METAL-CHELATED PHOSPHOLIPID COMPLEX, METAL-CHELATED PHOSPHOLIPID COMPLEX NANOPARTICLE, AND DRUG-LIPID PARTICLE, AND PREPARATION METHODS, AND USES THEREOF

Cross Reference to Related Applications

[0001]The present disclosure claims priority to and the benefit of Chinese Patent Application No. 202210951941.8, "Drug-Lipid Particle, Preparation Method for The Same, And Use Thereof", filed on August 09, 2022, Chinese Patent Application No. 202210950392.2, "Metal-Chelated Phospholipid complex Nanoparticles, Preparation method for The Same, And Use Thereof", filed on August 09, 2022, and Chinese Patent Application No. 202210950391.8, "Metal-chelated phospholipid complex, Preparation Method for The Same, And Use Thereof", filed on August 09, 2022, in the Chinese Intellectual Property Office, the entire content of which is incorporated herein by reference.

Technical Field

[0002]The disclosure relates to the field of biotechnology, and in particular relates to a metal-chelated phospholipid complex, a metal-chelated phospholipid complex nanoparticle, a drug-lipid particle, and preparation methods and uses thereof.

Background

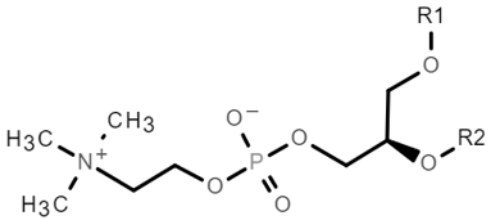
[0003]With the continuous development of molecular biology technology, the association between genes and diseases is more and more deeply known. Nucleic acid drugs refer to artificially synthesized DNA or RNA fragments with disease treatment functions, which are of great interest because they show great application potential in disease diagnosis and therapeutic process. Such drugs can act directly on pathogenic target genes or mRNA, having a therapeutic effect at the gene level. Compared with the traditional small-molecule drugs and antibody drugs, the nucleic acid drug is not limited by the druggability of the target protein, can treat a broader range of diseases, and can regulate the expression of pathogenic genes radically. The nucleic acid drug also has the outstanding advantages of high efficiency, low toxicity, high specificity and the like, and is expected to become the third largest type of drugs after small-molecule drugs and antibody drugs.

[0004]However, nucleic acid drugs are susceptible to degradation by nucleases in vivo, and due to their large molecular weight and their negatively charged nature, they have difficulty in crossing cell membranes to function. Therefore, the search for a safe and effective nucleic acid drug delivery system is a bottleneck problem to be solved urgently in the development of nucleic acid drugs. Currently, vectors capable of delivering nucleic acid drugs can be mainly classified into viral vectors and non-viral vectors. Viral vectors

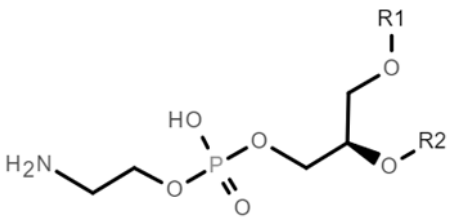
[0008]An embodiment of the present disclosure provides a metal-chelated phospholipid complex forming by reaction of a phospholipid molecular moiety, a linker molecular moiety, and a metal ion moiety, wherein the phospholipid molecular moiety is linked to the linker molecular moiety, the linker molecular moiety is linked to the metal ion moiety through a coordination bond, and the metal-chelated phospholipid complex is not a cationic lipid or an ionizable lipid.

[0009]Further, the phospholipid molecular moiety may be selected from the group consisting of lecithin (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidic acid (PA), phosphatidyl glycerol (PG), ceramide-1-phosphate (CP), phosphatidyl inositol (PI), phosphatidyl threonine (PT), sphingomyelin (SM), lysolecithin (LPC), lysophosphatidyl ethanolamine (LPE), lysophosphatidylserine (LPS), lysophosphatidic acid (LPA), lysophosphatidyl glycerol (LPG), lysophosphatidylinositol (LPI), lysophosphatidylthreonine (LPT), lysosphingomyelin (LSM), sphingosine 1-phosphate (S1P), derivatives thereof, and combinations thereof. Wherein the term “thereof” in “derivatives thereof” refers to “lecithin (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidic acid (PA), phosphatidyl glycerol (PG), ceramide-1-phosphate (CP), phosphatidyl inositol (PI), phosphatidyl threonine (PT), sphingomyelin (SM), lysolecithin (LPC), lysophosphatidyl ethanolamine (LPE), lysophosphatidylserine (LPS), lysophosphatidic acid (LPA), lysophosphatidyl glycerol (LPG), lysophosphatidylinositol (LPI), lysophosphatidylthreonine (LPT), lysosphingomyelin (LSM), sphingosine 1-phosphate (S1P)”. In the present disclosure, " derivatives thereof " may be interpreted similarly. The phospholipid molecular moiety may be, for example, but not limited to, lecithin (PC), lecithin (PC) derivatives, phosphatidyl ethanolamine (PE), phosphatidyl ethanolamine (PE) derivatives, phosphatidyl glycerol (PG), phosphatidyl glycerol (PG) derivatives, phosphatidyl glycerol (PG) and lecithin (PC), lecithin (PC) and lecithin (PC) derivatives, and the like.

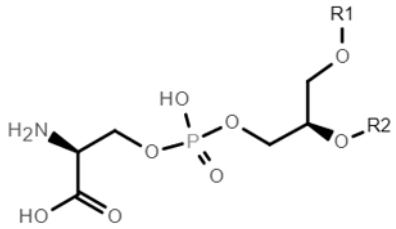
[0010]Further, the phospholipid molecular moiety may be selected from the group consisting of



lecithin (PC) (Formula 1) ,



phosphatidyl ethanolamine (PE) (Formula 2) ,

phosphatidyl serine (PS) (Formula 3) 

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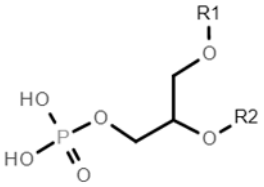
phosphatidic acid (PA) (Formula 4) 

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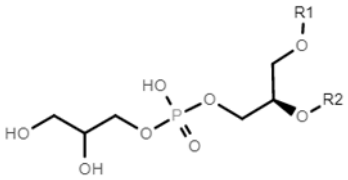
phosphatidyl glycerol (PG) (Formula 5) 

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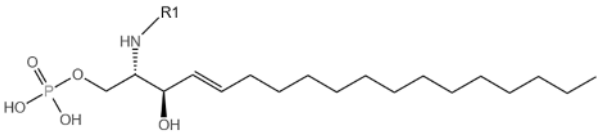
ceramide-1-phosphate (CP) (Formula 6) 

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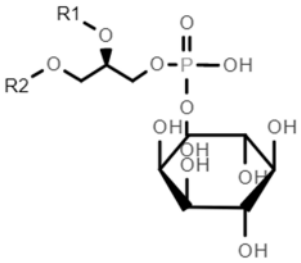
phosphatidyl inositol (PI) (Formula 7) 

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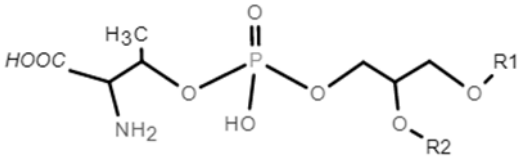
phosphatidyl threonine (PT) (Formula 8) 

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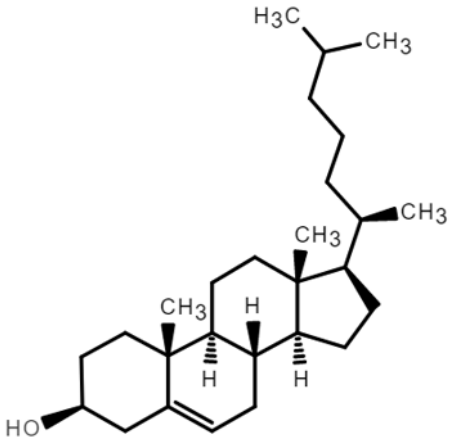
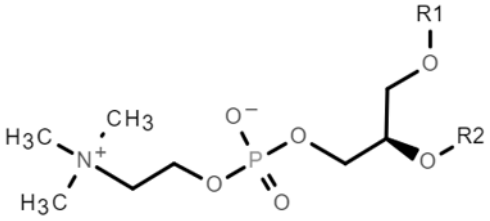
(iii) may be selected from cholesterol (Formula 40) , its derivatives, and combinations thereof.

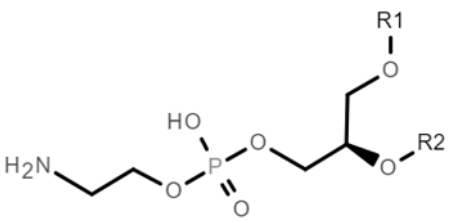
[0047]Further, the non-cationic lipid or the non-ionizable lipid in (iii) may be cholesterol (Formula 40).

[0048]Further, the non-cationic lipid or the non-ionizable lipid other than the metal-chelated phospholipid complex and the particle aggregation-inhibiting conjugated lipid of (iii) may further comprise, in addition to cholesterol, one or more selected from the group consisting of lecithin PC, phosphatidyl ethanolamine PE, phosphatidyl serine PS, phosphatidic acid PA, phosphatidyl glycerol PG, ceramide-1-phosphate CP, phosphatidyl inositol PI, phosphatidyl threonine PT, sphingomyelin SM, lysolecithin LPC, lysophosphatidyl ethanolamine LPE, lysophosphatidylserine LPS, lysophosphatidic acid LPA, lysophosphatidyl glycerol LPG, lysophosphatidylinositol LPI, lysophosphatidylthreonine LPT, lysosphingomyelin LSM, sphingosine 1-phosphate S1P, cholesterol sulfate, and derivatives thereof.

[0049]Further, the non-cationic lipid or the non-ionizable lipid other than the metal-chelated phospholipid complex and the particle aggregation-inhibiting conjugated lipid in (iii) may further comprise, in addition to cholesterol, one or more selected from the group consisting of



lecithin (PC) (Formula 1) ,



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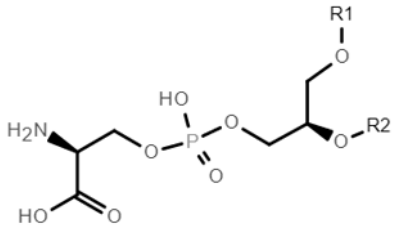
phosphatidylserine (PS) (Formula 3) 

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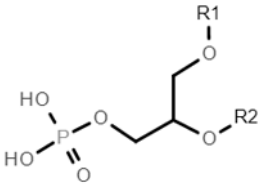
phosphatidic Acid (PA) (Formula 4) 

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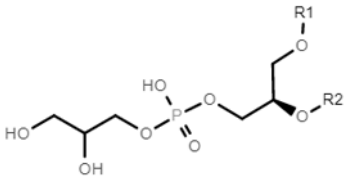
phosphatidyl glycerol (PG) (Formula 5) 

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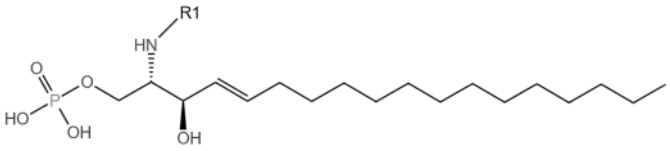
ceramide-1-phosphate (CP) (Formula 6)



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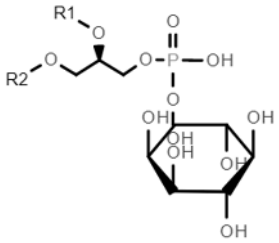
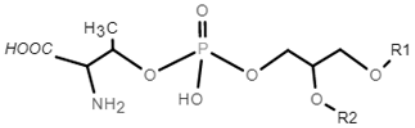


phosphatidyl inositol (PI) (Formula 7) ,



phosphatidyl threonine (PT) (Formula 8) ,

agarose gel was at a concentration of 1.5%, where the void of gel permitted only free nucleic acids to pass through, blocking lipid nanoparticles. Electrophoresis was halted once the free nucleic acid bands were distinctly separated and visible. The gray values of free nucleic acids in different groups were calculated by software Image J, the positive control group was set as 100%, and the proportion of free nucleic acids in each group to the positive control was a relative amount of free nucleic acids, and then the encapsulation efficiency of each group was calculated as (100- relative amount of free nucleic acids)%. A nucleic acid encapsulation efficiency of more than 50% is considered to be acceptable.

[0263]Cell culture method: Human embryonic kidney cell line 293T was cultured with DMEM medium containing 10% FBS and 1% penicillin-streptomycin at a condition of 37°C and 5% CO_2.

[0264]A method to analyze the percentage of eGFP-positive cells by a flow cytometry method: 293T cells were seeded on 24-well plates at a density of 5×10^5 cells/well, and when the cell density reached 80%, cells were incubated with 1 mL of MPP or eGFP-mRNA@MPP wherein the eGFP-mRNA@MPP has a concentration of 2 μg/mL. After 48 hours, the cell suspension was collected, 20,000 cells were collected with FITC channel of the flow cytometry, and the percentage of eGFP-positive cells was analyzed, and the calculation Formula was as follows: eGFP-positive cell rate = number of eGFP-expressing cells/total number of cells ×100%. A percentage of eGFP-positive cells above 40% was considered acceptable.

[0265]The principle of loading nucleic acids in metal-chelated phospholipid complex nanoparticles (MPP) assembled by metal-chelated phospholipid complex is as follows: curcumin is connected to DSPC through hydrogen bond, meanwhile curcumin is connected to Fe^{3+} or Al^{3+} through coordination bond, forming the metal-chelated phospholipid complex, and the Fe^{3+} or Al^{3+} of the metal-chelated phospholipid complex is linked to nucleic acids through coordination bond. This ensures that the metal-chelated phospholipid complex and other components self-assemble into MPPs while loading nucleic acids into nanoparticles. There are two possibilities for the contribution of curcumin in the loading of nucleic acids in MPP: (1) curcumin interacts with nucleic acids to assist in the loading of nucleic acids in MPPs, for example, curcumin assists in loading nucleic acids by being inserted into minor grooves of nucleic acids; (2) curcumin may also not interact directly with nucleic acids.

[0266]Example 3.1 Feeding ratios of components of metal-chelated phospholipid complex

[0267]DSPC, curcumin and $FeCl_3$ in Example 3 were fed into different feeding ratios (1: 1:1, 3: 3:2, 2:2: 1), and following the other procedures which are same as in Example 3, different eGFP-mRNA@MPP were prepared and their nucleic acid encapsulation rates were detected respectively.

[0268]Result analysis: as shown in Table 1-1, when the feeding ratio among DSPC, curcumin and $FeCl_3$ was 1:1:1, the eGFP-mRNA encapsulation efficiency of the prepared drug-lipid particles was 87%; when the feeding ratio among DSPC, curcumin and $FeCl_3$ was

3:3:2, the eGFP-mRNA encapsulation efficiency of the prepared drug-lipid particles was 70%; when the feeding ratio among DSPC, curcumin and $FeCl_3$ was 2:2:1, the eGFP-mRNA encapsulation efficiency of the prepared drug-lipid particles was 60%. The function of Fe^{3+} in drug-lipid particles is to connect phospholipid complex and nucleic acids, and each Fe^{3+} has up to three complexing sites, so the feeding ratio among DSPC, curcumin and $FeCl_3$ in the drug-lipid particles should be 1:1:1, so as to ensure that the drug-lipid particles can encapsulate as many nucleic acids as possible. The experimental results also confirmed that when the feeding ratio among DSPC, curcumin and $FeCl_3$ was 1:1:1, the mRNA encapsulation efficiency of drug-lipid particles prepared therefrom was the highest. When the feeding ratios among DSPC, curcumin and $FeCl_3$ were in the range of 1:1:1 to 2:2:1, the nucleic acid encapsulation efficiency of the drug-lipid complex nanoparticles were all more than 60%.

[0269]Table 1-1 Feeding ratios of components of metal-chelated phospholipid complexes and the functions of the drug-lipid particles prepared therefrom

feeding ratio among DSPC, curcumin and $FeCl_3$	mRNA encapsulation efficiency of drug-lipid particles
1: 1: 1	87%
3: 3: 2	70%
2: 2: 1	60%

[0270]The DSPC, curcumin and Al $(NO_3)_3 \cdot 9H_2O$ in Example 3 were added in different ratios (1:1:1, 3:3:2, 2:2:1), and the other steps were the same as in Example 3 to prepare different eGFP-mRNA@MPPs, and their nucleic acid encapsulation efficiency were detected respectively.

[0271]Result analysis: as shown in Table 1-2, when the ratio among DSPC, curcumin and $Al(NO_3)_3 \cdot 9H_2O$ was 1:1:1, the eGFP-mRNA encapsulation efficiency of the prepared metal-chelated phospholipid complex nanoparticles was 92%; when the ratio among DSPC, curcumin and $Al(NO_3)_3 \cdot 9H_2O$ was 3:2:2, the eGFP-mRNA encapsulation efficiency of the prepared metal-chelated phospholipid complex nanoparticles was 72%; when the ratio among DSPC, curcumin and $Al(NO_3)_3 \cdot 9H_2O$ was 2:2:1, the eGFP-mRNA encapsulation efficiency of the prepared metal-chelated phospholipid complex nanoparticles was 58%. The function of Al^{3+} in the metal-chelated phospholipid complex nanoparticles is to connect the phospholipid complex with the nucleic acids, and each Al^{3+} has a maximum of three complexing sites, so the feeding ratio among DSPC, curcumin and $Al(NO_3)_3 \cdot 9H_2O$ in the drug-lipid particles should be 1:1:1 to ensure that the metal-chelated phospholipid complex nanoparticles can encapsulate as many nucleic acids as possible. The results of the experiment also confirmed that when the ratio feeding ratio among DSPC, curcumin and $Al(NO_3)_3 \cdot 9H_2O$ was 1:1:1, the eGFP-mRNA encapsulation efficiency of metal-chelated phospholipid complex nanoparticles prepared from the same was the highest. When the ratio among DSPC, curcumin and $Al(NO_3)_3 \cdot 9H_2O$ was in the range of 1:1:1 to 2:2:1, the nucleic acid encapsulation efficiency of the metal-chelated phospholipid complex nanoparticles was above 60%.