Original Article: Synthesis of Graphene Oxide Nano Carriers Containing Alcoholic Extracts of Turmeric, Sedum, and Rosemary in Order to Treat Breast Cancer in Dogs

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ABSTRACT

Graphene oxide (GO) produced by intense oxidation of graphite in Hamer method is an ideal nano carrier for drug and gene delivery. The number of graphene oxide layers used for drug delivery is between one and three layers (the total thickness of the layers is 1 to 2 nm) and their size is between a few nanometers and several hundred nanometers. This material has unique structural features such that its specific contact surface is very high (2630 square meters per gram) and on the other hand, it is full of oxygen-rich groups; For this reason, this two-dimensional material has good biocompatibility and physiological solubility, and its stability is also suitable. The mentioned characteristics have made this material a suitable option for loading drugs and genes through chemical correlation. The presence of hydroxyl (OH) and carboxyl (COOH) functional groups in the structure of graphene oxide has caused GO to have good correlation with various systems such as polymers, biomolecules, quantum dots, iron oxide nanoparticles (Fe_3O_4), etc. It is possible to predict various applications for graphene oxide in this field used graphene oxide for water-insoluble aromatic anticancer drugs.

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Introduction

Graphene oxide was first coupled with polyethylene glycol (PEG) molecule, which has six amino arms, and then conjugated with a water-insoluble anticancer drug (SN_{38} was applied to the surface of graphene oxide by simple π-π non-covalent adsorption). Polyethylene glycol functionalized graphene oxide loaded with SN_{38} showed 1000 times more cytotoxicity to HCT-116 cells than CPT-11. In another research, the same group investigated the targeted release of chemical drugs in cells, using GO-PEG, which has formed a correlation with Rituxan (CD20+ antibody) [1-3]. In this research, it was found that the drug release rate from graphene oxide depends on PH is dependent; therefore, by controlling the PH, the release rate of the drug can be controlled. The proper performance of graphene oxide in drug delivery for cancer treatment has caused the application of this substance to be extended to non-cancer drug delivery systems [4]. The release of anti-inflammatory drug ibuprofen was investigated using graphene oxide grafted with chitosan. The loading rate of this drug on graphene oxide was 9.7%. In addition, in this research, it is claimed that the release rate of this drug can be controlled by controlling the PH value. Cancer is a group of diseases that includes abnormal cell growth in the body. Cancer can start in one place and spread to other parts of the body. Many animal species get cancer, but some never get it or the prevalence of cancer among them is very low. Previously, scientists believed that any potential body cell can become a cancer cell and if the body's defense system does not identify and destroy it, this cell can create a cancerous mass. For this reason, it was thought that larger animals with higher body mass and more cells are more at risk of developing cancer. In humans, taller and larger people are more prone to cancer than shorter and smaller people. Also, age is an important factor in getting cancer because, on the one hand, genetic repair mechanisms become less efficient in old age and the organism's defense system also becomes weaker. However, research in recent decades indicates that there is no relationship between body size and the prevalence of cancer in animal species [5].

All types of cancers are very common especially in old dogs (over 10 years old).

Both male and female dogs have 10 mammary glands in the form of 5 pairs facing each other, which are located from under the chest to the back of the abdomen. Like humans, these animals can get mammary gland tumors (both benign and malignant).

99 percent of cases of breast tumors are seen in females. The easiest way to prevent the development of these tumors is to sterilize (remove the uterus and ovaries) female dogs and cats. One of the important reasons why veterinarians recommend early sterilization of dogs is to prevent the development of these tumors. Almost half of mammary tumors in dogs are either malignant or become malignant and can spread (metastasize) to lymph nodes, lungs and other areas of the body, in which case surgery and treatment will be very difficult. The advent of nanotechnology has shown the possibility of treating or targeting breast cancer. Technological research progress in the veterinary field has continued for decades, but there has been no significant progress in the treatment of cancer [5].

Zirfon plant

Antioxidants in Zirfon leaves fight against free radicals. We have known for years that free radicals are the strongest causes of cancer. In addition, Zirfon leaf has anti-inflammatory properties that can prevent the growth of cancer cells that are caused by inflammation in the cells. A substance called P-coumaric in the Zirfon leaf helps to detoxify the body. Consuming Zirfon leaves can increase the production of sweat and urine. This function is attributed to a substance called P-coumaric. Zirfon leaf helps to detoxify the body. As we know, our body removes toxins through sweat and urine.

Rosemary plant

The effective ingredients of this plant are anti-bacterial and anti-fungal and have strong antioxidant properties. Its scientific name

(Rosmarinus) means sea dew because the natural habitat of the plant is mostly in the coastal areas of the Mediterranean Sea. Rosemary has been recognized as a valuable medicinal plant in the pharmaceutical and medical industry due to its antimicrobial, anti-mutagenic and chemical preventive properties. Rosemary plant extract and oil are used due to their liver protection properties, effective in treating Alzheimer's disease, antimicrobial, anti-inflammatory and anti-cancer effects. The fresh leaves and flowers of the rosemary plant contain amounts of vitamins, including vitamin C. This aromatic plant is very useful for strengthening the immune system. In addition, the active components of rosemary are antioxidant and anti-inflammatory compounds [6].

Turmeric: curcumin, the effective substance of the rhizome of the turmeric plant, is chemically called difeouloy imethane with the chemical formula $C_{12}H_{20}O_6$. In addition to curcumin, there are many chemical compounds such as volatile oil, zingiburn, alpha and beta tourmarin and other substances such as arabinose, fructose, glucose and starch are present in the rhizome of turmeric plant. The color of turmeric is related to color substances such as curcumin, desmethoxy curcumin curcumin and desmethoxy bis.

It has been shown in many researches that the consumption of certain foods and medicinal plants can inhibit the growth of cancer cells. Preliminary studies have shown that the compounds of this plant fight cancer cells. The development of nano medicine increases the possibility of specific targeted delivery that overcomes tumor barriers. It is expected to facilitate better drug delivery and increase efficacy while minimizing side effects of anticancer drugs. Nanomaterials have now become an interesting topic for research in various fields, especially in the field of medicine. Nanomaterials have now become an interesting topic for research in various fields, especially in the field of medicine. Nano medicine is one of the most active research areas in the field of nanotechnology, which has a great impact on the future formation of cancer treatment. Antioxidant, antitoxic, anti-inflammatory and anticancer effects of curcumin have been reported. And the consumption of this substance with a high dose prevents the proliferation of cancer cells, but does not harm healthy cells.

Materials and methods of work

Graphene oxidation

At first, the amount of 0.2 grams of graphene is weighed in a beaker, poured into an ice bath and placed on a stirrer. Then 3 ml of phosphoric acid and 20 ml of sulfuric acid (98%) are added to it and it is allowed to ferment for 30 minutes at a temperature below 0C. Then add 0.25 grams of sodium nitrate salt to the solution and add 0. Then, the amount of 1.5 grams of potassium permanganate is allowed for 30 minutes at a temperature below 0C and is slowly added to the solution by controlling the temperature (the temperature must be below 0C) over a period of one hour. At this stage, the color of the solution is dark muddy green. After this stage, the material is stirred for 3 hours at a temperature of 35°C by a stirrer. At this stage, the color of the solution is dark brown. After completing the above steps with temperature control (temperature must be below 0C). At this stage, purple colored vapors are removed from the container after each addition of water, and pouring distilled water continues until no purple colored vapors are seen. After completing this step, the material is placed in an ice bath and placed on the stirrer, and gradually and slowly, 120 ml of a solution containing hydrogen peroxide and distilled water (20 ml of hydrogen peroxide, 120 ml of distilled water) is added to It is slowly added to the solution, which causes the color of the solution to change from brown to yellow. After completing this step, the resulting substance is placed in an ice bath and temperature control (the temperature must be below 0C) and allowed to be eaten for 3 hours.

After this step, the resulting sediment is allowed to settle. After that, the water on the sediment is separated and the remaining sediment is washed with 50 ml of 5% hydrochloric acid and centrifuged. Then the sediment obtained is washed 6-7 times with distilled water to neutralize the high PH of the sediment. After complete washing, the sediment is poured into a container and placed in the oven at a temperature of 60°C to dry [7].

Preparation of phosphate buffer: First, 8 grams of sodium chloride (NaCl), 0.2 grams of potassium chloride (KCl), 0.25 grams of potassium dihydrogen phosphate (KH_2PO_4) and 1.44 grams of dihydrogen phosphate (Na_2HPO_4) respectively 900 mL of added deionized water is placed on the stirrer and allowed to elute until no solid particles are visible in the container. Then the acidity of the solution is measured with a PH meter, which at this stage should be around 7.4. Then about two to three drops of our 1 normal HCl are added to the solution and it is allowed to sit on the stirrer for a few minutes.

Then the PH of the solution is measured again, which should be around 2.7. At the end, 100 ml of deionized water is added to the solution. Extraction of plants: In this step, for extracting from each plant sample, first, 2 grams of dry plant is poured into 10 ml of absolute ethanol and the obtained sample is heated indirectly at 30 degrees Celsius for 15 minutes and stirred. Then the resulting sample is placed in the ultrasonic device for 5 minutes, temperature 25 degrees Celsius and power 16 Hz. Then the obtained sample is filtered by filter paper and the obtained liquid extract is made into powder form by freeze dryer. Loading the plant extract from the prepared carrier, 0.02 g is removed and poured into an Erlenmeyer flask.

2 mg of mixed plant extract (equal proportions) are added to it, and 50 ml of prepared phosphate buffer is added to each. The flasks are placed on the stirrer and stirred for 48 hours. After 1 hour of every 24 hours, the substances are ultra-sonicated and after centrifugation, the UV spectrum is taken: Drug release. To release the drug, at first, all the solutions are centrifuged until the phosphate buffer is completely separated from the sediment. Then the sediments are completely dried, and 50 ml of phosphate buffer with PH 2.7 is poured on it. After that, the resulting solution is stirred for 24 hours by stirrer and at the end Each time interval is taken from that UV spectrum. After that, the solutions are completely centrifuged and the remaining sediment is dried. Then, 50 ml of phosphate buffer with PH 5.4 is poured on each and it is allowed to eat for 1 and 24 hours. After the end of each time interval, the UV spectrum is taken. Preparation of the sample for taking SEM images and IR spectrum: To prepare the sample for the IR spectrum, first, 0.001 grams of the sample is mixed with 0.009 grams of KBr and it is made into tablets by a tablet maker with a pressure of 10 atm. At this stage, the sample is ready for spectroscopy [8].

Discuss

Graphene derivatives are attractive carriers for loading various cargoes, including anticancer drugs, because they have extremely high surface area, strong interaction, and diverse reaction capacity with a variety of materials. In addition, graphene derivatives can absorb NIR laser and transfer the energy to heat, which can be used for controlled drug release (thermal release).

Oxidation of graphene to GO often serves as a precursor for further functionalization with various bioactive molecules, enabling their use in a variety of biomedical applications. Like many other nanomaterials, the absorption of GO in the mononuclear phagocyte system (MPS, such as liver and spleen) is inevitably high. To improve in vivo pharmacokinetics, the attachment of engineered antibody fragments (e.g., Fab, F(ab')2, diabetes, nano body, etc.) can potentially reduce non-specific binding, increase tumor penetration, and have time adjustable circulation, which is one of us researches in the future. Various combinations of drug/genes/ therapeutic isotopes (for example Cu67) can be loaded onto the GO conjugates designed in this study for future therapy. The future of nano medicine lies in multifunctional nano platforms that have both therapeutic components and imaging tags. Other inorganic nanomaterials, such as magnetic iron oxide (IONPs) and silica-based nanoparticles, can be grown on the GO surface to obtain hybrid graphene nanomaterials for multimodal imaging. At the same time, graphene-based nanomaterials are usually considered as "Low degradability" in the body. Therefore, combination with other substances/molecules is a strategy to adjust their pharmacokinetics in test subjects. With further optimization these GO conjugates can be used as a "Smart" actor for cancer therapy imaging [9].

Conclusion

In this study, we demonstrated efficient tumor (metastatic) targeting of GO conjugates in an experimental model of breast cancer lung metastasis in which FSHR was used as the target. FSHR is ubiquitously expressed in vasculature from various cancer types, making it suitable for nanomaterial-based tumor targeting. Based on various in vivo/in vitro/ex vivo studies, GO conjugate showed excellent stability and high specificity for FSHR. NOTA-GO-FSHR-mAb can also serve as a highly efficient drug delivery vector in metastatic breast cancer.

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Conflict of Interest

There are no conflicts of interests.

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