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Graphene and Derivates: Physico-Chemical and Toxicology Properties in the mRNA Vaccine Manifacturing Strategy

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1. Abstract

Aim of this work is to verify the state of the art related the use of graphene and its derivates in some vaccine technology (m RNA), to show the chemical properties of this kind of carriers (or extractive agent) and to list the evidence at today related peculiarity involved in some covid-19 vaccine. The researchers will produce their own opinion about this topic.

Of great relevance to verify the manifacturing procedure of this innovative product (m- Rna vaccine) and the tecnology and material used in purification phases.

2. Introduction

It is interesting to observe some interesting article and research published recently related graphene and derivates in some covid-19 -vaccine or in blood of vaccinated patients.

The works published of CAMPRA P (1) , YOUNG R O (2) but also YOUNG Mi LEE (3) et al and GIOVANNINI F et al (4) need an depply investigationa about the chemical – phisical properties of this carriers for vaccine tecnology.

The same the chemical – pharmaceutical and toxicological point of view is relevant.

In legal –jurisditionsl languages there is an interesting sententia: 3 clues make a proof.

Every one, reading this article can produce an proper opinion about a relevant and crucial topic in this last 2 year after introduction of some covid-19 vaccine.

Some article related toxiciy of graphene products was writtenbefore covid-19 pandemia and related new vaccine production.

3. 26 August 2021 BBC news

3.1. Coronavirus Pandemic

Japan suspends 1.6 million Moderna doses over contamination fears

A staff of Japan"s supermarket group Aeon receives a dose of the Moderna vaccine

"Japan has suspended the use of about 1.6 million doses of the Moderna- vaccine due to contamination.

The health ministry said HM "foreign materials" were found in some doses of a batch of roughly about 560,000 vials.

Takeda Pharmaceutical, which sells and distributes the vaccine in Japan, said Moderna had put three batches on hold "out of an abundance of caution".

It said an issue at a manufacturing- contract site in Spain was the likely cause, but did not elaborate.

"To date, no safety or efficacy issues have been identified," Moderna said, adding that it would work with regulators and Takeda to investigate the matter further.

There are no details of what the "foreign objects" are, but Takeda described it as particulate matter PM, after which it said conducted an emergency examination.

Reports of contamination also came from seven other vaccination centres, according to the Japan Times new-spaper, with 39 vials - or 390 doses - found to have been affected."

From https://www.europeanconsumers.it/2021/08/29/grafene-rischi-di-una-tecnologia-emergente

Related: Japan suspends 1.6 million Moderna doses over contamination fears

"A ministry of health MH, work and welfare manager, said "it is a substance that react with magentes and it is a metal. Is seem that it was introduced during the manifacturing process".

3.2. And In article

Foreign Materials in Blood Samples of Recipients of COVID19 Vaccines

Young Mi Lee etal

"Recently, there have been reports (Broudy, Kyrie, 2021; Wilson, 2021) that some of the denied contents (Figure reported of the statement by the US CDC) are actually included in the COVID-19 vaccines: La Quinta Columna reported poisonous Graphene Oxide (GO) nano-particles in COVID-19 vaccines (Campra, 2021), and Robert O. Young, reported a vade- mecum of evidence of toxic Nano-metallic particles, graphene- oxide GO structures, and parasites in the COVID-19 vaccines (Young, 2021).

The KoVeDocs also found foreign materials and, as the vaccines were warmed to room temperature, moving and living micro-organisms and parasites could be detected in the Pfizer and Modern mRNA COVID-19 vaccines (Jeon, 2022). After the KoVeDocs held 2 nation wide Press Conferences — Dec 30, 2021 and Jan 13, 2022 many vaccinated Koreans volunteered their personal fluid samples to enable further indirect assessment of the contents of COVID-19 vaccines contents detected after vaccination for COVID-19 in their blood and in materials extracted from their bodies by foot immersion bathing.

In our own research work, as detailed above in Figure reported, we found various sizes and shapes of foreign materials and metal-like particles in the plasma samples of recipients of the Pfizer and Moderna COVID-19 vaccines.

In Figure reported we show similarities between foreign materials we found in blood- plasmas from Pfizer (Comirnaty) and Moderna COVID-19 mRNA vaccine recipients, respectively, and similar foreign- materials found in a separate prior study by Jeon (2022) in the vaccines themselves. Although we could not identify the specific components of any of the foreign objects in this study on account of the limitations of our laboratory equipment (we note that foreign materials and shapes of objects in COVID-19 vaccines were analyzed by Robert O. Young and his colleagues with more powerful equipment and procedures, and they identified certain of the announced ingredients such as histidine, sucrose, PEG (Poly-ethylene glycol), and ethylene alcohol in all Pfizer, Moderna, and AstraZeneca vaccines, but they also found components that the CDC had claimed were not used — including reduced graphene- oxide (rGO), or graphene hydroxide (GH), in the Moderna vaccine. The diameter they observed at 100µm was apparently the same as the one we have pictured above in Figure reported. We also showed a slightly smaller very similar structure in Figure reported. (see original article) In addition to those foreign- materials, we found various crystalline shaped, metal-plate like foreign structures in the plasma samples from recipients of the Pfizer COVID-19 vaccine as seen in Figure reported, and in the plasma samples from recipients of the Moderna COVID-19 vaccine as seen in Figure reported, and I. These metal-like materials also resemble closely ones found earlier with more powerful microscopes and examination protocols.

Pablo Campra, a full professor of chemical sciences at the University of Almeira in Madrid reported finding nano-structures of

graphene oxide (GO) and other materials later confirmed by Robert O. Young (2021) and other doctors (Wilson, 2021). Campra used Electron- Scanning Microscopy (SEM) and Transmission Electron -Microscopy (TEM) to find graphene nano-particles and nano-sheets in the Pfizer Comirnaty vaccine. In follow-up work, Robert O. Young and his colleagues found rGO, GH, and Trypanosoma cruzi parasites in the COVID-19 vaccines. We ourselves also found, in the present study, circular and oval disc-like plates and various-sizes of metal-like plates in the plasma of COVID-19 vaccine recipients. At such a time as this, and in the present context of world-wide unrest and the threat of World War III, international co-operation is urgently needed to confirm the presence of rGO, GH, and parasites in the COVID-19 vaccines and to discern their relationship to the foreign materials we have documented here in the blood -plasmas of COVID-19 vaccine recipients.

In South Korea, not only medical doctors, but non-medical volunteers are reporting foreign materials in blood samples of individuals who had received one or more COVID-19 vaccine doses. After the KoVeDocs made a public presentation at the Korea Press Center on January 13th, 2022, some of those volunteers up-loaded their own findings in the group blog shown in Figure reported.

In addition to all of the foregoing, we found that United States US Patent No. US2012/0265001 A1 signifies a composite magnetic nano-particle drug delivery system using a magnetic nano-particle to deliver a therapeutic pharmaceutical composition to a targeted site of a human body in a time-controlled manner. The multi-layered disc-like foreign materials seen in our Figure may well be exemplars of such a composite magnetic nano-particle drug delivery system. In any case it seems that the various-sized metal-plate-like particles are foreign to the body's blood plasma and are almost certainly involved in the adverse reactions ADR of recipients of the COVID-19 vaccines. It is interesting that some internet sites are recommending ways to detoxify (Daily News Break, 2021; Health Wellness Daily, 2021; Holistic Health Online, 2021) the harmful substances now known to be present in the COVID-19 vaccines as shown in the studies by Pablo Campra, Robert O. Young, and others (Föhse et al., 2021; Jiang & Mei, 2021; Jeon, 2022).

Generalized detoxifying methods may be applied to the known and yet to be identified foreign materials found in COVID-19 vaccine recipients: among those believed to be effective are Vit. C, vit. D3, N-Acetylcysteine, glutathione, suramin, pine needle tea, shikimate, aspirin, melatonin, zinc, quercetin, magnesium citrate, and hydration are recommended unless contra-indicated (Campra, 2021)." (3) (Figure 1).

Edouard Alphandéry J. Phys. Mater. 3 (2020) 034009 https://doi.org/10.1088/2515-7639/ab9317

Journal of Physics: Materials Graphene, other carbon nano-materials and the immune system: toward nanoimmunity-by-design Arianna Gazzi et al:

"Graphene is one of the most renowned 2-dimensional (2D) materials, characterized by a planar sheet of sp2–carbons arranged in a hexagonal lattice . This outstanding material has attracted increasing interest and expectation in the scientific community due to its unique physico-chemical properties, including high

surface-area-to-volume ratio, mechanical resistance, light-weight, flexibility, chemical inertia with respect to water and organic solvents, reduced atomic thickness, optical transparency, as well as high thermal ,electrical -conductivity" (5)

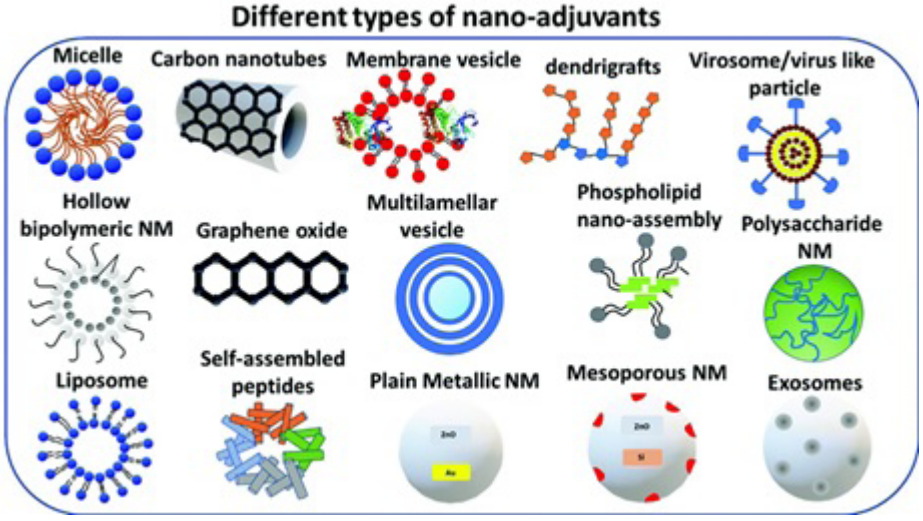

Figure 1: from Nano dimensions/adjuvants in COVID-19 vaccines

4. Material and Methods

Whit an observational point of view interesting and relevant literature is analyszed. Al reported article comes form scientific database or comes from university works. Table reported (n1) and figure form 1 to 15 are integrative material for the scope of this work. After this review part and experimental hypotesys project is submitted to the researcher in order to produce a global conclusion related the topics of this work

5. Results

5.1. From Literature

5.1.1. Ligeng Xu et al: "Benefiting from their unique physico-chemical properties, graphene derivatives have attracted great attention in biomedicine. In this study, we carefully engineered graphene- oxide (GO) as a vaccine adjuvant for immuno-therapy using urease B (Ure B) as the model antigen. Ure B is a specific antigen for Helicobacter -pylori HE-PY, which is a class I carcinogen for gastric cancer. Polyethylene glycol (PEG) and various types of polyethylenimine (PEI) were used as coating polymers. Compared with single-polymer modified GOs (GO-PEG and GO-PEI), certain dual-polymer modified GOs (GO-PEG-PEI) can act as a positive modulator to promote the maturation of dendritic cells (DCs) and enhance their cytokine secretion through the activation of multiple toll-like receptor (TLR) pathways while showing low toxicity.This GO-PEG-PEI can serve as an antigen carrier to effectively shuttle antigens into DCs. These 2 advantages enable GO-PEG-PEI to serve as a novel vaccine adjuvant. In the subsequent in vivo experiments, compared with free Ure B and clinically used aluminum-adjuvant-based vaccine (Alum-Ure B), GO-PEG-PEI-Ure B induces stronger cellular- immunity via intra-dermal administration, suggesting promising applications in cancer immuno-therapy IT. Our research work not only presents a novel, highly effective GO-based vaccine nano-adjuvant, but also highlights the critical roles of surface chemistry for the rational design of nano-adjuvants." (6)

5.1.2. Ya Liu et al: "During the last decades period, there has been growing interest in using therapeutic messager RNA (mRNA) together with drug delivery systems. Naked, un-formulated mRNA is, unable to cross the cell membrane and is susceptible to degradation. Here we use in, this work, graphene quantum dots (GQDs) functionalized with polyethyleneimine (PEI) as a novel mRNA delivery system. Our results show that these modified GQDs can be used to deliver intact and functional mRNA to Huh-7 hepato-carcinoma cells at low doses and, that the GQDs are not toxic, although cellular- toxicity is a problem for these first-generation modified particles. Functionalized GQDs represent a potentially interesting delivery- system that is easy to manufacture, stable and effective." (7)

The new era of vaccines: the "nanovaccinology

5.1.3. A Facciolà et al: European Review for Medical and Pharmacological Sciences 2019; "Carbon Nano-particles: Many research studies have been conducted to evaluate the use of carbon nano-materials as adjuvants or carriers for different kinds of vaccines especially because they are internalized into a wide variety of cell types. Many structural and physical features of these nano-systems effect the capacity of carrying antigens and stimulating immune- responses, among which their surface modifications.

Among carbon nano-particles, carbon nano-tubes (CNTs) have received a great attention because of their exceptional features that make them usable in many industrial and research fields. CNTs are engineered nano-particles formed by a thick sheet of graphene that rolls up to form a hollow cylinder named single-walled CNT (SWCNT). If more graphene sheets are present, the CNT it will be formed by concentric multiple sheets (from 2 to 50, linked together by van der Waals interactions); this CNT is named multi-walled CNT (MWCNT).

Their lengths vary from 100 to 1000 nm and their diameter range between 0.4-2 nm and 2-100 nm for SWCNTs and MWCNTs, respectively. "(8)

5.1.4. Qianqian Zhou et al: "Dendritic cell (DC) vaccines are used for cancer and infectious diseases, albeit with limited efficacy. Modulating the formation of DC–T-cell synapses may greatly increase their efficacy. The effects of graphene oxide (GO) nano-sheets on DCs and DC–T-cell synapse formation are evaluate. In particular, size-dependent interactions are observed between GO nanosheets and DCs. GOs with diameters of >1 μm (L-GOs) demonstrate strong adherence to the DC surface, inducing cyto-skeletal reorganization via the RhoA-ROCK-MLC pathway, while relatively small GOs (≈500 nm) are predominantly internalized by DCs. L-GO treatment enhances DC–T-cell synapse formation via cytoskeleton-dependent membrane positioning of integrin ICAM-1. L-GO acts as a "nano-zipper," facilitating the aggregation of DC–T-cell clusters to produce a stable micro-environment for T cell activation. Importantly, L-GO-adjuvanted DCs promote robust cytotoxic T cell immune responses against SARS-CoV-2 spike 1, leading to >99.7% viral RNA clearance in mice infected with a clinically isolated SARS-CoV-2 strain. These research findings highlight the potential value of nano-materials as DC vaccine adjuvants for modulating DC–T-cell synapse formation and provide a basis for the development of effective COVID-19 vaccines." (9)

5.1.5. Arjun Sharma et al: "Graphene Oxide Nano-particles (GO-NPs) have been shown to increase leukocyte numbers such as macro-phages and T cells. This effect boosts adaptive- immunity, thus allowing for a better immune response and viral clearance, or a possible use as vaccine adjuvants. In the scenario of un-controlled hyper-inflammation, nano-diamonds elicit an anti-inflammatory state in macrophages, while carbon and graphene sheets can be repurposed to remove pro-inflammatory cytokines and interleukins from the blood of patients" (10) (Figure 2).

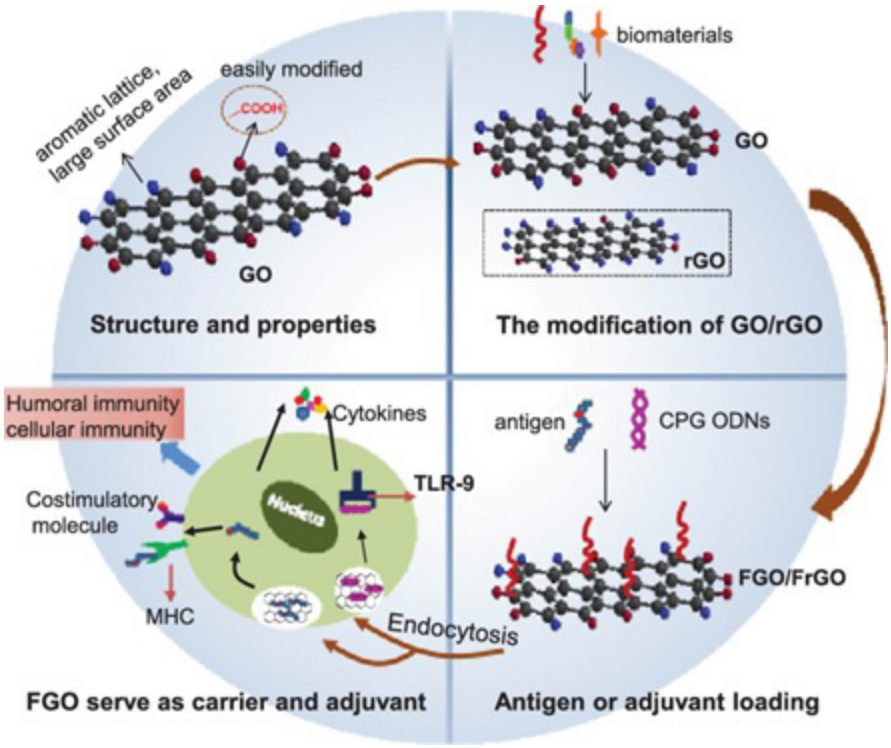

Figure 2: from Review article Recent progress of graphene oxide as a potential vaccine carrier and adjuvant Weidong Caob et al.

5.1.6. Chiara Rinoldi et al: "Carbonaceous Nano-materials

The unique structural properties of carbonaceous nano-materials make them promising in many fields, including energy storage and electro-chemistry research. The encouraging potential of these nano-materials, such as graphene oxide (GO) and carbon nano-tubes (CNTs), has been recently explored in bio-medicine and tissue engineering. Therapeutic molecules can bind non-covalently to GO and CNTs through π–π stacking. CNTs are particularly advantageous since they can benefit from the high -surface area. In this frame, for the successful integration of CNTs in a biological system, surface functionalization of nano-tubes should be practiced to break the relatively high number of nano-tube agglomerates in suspension and to improve their low bio-compatibility. The addition of cell-targeting agents to the design of CNT carriers is another requirement of RNA delivery for high cell recognition and efficient internalization. Cao et al. developed functionalized single-walled CNTs for the codelivery of surviving siRNA and 4-substituted-2, 5-dimethoxy-amphetamines (DOx). A combination of a poly (ethylenimine) (PEI)-betaine conjugate and a targeting peptide was further reacted with oxidized CNTs to grant cell penetration and pH-sensitive endosomal escape characteristics to the carrier. The undesirable size of pristine CNTs was decreased to 250 nm after polymeric modification. A similar approach method was used in the study conducted by Edwards et al. In their report, poly-amidoamine dendrimer and CNT suspension were placed under sonication to modify the surface of CNTs and improve their functional properties. On the other hand, GO is a sheet of oxidized carbon atoms with a hexagonal conformation resembling a honey-comb, which has a higher specific surface area, suspension stability, and bio-compatibility than CNTs. The carrying capacity of bare GO was evaluated via the intra-cellular delivery of siRNA. The small interfering RNA was complexed at different mass ratios with pristine GO, maintaining an average lateral -size of one μm and thickness of two nm. GO accumulated and isolated in large- vesicles and the intra-cellular trafficking was hindered, most probably due to the formation of GO agglomerates. The charges between cargo and carrier were cancelled out when the complex was introduced into the cell culture medium, which led to low transaction efficiency. In another research study, GO with an average diameter of approximately 200 nm was exfoliated under sonication and mixed with a complementary strand of miR-21 and doxorubicin hydrochloride as an anti-cancer drug. Results showed a quick cellular uptake of the carrier and desirable gene silencing by the cargo in cancer cells. These findings show that although GO benefits from several functional groups, effective delivery of RNA can be further enhanced by polymer and cationic lipid coating of the sheets. These coatings have the capability to extend the presence of nano-particles in blood circulation, by circumventing immune -system recognition, and improving functionalization for a targeted delivery of therapeutic agents. Dense polymer brushes were fabricated from fluorescent conjugated poly-electrolyte macro initiators on the surface of GO sheets with a thickness of 1.3 nm, which permitted the cellular tracking of the nano-carrier. Qu et al. constructed a composite of GO and poly (amidoamine) dendrimer incorporated with a PEG-modified glycyrrhetinic acid as targeting ligand, and complexed it with siRNA to demonstrate an active targeting of cancer liver cells. A satisfactory cell uptake of

nanocomplex by HepG2 cells and a decreased expression of VEGFA in mRNA and protein levels were observe, and the effective in vitro gene silencing was demonstrate. In a recent research study, Saravanabhavan et al. introduced a functionalized chitosan GO nano-particle into traditional pristine GO carriers and developed a suitable tumor-targeted material with good bio-compatibility and the potential to regulate the B-cell lymphoma-2 (Bcl-2) expression. In this experiment, chitosan was mix with siRNA prior to GO addition. A composite of loaded chitosan nano-particles and GO, formed at the weight ratio of 1:1, were complexed with siRNA, and used to reduce the survivability of tumor cells. It was apparent that chitosan prevented immuno-genicity, while the lateral size of GO reduced the inflammatory response. The Fickian pH-sensitive diffusion of siRNA from the carrier complex showed the controlled release required to target tumor cells. An advanced structure of GO and a porous zeolitic imidazolate framework were designed to enhance RNA-delivery efficiency. The complex of siRNA and positively charged GO-zeolite composite improved the cell transfection and demonstrated adequate in-vitro gene knockdown. A prime obstacle in the delivery of nano-materials to the targeted cells is represented by the liver and spleen sequestration of carriers. Carbonaceous platforms are not exempt from this process. The yet unclear CNT toxicity at the molecular and cellular level makes a future endorsement of this material un-certain and doubtful. Specifically, adopting CNT as a carrier of genetic molecules may carry more risks of malignant transformation, DNA damage, mutation. The adverse effects of accumulated sequestrated CNTs and GO on zonation, epigenetic changes, and liver function should be studied and addressed. Wu et al. set out to find that the controlling mechanisms related to GO liver retention and nano-bio interaction stemmed from the un-avoidable liver sequestration of GO nano-sheets. GO oxidation level, average lateral size, and the frequency and type of surface functional groups dictated the in vivo behavior of GO. The pattern of liver functional zonation appeared to be strikingly disrupted by GO and some notable changes in representative liver gene expression were found. In spite of the minute changes in the liver function, the study showed that the transcription and epigenetics of liver cells were largely affected by GO. These pieces of evidence prompted researchers to take cautionary steps toward the consideration of carbonaceous nano-materials as RNA carriers." (11) (Figure 3).

Worse Than the Disease? Reviewing Some Possible Unintended Consequences of the mRNA Vaccines

Against COVID-19 Stephanie Seneff, Greg Nigh IJVTPR

"Several studies on mRNA-based vaccines have confirmed independently that the spleen is a major center of activity for the immune- response. A study on an mRNA-based influenza virus vaccine is extremely relevant for answering the question of the bio-distribution of the mRNA in the vaccine.

This vaccine, like the SARS-CoV-2 vaccines, was designed as lipid nano-particles with modified RNAcoding for hemagglutinin (the equivalent surface fusion protein to the spike protein in corona viruses), and was administered through muscular injection IM. The concentration of mRNA was trackedover time in various tissue samples, and the maximum concentration observed at each site was record. Not surprisingly, the concentration was highest in the muscle at the injection site (5,680 ng/mL). This level decreased slowly over time, reaching half the original value at 18.8 hours following injection. The next highest level was observed in the proximal lymph node, peaking at 2, 120 ng/mL and not dropping to half this value until 25.4 hours' time later. Among organs, the highest levels by far were found in the spleen (86.69 ng/mL) and liver (47.2 ng/mL). Elsewhere in the body the concentration was at 100- to 1,000-fold lower levels. In particular, distal lymph- nodes only had a peak -concentration of 8 ng/mL. They concluded that the mRNA distributes from the injection site to the liver and spleen via the lymphatic system, ultimately reaching the general circulation. This likely happens through its transport inside macro-phages and other immune- cells that take it up at theme la proteina per un tempo che va da alcuni giorni ad alcune settimane. muscular injection site. Disturbingly, it also reaches into the brain, although at much lower levels.

The EMA report for Moderna vaccine also noted that mRNA could be detected in the brain following intramuscular IM administration at about 2% of the level found in the plasma (2021).

In another experiment work conducted to track the bio-distribution pathway of RNA vaccines, a rabies RNA vaccine was administered IM to rats in a single dose. The vaccine included a code for an immuno-genic rabies protein as well as the code for RNA -polymerase and was formulated as an oil-in-water nano-emulsion. Thus, it is not entirely representative of the SARS-CoV-2 mRNA vaccines. Nevertheless, its intra-muscular administration and its dependence on RNA uptake by immune cells likely means that it would migrate through the tissues in a similar pathway as the SARS-CoV-2 vaccine. The researcher authors observed an enlargement of the draining lymph- nodes, and tissue studies revealed that the rabies RNA appeared initially at the injection site and in the draining lymph- nodes within one day, and was also found in blood, lungs, spleen and liver.

These results are consistent with the above study on influenza mRNA vaccines.

A study comparing luciferase-expressing mRNA nano-particles with luciferase-expressing mRNA dendritic cells as an alternative approach to vaccination revealed that the luciferase signal reached a broader range of lymphoid sites with the nano-particle delivery mechanism.The luciferase signal was concentrated in the spleen for the nano-particles compared to dominance in the lungs for the dendritic cells " (12).

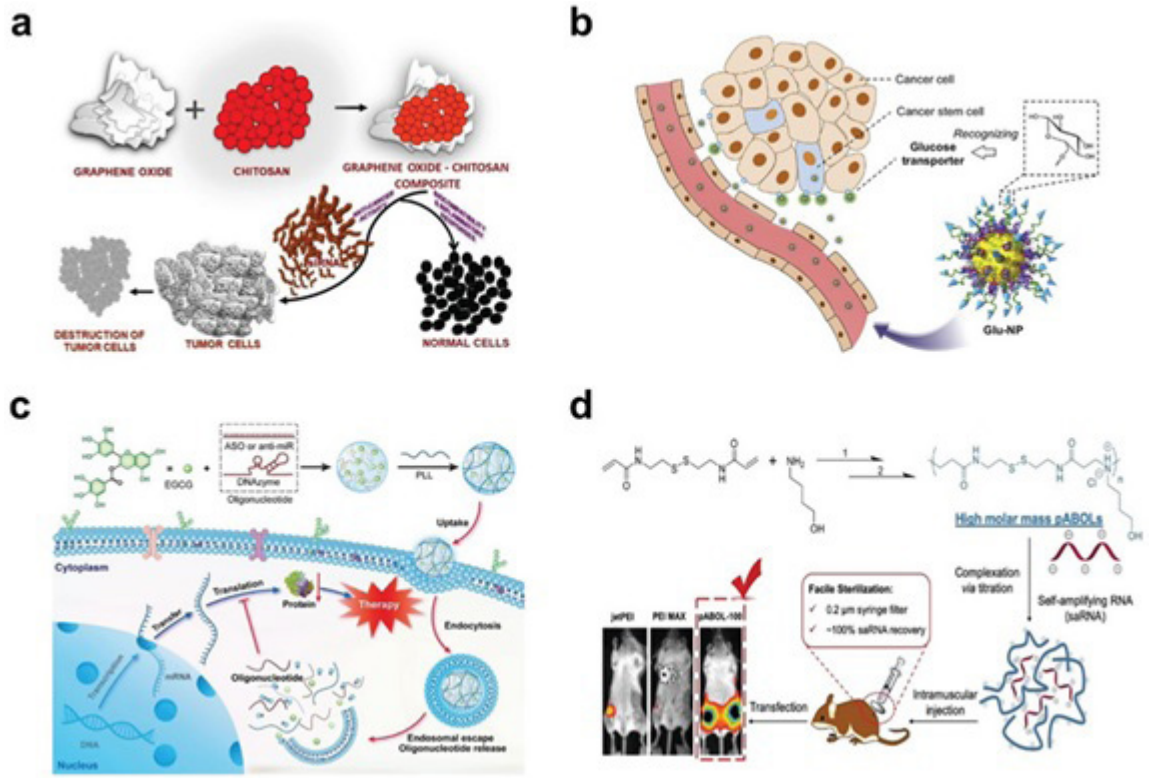

Figure 3: RNA delivery based on carbonaceous, inorganic, and polymer nano-carriers. a) Graphene oxide functionalized with chitosan nano-particles as a carrier of siRNA for regulating the Bcl-2 expression. b) Glucose-linked gold nano-particles for targeted siRNA delivery to breast cancer stem-like cells. Glucose ligands endow the nano-particles with target ability toward the breast. c) Green nano-particles for siRNA delivery. Natural polyphenol from green tea -catechin was complexed with siRNA to form negatively (-) charged nano-particles, followed by surface coating with PLL. d) Higher-molecular weight, bio-reducible, cationic polymer enhanced saRNA delivery. High-molecular weight pABOLs were achieved by improved aza-Michael addition. Complexation with saRNA happened via titration method and transfection efficacy of the pABOL-100 polyplexes were compared to jetPEI and PEIMAX. From Rinoldi C et al.

5.1.7. Ioannis P. Trougakos et al: "In line with a plausible systemic distribution of the antigen AG, it was found that the S protein circulates in the plasma of the BNT162b2 or mRNA-1273 vaccine recipients as early as day 1 after the first vaccine injection. Reportedly, antigen clearance is correlated with the production of AG-specific immuno-globulins or may remain in the circulation (in exosomes) for longer periods of time , providing one reasonable explanation (among the others) for the robust and durable systemic immune- responses found in vaccinated recipients. There is likely to be an extensive range of expected interactions between free-floating S protein/ subunits/peptide fragments and ACE2 circulating in the blood (or lymph), or ACE2 expressed in cells from various tissues/organs. This notion is further supported by the finding that in adenovirus-vectored vaccines (Box 2), the S protein produced upon vaccination has the native-like mimicry of SARS-CoV-2 S protein's receptor binding functionality and prefusion structure.

Additional interactions with human- proteins in the circulation, or even the presentation to the immune system of S protein antigenic epitopes mimicking human proteins may occur. Reportedly, some of the near-germline SARS-CoV-2-NAbs against S receptor-binding domain (RBD) reacted with mammalian self-antigens, and SARS-CoV2 S antagonizes innate antiviral- immunity by targeting multiple pathways controlling interferon production . Also, a sustained elevation in T -cell responses to SARS-CoV-2 mRNA vaccines has been found (data not yet peer-reviewed) in patients who suffer from chronic neurologic symptoms after acute SARS-CoV-2 infection as compared with healthy COVID-19 convalescents "(13)

5.1.8. Another study by Dr. Robert Young:

(https://www.drrobertyoung.com/post/transmission-electron-microscopy-reveals-graphene-oxide-in-cov-19vaccines) trough electronic microscopy it whould have found a graphene and derivates, nano-metallic particle , citotoxic and genotoxic and also a parassite.

"It must be consider that the presence of graphene in vaccine and gene product is a crime because not reproted in list of ingredients and the graphene oxide ha salso toxicological profile".

GRAFENE: Risk of an emerging technology

https://www.europeanconsumers.it/2021/08/29/grafene-rischi-di-una-tecnologia-emergente/

"graphene presence whould represent a crime because it is not reported in the ingrediants of genic therapy and its oxide has also a toxicological interest. But the identification of this substantie by electronic microscope would need, also of appropriate standardized chemical analisys of serum and blood of inoculated Graphee and ite derivates represent an emerging technology in every way but the possibility and the risk must to be evaluated with attention in all the areas of use, not only medical.

I graphene base materials (GBM) are a family of new materials like Graphene at few strates (FLG), Graphene Oxide (GO), reduced graphene oxide (rGO) graphene nanoplatelet (GNP).

This are used and under current experimentation for nuomerous applications: industrial and biomedical because at their rigidity and very high mechanical resistance, excellent electrical conductivity, high optical transparency and good biocompatibility.

This nanomaterials show a great relevance as "carriers" in drug-targeted delivery and the GO is reported use in some vaccine.

They also show a great potential release drugs through the BEE. Even if the nanoparticle production in the world is increasing in a logarithmic way the toxicological impact and possible risk of this nanoparticle for human safety and the environment are to be more investigate.

In 2021, it was with drawled form the public use by ministry of Health Canada, "Face masks that contain graphene "because this may pose health risks". This product was written, that are made of nanographene implicate a risk of pulmonary toxicity in inalated this nanoparticle as reported in lab animal tests. Into the phenomena related graphene some videos seem to show that the arm of some people become magnetic in the injection site.

Phenomena not only related to the arm and in few days it moves to chest, neck a superior part of the back column.

Officially, inoculated serum are not present magnetic material, and not graphehe based according the technical sheet publiced (ingradients). However, at technological level this are proposed to improve the genic production. Efficiency in subministration of DNA vaccine is relatively lower versus proteic vaccine.

The use of SUPERPARAMAGNETIC ferrum oxide nanoparticle (SPION) show promising results in improving genic production in vitro and in vivo.

Unfortunately, industries not provide information related production methods. However, the possible presence of impurity must to be report in the security sheet moreover are not presenta t today, a part photo foto and video on internet, study with technical scientific validity on this phenomenon."

5.1.9. Lingling Ou et al: "Due to their unique physico-chemical properties, graphene-family nano-materials (GFNs) are widely used in many fields, especially in bio-medical applications. Currently, many studies have investigated the bio-compatibility and toxicity of GFNs in vivo and in intro. Generally, GFNs may exert different degrees of toxicity in animals or cell models by following with different administration routes and penetrating through physiological -barriers, subsequently being distributed in tissues or located in cells, eventually being excreted out of the bodies. This review collects studies on the toxic- effects of GFNs in several organs and cell models. We also point out that various factors determine the toxicity of GFNs including the lateral size, surface -structure, functionalization, charge, impurities, aggregations, and corona effect ect. Several typical mechanisms underlying GFN toxicity have been revealed, for instance, physical destruction, oxidative stress, DNA damage, inflammatory response, apoptosis, autophagy, and necrosis. In these mechanisms, (toll-like receptors-) TLR-, TGF-β and TNF-α dependent-pathways are involved in the signalling- pathway network, and oxidative stress plays a crucial role in these pathways. In this review work, we summarize the available information on regulating factors and the mechanisms of GFNs toxicity, and propose some challenges and suggestions for further investigations of GFNs, with the aim of completing the toxicology mechanisms, and providing suggestions to improve the biological safety of GFNs and facilitate their wide- application. Toxicity of GFNs (in vivo and in vitro) GFNs penetrate through the physiological barriers or cellular- structures by different exposure ways or administration routes and entry the body or cells, eventually resulting in toxicity in vivo and in vitro. The varying administration routes and entry paths, different tissue distribution and excretion, even the various cell uptake patterns and locations, may determine the degree of the toxicity of GFNs. So, to make them clear may be helpful to better understand the laws of the occurrence and development of the GFNs toxicity.

5.1.10. GFNs entry paths: GFNs reach various locations through blood circulation or biological barriers after entering the body, which results in varying degrees of retention in different organs. Due to their nano-size, GFNs can reach deeper organs by passing through the normal physiological barriers, such as the blood-air barrier, blood-testis barrier, blood-brain barrier and blood-placental barrier. Influence of haemo-compatibility GO release into the blood is ineluctable. The haemo-compatibility of GO was found to be dependent on the functional coating and the exposure conditions. GO with submicron size resulted in the greatest haemolytic activity, while aggregated graphene induced the lowest haemolytic reaction. Pristine graphene and GO demonstrated haemolytic- effect up to 75 μg/mL. GO-polyethylenimine (GO-PEI) exhibited notable toxicity by binding to HSA, even at 1.6 μg/mL. Carboxylated graphene oxide (GO-COOH) showed significant cytotoxicity toward T- lymphocytes at concentrations above 50 μg/mL and had good bio-compatibility below 25 μg/mL, whereas GO-chitosan nearly inhibited haemolytic activity. Until now, the corresponding risk of haemo-compatibility has remained largely unknown." (14) (Figure 4 and 5) (Table 1).

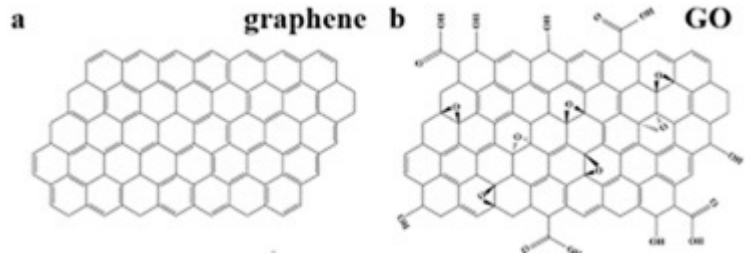

Figure 4: graphene and graphene oxide GO

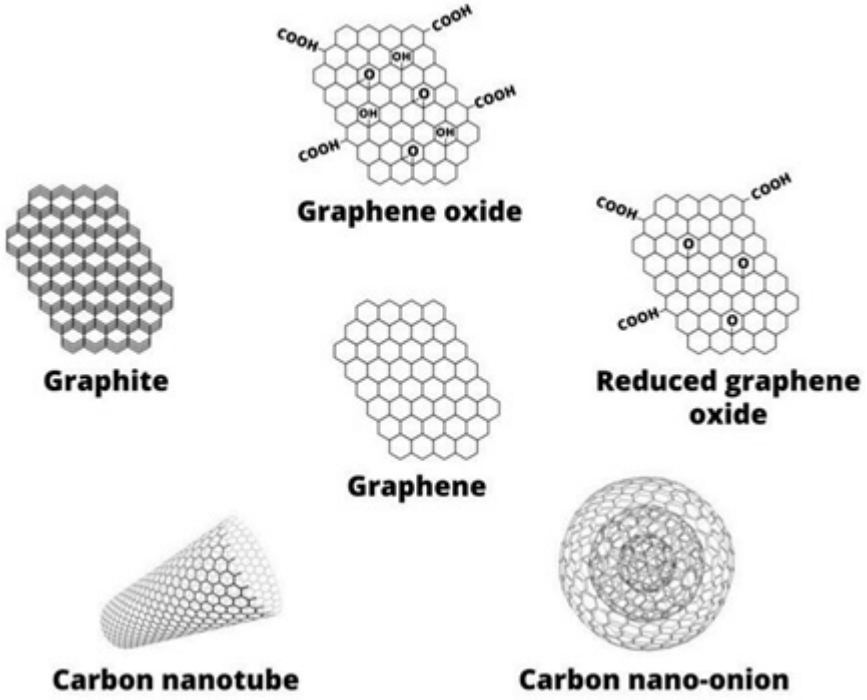

Figure 5: form Extrusion of Polymer Nanocomposites with Graphene and Graphene Derivative Nanofillers: An Overview of Recent Developments by José Sanes et al.

Table 1: form Kausar A. 2018. Advances in Polymer/Graphene Nanocomposite for Biosensor Application.

Physical Properties	Graphene	Carbon nanotube	Si	Cu
Melting point (K)	3800	3800	1687	1357
Thermal conductivity (10^3 W/mK)	3-5	1.75-5.8	0.15	0.385
Current density (A/cm^2)	>10^8	>10^9	-	10^7
Electron mobility (cm^2/(V.s))	>10,000	>10,000	1400	-
Mean free path (nm)	1 X 10^3	>10^3	20-30	40

5.1.11. Ken-Hsuan Liao et al: "2-dimensional carbon-based nano-materials, including graphene oxide and graphene, are potential candidates for bio-medical applications such as sensors, cell labeling, bacterial inhibition, and drug delivery. We explore the bio-compatibility of graphene-related materials with controlled physical and chemical properties. The size and extent of exfoliation of graphene oxide sheets was varied by sonication intensity and time. Graphene -sheets were obtained from graphene oxide by a simple (hydrazine-free) hydro-thermal route. The particle size, morphology, exfoliation extent, oxygen content, and surface charge of graphene oxide and graphene were characterized by wide-angle powder X-ray diffraction, atomic force microscopy, X-ray photo-electron spectroscopy, dynamic light scattering, and zeta-potential. One method of toxicity assessment was based on measurement of the efflux of hemoglobin HB from suspended red blood -cells RBC. At the smallest size, graphene oxide showed the greatest hemolytic activity, whereas aggregated graphene sheets exhibited the lowest hemolytic activity. Coating graphene -oxide with chitosan nearly eliminated hemolytic activity. Together, these results demonstrate that particle size, particulate state, and oxygen content/surface charge of graphene have a strong impact on biological/toxicological responses to red blood cells. The cytotoxicity of graphene oxide and graphene sheets was investigated by measuring mitochondrial activity in adherent human skin fibroblasts using 2 assays. The methyl-thiazolyldiphenyl-tetrazolium bromide (MTT) assay, a typical nano-toxicity assay, fails to predict

the toxicity of graphene oxide and graphene toxicity because of the spontaneous reduction of MTT by graphene and graphene oxide, resulting in a false positive signal. Appropriate alternate assessments, using the water-soluble tetrazolium salt (WST-8), trypan blue exclusion, and reactive oxygen species assay reveal that the compacted graphene- sheets are more damaging to mammalian fibroblasts than the less densely packed graphene oxide. Clearly, the toxicity of graphene and graphene- oxide depends on the exposure environment (whether or not aggregation occurs) and mode of interaction with cells (suspension versus adherent cell types). Intra-venous injection IV is also widely used to assess the toxicity of graphene nano-materials, and graphene circulates through the body of mice in 30 min, accumulating at a working concentration in the liver and bladder." (15)

5.1.12. Kai-Ping Wen et al: "Graphene and its functionalized derivatives have recently emerged as interesting nano-materials with promising applications in bio-medicine. In this study, the long-term in vivo biodistribution of intravenously injected nano-graphene oxide (NGO) functionalized with poly sodium 4-styrenesulfonate (PSS) was systematically examined and the potential toxicity over 6 months of NGO-PSS nano-particles was investigated. Our results showed that the nano-particles mainly accumulate in the lung, liver and spleen, where they persist for at least 6 months. These nano-particles result in acute liver injury and chronic inflammation of the lung, liver and spleen, as evidenced by blood bio-chemistry results and histological examinations". (16)

5.1.13. Sunil K Singh et al: "Graphene oxide (GO), the new 2-dimensional carbon nano-material, is extensively investigated for potential bio-medical applications. Thus, it is pertinent to critically evaluate its untoward effects on physiology of tissue systems including blood platelets, the cells responsible for maintenance of hemostasis and thrombus formation.We report for the first time that atomically thin GO sheets elicited strong aggregatory response in platelets through activation of Src kinases and release of calcium from intracellular stores. Compounding this, intravenous IV administration of GO was found to induce extensive pulmonary thrombo-embolism in mice. Prothrombotic character of GO was dependent on surface charge distribution as reduced GO (RGO) was significantly less effective in aggregating platelets. Our findings raise a concern on putative bio-medical applications of GO in the form of diagnostic and therapeutic tools where its pro-thrombotic property should be carefully investigated." (17)

5.1.14. Hua Yue et al: "We explored an intelligent vaccine system via facile approaches using both experimental and theoretical techniques based on the 2-dimensional graphene oxide (GO). Without extra addition of bio/chemical stimulators, the microsized GO imparted various immune activation tactics to improve the antigen immuno-genicity. A high antigen adsorption was acquired, and the mechanism was revealed a combination of electrostatic, hydrophobic, and π–π stacking interactions. The "folding GO" acted as a cytokine self-producer and antigen AG reservoir and showed a particular autophagy, which efficiently promoted the activation of antigen presenting cells (APCs) and subsequent antigen cross-presentation. Such a "One but All" modality thus induced a high level of anti-tumor responses in a programmable way and resulted in efficient tumor regression in vivo. This work may shed light on the potential use of a new dimensional nano-platform in the development of high-performance cancer- vaccines." (18) (Figure 6).

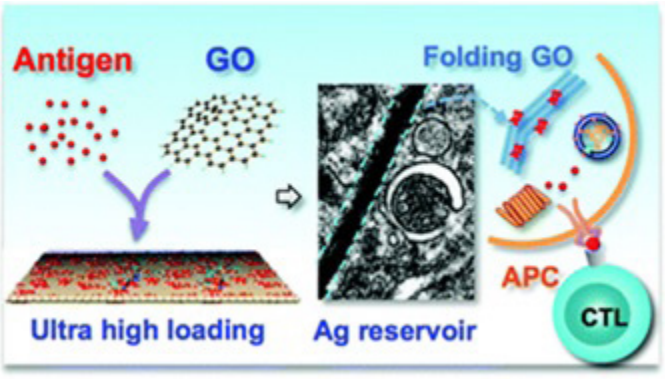

Figure 6:

5.1.15. Ligeng Xu et al: "Benefiting from their unique physico-chemical properties, graphene derivatives have attracted great attention in bio-medicine. In this study, we carefully engineered graphene oxide (GO) as a vaccine- adjuvant for immuno-therapy using urease B (Ure B) as the model antigen. Ure B is a specific antigen for Helicobacter pylori, which is a class I carcinogen for gastric cancer. Polyethylene -glycol (PEG) and various types of poly-ethylenimine (PEI) were used as coating polymers. Compared with single-polymer modified GOs (GO–PEG and GO–PEI), certain dual-polymer modified GOs (GO–PEG–PEI) can act as a positive modulator to promote the maturation of dendritic cells (DCs) and enhance their cytokine secretion through the activation of multiple toll-like receptor (TLR) pathways while showing low -toxicity. This GO–PEG–PEI can serve as an antigen

-carrier to effectively shuttle antigens into DCs. These 2 advantages enable GO–PEG–PEI to serve as a novel vaccine adjuvant. In the subsequent in vivo experiments, compared with free Ure B and clinically used aluminum-adjuvant-based vaccine (Alum-Ure B), GO–PEG–PEI–Ure B induces stronger cellular immunity via intradermal administration, suggesting promising applications in cancer immuno-therapy. Our reseach work not only presents a novel, highly effective GO-based vaccine nano-adjuvant, but also highlights the critical roles of surface chemistry for the rational design of nano-adjuvants." (19)

5.1.16. Chunhong Dong et al: "A non-invasive intranasal (i.n.) influenza vaccine can induce mucosal immune responses in respiratory tracts, preventing infection at the portal of virus- entry. The absence of appropriate mucosal adjuvants at present hinders the development of such a vaccine. We developed polyethylene-imine-functionalized 2-dimensional graphene oxide nano-particles (GP) that showed high antigen-loading capacities and superior immuno-enhancing properties. Robust and broadly reactive immune- responses were induced with i.n. immunization with GP-HA nano-particles, conferring protection against homologous and heterologous viruses. With versatility and flexibility, GP nano-particles can be easily adapted for constructing mucosal- vaccines of different respiratory pathogens." (20) (Figure 7-11).

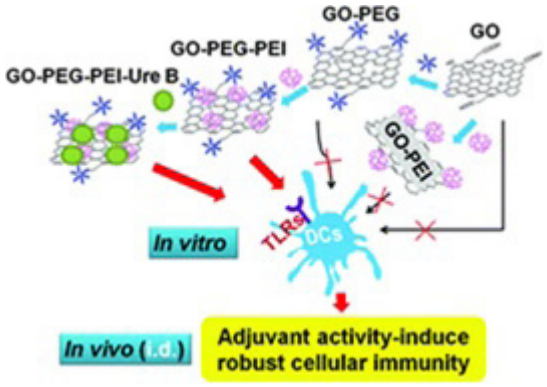

Figure 7:

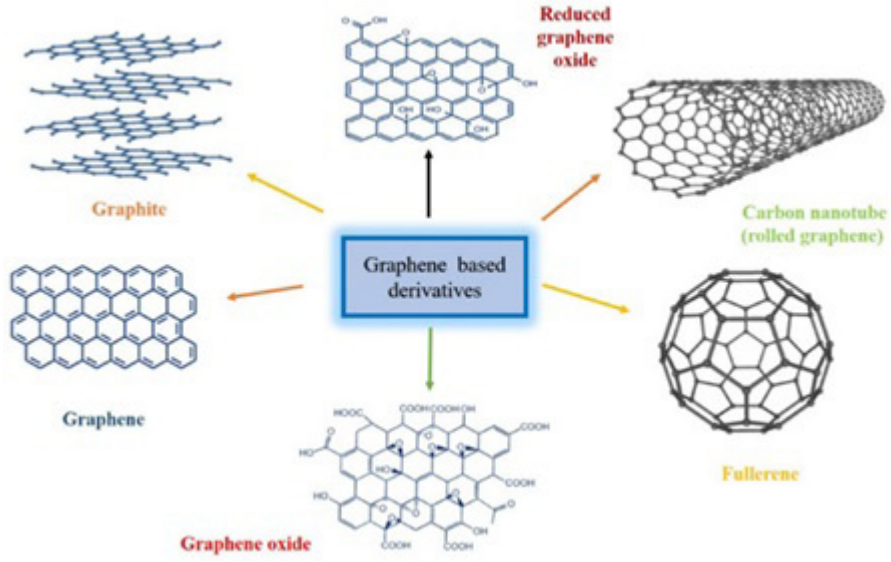

Figure 8: from Materials Today Advances

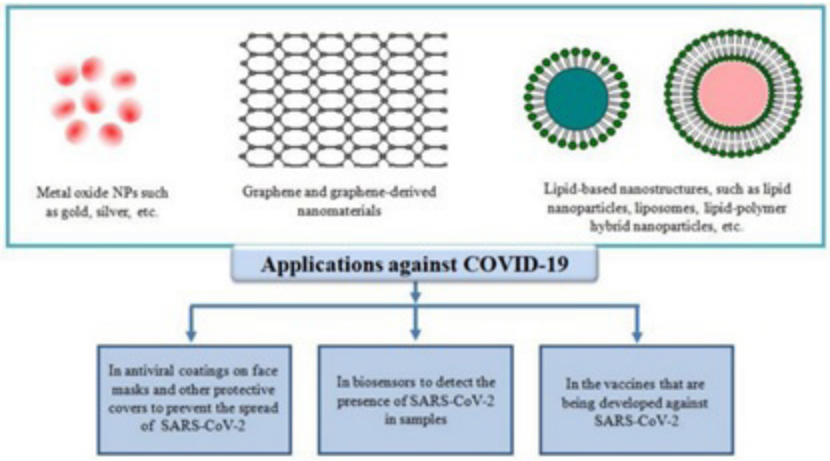

Figure 9: from AIMS Microbiology

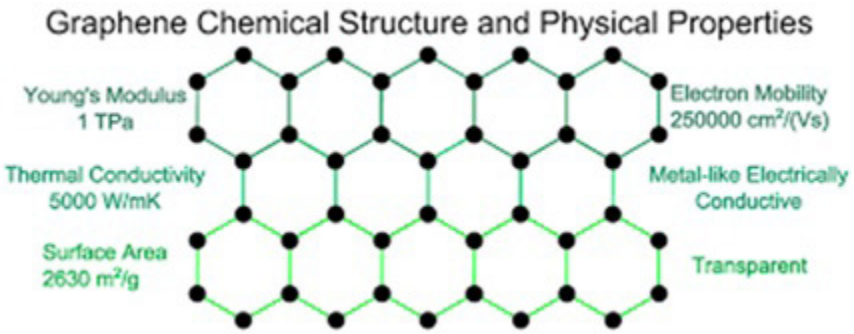

Figure 10: from Graphene Nanoplatelets-Based Advanced Materials and Recent Progress in Sustainable Applicationsby Pietro Cataldi et al.

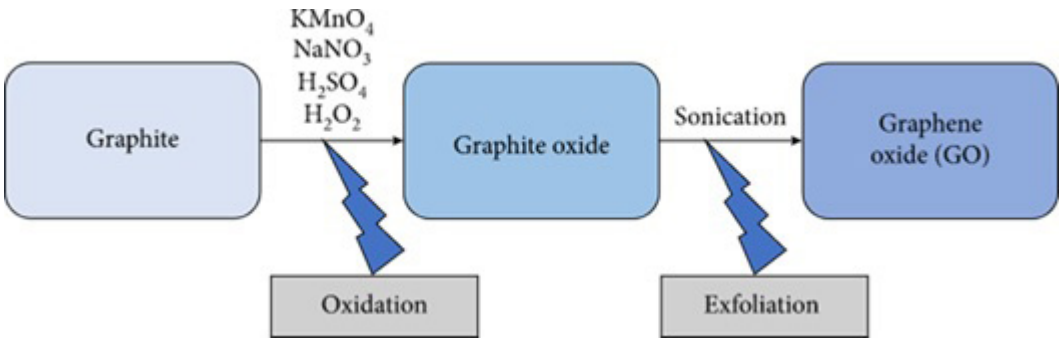

Figure 11: from Environmental Mutagenicity and Carcinogenicity of Nano-materials1

5.1.17. Federica Valentini et al: "This review work focus on several aspects essential to consider for new nano-materials, when they are applied as new nano-carriers in drug delivery.The key point is the synthetic strategies forgraphene growth because they mainly influence all the resulting properties Associated to the graphene final products. Chemicaland Electro-chemical synthetic strategies provide oxygenated functionalized graphene nano-sheets. The characterization study (under a morphological/topographic and structural point of view) is a fundamental step to unequivocally identify graphene, with all the suitable characteristics, including the presence of structural defects, as the functional groups on nano-sheet surfaces.

Functionalization is essential to guarantee the best dispersibilityof graphene nano-sheets in the working cellular environment,and this is a crucial point for in vitro and in vivo experiments (improving the bio-distribution of graphene in the cellular compartments). At the same time, functionalization carried out by the introduction of oxygenated functional groups (mainly COOH groups) could also provoke in vitro cytotoxicity on human cell lines. For this reason,to improve in vitro biocompatibility of graphene, used as nanocarrier of drugs and therapeutics, several different bio-coatings (mainly GO/PEI and GO/PEG nano-composite materials) have been developed and detailed described herein. Many authors, cited in this review, show that the bio-polymer coating increases cyto-toxicity. At the state of the art, what can be proposed for graphene is a broad-spectrum study with different functionalized grafenes, tested on different human cell lines (normal and cancerous cell lines). So, it is possible to create a very useful database, able to explore the properties of graphene not only in the medical field but also with regard to the environmental impact and the safe-guarding of the state of health, in the work-places" (21)

5.1.18. Hye Yeon Choi et al: "Clinical applications of induced pluripotent stem cells (iPSCs) require development of technologies for the production of "footprint-free" (gene integration-free) iPSCs, which avoid the potential risk of insertional- mutagenesis in humans. Previously, several studies have shown that mRNA transfer can generate "footprint-free" iPSCs, but these studies did not use a delivery vehicle and thus repetitive daily transfection was required because of mRNA degradation.We report an mRNA delivery system employing graphene oxide (GO)-polyethylenimine (PEI) complexes for the efficient generation of "footprint-free" iPSCs. GO-PEI complexes were found to be very effective for loading mRNA of re-programming transcription factors and protection from mRNA degradation by RNase. Dynamic suspension cultures of GO-PEI/RNA complexes-treated cells dramatically increased the re-programming efficiency and successfully generated rat and human iPSCs from adult adipose tissue-derived fibroblasts without repetitive daily transfection. The iPSCs showed all the hallmarks of pluripotent stem cells including expression of pluripotency genes, epigenetic re-programming, and differentiation into the three germ layers. These results demonstrate that mRNA delivery using GO-PEI-RNA complexes can efficiently generate "footprint-free" iPSCs, which may advance the translation of iPSC technology into clinical-settings." (22)

Preprint Detection of Graphene in COVID19 Vaccines

November 2021 Pablo Campra

"We present here our research on the presence of graphene in covid vaccines. We have carried out a random screening of graphene-like nano-particles visible at the optical microscopy in seven random samples of vials from four different trade-marks, coupling images with their spectral signatures of RAMAN vibration. By this technique, called micro-RAMAN, we have been able to determine the presence of graphene in some of these samples, after screening more than 110 objects selected for their graphene-like appearance under optical -microscopy. Out of them, a group of 28 objects have been selected, due to the compatibility of both images and spectra with the presence of graphene derivatives, based on the correspondence of these signals with those obtained from standards and scientific- literature. The identification of graphene oxide structures can be regarded as conclusive in 8 of them, due to the high spectral correlation with the standard. In the remaining 20 objects, images coupled with Raman signals show a very high level of compatibility with un-determined graphene structures, different than the standard used here. This research remains open and is made available to scientific community for discussion. We make a call for independent researchers, with no conflict of interest or coaction from any institution to make wider counter-analysis of these products to achieve a more detailed knowledge of the composition and potential health risk of these experimental -drugs, reminding that graphene materials have a potential toxicity on human beings and its presence has not been declared in any emergency- use authorization. But the same author in the article conclusion affermano che: Rilevazione di grafene in campione in sospensione acquosa.

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" the miscroscope assay of the sample provide strong proof for the probable presence of graphene derivates , even if this microscopy not provide conclusive proof.

The definitive identification of the graphene nd graphene GO or graphene oxidated reduced (rGO) in the sample RD1 require STRUCTURAL CHARACTERIZATION trought analisys of specific Spettral pattern comparable with that published in literature and to one obtained from a standard sample , obtained with spettroscopic technique like XPS, EDS, NMR, FTIR o Raman, between other .

The analisys in this reportcorrespond to an unique sample, l limited in total volume available for the processing So in order to draw a general conclusion it is necessary execute sampling of a significative number of vials , registering origin,traceability and quality control during storage and transport before the analysys".

GOOGLE PATENT https://patents.google.com/patent/CN112220919A/en

Nano coronavirus recombinant vaccine taking graphene oxide as carrier

Current Assignee

Google has not performed a legal analysis and makes no representation or warranty as to the accuracy of the list.Shanghai National Engineering Research Center for Nano-technology Co Ltd

"The invention belongs to the field of nano materials and biomedicine, and relates to a vaccine, in particular to development of 2019-nCoV coronavirus nuclear recombinant nano -vaccine. The invention also comprises a preparation method of the vaccine and application of the vaccine in animal- experiments. The new corona vaccine contains graphene oxide, carnosine, CpG and new corona virus RBD; binding carnosine, CpG and neo-coronavirus RBD on the backbone of graphene oxide; the CpG coding sequence is shown as SEQ ID NO 1; the novel coronavirus RBD refers to a novel coronavirus protein receptor binding region which can generate a high-titer specific antibody aiming at the RBD in a mouse body, and provides a strong support for prevention and treatment of the novel- coronavirus."

5.1.19. Gaurav Lalwani et al: "Dose, time, and morphology dependent cytotoxicity. Zhang et. al. investigated the interactions of graphene (diameter 100–110 nm, thickness 3–5 nm) with rat pheochromocytoma PC12 cells using 3-(4,5-dimethylth-

iazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and Lactate Dehydrogenase (LDH) assays and compared the results with single-walled carbon nano-tubes (SWCNTs) . More than 70% cell death was observed for 100 µg/ml treatment concentration of SWCNTs whereas no cell death was observed for 0.01–10 µg/ml concentrations of graphene. Nearly 15–20% cell death was observed for graphene treatment at 100 µg/ml. The observed cyto-toxicity was attributed to the agglomeration of graphene, generation of reactive -oxygen species (and an increased caspase-3 activation resulting in apoptosis. These results show a dose dependent cyto-toxicity trend that is dependent on the morphology (shape and composition) of the nano-material, with graphene exhibiting an overall lower toxicity compared to single-walled carbon nano-tubes (SWCNTs). Vallabani et. al. investigated the toxicity of graphene oxide using normal human lung cells (BEAS-2B) after 24 and 48 hours of exposure at concentrations between 10–100 µg/ml. A significant dose- and time- dependent decrease in cell viability and an increase of early and late apoptotic- cells was observed using MTT assay. Hemolytic potential of graphene is dependent on the size and aggregation state of individual nano-sheets. Liao, et al. investigated the cyto-toxicity of graphene and GO using human erythrocytes (RBCs). Hemolysis was quantified by measuring the amount of hemoglobin released due to RBC membrane damage upon incubation with graphene and GO at 3–200 µg/ml for 3 hours. At 200 µg/ml, individually dispersed GO sheets showed ~60% hemolysis, significantly higher than graphene dispersions which showed ~20% hemolysis. The aggregation of graphene in DI water results in fewer cell-contractable ROS groups on the surface of graphene. Cells interact with several ROS- species present on the surface of individually dispersed GO, leading to greater hemolysis. Chitosan coated GO aggregate in DI water due to pH dependent conformational change of chitosan resulting in no hemolytic- toxicity of GO.

Singh et al. have reported the in vitro hemolytic toxicity of GO and rGO using human platelets. Freshly isolated suspension of platelets exposed to GO (2 µg/ml) show aggregation and platelet activation at levels greater than induction by thrombin (1 U/ml, a strong platelet agonist). Exposure of platelets to GO resulted in the activation of Src kinases and release of calcium, leading to thrombus formation. In comparison, rGO at 2 µg/ml induced minor platelet- aggregation, only 10% of aggregation induced by GO. In another study, Singh et al. showed that amine functionalized GO does not induce lysis of erythrocytes and has no stimulatory effects on platelets highlighting their non-thrombotoxic properties . These results suggest that surface modifications of graphene nano-particles play an important role towards defining their hemolytic- activity.

5.2. Mechanisms of Toxicity

The interactions of graphene with cells, proteins, and other biomolecules is influenced by its physio-chemical properties such as shape, size, functional group density and charge transfer abilities. The main mechanism of graphene toxicity is associated with the generation of intra-cellular reactive oxygen species that cause damage to proteins and DNA leading to cell death via apoptotic or necrotic pathways. Graphene can be internalized into cells via passive internalization (endocytosis) or active internalization (clathrin mediated energy dependent endocytosis or actin-dependent macro-pinocytosis). Research Studies have elucidated 2 mechanism of graphene mediated ROS damage: (1) Upon cellular internalization, GO interferes with the electron- transport system, induces overproduction of H2O2 and hydroxyl radicals. This leads to the oxidization of cardiolipin and the release and translocation of hemo-protein from mitochondrial inner membrane to the cytoplasm. This triggers release of cytochrome c complex (cyt c) which induces calcium release from endoplasmic reticulum and activates caspase 9 which in turn activates caspase 3 and 7 leading to cell death. (2) GO induces the activation of MAPK (JNK, ERK, p38) and TGF- β signaling pathways that lead to activation of Bcl-2 proteins which in turn activate mitochondria-induced apoptosis. In addition to ROS induced cell death, GO may also lead to the activation of toll-like receptors and induce autophagy via inflammatory- pathways. Post internalization; graphene may induce DNA cleavage due to interactions such as pi-pi stacking, hydrophobicity, and electrostatic interactions. Singh et al. have shown that surface charge distribution on graphene -sheets plays an important role in the activation of src kinases and release of calcium eventually leading to platelet aggregation." (23)

5.2.1. Arjun Sharma et al: "Graphene Oxide Nano-particles (GO-NPs) have been shown to increase leukocyte numbers such as macrophages and T cells. This effect boosts adaptive- immunity, thus allowing for a better immune response and viral clearance, or a possible use as vaccine adjuvants. In the scenario of un controlled hyper--inflammation, nanodiamonds elicit an anti-inflammatory state in macrophages, while carbon and graphene sheets can be repurposed to remove pro-inflammatory cytokines and interleukins from the blood of patients". (24) (Figure 12).

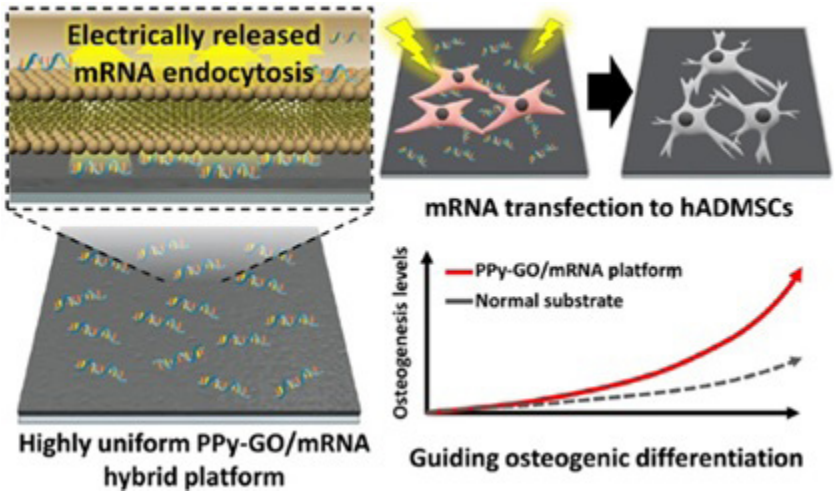

Figure 12: Electrically controlled mRNA delivery using a polypyrrole-graphene oxide hybrid film to promote osteogenic differentiation of human mesenchymal stem cells.

5.2.2. Ahn, M.; Song, J.; Hong, B.H. Facile Synthesis of N-Doped Graphene Quantum Dots as Novel

Transfection Agents for mRNA and pDNA. Nano-materials 2021, 11, 2816.

"In conclusion, we synthesized positively charged NGQDs to deliver genes such as mRNA and pDNA. NGQDs were synthesized using PEI and citric acid as precursors to give positive charges. The NGQDs synthesized via microwave-assisted hydro-thermal reactions were characterized by TEM, DLS, FT-IR, XPS, and Raman spectroscopy. Overall characterization data exhibit that NGQDs consist of a hydrophobic graphene domain and hydrophilic functional groups such as carboxylic acid and amine. It is confirmed that the positively charged NGQDs interact with the model mRNA and pDNA, the representative types of components for gene therapy, and transfect the cells, successfully. The gene transfection efficiency of NGQDs was measured to be comparable to Lipofectamine that is recognized as the "gold-standard" for in vitro gene transfection agents. Even in the case of mRNA transfection, the NGQDs exhibited a better performance than Lipofectamine. We expect that NGQDs can be utilized in clinical- field after further studies on their toxicity and metabolism in consideration of the previous studies on the intra-cellular distribution of-NGQDs." (25).

"Another study also demonstrated extensive pulmonary thrombo-embolism in Swiss male mice only 15 minutes after intravenously administrating 250 μg/kg body weight GO" (26)

Nano-materials (Basel). 2017

Chitosan-Functionalized Graphene Oxide as a Potential Immuno-adjuvant

5.2.3. Ting Yan et al: "The application of graphene oxide (GO) as a potential vaccine adjuvant has recently attracted considerable attention by researcher. Appropriate surface functionalization of GO is crucial to improve its bio-compatibility and enhance its adjuvant activity. In this study, we developed a simple method to prepare chitosan (CS)-functionalized GO (GO-CS) and further investigated its potential as a nano-adjuvant. Compared with GO, GO-CS possessed considerably smaller size, positive surface charge, and better thermal stability. The functionalization of GO with CS was effective in decreasing the non-specific protein adsorption and improving its biocompatibility. GO-CS significantly activated RAW264.7 cells and stimulated more cytokines for mediating cellular immune- response, which was mainly due to the synergistic immunostimulatory effect of both GO and CS. GO-CS exhibits strong potential as a safe nano-adjuvant for vaccines and immuno-therapy." (27)

5.2.4. Zhenguang Liu et al: "Graphene oxide (GO) and lentinan have received great attention because of their utility in bio-medical applications. Graphene oxide is utilized in drug- and vaccine-delivery systems due to its bio-compatibility, large surface area, and outstanding adsorption capability, while lentinan has immunity-enhancing effects. In this study, we synthesized and characterized GO grafted with lentinan (LNT) as an adjuvant and investigated how to impact the immune- responses. Lentinan-modified GO (GO-LNT) facilitated antigen uptake in macrophages and improved the efficiency of antigen application in vitro. In vivo, compared with GO/OVA, GO-LNT/OVA decreased the release rate of ovalbumin (OVA) to sustain long-term immune- responses and boost the levels of IgG and IgG subtypes. Hence, we can infer that the effects of GO-LNT were a result of the increased amounts of antigen uptake by cells. Overall, our studies demonstrated that GO-LNT could suffice for a safe and effective vaccine-delivery system as well as an excellent adjuvant that both elicits a long-term immune memory response and potentiates cellular and humoral -immunity." (28)

5.2.5. Cheng Xu et al: "Therapeutic cancer vaccines require robust cellular -immunity for the efficient killing of tumor cells, and recent advances in neoantigen discovery may provide safe and

promising targets for cancer vaccines. Elicitation of T cells with strong antitumor efficacy requires intricate multistep processes that have been difficult to attain with traditional vaccination approaches.A multi-functional nanovaccine platform has been developed for direct delivery of neoantigens and adjuvants to lymph nodes (LNs) and highly efficient induction of neoantigen-specific T cell responses. A PEGylated reduced graphene oxide nanosheet (RGO-PEG, 20-30 nm in diameter) is a highly modular and bio-degradable platform for facile preparation of neo antigen vaccines within 2 h. RGO-PEG exhibits rapid, efficient (15-20% ID/g), and sustained (up to 72 h) accumulation in LNs, achieving >100-fold improvement in LN-targeted delivery, compared with soluble vaccines. RGO-PEG induces intra cellular reactive oxygen species in dendritic cells, guiding antigen processing and presentation to T cells. Importantly, a single injection of RGO-PEG vaccine elicits potent neoantigen-specific T cell responses lasting up to 30 days and eradicates established MC-38 colon carcinoma. Further combination with anti-PD-1 therapy achieved great therapeutic improvements against B16F10 melanoma. RGO-PEG may serve a powerful delivery platform for personalized cancer -vaccination." (29)

Turkey developing intranasal COVID-19 vaccine. https://www.aa.com.tr/en/economy/turkey-developing-intranasal-covid-19-vaccine/2192492;

"Turkish scientists working with nano-technology firm Nanografi are developing the country's first intranasal COVID-19 vaccine, the industry and technology minister said on Tuesday.

Speaking at the opening ceremony of Nanografi's new graphene production plant in the capital Ankara, Mustafa Varank said the nasal spray is expected to be more effective.

The intranasa IN l vaccine will boost Turkey's efforts in its fight against the coronavirus, Varank said, adding the vaccine could be "remodeled" in case of virus mutations.

Phase-1 human trials will begin shortly for the first Turkish-made intranasal vaccine candidate as its pre clinical stages have successfully completed, he said.

"After all clinical stages successfully completed, we aim to launch administering intranasal vaccine this year," Varank stressed.

Pointing out the obstacles to commercial graphene- adoption, Varank said Turkey will be one of 10 countries which can produce graphene at large scale thanks to the new investment.

Graphene, which is made of a layer of tightly-packed carbon, is light, 200 times stronger than steel and more conductive than copper.

Purely carbon-based graphene is one of the most critical components of nano-technology with single-atom-thickness.

Varank stressed that graphene will help the production of longer-lasting materials, ultra-fast rechargeable batteries, faster -lighter aircraft, bionic devices that can connect to neurons in the body.

Bio-electronic medical technologies that provide real-time treatment by reading and changing body electricity will be developed, and corrosion, heating and transmission problems will be solved," he added.

Citing the latest survey from the Graphene Council, Varank noted that the cost of the material, the mass -production capability, standard and certification are the most prominent obstacles to graphene adoption.

The facility, which will produce graphene at low cost and at industrial- scale with environmental-friendly methods, will be one of the largest in the world thanks to its capacity". (30)

According ECHA Hazard classification & labellingHelp

Graphene: "According to the classification provided by companies to ECHA in REACH registrations this substance is harmful to aquatic life with long lasting effects."

Graphene OXIDE (GO) GHS07: Health hazard

Warning! According to the classification provided by companies to ECHA in CLP notifications this substance "causes serious eye irritation, causes skin irritation and may cause respiratory irritation".

Graphene, under the allowed way of use, not would result peculiar toxicity according ECHA, but GRAPHENE OXIDE it is considered NOCIVE.

It induce oxidative strass dose dependent into the cells, inducing a reduction of vitality as verified in fibroblastic cells (uman and mouse). (Figure 13).

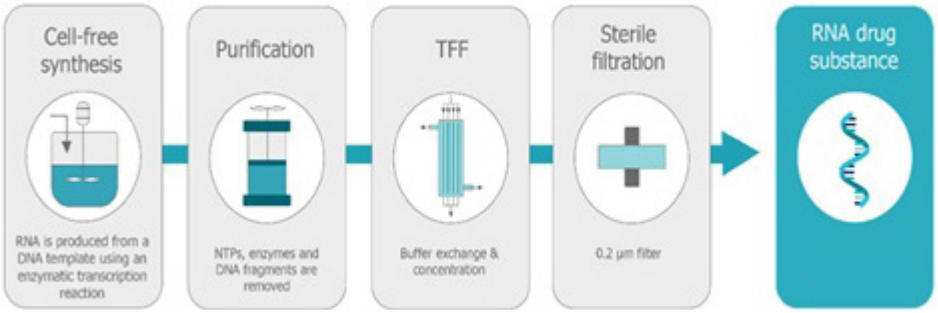

Figure 13: manufacturing process for the RNA drug substance. The process involves a cell-free enzymatic in-vitro transcription reaction followed by a purification phase n to remove the DNA template, then followed by tangential flow filtration (TFF) for buffer exchange and concentration, finally followed by sterile filtration through a 0.2 µm filter.

5.2.6. Anna K. Blakney et al: "Production of Self-Amplifying mRNA saRNA is produced in vitro using an enzymatic transcription in a similar process to the production of conventional shorter mRNA, although the reaction conditions need to be optimized to increase yields for this longer mRNA. The process for the synthesis of in vitro transcribed RNAs was established in the 1990 s, predominantly using phage RNA- polymerases, and is now a robust and well-established for the large-scale production of synthetic RNA. The production method avoids complex manufacturing and safety issues associated with cell culture production of live viral vaccines, recombinant subunit- proteins, and viral vectors. The enzymatic reaction is catalyzed by a phage RNA polymerase, and commercial in vitro transcription kits that produce milligram quantities of RNA for research purposes have been available for several years. Pharmaceutical grade mRNA is currently offered as a contract development and manufacturing organization (CDMO) service by several companies: TriLink, Aldevron, Eurogentec, Biomay, Creative Biolabs and several more will enable capacity in the near future. There are no publications describing the large-scale manufacture of saRNA, but Figure reported describes the unit operations that would be found in a typical cell-free RNA production process. Capped mRNA is produced enzymatically in a bio-reactor and the DNA template is digested. DNA fragments, transcription enzymes, reagents, and byproducts are removed using chromatographic purification followed by tangential flow filtration (TFF). During TFF, due to the large size of the saRNA, lower molecular weight species are removed if the appropriate molecular weight cut-off membrane is selected, and the RNA can dia-filtered into the appropriate buffer and adjusted to the required concentration. RNA is then sterile filtered and stored in bulk ready for further downstream processing and formulation. In addition to the polymerase enzyme, in vitro transcription reactions typically includes: A linearized DNA template with a promoter sequence (~23 bases) that has a high binding affinity for its respective polymerase; ribo-nucleotide triphosphates (rNTPs) for the four required bases (adenine, cytosine, guanine, and uracil); a ribonuclease inhibitor to inactivate any contaminating RNase; a pyrophosphatase to degrade pyrophosphate, which will inhibit transcription; MgCl2, which supplies Mg2+ as a co-factor for the polymerase; and a pH buffer, which also contains an anti-oxidant and a polyamine at the optimal concentrations. If co-transcriptional capping is utilized, the addition of a cap analogue as an initiator of transcription is required.

The recombinant plasmid is propagated in Escherichia coli, linearized using a unique restriction site down stream of the transcription cassette's 3′ end, and isolated and purified using standard molecular biology techniques. During the in vitro transcription reaction, the bacterio-phage polymerase binds the promoter sequence to initiate transcription, and the enzyme then moves along the template towards its 5′ end, elongating the RNA transcript as it travels. Termination of transcription occurs when the enzyme runs off the end of the template (run-off transcription). The poly (A) tail can be encoded into the DNA template, or, alternatively, it can be added enzymatically post-transcription. When the in vitro transcription reaction is complete, the DNA template is fragmented with a DNase, and RNA is recovered using several methods, including precipitation or chromatography. The quality and quantity of RNA produced in an in vitro transcription reaction depends upon a number of factors, including RNA transcript size, template concentration, reaction time and temperature, Mg2+ concentration, and NTP concentration. Typically, the conditions require some optimization for each type of construct being produced.

While there is no published data on a large-scale production process for saRNA, the following sections on capping, purification, immuno-stimulatory by-products, and stability highlight areas that should be consider during process development. Purification Strategies for saRNA mRNA has a negatively charged (-) phosphodiester backbone, and many of the purification techniques used for pDNA could potentially be adapted to the purification of this molecule. DNA purification techniques include: Size-exclusion chromatography (SEC), reversed-phase chromatography (RPC), anion-exchange chromatography (AIEX), hydrophobic interaction (HIC), and thiophilic adsorption chromatography (TOC) . For routine pre-clinical work and in vivo immunization studies, RNA can be precipitated. The polar nature of the negatively charged backbone makes RNA highly soluble in water and several cations (lithium chloride is the most widely used) in combination with ice-cold ethanol as a co-solvent can neutralize the backbone charges and decrease solubility to precipitate the RNA out of solution .Implementing such a process for GMP production would be extremely challenging. Self-amplifying mRNA with sizes in the order of 10,000 bases (MW ~3MDa), has additional challenges over smaller conventional mRNAs and no commercially viable scalable process has been disclosed to date, although likely rely on strategies such as tangential flow filtration (TFF). Review articles on RNA purification indicate that several techniques could be potentially be utilized and these include: Ion exchange (IE), affinity (AC) and SEC. Thus, there remains a need for improved RNA purification methods for saRNA that will enable cost and time efficient purification at an industrial scale with high yield and pharmaceutical grade purity, while retaining the stability, biological potency and functionality of the RNA. Large-scale chromato-graphic purification of saRNA is complex and is an active area of research for many companies and academic institutions. (31)

5.2.7. Norbert Pardi et al: Good manufacturing practice production mRNA is produced by in vitro reactions with recombinant enzymes, ribo-nucleotide triphosphates (NTPs) and a DNA- template; thus, it is rapid and relatively simple to produce in compar-

ison with traditional protein subunit and live or in activated virus vaccine production platforms. Its reaction yield and simplicity make rapid mRNA production possible in a small GMP facility footprint. The manufacturing process is sequence-independent and is primarily dictated by the length of the RNA, the nucleotide and capping chemistry and the purification of the product;It is possible that certain sequence properties such as extreme length may present difficulties (D.W., unpublished observations). According to current at today experience, the process can be standardized to produce nearly any encoded protein immunogen, making it particularly suitable for rapid response to emerging infectious- diseases.

All enzymes and reaction components required for the GMP production of mRNA can be obtained from commercial suppliers as synthesized chemicals or bacterially expressed, animal component-free reagents, thereby avoiding safety concerns surrounding the adventitious agents that plague cell-culture-based vaccine manufacture. All the components, such as plasmid DNA, phage polymerases, capping enzymes and NTPs, are readily available as GMP-grade traceable components; Some of these are currently available at only limited scale or high cost. As mRNA therapeutics move towards commercialization and the scale of production increases, more economical options may become accessible for GMP source materials.

GMP production of mRNA begins with DNA template production followed by enzymatic IVT and follows the same multi-step protocol that is used for research scale synthesis, with added controls to ensure the safety and potency of the product. Depending on the specific mRNA construct and chemistry, the protocol may be modified slightly from what is described here to accommodate modified nucleosides, capping strategies or template removal. To initiate the production process, template plasmid DNA produced in Escherichia coli is linearized using a restriction enzyme to allow synthesis of runoff transcripts with a poly (A) tract at the 3′ end. Next, the mRNA is synthesized from NTPs by a DNA-dependent RNA polymerase from bacterio-phage (such as T7, SP6, or T3). The template DNA is then degraded by incubation with DNase. The mRNA is enzymatically or chemically capped to enable efficient translation in vivo. mRNA synthesis is highly productive, yielding in excess of 2 g l−1 of full-length mRNA in multi-gram scale reactions under optimized conditions.

Once the mRNA is synthesized, it is processed though several purification steps to remove reaction components, including enzymes, free nucleotides, residual DNA and truncated RNA fragments. While LiCl precipitation is routinely used for laboratory-scale preparation, purification at the clinical scale utilizes derivatized microbeads in batch or column formats, which are easier to utilize at large scale. For some mRNA platforms, removal of dsRNA and other contaminants is critical for the potency of the final product, as it is a potent inducer of interferon-dependent translation inhibition. This has been accomplished by reverse-phase FPLC at the laboratory scale, and scalable aqueous purification approaches are being investigated. After mRNA is purified, it is exchanged into a final storage buffer and sterile-filtered for subsequent filling into vials for clinical use. RNA is susceptible to degradation by both enzymatic and chemical pathways. Formulation buffers are tested to ensure that they are free of contaminating RNases and may contain buffer components, such as antioxidants and chelators, which minimize the effects of reactive oxygen species and divalent metal ions that lead to mRNA instability159". (32)

5.2.8. Yuhui Liao et al: "It is well known that graphene oxide (GO), a planar nanomaterial, is endowed with the capacity to immobilize short ssRNA via π-π stacking, thus enhancing its stability. Whether large RNA molecules, such as total RNA, extracted from biological tissues can be protected using GO has not been investigated. It is usually believed that the protection of total RNA by GO is not effective because the lengths of total RNA, which range from a few to thousands of bases, are inclined to undergo desorption due to their complicated structure. Here in, the nanobiological effects of total RNA/GO are first investigated and demonstrate that the total RNA can be harbored on the surface of GO, thus resulting in a shield effect. This shield effect allows total RNA to highly resist RNase degradation and maintain RNA stability at room temperature up to 4 days, enabling the discovery of GO as the potential next-generation RNase nano-inhibitor. GO can be conjugated to nano-magnetic beads, defined as magnetic graphene oxide, enabling the rapid purification and protection of RNA from animal cells and tissues, whole blood, bacteria, and plant tissue." (34)

From Creative biogene website:

VT mRNA Purification –Services "There has been tremendous interest around in vitro-transcribed (IVT) mRNA for the research and the therapeutic applications in recent years. The manufacturing of pure mRNA products are on-demand as the importance of IVT mRNA is gradually rising. With years of experience in IVT mRNA synthesis and purification technology, Creative Biogene has developed robust and cost-effective protocols to purify IVT mRNA to help worldwide customers obtain efficient and high-quality synthetic mRNAs.

The importance of purification Schematic of IVT m RNA purification (Figure 14).

Creative Biogene' IVT mRNA purification protocols

Many purification techniques have been developed to enhance the concentration and quality of IVT mRNA, including high-performance liquid chromatography (HPLC), fast protein liquid chromatography (FPLC) and magnetic- beads technology.

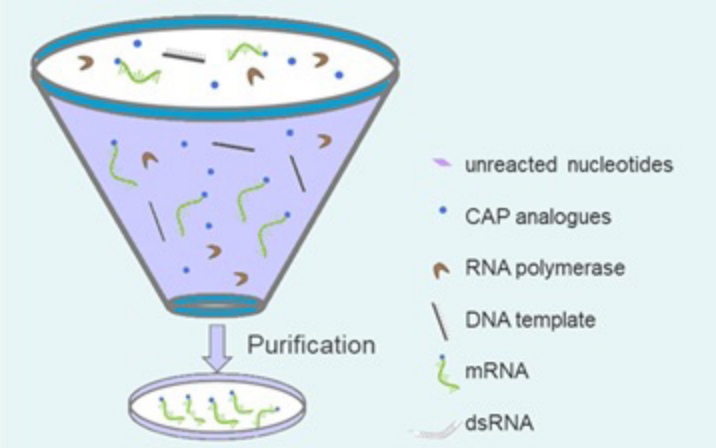

Figure 14: The purity of IVT mRNA is a key factor in determining the efficiency of target antigen AG expression. After in vitro transcription, the reaction solution contains m RNAs and remaining reagents. The remaining reagents of the in vitro synthesis process are comprised of RNA polymerase, un-reacted nucleotides, CAP analogues, DNA template as well as double-stranded RNA (dsRNA) side products. Previous studies have identified that the presence of certain byproducts can trigger cellular immune responses. Thus, purifying mRNA from these contaminant impurities is critical, especially for in vivo- applications.

5.3. HPLC purification of IVT mRNA

For large-scale purification and isolation, high performance liquid chromatography (HPLC) is most often used. Previous studies demonstrated that the translation efficiency of HPLC-purified mRNA is greatly enhanced. HPLC can be used to isolate synthesized mRNA from the remaining reagents via size exclusion or ion-exchange columns, thereby removing smaller or larger by-products, such as dsRNA, abortive transcripts and so on.

5.4. mRNA purification using magnetic beads technology

Magnetic beads are made up of tiny (20 to 30 nm) particles of iron oxides, which give them superparamagnetic properties. In contrast to prior purification methods, this method is fast, consistently reliable, and the most efficient in m RNA purification. Under optimized conditions, m RNA selectively binds to the surface of magnetic- beads, while other contaminants stay in in vitro transcription reaction solution".

5.4.1. Xuan-Hung Pham et al: "A magnetic material that consists of silica-coated magnetic beads conjugated with graphene oxide (GO) was successfully prepared for a facile ribonucleic acid (RNA) extraction. When the GO-modified magnetic beads were applied to separate the RNA from the lysed cell, the cellular RNAs were readily adsorbed to and readily desorbed from the surface of the GO-modified magnetic beads by urea. The amount of RNA extracted by the GO-modified magnetic beads was ≈170 % as much as those of the control extracted by a conventional phenol-based chaotropic solution. These results demonstrate that the facile method of RNA separation by using GO-modified magnetic beads as an adsorbent is an efficient and simple way to purify intact cellular RNAs and/or microRNA from the cell -lysates. (33)" (Figure 15).

In a Preprint: A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic and prevents lung infection in non-human primates bioRxiv preprint doi: https://doi.org/10.1101/2020.09.08.280818;

Magnetic particles for the separation and purification of nucleic acidsSonja Berensmeier

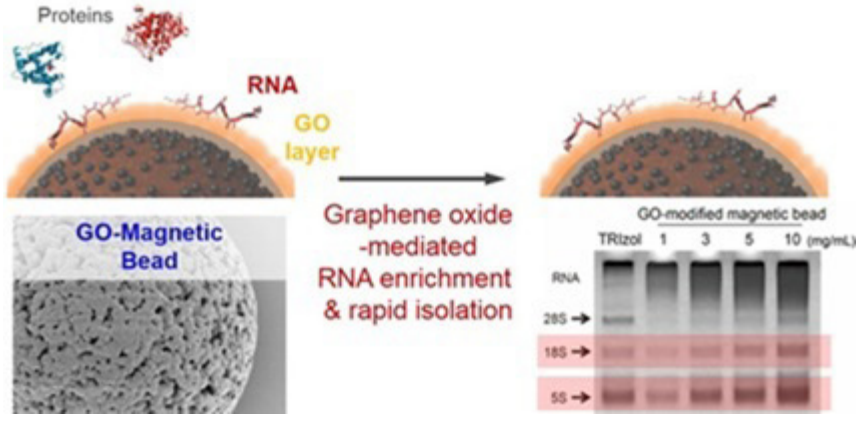

Figure 15: Magnets! A magnetic material that consists of silica-coated magnetic beads conjugated with graphene oxide (GO) was prepared. The material was tested in the separation of ribonucleic acid (RNA) in a lysed- cell, and the results show that the GO-modified magnetic beads can be used as mobile solid-phase particle candidates, and used to directly separate the RNA in bio-materials.

5.5. Applied Microbiology and Biotechnology

"Nucleic acid separation is an increasingly important tool for molecular biology. Before modern technologies could be used, nucleic acid separation had been a time- and work-consuming process based on several extraction and centrifugation steps, often limited by small yields and low purities of the separation products, and not suited for automation and up-scaling. During the last few years, specifically functionalised magnetic- particles were developed. Together with an appropriate buffer system, they allow for the quick and efficient purification directly after their extraction from crude cell extracts. Centrifugation steps were avoided. The new approach provided for an easy automation of the entire process and the isolation of nucleic acids from larger sample volumes. This review work describes traditional methods and methods based on magnetic particles for nucleic acid purification. The synthesis of a variety of magnetic particles is presented in more detail. Various suppliers of magnetic -particles for nucleic acid separation as well as suppliers offering particle-based kits for a variety of different sample materials are listed. Commercially available manual magnetic separators and automated systems for magnetic- particle handling and liquid handling are mentioned."(36)

Goole patent https://patents.google.com/patent/KR101800004B1/en

Graphene oxide modified magnetic bead, process for preparing the same and process for nucleic acid extraction using the same

"The present invention provides magnetic -beads modified with graphene oxide, a production method of the magnetic beads, and a separation method for nucleic acids using the same. The magnetic- beads modified with oxidized graphene of the present invention can be usefully used for a simple and convenient method for purifying single stranded DNA or RNA in bio-technology where extraction of small molecule RNA such as miRNA is essential. "

Reduced Graphene Oxide-Based Solid-Phase Extraction for the Enrichment and Detection of microRNA

5.5.1. He Yan et al Anal. Chem. September 21, 2017: "MicroRNAs (miRNAs) are endogenous molecules with regulatory functions. The purification and enrichment of miRNA are essential for its precise and sensitive detection. miRNA isolated using commercial kits contains abundant interfering RNAs, and the concentration of miRNA may not be adequate for detection.We prepared a reduced graphene oxide (rGO)-based magnetic solid-phase extraction material for the enrichment and ultra-sensitive detection of miRNA from intricate nucleic acid solutions. In situ reverse transcription (RT) was developed as the most efficient approach to desorb miRNA from rGO among the methods that are compatible for the subsequent amplification reported thus far.Rolling circle amplification and qPCR were used to detect let-7a with a decrease of the limit of detection by 24.7- and 31.3-fold, respectively. This material was also successfully used to extract and detect miRNA from total RNA isolated from human plasma. Our results show that the material prepared in this study has the potential for cancer-biopsy in clinics and the discovery of new miRNAs in scientific research."(37) (Figure 16).

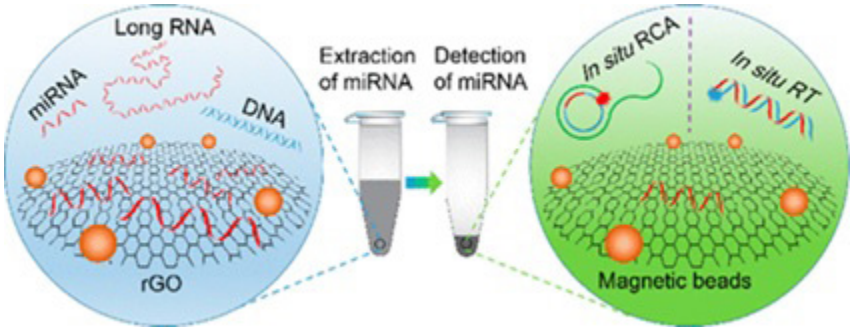

Figure 16:

5.5.2. Oxana Vasilievna Kharissova et al: "Environmental problems of contamination with heavy- metals and other pollutants (As, Cr) can be partially solved using iron/graphene composites, in particular those containing iron in both metallic and oxidized form. A one-pot thermal decomposition method was used for the preparation of graphene nano-platelet composites decorated with core-shell Fe-Fe2O3 nano-particles. These nano-composites could be separated from the liquid-phase mixture with the aid of a permanent- magnet. Efficient and effective adsorption of arsenic (III) from the polluted water was observed for this material (nearly complete As (III) removal within 1 ppb) and attributed to the increased adsorption sites existing in the presence of magnetic nano-particles. Magnetic- graphene nano-composites (MGNCs), consisting of a core@double-shell structure of the nano-particles with crystalline Fe as the core, iron oxide as the inner shell, and amorphous Si-S-O compound as the outer shell, were prepared by a thermo-decomposition process . These composites were highly stable even in 1 M HCl aqueous acid and showed a fast and highly-efficient removal of Cr(VI) from waste-water after 5 min (Figure 10), in contrast to several other materials (like carbon, waste biomass), the use of which require several hours or days and are not able to achieve 100% removal of Cr(VI)". (38) (Figure 17).

DNA and RNA extractions from eukaryotic and prokaryotic cells by graphene nanoplatelets. Ehsan Hashemi, Omid Akhavan, Mehdi Shamsara, Sepideh Valimehra, Reza Rahighib

"Graphene nano-platelets with lateral dimensions of ~50–200 nm and thicknesses <2 nm were utilized for the extraction of nucleic acids (NAs) from eukaryotic and prokaryotic- cells. The graphene nano-platelets (both chemically exfoliated graphene oxide nano-platelets and hydrazine-reduced graphene oxide nano-platelets) successfully extracted plasmid DNA (pDNA) from Escherichia coli bacteria, comparable to a conventional phenol–chloroform (PC) method. It was found that the yield of graphene nano-platelets in genomic DNA (gDNA) and RNA extractions from embryonic stem- cells (ESCs) was also comparable to the yield of the conventional methods. The effects of the graphene nano-platelets on restriction enzyme digestion of the pDNA and gene amplification of all the extracted NAs (including pDNA, gDNA and RNA) were also investigated in order to confirm the quality of the extractions. These results not only demonstrated an easy gene extraction capability of graphene nano-platelets with a high gene amplification, but also provide an easy, fast, inexpensive and bio-compatible DNA/RNA extraction method"(39) (Figure 18).

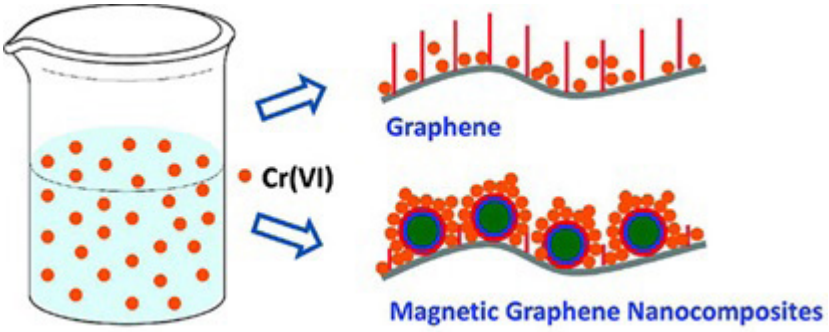

Figure 17: Schematic adsorption mechanisms on graphene and MGNCs

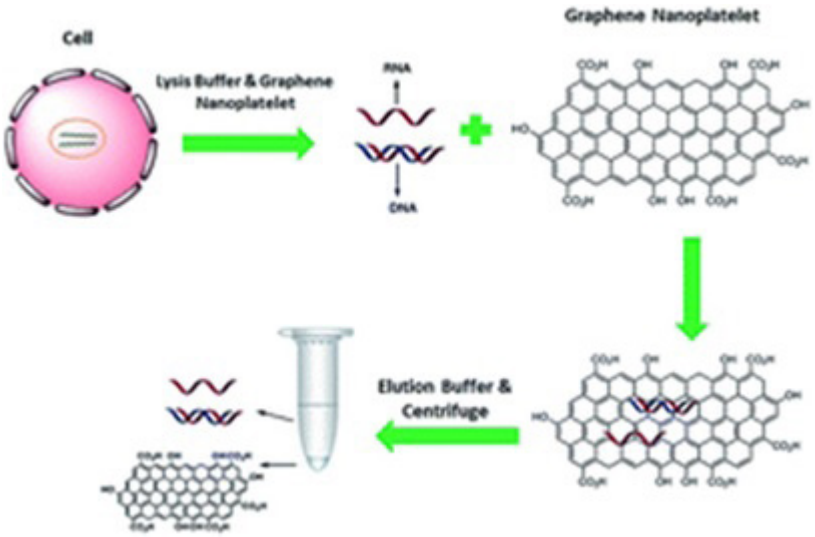

Figure 18:

6. Experimental Project Hypotesys

In order to verify the presence / absence of graphene and derivates in various kind of covid-19 vaccine it is needed to test in analytical way (using the official chemical instrumental methods officially in use and with the sensibility and accurance levele needed). 100 vial of each vaccine blinded whit other 100 vials of non covid-19 vaccine 10 vial must to be sended to one certified laboratory and 10 to other and so on. All data must to be collected and statistycally analized.

7. Results

Presence on graphene or GO in sample related vaccine in significative way (p> 0,0,5) versus the control is considered suspicious.

8. Discussion

Related all the literature and the data reported it is clear the various covid-19 vaccine not show in technical sheet graphene and derivates and international authorities for DRUGS SAFETY gived us confirm of this.

Even if generally, this is not recognized by public authorizative international org. Some interesting evidence seem proof in the sample analyzed something of interest (see the literature in the reference).

Of interest also the microscope images related blood of patient after vaccination (see images as reported).

The fact that some of this data comes from university is crucial.

Also chemical -phisical properties of this class of carrier (and extraction agent of RNA) are of interest in vaccine strategy design since 2016 (before covid vaccine design) .

The The use in manifacturing procedure (extraction of RNA) is reported in many literature published .(magnetics microbeads).

Related this properies it is interesting to observe the profile of toxicity of this molecule and to cross this with some relevant ADR registered in some covid-19 vaccine (see all the SEVERE, ADR registered in official.

Public database: trombosys, miocarditis and other relevant even rare effect)

In some italian judge sententia (2022) related casue effect relationship (PISA) between a covid-19 vaccine m-RNA vaccine and an severe trombocitemia in a 16 year old patient.

In another italian judge sentantia in 2022 (firenze) was reported that the the covid vaccine are sperimental and invasive Of human DNA, also it is possibile in irreversible way and whit unprevedible effetc at today know so it is not possible to oblige someone healthcare professional to Be vaccinated whit this.

In referencen n 33 in 2017 (before covid pandemia) was reported the use of Graphene GO in extraction of RNA durign the manifacturing process.

In the experimental hypotesys project it is reportet a simply method to verify the presence/ absence of graphene and derivates in the various vials of covid-19 vaccine (all types).

9. Conclusion

Related the recent evidence, the profile of toxicicy of graphene products, the clinical aspects of some rare ADR of some covid -19 vaccine and the chemical physical properties of this carriers need to be deeply investigated.

The use in vaccine strategy started before covid-19 pandemia also related cancer vaccine but at today various project test, this material in an intranasal vaccine so it is possible to say that probably the Efficacy/ tocicity profile depend also on the way of submini-stration.

Because this product, as reported in literature, increase some immune response it is interesting to continue the research to find the real pharmaceutical/ toxicological profile.

In every way it is fundamental to observe the manifacturing process, the technologies and material used to Produce and purify this m- RNA vaccine: this can make possibile to better understant the impurity profile even if this are not reported in the technical sheet of some approved m-Rna vaccine related the graphene and derivated presence.

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