
60. Jia PP, Sun T, Junaid M, *et al.* Nanotoxicity of different sizes of graphene (G) and graphene oxide (GO) in vitro and in vivo. Environmental Pollution 2019; 247: 595–606. doi: 10.1016/j.envpol.2019.01.072.
61. Gu Y, Guo Y, Wang C, *et al.* A polyamidoamne dendrimer functionalized graphene oxide for DOX and MMP-9 shRNA plasmid co-delivery. Materials Science and Engineering: C 2017; 70: 572–585. doi: 10.1016/j.msec.2016.09.035.
62. Wang B, Su X, Liang J, *et al.* Synthesis of polymer-functionalized nanoscale graphene oxide with different surface charge and its cellular uptake, biosafety and immune responses in Raw264.7 macrophages. Materials Science and Engineering: C 2018; 90: 514–522. doi: 10.1016/j.msec.2018.04.096.
63. Xu L, Xiang J, Liu Y, *et al.* Functionalized graphene oxide serves as a novel vaccine nano-adjuvant for robust stimulation of cellular immunity. Nanoscale 2016; 8(6): 3785–3795. doi: 10.1039/c5nr09208f.
64. Wang X, Cao F, Yan M, *et al.* Alum-functionalized graphene oxide nanocomplexes for effective anticancer vaccination. Acta Biomaterialia 2019; 83: 390–399. doi: 10.1016/j.actbio.2018.11.023.
65. Liao Y, Zhou X, Fu Y, Xing D. Graphene oxide as a bifunctional material toward superior RNA protection and extraction. ACS Applied Materials & Interfaces 2018; 10(36): 30227–30234. doi: 10.1021/acsami.8b12522.
66. Rhazouani A, Gamrani H, El Achaby M, *et al.* Synthesis and toxicity of graphene oxide nanoparticles: A literature review of in vitro and in vivo studies. BioMed Research International 2021; 2021: 5518999. doi: 10.1155/2021/5518999.

67. Liang J, Zhao X. Nanomaterial-based delivery vehicles for therapeutic cancer vaccine development. Cancer Biology & Medicine 2021; 18(2): 352–371. doi: 10.20892/j.issn.2095-3941.2021.0004.

68. Reina G, González-Domínguez JM, Criado A, *et al.* Promises, facts and challenges for graphene in biomedical applications. Chemical Society Reviews 2017; 46(15): 4400–4416. doi: 10.1039/c7cs00363c.