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(54) **BIONIC DRUG-LOADED NANOPARTICLE EXPRESSING CXCR4, AND PREPARATION METHOD THEREFOR AND USE THEREOF.**

(57) The invention discloses a preparation method for a bionic drug-loaded nanoparticle expressing CXCR4, comprising: mixing PLGA (poly lactic-co-glycolic acid) and an immunosuppressant at a certain ratio, and preparing a resulting mixture into a drug-loaded nanoparticle by using a microfluidic technology; preparing a MSC stably expressing CXCR4 membrane protein, lysing the MSC and extracting a cell membrane to obtain an engineered stem cell membrane; and fusing the engineered stem cell membrane with the drug-loaded nanoparticle to obtain a bionic drug-loaded nanoparticle expressing CXCR4, the bionic drug-loaded nanoparticle expressing CXCR4 being a bionic cell membrane drug-loaded nanoparticle with chemotactic properties. In the invention, the engineered stem cell membrane is transformed by a genetic engineering technology, and encapsulating a clinical therapeutic drug, migrating to the site of injury in the body and target specific cells, the drug distribution in the body is improved and the toxic and side effects of the immunosuppressive drug is reduced.

Fig. 2

BIONIC DRUG-LOADED NANOPARTICLE EXPRESSING CXCR4, AND PREPARATION METHOD THEREFOR AND USE THEREOF

Field of the Invention

The invention relates to the field of bionic cells, and in particular to a bionic drug-loaded nanoparticle expressing CXCR4, and a preparation method therefor and a use thereof.

Background of the Invention

Mesenchymal stem cells (MSCs) have been widely used in the clinical treatment of various diseases due to their low immunogenicity and broad-spectrum immunoregulatory functions. Thanks to the lack or low expression of major histocompatibility complex (MHC) class I molecules, MHC class II molecules and co-stimulatory molecules on the cell membrane, the MSCs have naturally low immunogenicity, allowing them to evade clearance by the body's immune cells after entering the body, thus prolonging blood circulation time. In recent years, the research on "carrier stem cells" based on stem cells has gradually come into people's view. The MSCs express many receptors and cell adhesion molecules that can help the MSCs migrate and home to target tissues. Among them, a CXCR4/SDF-1 signaling axis composed of a membrane chemokine receptor CXCR4 and the high concentration of stromal-derived factor-1 (SDF-1) in the inflammatory injury site is one of the most important mechanisms for the chemotaxis and homing of MSCs. This is the basis for stem cell-delivery nanoparticles for targeted therapy of tumors and inflammatory diseases. Dysregulation of the SDF-1/CXCR4 axis plays a key role in the differentiation, migration, recruitment and migration, as well as survival and proliferation of bone marrow mesenchymal stem cells.

Although stem cell drug delivery systems can improve the environmental adaptability of nano drugs, the microsize of MSCs themselves also limits their precise homing to target tissues and organs. Many studies have shown that intravenously infused MSCs are mostly distributed in the lungs, liver, and spleen. In addition, the migration and homing of MSCs to injured tissues are affected by many factors, including cell age and passage number, culture conditions, and delivery methods, all of which limit the application of MSC delivery carriers in the biomedical field.

Currently, immunosuppressants are mainly used for organ transplant anti-rejection and

treatment of autoimmune diseases and malignant tumors. However, patients have significant toxic and side effects to high-dose immunosuppressants, while low-dose treatments severely limit their efficacy. The design of nano drug delivery systems can solubilize drugs, increase drug half-life, improve drug distribution in the body, enhance targeting, and reduce toxic side effects, and has great application potential in the field of disease diagnosis and treatment. The cells in the capillaries at the site of injury of a patient proliferate, and the lymphatic system is blocked, so the nano drugs tend to accumulate at this site. Most studies believe that one of the main reasons for the poor efficacy of nano drugs is that the nanoparticles are not targeted enough for arthritis, but the safety is relatively low.

Summary of the Invention

In order to solve the problems of low homing rate, large toxic and side effects, unstable therapeutic effect, etc., of traditional nano drugs for treating autoimmune diseases, the invention provides a bionic drug-loaded nanoparticle expressing CXCR4 and a preparation method therefor and a use thereof. A nano drug is prepared by using a biocompatible and degradable nanomaterial loaded with an immunosuppressant, and the surface of the nano drug is coated with the mesenchymal stem cell membrane overexpressing CXCR4 to further coordinate the immune escape and inflammatory chemotaxis functions of stem cells, thus obtaining a cell membrane-coated bionic nano drug. In this way, the drug distribution in the body is improved and the toxic and side effects of the immunosuppressive drug is reduced, thereby realizing an effective strategy for efficient drug delivery and targeted lesion site delivery.

A preparation method for a bionic drug-loaded nanoparticle expressing CXCR4, comprising:

mixing PLGA (poly lactic-co-glycolic acid) and an immunosuppressant at a certain ratio, and preparing a resulting mixture into a drug-loaded nanoparticle by using a microfluidic technology;

preparing a MSC stably expressing CXCR4 membrane protein, lysing the MSC and extracting a cell membrane to obtain an engineered stem cell membrane; and

fusing the engineered stem cell membrane with the drug-loaded nanoparticle to obtain a

bionic drug-loaded nanoparticle expressing CXCR4, the bionic drug-loaded nanoparticle expressing CXCR4 being a bionic cell membrane drug-loaded nanoparticle with chemotactic properties;

wherein a method for fusing the engineered stem cell membrane with the drug-loaded nanoparticle comprises: blending the engineered stem cell membrane with the drug-loaded nanoparticle at a mass ratio of 0.8-1: 0.8-1, and performing ultrasonic fusion at a frequency of 10-30 kHz, a time of 30-60 s, and a temperature of 5-10 °C.

In order to optimize the described technical solution, the specific measures/limitations taken also include:

The immunosuppressant is at least one of cyclosporine, methotrexate and cyclophosphamide.

The PLGA and the immunosuppressant are mixed at a mass ratio of 5-10:1.

A method for preparing the resulting mixture into a drug-loaded nanoparticle by using a microfluidic technology comprises: mixing the PLGA and the immunosuppressant and dissolving a resulting mixture in DMSO (dimethyl sulfoxide) to obtain an aqueous mixture, wherein a mass/volume ratio of the PLGA and the immunosuppressant to the DMSO is 5-15:1 mg/mL; dropwise adding the aqueous mixture to ultrapure water, wherein a volume ratio of the ultrapure water to the aqueous mixture is 6-10:1; and stirring a resulting mixture at room temperature for 0.5-3 h.

Purification of the drug-loaded nanoparticle: the prepared drug-loaded nanoparticle is transferred to a dialysis bag to remove excess organic phase and reagents, and distilled to remove water; after dialysis for 30 h, a resulting suspension is freeze-dried to obtain a purified nanoparticle. The molecular weight cutoff of a filter membrane is within a range of 3-7 kDa.

The MSC is at least one of human umbilical cord MSC, human adipose MSC and human bone marrow MSC.

A method for preparing a MSC stably expressing the CXCR4 membrane protein comprises:

culturing P3 MSCs until confluence reaches 80- 90%, the MSC is transfected with a culture medium containing a Lv-CXCR4-overexpressed lentivirus and cultured at 37 °C for 12-24 h, and then replacing the Lv CXCR4-overexpressed lentivirus with DMEM to further culture the cells for 36-48 h; then, performing resistance screening to obtain a MSC stably expressing the CXCR4 membrane protein.

A method for lysing the MSC and extracting a cell membrane to obtain an engineered stem cell membrane comprises: after being rinsed for two to three times with a pre-cooled phosphate buffer solution, resuspending the MSC with a hypotonic lysis solution, and repeatedly frozening and thawing the MSC in liquid nitrogen for four to five times to fully lyse the MSC; centrifuging the MSC to extract a cell membrane precipitate; resuspending the cell membrane precipitate in a phosphate buffer solution, the MSC is repeatedly extruded in a polycarbonate membrane for 8-13 times to obtain an engineered stem cell membrane. The polycarbonate membrane has a pore size of 400 nm, 200 nm or 100 nm, preferably 200 nm.

A hypotonic lysis solution includes a Tris-HCl buffer solution, a protease inhibitor, and a phosphatase inhibitor.

The invention further includes a bionic drug-loaded nanoparticle expressing CXCR4 prepared by the method.

The invention further includes a use of the bionic drug-loaded nanoparticle expressing CXCR4 in the preparation of an immunosuppressant drug.

Preferably, the use comprises a use of the bionic drug-loaded nanoparticle expressing CXCR4 in the preparation of drug delivery for rheumatoid arthritis (RA).

Compared with the prior art, the invention has the beneficial effects as follows:

In the invention, the in vitro 2D cultured stem cells with weakened chemotaxis are transformed so that they can "home" to an inflammatory injury or tumor microenvironment, and the separated CXCR4 stem cell membrane is used to encapsulate a clinical therapeutic drug. The obtained bionic cell membrane drug-loaded nanoparticle can migrate to the site of injury in the body and target specific cells.

In the invention, the characteristics of the MSC membrane are firstly analyzed, and in combination with the properties of a biomaterial, a stem cell that overexpresses CXCR4 is constructed by a genetic engineering technology, the PLGA drug-loaded nanoparticle is prepared by means of nanoprecipitation and coated with the CXCR4 stem cell membrane to form a bionic nano drug, so as to achieve systemic delivery of the bionic membrane-coated nano drug to the site of injury and alleviate the course of the disease. The method is simple and easy to operate.

In the invention, the method of fusing the engineered stem cell membrane with the drug-loaded nanoparticle and preparing the drug-loaded nanoparticle by a microfluidic technology is critical to the successful acquisition of the product CMPNs and the impact on the performance of the product. The process method of the invention can achieve a relatively ideal experimental effect.

Further studies have found that the engineered MSC of the invention highly express CXCR4, thereby causing the cells to acquire a stronger migration ability and reducing the proportion of co-cultured inflammatory cells. Therefore, when an engineered MSC membrane-coated nano drug is injected during the progression of a disease, the bionic nanoparticle has an enhanced ability of directional migration to injured organs and immune regulation, which is beneficial to further improving the disease treatment effect of the bionic MSC membrane.

The invention further provides its related medical and pharmaceutical applications, such as organ transplantation anti-rejection and treatment of autoimmune diseases and malignant tumors, alleviating the problem of systemic toxicity of nano drugs, and ensuring that the carried therapeutic drugs survive for a long time at the site of injury and stably exert biological functions, so that immunosuppressive drugs can reach the minimum therapeutic dose in vivo.

The invention has good application prospects in the treatment research of bionic nano drugs, such as the development of bionic intravenous preparations and is safe, feasible and effective.

Brief Description of the Drawings

Fig. 1 is a transmission electron micrograph of a bionic drug-loaded nanoparticle expressing CXCR4 according to the invention.

Fig. 2 is a schematic diagram and identification of the preparation of the bionic drug-loaded nanoparticle expressing CXCR4 according to the invention.

Fig. 3 shows a hemolytic experiment of the bionic drug-loaded nanoparticle expressing CXCR4 according to the invention.

Fig. 4 shows a verification of the chemotaxis of the bionic drug-loaded nanoparticle expressing CXCR4 according to the invention.

Fig. 5 shows photos of joints treated with the bionic drug-loaded nanoparticle expressing CXCR4 according to the invention for RA.

Fig. 6 is a H&E pathological picture after the treatment with the bionic drug-loaded nanoparticle expressing CXCR4 according to the invention for RA.

Detailed Description of the Invention

The above contents of the invention are further described in detail in the form of embodiments below, but this should not be understood as the scope of the described subject matter of the invention is limited to the following embodiments. All technologies realized based on the above contents of the invention fall within the scope of the invention.

The experimental methods used in the following examples are conventional methods unless otherwise specified, and the reagents, methods and equipment used are conventional reagents, methods and equipment in the art unless otherwise specified.

Example 1

A preparation method for a bionic drug-loaded nanoparticle expressing CXCR4 includes the following steps:

1. Preparation of a drug-loaded nanoparticle by using a microfluidic technology: 10 mg of PLGA (poly lactic-co-glycolic acid) was dissolved in 1 mL of DMSO (dimethyl sulfoxide) and 1 mg of MTX (methotrexate) was then added, thus obtaining an aqueous mixture; the obtained aqueous mixture was dropwise added to 8 ml of ultrapure water and a resulting solution was stirred at 25 °C for 1 h; a resulting product was transferred to a dialysis tube (MW: 3500 Da) to remove excess organic phase and reagents; after dialysis with distilled deionized water for 24 h, the resulting suspension was freeze-dried to obtain a purified drug-loaded nanoparticle.

2. Preparation of a MSC stably expressing CXCR4 membrane protein: P3 MSCs were cultured until confluence reached 80%, then transfected with a culture medium containing a Lv-CXCR4-overexpressed lentivirus and cultured at 37 °C for 12-24 h; the culture medium containing a Lv-CXCR4-overexpressed lentivirus was then replaced with DMEM to further culture the cells for 36-48 h; then, resistance screening was performed by using a culture medium containing 1-3 mg/ml puromycin to obtain stably transfected cells that can stably express the CXCR4 membrane protein.

3. Identification of a CXCR4 engineered stem cell membrane: flow cytometry (FCM) was performed to detect cell growth cycle and stem cell surface markers; the human-derived CXCR4 gene was aligned by using BLAST software to obtain a gene sequence and a pDsRed-CXCR4 plasmid was constructed and packaged into a lentivirus; the MSC was subjected to gene transfection, and the expression of the CXCR4 receptor was detected by laser scanning confocal microscope (LSCM), reverse transcription PCR (RT-PCR), Western blot (WB), immunofluorescence, FCM and other techniques; the Transwell experiment was performed to evaluate the changes in the in vitro migration ability of the MSC after transfection.

4. Preparation of the CXCR4 engineered stem cell membrane: after being rinsed for two to three times with a pre-cooled phosphate buffer solution, the MSC stably expressing the CXCR4 membrane protein prepared in step 2 was resuspended with a hypotonic lysis solution, and repeatedly frozen and thawed in liquid nitrogen for five times; after being fully lysed, the Trans well MSC was centrifuged at 4 °C, 1850 g for 10 min to extract a cell membrane precipitate; finally, the cell membrane precipitate was resuspended in a phosphate buffer solution, and the cell membrane suspension was extruded repeatedly for 11 times in a

200 nm polycarbonate membrane by using a micro extruder to obtain a CXCR4 engineered stem cell membrane.

5. Preparation of a bionic cell membrane drug-loaded nanoparticle CMPNs with chemotactic properties: After being subjected to ultrasonic treatment, the engineered cell membrane prepared in step 3 was blended with the drug-loaded nanoparticle prepared in step 1 at a mass ratio of 1:1, and ultrasonic fusion was performed at a frequency of 20 kHz, a time of 30 s, and a temperature of 8 °C to obtain a bionic cell membrane drug-loaded nanoparticle with chemotactic properties. Referring to Fig. 1, the results show that the bionic cell membrane drug-loaded nanoparticle obtained has an obvious core-shell structure, is round and uniform, has a particle size of about 121.4 nm, a charge of about -20 mV, a MCPNs drug loading of 4.3%, and an encapsulation rate of 93.2%, and achieves effective loading of methotrexate.

Example 2

Study on in vitro characteristics of the bionic drug-loaded nanoparticle expressing CXCR4.

To test the long-term stability of the prepared bionic drug-loaded nanoparticle expressing CXCR4, the nanoparticles were dispersed in a PBS buffer to a final concentration of 1 mg/mL, and the particle size of the nanoparticles was observed by dynamic light scattering. The particle size of the particles did not change much within one week, indicating that the bionic drug-loaded nanoparticle expressing CXCR4 has good solution stability.

The in vitro drug release experiment studied the drug release capacity of the bionic drug-loaded nanoparticle expressing CXCR4. The red blood cell hemolysis experiment was performed to evaluate the blood compatibility of the bionic drug-loaded nanoparticle expressing CXCR4. The results show that the bionic drug-loaded nanoparticle expressing CXCR4 has good blood compatibility, as shown in Fig. 3.

Example 3

Use of the bionic drug-loaded nanoparticle expressing CXCR4 in targeted therapy of RA joint. In order to evaluate the in vivo therapeutic effect of bionic drug-loaded nanoparticle expressing CXCR4, a collagen adjuvant-induced rheumatoid arthritis (RA) mouse model was

used in the experiment, where a model group, an MTX group, an MPNs group, and a CMPNs group were set up. The model group did not receive any treatment, the MTX group was directly injected with an MTX solution (methotrexate injection), the MPNs group was injected with MPNs (PLGA & MTX coated with a stem cell membrane), and the CMPNs group was injected with CMPNs (PLGA & MTX coated with a CXCR4-modifed stem cell membrane). The concentration of each group was 100 ul, 2.5 mg MTX/kg; the mice were injected twice through the tail vein on day 28 and day 35, and the mice were killed on day 42 to observe the therapeutic effect, and peripheral blood, synovium and joint tissues were collected at the same time. As shown in Fig. 5 are joint tissues, from which it can be found that the redness and swelling of the joints of CIA mice were significantly inhibited after treatment with the stem cell membrane nanoparticle, and the swelling of the mouse paws was significantly alleviated. Pathological analysis of the knee joint (HE staining) showed that the inflammation and bone destruction in the joints were significantly alleviated after treatment with the nanoparticle, as shown in Fig. 6.

The above description is only a preferred embodiment of the invention and does not limit the invention in any form. Any simple modification, equivalent replacement and improvement made by any person skilled in the art to the above embodiments without departing from the scope of the technical solution of the invention and based on the technical essence of the invention shall still fall within the scope of the technical solution of the invention.

CLAIMS

1. A preparation method for a bionic drug-loaded nanoparticle expressing CXCR4, comprising:

   mixing PLGA (poly lactic-co-glycolic acid) and an immunosuppressant at a certain ratio, and preparing a resulting mixture into a drug-loaded nanoparticle by using a microfluidic technology;

   preparing a MSC stably expressing CXCR4 membrane protein, lysing the MSC and extracting a cell membrane to obtain an engineered stem cell membrane; and

   fusing the engineered stem cell membrane with the drug-loaded nanoparticle to obtain a bionic drug-loaded nanoparticle expressing CXCR4, the bionic drug-loaded nanoparticle expressing CXCR4 being a bionic cell membrane drug-loaded nanoparticle with chemotactic properties;

   wherein a method for fusing the engineered stem cell membrane with the drug-loaded nanoparticle comprises: blending the engineered stem cell membrane with the drug-loaded nanoparticle at a mass ratio of 0.8-1: 0.8-1, and performing ultrasonic fusion at a frequency of 10-30 kHz, a time of 30-60 s, and a temperature of 5-10 °C.

2. The preparation method for a bionic drug-loaded nanoparticle expressing CXCR4 according to Claim 1, wherein the immunosuppressant is at least one of cyclosporine, methotrexate and cyclophosphamide.

3. The preparation method for a bionic drug-loaded nanoparticle expressing CXCR4 according to Claim 1, wherein the PLGA and the immunosuppressant are mixed at a mass ratio of 5-10:1.

4. The preparation method for a bionic drug-loaded nanoparticle expressing CXCR4 according to Claim 1, wherein a method for preparing the resulting mixture into a drug-loaded nanoparticle by using a microfluidic technology comprises: mixing the PLGA and the immunosuppressant and dissolving a resulting mixture in DMSO

(dimethyl sulfoxide) to obtain an aqueous mixture, wherein a mass/volume ratio of the PLGA and the immunosuppressant to the DMSO is 5-15:1 mg/mL; dropwise adding the aqueous mixture to ultrapure water, wherein a volume ratio of the ultrapure water to the aqueous mixture is 6-10:1; and stirring a resulting mixture at room temperature for 0.5-3 h.

5. The preparation method for a bionic drug-loaded nanoparticle expressing CXCR4 according to Claim 1, wherein the MSC is at least one of human umbilical cord MSC, human adipose MSC and human bone marrow MSC.

6. The preparation method for a bionic drug-loaded nanoparticle expressing CXCR4 according to Claim 1, wherein a method for preparing a MSC stably expressing the CXCR4 membrane protein comprises: culturing P3 MSCs until confluence reaches 80-90%, then transfecting the MSCs with a culture medium containing a Lv-CXCR4-overexpressed lentivirus and then replacing the Lv-CXCR4-overexpressed lentivirus with DMEM to further culture the cells; then, performing resistance screening to obtain a MSC stably expressing the CXCR4 membrane protein.

7. The preparation method for a bionic drug-loaded nanoparticle expressing CXCR4 according to Claim 1, wherein a method for lysing the MSC and extracting a cell membrane to obtain an engineered stem cell membrane comprises: rinsing the MSC with a pre-cooled phosphate buffer solution, resuspending the MSC with a hypotonic lysis solution, and repeatedly frozening and thawing the MSC in liquid nitrogen to fully lyse the MSC; centrifuging the MSC to extract a cell membrane precipitate; resuspending the cell membrane precipitate in a phosphate buffer solution, and extruding a resulting suspension repeatedly to obtain the engineered stem cell membrane.

8. A bionic drug-loaded nanoparticle expressing CXCR4 prepared by the method according to any one of Claims 1-7.

9. A use of the bionic drug-loaded nanoparticle expressing CXCR4 according to Claim 8 in the preparation of an immunosuppressant drug.

10. The use according to Claim 9, comprising a use of the bionic drug-loaded nanoparticle expressing CXCR4 in the preparation of drug delivery for rheumatoid arthritis (RA).

Ansprüche

1. Herstellungsverfahren für mit bionischen Arzneistoffen beladene Nanopartikeln in der Expression von CXCR4, dadurch gekennzeichnet, dass es Folgendes umfasst:
   Milchsäure-Glykolsäure-Copolymer und Immunsuppressivum werden in einem bestimmten Verhältnis gemischt und die Mikrofluidik-Technologie wird verwendet, um die mit Arzneistoffen beladenen Nanopartikeln herzustellen;
   MSC-Zellen in der stabilen Expresson von CXCR4-Membranprotein werden hergestellt, um die Zellmembran zu extrahieren und die manipulierte Stammzellmembran zu erhalten; und
   die Fusion von manipulierten Stammzellmembranen und mit Arzneistoffen beladenen Nanopartikeln wird durchgeführt, um die mit bionischen Arzneistoffen beladenen Nanopartikeln in der Expression von CXCR4 zu erhalten, wobei die mit bionischen Arzneistoffen beladenen Nanopartikeln in der Expression von CXCR4 mit Arzneistoffen beladenen Nanopartikeln für bionische Zellmembranen mit chemotaktischen Eigenschaften sind;
   wobei die Methode für die Fusion der manipulierten Stammzellmembran und der mit Arzneistoffen beladenen Nanopartikeln darin besteht, die manipulierte Stammzellmembran und die mit Arzneistoffen beladenen Nanopartikeln in einem Massenverhältnis von 0,8–1:0,8–1 zu mischen und durch Ultraschall die Fusion durchzuführen, wobei die Ultraschallbehandlungsfrequenz 10 bis 30 kHz, die Zeit 30 bis 60 s und die Temperatur 5 bis 10 °C beträgt.

2. Herstellungsverfahren für mit bionischen Arzneistoffen beladene Nanopartikeln in der Expression von CXCR4 nach Anspruch 1, dadurch gekennzeichnet, dass das Immunsuppressivum aus mindestens einem von Cyclosporin, Methotrexat und Cyclophosphamid ausgewählt wird.

3. Herstellungsverfahren für mit bionischen Arzneistoffen beladene Nanopartikeln in der Expression von CXCR4 nach Anspruch 1, dadurch gekennzeichnet, dass Milchsäure-Glykolsäure-Copolymer und Immunsuppressivum in einem Massenverhältnis von 5 bis 10:1 gemischt werden.

4. Herstellungsverfahren für mit bionischen Arzneistoffen beladene Nanopartikeln in der

Expression von CXCR4 nach Anspruch 1, dadurch gekennzeichnet, dass das spezifische Verfahren zur Herstellung von mit Arzneistoffen beladenen Nanopartikeln durch die Mikrofluidik-Technologie darin besteht, Milchsäure-Glykolsäure-Copolymer und Immuninhibitor zu mischen und in Dimethylsulfoxid zu lösen, um ein Wasserphasengemisch zu bilden, wobei das Masse/Volumen-Verhältnis von Milchsäure-Glykolsäure-Copolymer und Immunsuppressivum zu Dimethylsulfoxid 5–15:1 mg/ml beträgt, wobei das Wasserphasengemisch in hochreines Wasser getropft wird, wobei das Volumenverhältnis von Reinstwasser zum Wasserphasengemisch 6–10:1 beträgt und es für 0,5–3 Stunden bei Raumtemperatur gerührt wird.

5. Herstellungsverfahren für mit bionischen Arzneistoffen beladene Nanopartikeln in der Expression von CXCR4 nach Anspruch 1, dadurch gekennzeichnet, dass die MSC-Zellen aus mindestens einer Art von menschlichen Nabelschnur-MSC-Zellen, menschlichen Fett-MSC-Zellen oder menschlichen Knochenmark-MSC-Zellen ausgewählt werden.

6. Herstellungsverfahren für mit bionischen Arzneistoffen beladene Nanopartikeln in der Expression von CXCR4 nach Anspruch 1, dadurch gekennzeichnet, dass das Herstellungsverfahren für MSC-Zellen in der Expression von CXCR4-Membranprotein darin besteht, die MSC-Zellen der P3-Generation zu kultivieren, bis die Fusion 80–90 % erreicht, wobei nach der Transfektion von MSC-Zellen mit dem Medium, das Lv-CXCR4-Überexpressions-Lentivirus enthält, DMEM-Medium verwendet wird, um das Medium zu ersetzen, das Lv-CXCR4 überexprimierendes Lentivirus enthält, wonach die Kultivierung forgesetzt wird und dann ein Resistenzscreening durchgeführt wird, um die MSC-Zellen für eine stabile Expression von CXCR4-Membranprotein zu erhalten.

7. Herstellungsverfahren für mit bionischen Arzneistoffen beladene Nanopartikeln in der Expression von CXCR4 nach Anspruch 1, dadurch gekennzeichnet, dass die Zellmembran lysiert wird, um die Zellmembran zu extrahieren, wobei das Verfahren zur Gewinnung der manipulierten Stammzellmembran darin besteht, die Zellen mit vorgekühltem Phosphatpufferlösung zu spülen und in hypotonischer Lyselösung zu resuspendieren, wonach sie in flüssigen Stickstoff gegeben und wiederholt eingefroren und aufgetaut werden, um die Zellen vollständig zu lysieren und das Zellmembranpellet zentrifugal zu extrahieren, wonach sie in phosphatgepufferter Kochsalzlösung resuspendiert und

wiederholt extrudiert werden, um manipulierte Stammzellmembranen zu erhalten .

8. Mit bionischen Arzneistoffen beladene Nanopartikeln in der Expression von CXCR4, die mit dem Verfahren nach einem der Ansprüche 1 bis 7 hergestellt werden.

9. Anwendung der mit bionischen Arzneistoffen beladenen Nanopartikeln nach Anspruch 8 zur Herstellung von immunsuppressiven Arzneimitteln.

10. Anwendung nach Anspruch 9, dadurch gekennzeichnet, dass die Anwendung der mit bionischen Arzneistoffen beladenen Nanopartikeln zur Herstellung von Arzneimitteln zur Verabreichung gegen rheumatoide Arthritis.

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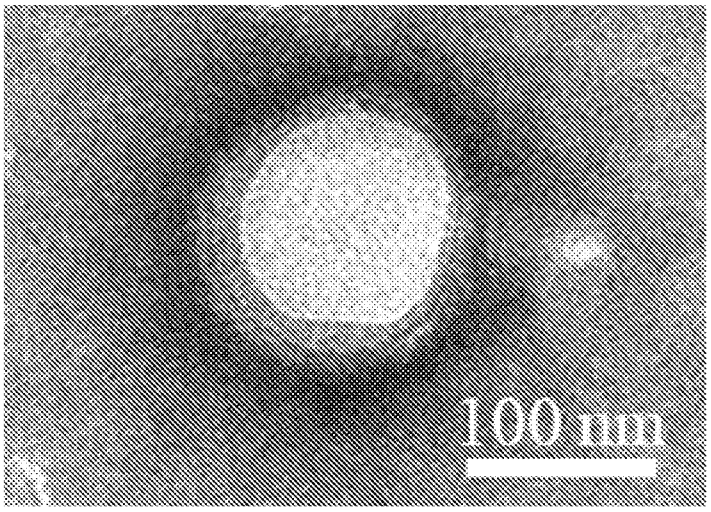

Fig. 1

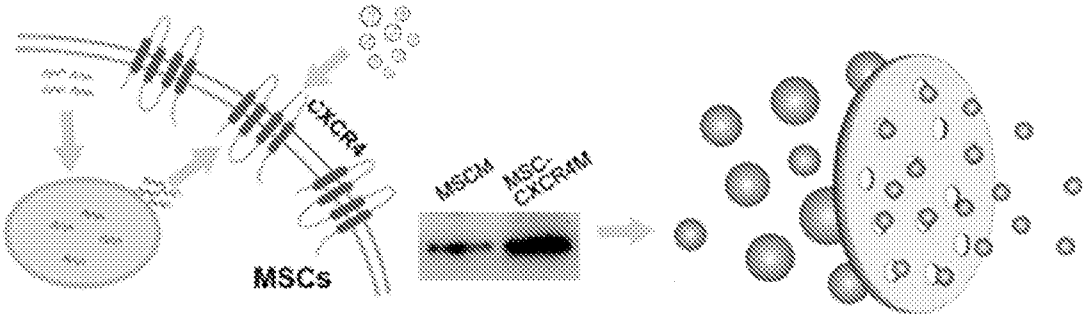

Fig. 2

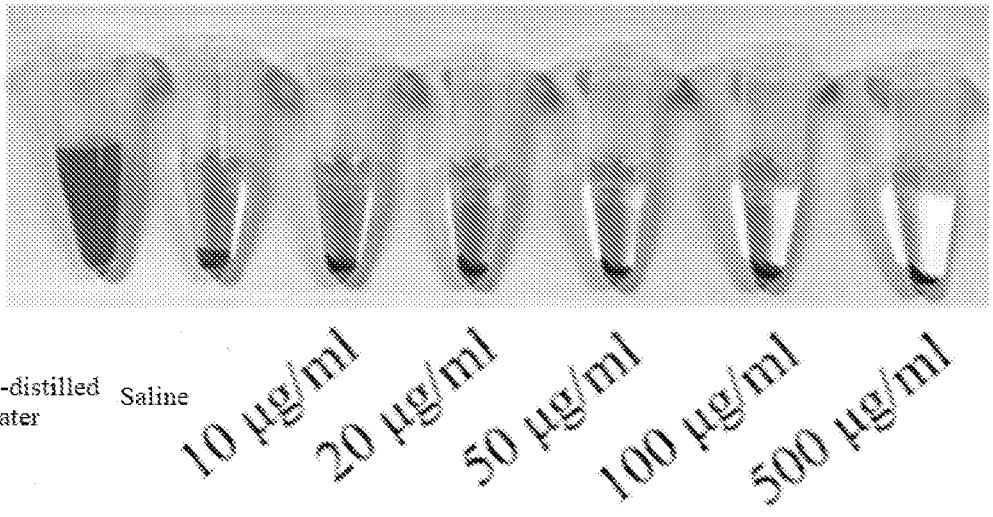

Fig. 3

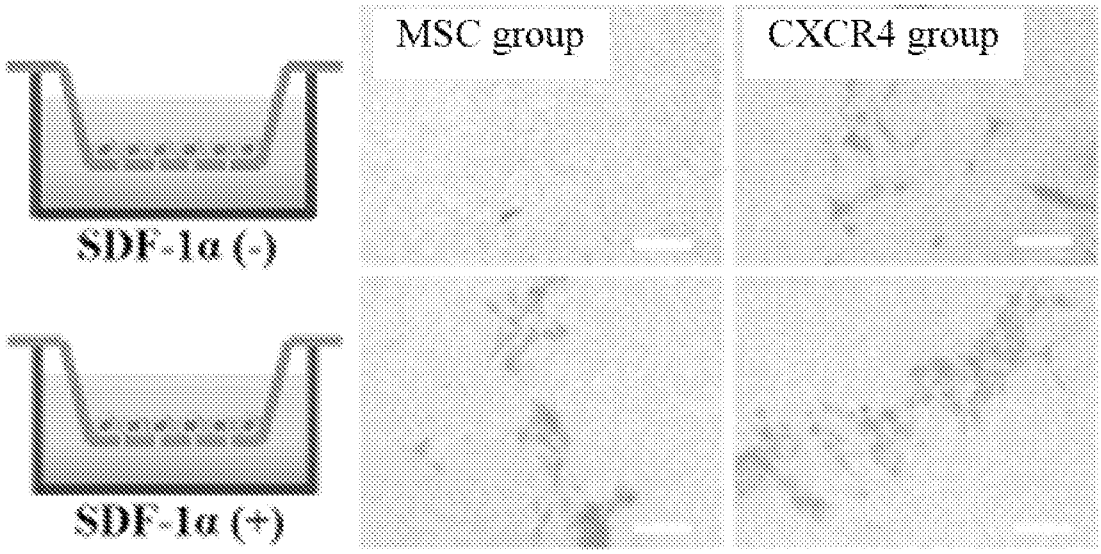

Fig. 4

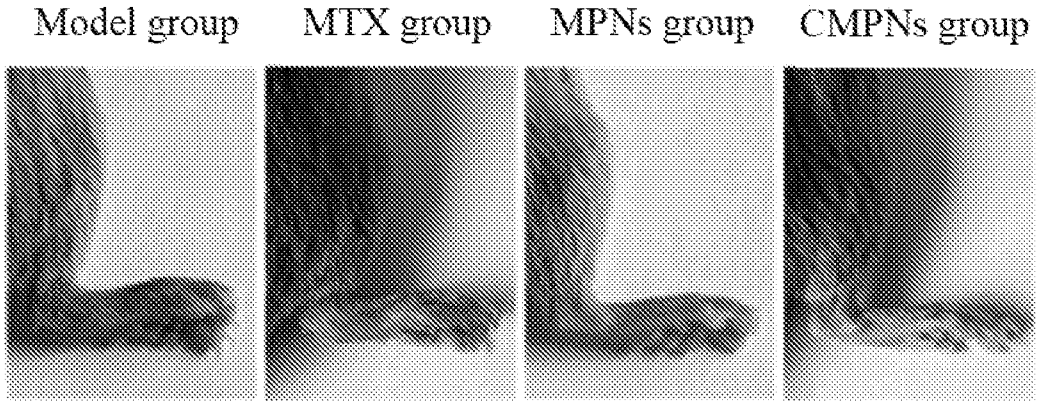

Fig. 5

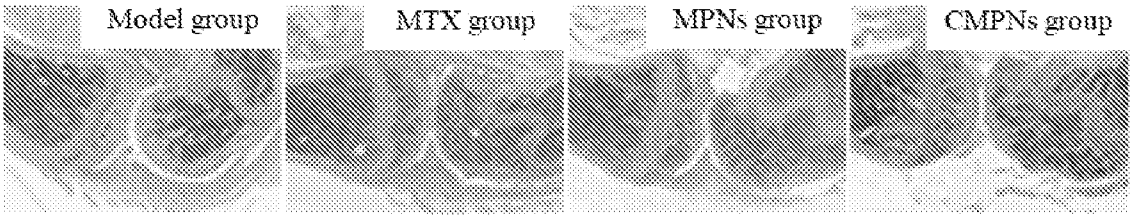

Fig. 6