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(54) Title of Invention

Nano-coronavirus reconstitution based on graphene oxide vaccine

(57) Abstract

The present invention belongs to the field of nanomaterials and biomedicine. It involved A vaccine, Specifically, involving the 2019-nCoV coronavirus The invention also includes the preparation of the vaccine. Preparation method and application in animal experiments. The seedlings contain graphene oxide, carnosine, CpG, and SARS-CoV-2 RBD; exist Carnosine, CpG and the new coronavirus are combined on the skeleton of graphene oxide RBD; The coding sequence of the CpG is shown in SEQ ID NO 1; The novel coronavirus RBD refers to the novel coronavirus protein receptor The binding region can produce high titers against RBD in mice Specific antibodies Provides strong support for the prevention and treatment of new coronavirus Strong support.

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1. A coronavirus vaccine, It is characterized in that The coronavirus contains graphene oxide, Carnosine, CpG and COVID-19 Viral receptor binding region; Combine carnosine, CpG and the novel coronavirus receptor binding region on the graphene oxide backbone; Said The coding sequence of CpG is shown in SEQ ID NO 1; The novel coronavirus receptor binding region is the novel coronavirus S Protein receptor binding region.

2. The coronavirus according to claim 1, It is characterized in that The coronavirus is expressed in activated graphite oxide Ethylene-linked carnosine, CpG and coronavirus receptor binding regions.

3. The method for preparing the coronavirus according to claim 1, It is characterized in that The preparation method comprises the following steps: Steps:

Get CpG, Recombinant protein of receptor binding domain and carnosine The coding sequence of CpG is shown in SEQ ID NO 1;

Graphene oxide freeze-dried powder was added to phosphate buffer. Ultrasonic treatment;

Add EDC and NHS to activate the graphene oxide solution. The excess EDC/sulfo-NHS, Adjusting the pH of the reaction solution to neutral;

Carnosine, CpG and receptor binding domain recombinant proteins were added to the reaction solution. incubation with activated graphene oxide;

Remove excess uncoupled protein from the reaction solution. Sterilization, spare.

4. The preparation method according to claim 3, It is characterized in that The ultrasound lasts 2-3 hours.

5. The preparation method according to claim 3, It is characterized in that The pH value of the phosphate buffer is 6.8-7.6.

6. The preparation method according to claim 3, It is characterized in that Remove excess EDC/Sulfo-NHS or remove un The method of coupling the protein is ultrafiltration.

7. The preparation method according to claim 3, It is characterized in that The amount of carnosine used is more than 1.5 times that of graphene oxide by The amount of the binding region is 2-10 times that of CpG. The amount of CpG used is one ten-thousandth of graphene oxide. Quality ratio.

8. The preparation method according to any one of claims 3 to 7, It is characterized in that The reaction temperature is between 20 and 28 °C.

9. The use of the coronavirus according to claim 1, It is characterized in that The new crown vaccine is used to prevent coronavirus disease Application of toxic drugs.

10. The use according to claim 9, It is characterized in that The coronavirus causes the body to produce receptor binding Antibodies to regional recombinant proteins.

Nano coronavirus recombinant vaccine based on graphene oxide

Technical Field

[0001] The present invention belongs to the field of nanomaterials and biomedicine. It involves the development of a vaccine development platform. Specifically, The invention relates to the development of a 2019-nCoV coronavirus nuclear recombinant nano vaccine. The invention also includes the use of the vaccine in animal experiments.

Technical Background

[0003] Vaccines are the ultimate weapon to eliminate major infectious diseases. Vaccines have the lowest cost compared to other treatments. More first Develop the advantage over the ering, has undoubtedly become the public's hope. Through vaccination, humans have eradicated smallpox. Poliomyelitis The number of cases has also been reduced by 99%. Infectious diseases such as diphtheria are rare. measles, The incidence of diseases such as neonatal tetanus has dropped significantly. The impact of antibiotics on human health cannot be overstated. The birth of each new vaccine is humanity's victory over an infectious disease. A great victory! So far, no medical measure has had such an important and lasting impact on human health as vaccines. lasting and far-reaching impact; There is no therapeutic drug that can eliminate a disease from the earth at an extremely low cost like a vaccine.

[0004] Soon after the SARS-CoV-2 outbreak, Different laboratories in China have completed the isolation of virus strains. To fight against the epidemic The research and development of seedlings has taken a big step forward. I believe we will soon have the ultimate weapon to eliminate SARS-CoV-2. However, Until now So far, There is no approved vaccine or drug for the treatment of CoV infection. Therefore, it is very urgent to develop an effective Drugs to treat or prevent coronavirus infections and outbreaks.

[0005] According to research on coronavirus vaccines such as SARS and MERS, The main targets for coronavirus vaccines The S protein of the coronavirus. Vaccines need to induce not only humoral and cellular immune responses, It is also necessary to induce a mucosal immune response, and with the help of adjuvants to induce balanced Th1 and Th2 pathways, Only then can a truly effective vaccine be produced. The research on MERS vaccines mainly focuses on viral vector vaccines and subunit vaccines. Numerous studies have shown that SARS and MERS are difficult to The problem is that it cannot stimulate the production of long-term memory B cells. In recovered SARS and MERS patients, long-term memory cells can only last Continue for 2-3 years, Failure to generate immune memory The failure of vaccine development Only six potential coronavirus vaccines are in development Entering the clinical research stage, However, no effective vaccine has been approved for marketing.

Summary of the invention

[0006] The present invention aims to provide a coronavirus recombinant vaccine.

[0007] Another object of the present invention is to provide a method for preparing the virus recombinant vaccine. Another

[0008] object of the present invention is to provide an application of the virus recombinant vaccine. In view of the various

[0009] problems existing in the current traditional vaccines, How to change the problems of existing vaccines? Enhance immune response This should be the question we have been thinking about. In order to enhance the immune activity of the immunogen, Strengthen the body's immune response ability, The most basic The original method is to mix the immunogen with an adjuvant. Adjuvants are a class of agents that can enhance the body's immune response to an immunogen. CpG oligodeoxynucleotide (ODN) is a very promising adjuvant discovered in recent years. CpG ODN has a very good effect in animals, In vitro and clinical studies have shown that It has good adjuvant activity. The most well-studied CpG7909 and CpG1018. November 9, 2017 Dynavax Technologies approved by US FDA for CpG1018 The adjuvanted hepatitis B vaccine was launched on the market. The vaccine is the world's first approved CpG ODN adjuvant vaccine for the prevention of

Adults with HBV infection In addition, different types of CpG ODN have been used as adjuvants in multiple clinical trials. By binding to TLR9, Activate immature pDCs. Inducing innate and adaptive immune responses, However, a single CpG The activation effect of the structure on immune cells is limited. At the same time, it is easily hydrolyzed by exonucleases. The lack of stability in the body return Can cause side effects; Synthetic CpG oligodeoxynucleotides (ODN) in the sequence can also enhance the stimulation. CpG and antigen Other proteins can be used after coupling It has a very significant immune activating effect.

[0010] Graphene is a carbon atom in the form of sp^2 The hybrid orbitals form a two-dimensional carbon nanomaterial with a hexagonal honeycomb lattice. Its basic structural unit is the most stable benzene six-membered ring in organic materials. It is the most ideal two-dimensional material at present. Graphene oxide (GO) is a derivative of graphene. It is the exfoliation of graphite oxide Because of its unique The characteristics of SP2 hybridization and perfect two-dimensional structure as well as high reactivity of the edge, So that in the design and development based on it The therapeutic platform can serve as an ideal loading and grafting carrier. In nano drug delivery systems, Biological detection, tumor treatment and It plays an important role in cell imaging.

[0011] The present invention is completed based on the above research.

[0012] The present invention is based on graphene oxide material as a skeleton to load CpG molecules and recombinant proteins. Developed a new Vaccine development method. A new nano COVID-19 vaccine was prepared based on this technology platform combined with the recombinant protein of the RBD region of the Spike protein of SAR-CoV-2. The prepared nano COVID-19 vaccine showed strong immunogenicity in mouse experiments. Can produce Produce high titer antibodies.

[0013] on the one hand, The present invention provides a coronavirus vaccine. Contains graphene oxide, carnosine, CpG, RBD. In a preferred embodiment of the invention, It is called GO-Car-carnosine-CpG-RBD vaccine.

[0014] Graphene oxide (GO, Graphene oxide) is the oxide of graphene. After oxidation, Oxygen-containing functional The increase in clusters makes the properties more active than graphene. For example, Randomly distribute hydroxyl and epoxy groups on a graphene oxide sheet. and Carboxyl and carbonyl groups are introduced at the edge of the single sheet. Common commercial products of graphene oxide are in powder, flake and solution form. The color is brownish yellow.

[0015] Carnosine, Scientific name: β-alanyl-L-histidine, It is composed of two amino acids, β-alanine and L-histidine. The dipeptide Crystalline solid. Carnosine has strong antioxidant capacity. It can remove the cell membrane during oxidative stress Reactive oxygen free radicals (ROS) and α-β unsaturated aldehydes are formed by excessive oxidation of fatty acids.

[0016] CpG motifs have the function of activating the body's immune system. Can be used as an adjuvant. The CpG coding The sequence is shown as SEQ ID NO 1.

[0017] RBD (spike receptor binding domain) is the receptor binding region. The RBD in the present invention refers specifically to crown The receptor binding domain (RBD) of the virus protein (S protein). For example, You can choose RBD proteins with the following sequences:

PNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFV IRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGS TPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAP (SEQ ID NO 2).

[0018] The coronavirus vaccine of the present invention combines carnosine, CpG and SARS-CoV-2 RBD And obtain.

[0019] In the coronavirus vaccine of the present invention, GO is used as the skeleton basis. Usually overdose, The dosage of carnosine can be GO CpG and SARS-CoV-2 RBD are biological macromolecules. Its dosage is small, The dosage of both is usually 100% of GO. One of them is the mass ratio. The amount of RBD is more than twice that of CpG. For example, CpG: RBD=1:2-10, Better, The dosage of RBD is 3-6 times that of CpG.

[0020] on the other hand, The present invention provides a method for preparing the coronavirus vaccine. The preparation method comprises

Follow these steps:

Get CpG, RBD recombinant protein and carnosine;

GO freeze-dried powder was added to phosphate buffer. Ultrasonic treatment;

EDC and NHS were added to activate the GO solution. The excess EDC/sulfo-NHS in the reaction solution was removed by ultrafiltration. Will react The pH of the solution was adjusted to neutral;

Carnosine, CpG and RBD recombinant proteins were added to the reaction solution. Incubation with activated GO;

Remove excess uncoupled protein from the reaction solution. Sterilization, spare.

[0021] Better, The duration of ultrasound is 2-3 hours. The ultrasound condition is 200 W. 40 kHz.

[0022] Better, The pH value of the phosphate buffer is neutral. For example, 6.8-7.6, The better one is 7.0-7.4, or 7.2.

[0023] Better, The method for removing excess EDC/sulfo-NHS or uncoupled protein is ultrafiltration. In a preferred

[0024] embodiment of the present invention, The ratio of graphene oxide, carnosine, CpG and RBD is: 26mg:40mg: 1.2μg:(3-6) μg.

[0025] Better, The reaction temperature is 20-28°C. For example, Use room temperature.

[0026] In a preferred embodiment of the present invention, The preparation method of GO-Car-Carnosine-CpG-RBD vaccine is as follows: use An improved method of EDC-NHS reaction was used to couple GO with carnosine. Add 26 mg of GO freeze-dried powder to 5.20 mL of phosphate Buffer (PBS, pH = 7.4), In 25°C (200 W, 40 kHz) for 3 h.° C added 6.82 mg EDC (N1-((ethylimino)methylene)-N3,N3-dimethylpropane-1,3-diamine, Chinese:1-(3-二 The GO solution was activated by adding 7.73 mg of EDC/sulfo-NHS (3-(4-(4-aminopropyl)-3-ethylcarbodiimide) and 7.73 mg of NHS (NN-hydroxysuccinimide). The excess EDC/sulfo-NHS was removed from the reaction solution by ultrafiltration. The pH of the solution was then adjusted to 7.4. 40 mg of carnosine, 1.2ug CpG, and different concentrations of RBD recombinant protein were added to the solution. In 25°C and reacted with activated GO 2 h. Then, The excess uncoupled protein was removed from the reaction solution by ultrafiltration. The prepared product was labeled as GO-Car-Carnosine-CpG-RBD vaccine. Finally, Contact GO-Car-Carnosine-CpG-RBD with a sterile filter (0.22 μm). Seedling solution, and store it in 4°C in a sterile container for subsequent experiments.

[0027] The present invention has established a nano-recombinant protein vaccine preparation technology platform that can quickly stimulate the human immune system. tower, After the infectious virus is confirmed, a large amount of preventive vaccine can be quickly produced. This technology platform makes full use of the oxide The surface of graphene contains COOH, The characteristics of hydroxyl groups, Using the interaction between π-π bonds, The weight of the selected RBD Histones are assembled with CpG molecules and carnosine. A nano-recombinant protein vaccine based on graphene oxide as the skeleton is prepared. The vaccine can stimulate the body to produce high-titer RBD neutralizing antibodies against SAR-CoV-2, laying a To prevent and treat coronavirus infection and good technical foundation for future outbreaks of similar epidemics.

[0028] On the other hand, The present invention provides the application of the above-mentioned GO-Car-carnosine-CpG-RBD vaccine, That is GO-Car-Carnosine-The application of CpG-RBD vaccine in the preparation of drugs for preventing new coronavirus.

[0029] Better, The application is to improve the body's immunity to the new coronavirus.

[0030] Better yet, The above-mentioned GO-Car-carnosine-CpG-RBD vaccine can produce specific antibodies against RBD. And the The specific antibody titer is high. In an embodiment of the present invention, Nano COVID-19 vaccine shows strong immunity in mouse tests Originality, High titer antibodies can be produced.

[0031] The beneficial effects of the present invention are:

A new vaccine technology platform was developed based on graphene oxide materials as the backbone loading CpG molecules and recombinant proteins. A new nano coronavirus vaccine was prepared by combining the recombinant protein of the RBD region of the Spike protein of SAR-CoV-2. Can

Produce high titer of RBD-specific antibodies in mice. Provides a powerful tool for the prevention and treatment of the new coronavirus support.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] In order to more clearly illustrate the technical solution in the embodiments of the present application, The following will describe the The attached figure is briefly introduced.Obviously, The drawings described below are only some embodiments of the present application. For the ability For ordinary technicians in this field,Without creative labor, Other additional information can also be obtained based on these drawings. picture.

[0033] Figure 1 is a schematic diagram and timeline of immunization of mice with the GO-Car-carnosine-CpG-RBD vaccine; Figure 2 shows the changes in specific RBD antibodies in the serum of mice 28 days after immunization and the changes in cytokine production by spleen cells at 42 days.

DETAILED DESCRIPTION

[0034] The technical solutions in the embodiments of the present application will be explained below. Complete description,Obviously,The implementation described The examples are only some of the embodiments of this application. Rather than all embodiments. Based on the embodiments in this application, General in this field All other embodiments obtained by technicians without creative work, All of them belong to the scope of protection of this application For methods and techniques not specifically described, All of the operations can be performed using conventional techniques known in the art. For example,Ginseng According to the Cold Spring Harbor Manual of Molecular Cloning, etc.

[0035] Example 1

The preparation process of graphene oxide (GO)-carnosine-CpG-RBD recombinant protein vaccine preparation uses the TLR9 receptor nucleic acid sequence CpG ODN M362 which has cross-reactions to both humans and mice. The specific sequence is as follows:5'-TCGTCGTCGTTC:GAACGACGTTGAT-3' (25 mer, SEQ ID NO 1), Improved method using EDC-NHS reaction The process of coupling GO with carnosine,26 mg of GO freeze-dried powder was added to 5.20 mL of phosphate buffered saline (PBS). pH = 7.4), at 25° C for 3 h.° C, 6.82 mg EDC (N1-((ethylimino)methylene)-N3,N3-dimethylpropane-1,3-diamine, Chinese:1-(3-dimethylamino 7.73 mg of NHS (NN-hydroxysuccinimide) and 7.73 mg of EDC/sulfo-NHS were added to activate the GO solution. The excess EDC/sulfo-NHS was removed from the reaction solution by ultrafiltration. The pH of the solution was then adjusted to 7.4.40 mg of intramuscular Peptide,1.2ug CpG, and different concentrations of RBD recombinant protein were added to the solution.In 25°C and reacted with activated GO for 2 h.Follow back,The excess uncoupled protein was removed from the reaction solution by ultrafiltration. The prepared product was labeled as GO-Car-Carnosine-CpG-RBD vaccine. Finally, the GO-Car-Carnosine-CpG-RBD vaccine solution was contacted with a sterile filter (0.22 um). and store it in 4°C in a sterile container for subsequent experiments.

[0036] Example 2

The experiment of immunizing mice with graphene oxide (GO)-carnosine-CpG-RBD recombinant protein vaccine was carried out at 0,14, 28 days after vaccination, 6-week-old female BALB/c were immunized by subcutaneous injection mice, Until the 28th and 42nd day,Blood was collected by taking blood from the lower abdomen.Separate serum,Detection of specific RBD in serum The mice were killed on day 42. Isolation of splenocytes,Detection of specific T cell immune response and cytokine secretion condition.

[0037] Grouping and dosage determination of immunized mice:

1. (Graphene oxide + carnosine) + 1.2ug cpG + 3ug RBD

2. (Graphene oxide + carnosine) + 1.2ug cpG + 6ug RBD

3. Aluminum hydroxide + 6ug RBD (1: 1)

4. 6ug RBD

5. Liposome (lipo) + 6ug RBD group

Mouse strain: BALB/c mice (n=6).

[0038] The schedule for immunizing mice with the GO-Car-Carnosine-CpG-RBD vaccine is: Blood sampling and first immunization, As immunized mice Starting point. Second blood draw on the 7th day, Investigate the new crown virus system, Grasp the principle. The third blood draw on the 14th day, Strengthen immunity. The fourth blood draw on the 28th. Strengthen immunity, Detection of antibodies in serum, If positive, Prepare to collect spleen cells. Day 42, Day 5 Blood draw, Afterwards, they were killed and the abdominal cavity was opened, Isolation of splenocytes, Perform cytokine experiments.

[0039] The test results show that: After immunization of mice with GO-Car-carnosine-CpG-RBD vaccine, both the 3ug and 6ug groups produced high The titers of specific antibodies against RBD were significantly different from those in the traditional adjuvant group, the RBD group, and the liposome group (Figure 2). Further analysis of the specific immune response of T cells isolated from the spleen revealed that: The results showed that GO-Car-Carnosine CpG-RBD vaccine can stimulate the body to produce specific IFN-gamma cytokines. Improve the body's immunity, Fight against the new The coronavirus epidemic.

[0040] As mentioned above, This is only a specific implementation of this application. However, the protection scope of this application is not limited to this. any Those skilled in the art are within the technical scope disclosed in this application. Changes or substitutions that can be easily thought of, Should It is included in the protection scope of this application. The protection scope of this application shall be the protection scope of the claims. allow.

SEQUENCE LISTING

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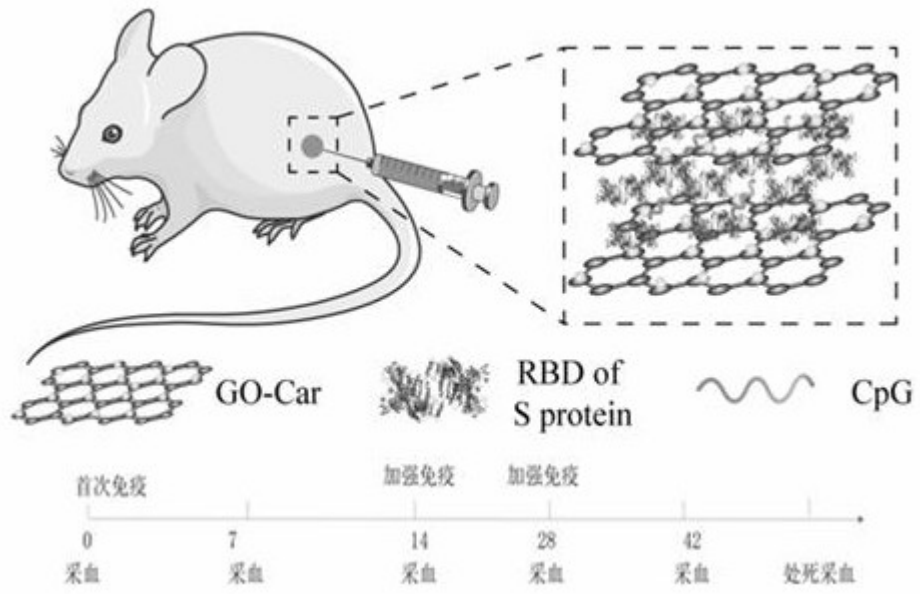

Figure 1

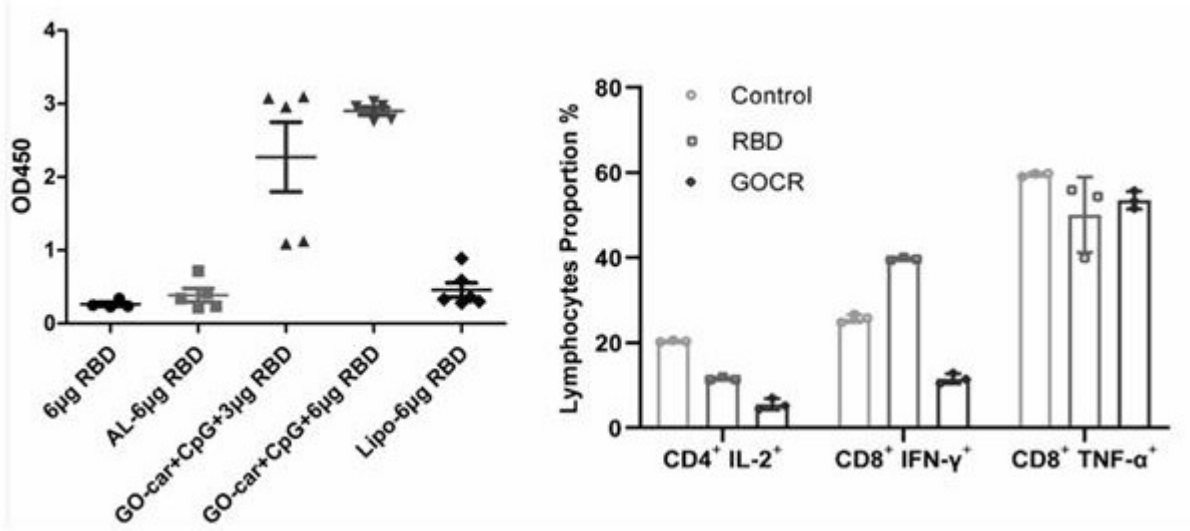

Figure 2