Notice

DESCRIPTION CN111481678A

A nanomaterial targeting the acidic blocking region of osteoclasts and its preparation method

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一种针对破骨细胞酸性封闭区的纳米材料及其制备方法

[0001]

Technical Field

技术领域

[0002]

This invention belongs to the field of bone tissue drug therapy, specifically relating to a nanomaterial targeting the acidic blocking region of osteoclasts and its preparation method.

本发明属于骨组织药物治疗领域，具体涉及一种针对破骨细胞酸性封闭区的纳米材料及其制备方法。

[0003]

Background Technology

背景技术

[0004]

Osteoporosis is a global chronic disease that can lead to severe bone loss and fractures, causing suffering and a significant decline in quality of life for patients.

骨质疏松症是一种全球性的慢性疾病，可导致严重的骨质流失和骨折，对患者造成痛苦，生活质量严重下降。

Abnormal activation of osteoclasts in bone tumors and bone metastases can also lead to osteoporosis and pathological fractures.

骨肿瘤及肿瘤骨转移中的破骨细胞异常活化也可造成破骨细胞异常活化，引起骨质疏松，从而导致病理性骨折。

Currently, the main treatments for abnormal osteoclast activation include bone resorption inhibitors such as calcium, vitamin D, calcitonin, bisphosphonates, and estrogen, and bone formation promoters such as fluoride, anabolic steroids, and parathyroid hormone. Although these treatments can affect osteoclast function or slow the disease progression by promoting bone formation, they are difficult to completely inhibit bone loss because the acidification and bone destruction caused by osteoclasts are irreversible.

目前对破骨细胞异常活化的治疗主要有钙剂、维生素D、降钙素、二磷酸盐、雌激素等抑制骨吸收药与氟化物、合成类固醇、甲状旁腺激素等促进骨形成药，这些治疗方法虽然可以影响破骨细胞的功能或通过促进骨生成来延缓病程，但难以完全抑制骨质流失，因为破骨细胞引起的酸化和骨质破坏是不可逆的。

Acidification of the osteoclast-bone interface is the root cause of bone mineral dissolution and organic degradation during osteoporosis. Therefore, there is an urgent need to develop new materials that precisely target the acidic blocking zone of osteoclasts to prevent abnormal osteoclast activation.

破骨细胞与骨接触界面的酸化是骨质疏松症期间骨矿物溶解和有机降解的根源，因此亟需研发新的精准针对破骨细胞酸性封闭区从而防治破骨细胞异常活化的材料。

[0005]

There is a wealth of nanomaterials available for drug therapy of bone tissue, including liposomes, polymer nanoparticles, silica particles, and nanocoatings. These nanomaterials can achieve therapeutic effects through specific targeting and drug release methods.

用于骨组织药物治疗的纳米材料十分丰富，主要有脂质体、聚合物纳米颗粒、二氧化硅颗粒及纳米涂层等，这些纳米材料可以通过一定的靶向方式和药物释放方式达到治疗效果。

In the treatment of osteoporosis, these nanomaterials can target bone tissue and affect osteoclast function in a certain way. However, common materials often have problems such as inaccurate targeting, insignificant efficacy, and large drug toxicity. Patent application number 201710283530.5 discloses a method for preparing dual-targeted drug-loaded nanoparticles with lipid-polymer for osteoporosis, which enhances the targeting effect of the

drug and reduces its side effects. Patent application number 201710841290.6 discloses the application of pH-responsive nanomaterials in the preparation of drugs for preventing osteoporosis and bone resorption, which utilizes pH-responsive graphene oxide, chitosan or hydrogel to selectively inhibit osteoclasts. These patents optimize the delivery or release process in certain aspects.

在治疗骨质疏松症层面，这些纳米材料可以达到靶向骨组织，并以一定方式影响破骨细胞功能的作用，但是常见的材料常出现靶向不精确、效用不显著、药物毒副作用大等问题。 申请号为201710283530.5的专利公开了一种用于骨质疏松的双靶向载药纳米颗粒脂-聚合物制备方法，加强了药物的靶向作用以减少药物的副作用。 申请号为201710841290.6的专利公开了一种pH响应纳米材料在制备防治骨质疏松抗骨吸收药物中的应用，利用pH响应型氧化石墨烯、壳聚糖或水凝胶选择性地抑制破骨细胞。 这些专利在一定维度上优化了递送或释放过程。

[0006]

However, existing materials still cannot solve two problems at the same time: the precision of osteoclast targeting and the physiological nature of the drug.

但既往的材料仍然难以同时解决两个问题，即破骨细胞靶向的精准性与作用药物的生理性。

In the progression of osteoporosis, bone destruction caused by bone resorption by mature osteoclasts is an important aspect, but other cell-mediated bone repair and homeostasis

maintenance are also very important aspects. Therefore, appropriate osteoporosis treatment materials should have the ability to target bone tissue, especially acidified osteoclasts, in circulation. At the same time, materials used for targeting and pH response should be widely used in clinical practice or be substances commonly found in vivo. Meeting the above two points can achieve the inhibition of osteoclasts while minimizing the toxic side effects on other cells, thereby achieving a better effect of resisting bone resorption and promoting bone formation.

在骨质疏松症的病程进展中，成熟破骨细胞骨吸收导致的骨质破坏是重要的一方面，但是其他细胞介导的骨修复和稳态维持也是十分重要的一方面。 因此，合理的骨质疏松治疗材料应具有在循环中对骨组织，尤其是酸化破骨细胞的靶向，同时，用于靶向和pH响应的材料应在临床上广泛使用或为体内常见物质。 符合上述两点，可实现对破骨细胞的抑制而其他细胞产生尽可能低的毒副作用，从而达到更优的抗骨吸收和促骨形成的效果。

[0007]

Summary of the Invention

发明内容

[0008]

This invention addresses the shortcomings of existing technologies by providing a nanomaterial targeting the acidic blocking region of osteoclasts and its preparation method.

本发明针对现有技术的不足，提供一种针对破骨细胞酸性封闭区的纳米材料及其制备方法。

Bone-targeting is achieved through commonly used clinical drugs, and the chemical reaction simultaneously achieves precise targeting and functional inhibition of osteoclasts.

通过临床常用药物进行骨靶向，并通过化学反应同时达到对破骨细胞的精准靶向与功能抑制。

[0009]

To achieve the above objectives, the present invention provides the following technical solution: a nanomaterial targeting the acidic blocking region of osteoclasts, comprising nanomaterials, bone-targeting molecules, and compounds that chemically react with the acidic region of osteoclasts; after modifying the nanomaterials with bone-targeting molecules, the compounds that chemically react with the acidic region of osteoclasts are encapsulated; the nanomaterials are encapsulable and modifiable nanomaterials; the bone-targeting molecules are molecules with a clear affinity for bone tissue; and the compounds that chemically react with the acidic region of osteoclasts are weakly basic or neutral bicarbonate salts.

为实现上述目的，本发明提供以下技术方案：一种针对破骨细胞酸性封闭区的纳米材料，包括纳米材料、骨靶向分子和对破骨细胞酸性区域产生化学反应的化合物；对纳米材料进行骨靶向分子修饰后，包载对破骨细胞酸性区域产生化学反应的化合物；所述的纳米材料为可包载、可修饰的纳米材料；所述的骨靶向分子为对骨组织具有明确亲和力的分子，所述的可对破骨细胞酸性区域产生化学反应的化合物为弱碱性或中性碳酸氢根盐。

[0010]

Preferably, the nanomaterial is a liposome, polymer nanoparticle, or mesoporous silica particle.

作为优选，所述纳米材料为脂质体、聚合物纳米颗粒或介孔氧化硅颗粒。

[0011]

Preferably, the bone-targeting molecule is a tetracycline, phosphonate, or aspartic acid polypeptide sequence.

作为优选，所述骨靶向分子为四环素、膦酸盐或天冬氨酸类多肽序列。

[0012]

Preferably, the compound that can chemically react with the acidic region of osteoclasts is sodium bicarbonate, potassium bicarbonate, or ammonium bicarbonate, wherein the sodium bicarbonate is 1 mol/L sodium bicarbonate.

作为优选，所述可对破骨细胞酸性区域产生化学反应的化合物为碳酸氢钠、碳酸氢钾、碳酸氢铵，所述的碳酸氢钠为1mol/L的碳酸氢钠。

[0013]

A method for preparing nanomaterials targeting the acidic blocking region of osteoclasts, the method specifically comprising: cross-linking a loadable and modifiable nanomaterial with bone-targeting molecules, dissolving it with lecithin and cholesterol in chloroform, controlling the pH value to 8.0-8.4, magnetically stirring and cross-linking at room temperature for 24-72 hours, thinning it in a rotary evaporator, then adding the solution to be loaded for shaking hydration, ultrasonic emulsification, and dialysis; wherein the molar ratio of the loadable and modifiable nanomaterials to the bone-targeting molecules is 1:1 to 1:2.

一种针对破骨细胞酸性封闭区的纳米材料的制备方法，该方法具体为：将可包载、可修饰的纳米材料与骨靶向分子交联后，与卵磷脂、胆固醇溶于氯仿，并控制PH值为8.0-8.4，室温下磁力搅拌交联24-72小时，在旋蒸仪中薄膜化，然后加入待包载溶液进行震荡水化，并超声乳化后进行透析；其中可包载、可修饰的纳米材料与骨靶向分子的摩尔比为1：1至1：2。

[0014]

The aforementioned loadable and modifiable nanomaterials are functionalized phospholipids, which are cross-linked with bone-targeting molecules to obtain bone-targeting functional phospholipids.

所述的可包载、可修饰的纳米材料为功能化磷脂，与骨靶向分子交联后得到骨靶向功能磷脂。

[0015]

The ultrasonic emulsification process is as follows: turn on for 1-2 seconds, turn off for 2-3 seconds, power 30-70%, time 5-20 minutes, and the dialysis time is 1-3 days.

所述的超声乳化流程为开1-2s，关2-3s，功率30-70%，时间5-20分钟，所述的透析时间为1-3天。

[0016]

The functionalized phospholipids used are DSPE-PEG-NHS, and the bone-targeting molecule is tetracycline, i.e., TC.

所述的功能化磷脂采用DSPE-PEG-NHS，所述骨靶向分子采用四环素，即TC。

[0017]

The beneficial effects of this invention are as follows: This invention provides nanomaterials targeting the acidic blocking region of osteoclasts and their preparation method. By precisely targeting mature osteoclasts and regulating the biological cascade reaction of physiological chemical reactions, it inhibits osteoclasts, providing new ideas and tools for the drug treatment of abnormal osteoclast activation.

本发明的有益效果：本发明提供了针对破骨细胞酸性封闭区的纳米材料及其制备方法，通过精确的成熟破骨细胞靶向和生理性化学反应调控的生物级联反应抑制破骨细胞，为破骨细胞异常活化的药物治疗提供了新思路和新工具。

[0018]

Compared to existing drugs or materials for treating osteoporosis, the significant advancement of this invention lies in:

相比现有治疗骨质疏松的药物或材料，本发明显著的进步在于：

[0019]

1) By employing bone tissue-targeting molecules and pH-responsive targeting of osteoclast-blocking regions, dual precision targeting is achieved, improving drug utilization and reducing side effects on other tissues and organs.

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1)采用骨组织靶向分子与针对破骨细胞封闭区域的pH响应进行双重精确靶向，提高药物利用率，降低对其他组织器官的副作用。

[0020]

2) The drugs used are physiological compounds that are present in the human body, ensuring low toxicity.

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2)所用药物为人体内存在的生理性化合物，确保低毒性。

It can act as both a therapeutic component and a pH-responsive component, thus having a dual function.

可同时作为治疗效应产生成分和pH迅速响应成分，具有双重作用。

[0021]

3) In vitro experiments verified a significant anti-osteoclast bone erosion effect, with a clear inhibitory effect on the number and size of osteoclasts, as well as the number and area of bone erosion regions.

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3)体外实验验证显著的抗破骨细胞骨侵蚀作用，对破骨细胞数量及大小、骨侵蚀区域数量及面积均有明确的抑制作用。

[0022]

4) In vitro experiments verified the significant osteoclast-promoting exosome effect. The exosome surface RANK achieves ineffective binding with serum RANKL, thereby achieving long-term osteoclast inhibition.

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4)体外实验验证显著的促破骨细胞外泌体作用，利用外泌体表面RANK实现与血清中RANKL的无效结合，继而实现远期破骨抑制作用。

[0023]

5) In vivo experiments have verified a significant anti-osteoporosis effect, with clear improvements in bone mass, trabecular bone quantity, and trabecular bone spacing in osteoporosis patients.

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5)在体实验验证显著的抗骨质疏松作用，对骨质疏松的骨量、骨小梁数量、骨小梁间隙均有明确的改善作用。

[0024]

6) As a nanomaterial model targeting the acidic blocking region of osteoclasts, each component is replaceable and has sufficient reference value.

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6)作为一种针对破骨细胞酸性封闭区的纳米材料模型，其中各组分均可替换，具有充足的可借鉴性。

[0025]

Therefore, nanomaterials targeting the acidic blocking region of osteoclasts can be used to prevent and treat abnormal osteoclast activation, and have a clear therapeutic effect.

因此，针对破骨细胞酸性封闭区的纳米材料可应用于防治破骨细胞异常活化，具有明确的治疗效果。

[0026]

Attached Figure Description

附图说明

[0027]

To make the objectives, technical solutions, and beneficial effects of this invention clearer, the following figures are provided for illustration:

为了使本发明的目的、技术方案和有益效果更加清楚，本发明提供以下附图进行说明：

[0028]

Figure 1 shows the preparation and characterization of nanomaterials targeting the acidic blocking region of osteoclasts.

图1针对破骨细胞酸性封闭区的纳米材料的制备和表征。

a is a schematic diagram of the mechanism of action of nanomaterials targeting the acidic blocking region of osteoclasts; b and c are schematic diagrams of the preparation process of nanomaterials targeting the acidic blocking region of osteoclasts; d-f are verifications of the cross-linking of bone-targeting molecules and functionalized phospholipids by MALDI-TOF, confocal microscopy, and fluorescence spectrophotometer; g is the characterization of

nanomaterials targeting the acidic blocking region of osteoclasts under cryo-electron microscopy.

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a为针对破骨细胞酸性封闭区的纳米材料作用机制示意图；b，c为针对破骨细胞酸性封闭区的纳米材料的制备流程示意图；d-f为MALDI-TOF、共聚焦显微镜、荧光光度计验证骨靶向分子与功能化磷脂的交联；g为针对破骨细胞酸性封闭区的纳米材料在冷冻电镜下的表征。

[0029]

Figure 2 shows the functional verification of nanomaterials targeting the acidic blocking region of osteoclasts.

图2针对破骨细胞酸性封闭区的纳米材料的功能验证。

a) Acid titration test to verify the acid resistance of nanomaterials targeting the acidic blocking region of osteoclasts; b) Measurement of particle size at different pH levels to verify the pH response of nanomaterials targeting the acidic blocking region of osteoclasts; c, d) In-situ liquid atomic force microscopy to verify the mechanical changes of nanomaterials targeting the acidic blocking region of osteoclasts at different pH levels; e) Cryo-electron microscopy to verify the release of nanomaterials targeting the acidic blocking region of osteoclasts under acidic conditions (pH=4); f, g) In vitro fluorescence microscopy to verify the long-term (7-day) stability and rapid pH response of nanomaterials targeting the acidic

blocking region of osteoclasts; h) In vivo fluorescence to verify the rapid accumulation of nanomaterials targeting the acidic blocking region of osteoclasts in the target bone of mice; i) Fluorescence confocal microscopy to verify the inhibitory effect of nanomaterials targeting the acidic blocking region of osteoclasts on osteoclast bone erosion.

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a为酸滴定试验验证针对破骨细胞酸性封闭区的纳米材料的抗酸能力；b为测定不同pH下粒径验证针对破骨细胞酸性封闭区的纳米材料的pH响应；c，d为原位液体原子力显微镜验证不同pH下针对破骨细胞酸性封闭区的纳米材料的力学变化；e为冷冻电镜验证针对破骨细胞酸性封闭区的纳米材料在酸性条件(pH＝4)下释放；f，g为体外荧光显微镜验证针对破骨细胞酸性封闭区的纳米材料的长期(7天)稳定性及快速pH响应；h为活体荧光验证针对破骨细胞酸性封闭区的纳米材料在小鼠体内靶向骨的快速富集；i为荧光共聚焦显微镜验证针对破骨细胞酸性封闭区的纳米材料对破骨细胞骨侵蚀的抑制作用。

[0030]

Figure 3 shows the inhibitory effect of nanomaterials targeting the acidic blocking region of osteoclasts on osteoclasts through a bio-cascade effect regulated by chemical reactions.

图3针对破骨细胞酸性封闭区的纳米材料通过化学反应调控的生物级联效应对破骨细胞的抑制作用。a) TRAP staining verifies the inhibitory effect of nanomaterials targeting the acidic blocking region of osteoclasts on osteoclasts; b) Scanning electron microscopy verifies the significant

improvement of osteoclast bone erosion by nanomaterials targeting the acidic blocking region of osteoclasts; c, d) Western blot and qPCR verify that nanomaterials targeting the acidic blocking region of osteoclasts can inhibit the time-dependent increase in the expression of NFATc-1, c-Fos, and CTSK in osteoclasts; e) Fluorescence confocal microscopy verifies that nanomaterials targeting the acidic blocking region of osteoclasts can inhibit the formation of the blocking region; f, g) Western blot and fluorescence confocal microscopy verify that nanomaterials targeting the acidic blocking region of osteoclasts can inhibit the time-dependent increase in RANK expression in osteoclasts; h, i) Western blot and extracellular vesicle flow cytometry verify that nanomaterials targeting the acidic blocking region of osteoclasts can promote the production of RANK-containing exosomes by osteoclasts; j, k) TRAP staining verifies that the extracellular vesicles containing RANK secreted by osteoclasts promoted by nanomaterials targeting the acidic blocking region of osteoclasts can further inhibit osteoclast formation.

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a为TRAP染色验证针对破骨细胞酸性封闭区的纳米材料对破骨细胞的抑制作用；b为扫描电子显微镜验证针对破骨细胞酸性封闭区的纳米材料对破骨细胞骨侵蚀的显著改善；c，d为Western-blot、qPCR验证针对破骨细胞酸性封闭区的纳米材料可抑制破骨细胞NFATc-1、c-Fos、CTSK表达随时间的递增效应；e为荧光共聚焦显微镜验证针对破骨细胞酸性封闭区的纳米材料可抑制破骨细胞封闭区形成；f，g为Western-blot、荧光共聚焦显微镜验证针对破骨细胞酸性封闭区的纳米材料可抑制破骨细胞RANK表达随时间的递增效应；h，i为Western-blot、细胞外囊泡流式计数验证针对破骨细胞酸

性封闭区的纳米材料可促进破骨细胞产生含RANK的外泌体；j，k为TRAP染色验证针对破骨细胞酸性封闭区的纳米材料促进破骨细胞分泌的含RANK的细胞外囊泡可进一步抑制破骨细胞形成。

[0031]

Figure 4 shows the inhibitory effect of nanomaterials targeting the acidic blocking region of osteoclasts on osteoporosis in OVX mice.

图4针对破骨细胞酸性封闭区的纳米材料对OVX小鼠骨质疏松的抑制作用。

A represents the construction of animal models and the grouping and evaluation methods; b and c represent micro-CT verification that nanomaterials targeting the acidic blocking region of osteoclasts can significantly improve bone mass, trabecular bone number, and trabecular gaps in the spine, femur, and tibia of OVX mice; d and e represent H&E staining and TRAP staining verification that nanomaterials targeting the acidic blocking region of osteoclasts can significantly improve bone mass, osteoclast number, and area in the bone tissue of OVX mice; f represents serological verification that nanomaterials targeting the acidic blocking region of osteoclasts can significantly inhibit osteoclast metabolic indicators in OVX mice.

A为构建动物模型及分组、评估方式；b，c为micro-CT验证针对破骨细胞酸性封闭区的纳米材料可对OVX小鼠脊柱及股骨、胫骨的骨量、骨小梁数量及骨小梁间隙起到显著改善作用；d，e为H&E染色及TRAP染色验证针对破骨细胞酸性封闭区的纳米材料可对OVX小鼠骨组织的骨量、破骨细胞数量

及面积起到显著改善作用；f为血清学指标验证针对破骨细胞酸性封闭区的纳米材料可对OVX小鼠的破骨细胞代谢指标起到显著抑制作用。

[0032]

Detailed Implementation

具体实施方式

[0033]

The following detailed description, in conjunction with embodiments, of a nanomaterial for targeting the acidic blocking region of osteoclasts and its preparation method provided by the present invention, should not be construed as limiting the scope of protection of the present invention.

下面结合实施例对本发明提供的一种针对破骨细胞酸性封闭区的纳米材料材料及其制备方法进行详细的说明，但是不能把它们理解为对本发明保护范围的限定。

[0034]

Figure 1(a) shows a schematic diagram of the mechanism of action of nanomaterials in the acidic blocking region of osteoclasts.

如图1(a)所示，破骨细胞酸性封闭区的纳米材料作用机制示意图；

[0035]

A preferred example of the nanomaterial for the acidic blocking region of osteoclasts in this invention is tetracycline-modified nanoliposomes loaded with sodium bicarbonate (abbreviated as NaHCO₃-TNLs), which are prepared by the following method, with the specific steps as follows: 20.00 mg of DSPE-PEG-NHS and 3.05 mg of tetracycline are dissolved in 10.00 mL of chloroform, triethylamine is added to adjust the pH to 8.2, and after crosslinking with magnetic stirring at room temperature for 48 hours, DSPE-PEG-TC is obtained. DSPE-PEG-TC is then dissolved in chloroform with 80.00-120.00 mg of lecithin and 12.00-20.00 mg of cholesterol. The mixture is thinned in a rotary evaporator, and then 10 mL of 1 mol/L sodium bicarbonate solution is added for shaking hydration. Finally, ultrasonic emulsification is performed with the following steps: 2 seconds on, 3 seconds off, 40% power, and 10 minutes.

本发明中的针对破骨细胞酸性封闭区的纳米材料的优选案例为包载碳酸氢钠的四环素修饰的纳米脂质体(简称NaHCO₃-TNLs)，由以下方法制备，具体步骤如下：将20.00mg的DSPE-PEG-NHS与3.05mg四环素溶于10.00mL氯仿，加入三乙胺调节pH至8.2，室温下磁力搅拌交联48小时后得到DSPE-PEG-TC，与80.00-120.00mg卵磷脂、12.00-20.00mg胆固醇溶于氯仿，在旋蒸仪中薄膜

化，然后加入10mL的1mol/L碳酸氢钠溶液进行震荡水化，然后进行超声乳化，超声乳化流程为开2s，关3s，功率40%，时间10分钟。

Finally, the patient was dialyzed in a dialysis bag for 72 hours, then filtered through a 0.22-micron filter and stored at 4 degrees Celsius.

最后于透析袋中透析72小时，取出后经0.22微米滤头过滤，4度保存。

The materials obtained are shown in Figures b and c in Figure 1.

制得的材料如图1中b、c所示。

[0036]

The present invention can also use other existing nanomaterials targeting the acidic blocking region of osteoclasts, such as tetracycline or alendronate-modified nanoliposomes loaded with ammonium bicarbonate or potassium bicarbonate, to achieve the same technical effect.

本发明还可以使用其他现有的针对破骨细胞酸性封闭区的纳米材料，如包载碳酸氢铵或碳酸氢钾的四环素或阿仑膦酸修饰的纳米脂质体等均可获得相同的技术效果。

[0037]

Example 1: Preparation of tetracycline-modified nanoliposomes loaded with sodium bicarbonate

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实施例1、包载碳酸氢钠的四环素修饰纳米脂质体的制备

[0038]

1. Dissolve 20.00 mg of DSPE-PEG-NHS and 3.05 mg of tetracycline in 10.00 mL of chloroform, add triethylamine to adjust the pH to 8.2, and crosslink at room temperature with magnetic stirring for 48 hours.

1、将20.00mg的DSPE-PEG-NHS与3.05mg四环素溶于10.00mL氯仿，加入三乙胺调节pH至8.2，室温下磁力搅拌交联48小时。

[0039]

2. Dissolve the product obtained in step 1 with 100.00 mg of lecithin and 16.00 mg of cholesterol in chloroform and thin film in a rotary evaporator.

2、将步骤1得到的产物与100.00mg卵磷脂、16.00mg胆固醇溶于氯仿，在旋蒸仪中薄膜化。

[0040]

3. Add 10 mL of 1 mol/L sodium bicarbonate solution to the flask and shake to hydrate.

3、在烧瓶中加入10mL的1mol/L碳酸氢钠溶液进行震荡水化。

[0041]

4. Perform ultrasonic emulsification. The ultrasonic emulsification process is as follows: on for 2 seconds, off for 3 seconds, power 40%, time 10 minutes.

4、进行超声乳化，超声乳化流程为开2s，关3s，功率40％，时间10分钟

[0042]

5. Dialyze in a dialysis bag for 72 hours, then remove and filter through a 0.22-micron filter and store at 4 degrees Celsius.

5、于透析袋中透析72小时，取出后经0.22微米滤头过滤，4度保存。

[0043]

Example 2: Preparation of alendronate-modified nanoliposomes loaded with sodium bicarbonate

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实施例2、包载碳酸氢钠的阿仑膦酸修饰纳米脂质体的制备

[0044]

1. Dissolve 20.00 mg of DSPE-PEG-NHS and 2.30 mg of sodium alendronate in 10.00 mL of chloroform, add triethylamine to adjust the pH to 8.2, and crosslink at room temperature with magnetic stirring for 48 hours.

1、将20.00mg的DSPE-PEG-NHS与2.30mg阿仑膦酸钠溶于10.00mL氯仿，加入三乙胺调节pH至8.2，室温下磁力搅拌交联48小时。

[0045]

2. Dissolve the product obtained in step 1 with 100.00 mg of lecithin and 16.00 mg of cholesterol in chloroform and thin film in a rotary evaporator.

2、将步骤1得到的产物与100.00mg卵磷脂、16.00mg胆固醇溶于氯仿，在旋蒸仪中薄膜化。

[0046]

3. Add 10 mL of 1 mol/L sodium bicarbonate solution to the flask and shake to hydrate.

3、在烧瓶中加入10mL的1mol/L碳酸氢钠溶液进行震荡水化。

[0047]

4. Perform ultrasonic emulsification. The ultrasonic emulsification process is as follows: on for 2 seconds, off for 3 seconds, power at 40%, for 20 minutes.

4、进行超声乳化，超声乳化流程为开2s，关3s，功率40％，时间20分钟

[0048]

5. Dialyze in a dialysis bag for 72 hours, then remove and filter through a 0.22-micron filter and store at 4 degrees Celsius.

5、于透析袋中透析72小时，取出后经0.22微米滤头过滤，4度保存。

[0049]

Example 3: Preparation of tetracycline-modified nanoliposomes loaded with potassium bicarbonate

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实施例3、包载碳酸氢钾的四环素修饰纳米脂质体的制备

[0050]

1. Dissolve 20.00 mg of DSPE-PEG-NHS and 3.05 mg of tetracycline in 10.00 mL of chloroform, add triethylamine to adjust the pH to 8.2, and crosslink with magnetic stirring at room temperature for 24 hours.

1、将20.00mg的DSPE-PEG-NHS与3.05mg四环素溶于10.00mL氯仿，加入三乙胺调节pH至8.2，室温下磁力搅拌交联24小时。

[0051]

2. Dissolve the product obtained in step 1 with 80 mg of lecithin and 16.00 mg of cholesterol in chloroform and thin film in a rotary evaporator.

2、将步骤1得到的产物与80mg卵磷脂、16.00mg胆固醇溶于氯仿，在旋蒸仪中薄膜化。

[0052]

3. Add 10 mL of 1 mol/L potassium bicarbonate solution to the flask and shake to hydrate.

3、在烧瓶中加入10mL的1mol/L碳酸氢钾溶液进行震荡水化。

[0053]

4. Perform ultrasonic emulsification. The ultrasonic emulsification process is as follows: on for 2 seconds, off for 3 seconds, power 40%, time 10 minutes.

4、进行超声乳化，超声乳化流程为开2s，关3s，功率40％，时间10分钟

[0054]

5. Dialyze in a dialysis bag for 72 hours, then remove and filter through a 0.22-micron filter and store at 4 degrees Celsius.

5、于透析袋中透析72小时，取出后经0.22微米滤头过滤，4度保存。

[0055]

Example 4: Preparation of alendronic acid-modified nanoliposomes loaded with potassium bicarbonate

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实施例4、包载碳酸氢钾的阿仑膦酸修饰纳米脂质体的制备

[0056]

1. Dissolve 20.00 mg of DSPE-PEG-NHS and 2.30 mg of sodium alendronate in 10.00 mL of chloroform, add triethylamine to adjust the pH to 8.2, and crosslink at room temperature with magnetic stirring for 48 hours.

1、将20.00mg的DSPE-PEG-NHS与2.30mg阿仑膦酸钠溶于10.00mL氯仿，加入三乙胺调节pH至8.2，室温下磁力搅拌交联48小时。

[0057]

2. Dissolve the product obtained in step 1 with 120.00 mg of lecithin and 16.00 mg of cholesterol in chloroform and thin film in a rotary evaporator.

2、将步骤1得到的产物与120.00mg卵磷脂、16.00mg胆固醇溶于氯仿，在旋蒸仪中薄膜化。

[0058]

3. Add 10 mL of 1 mol/L potassium bicarbonate solution to the flask and shake to hydrate.

3、在烧瓶中加入10mL的1mol/L碳酸氢钾溶液进行震荡水化。

[0059]

4. Perform ultrasonic emulsification. The ultrasonic emulsification process is as follows: on for 2 seconds, off for 3 seconds, power at 40%, time for 5 minutes.

4、进行超声乳化，超声乳化流程为开2s，关3s，功率40％，时间5分钟

[0060]

5. Dialyze in a dialysis bag for 72 hours, then remove and filter through a 0.22-micron filter and store at 4 degrees Celsius.

5、于透析袋中透析72小时，取出后经0.22微米滤头过滤，4度保存。

[0061]

Example 5: Preparation of tetracycline-modified nanoliposomes loaded with ammonium bicarbonate

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实施例5、包载碳酸氢铵的四环素修饰纳米脂质体的制备

[0062]

1. Dissolve 20.00 mg of DSPE-PEG-NHS and 3.05 mg of tetracycline in 10.00 mL of chloroform, add triethylamine to adjust the pH to 8.2, and crosslink at room temperature with magnetic stirring for 48 hours.

1、将20.00mg的DSPE-PEG-NHS与3.05mg四环素溶于10.00mL氯仿，加入三乙胺调节pH至8.2，室温下磁力搅拌交联48小时。

[0063]

2. Dissolve the product obtained in step 1 with 100.00 mg of lecithin and 12.00 mg of cholesterol in chloroform and thin film in a rotary evaporator.

2、将步骤1得到的产物与100.00mg卵磷脂、12.00mg胆固醇溶于氯仿，在旋蒸仪中薄膜化。

[0064]

3. Add 10 mL of 1 mol/L ammonium bicarbonate solution to the flask and shake to hydrate.

3、在烧瓶中加入10mL的1mol/L碳酸氢铵溶液进行震荡水化。

[0065]

4. Perform ultrasonic emulsification. The ultrasonic emulsification process is as follows: on for 1 second, off for 3 seconds, power at 40%, time for 10 minutes.

4、进行超声乳化，超声乳化流程为开1s，关3s，功率40％，时间10分钟

[0066]

5. Dialyze in a dialysis bag for 24 hours, then remove and filter through a 0.22-micron filter. Store at 4 degrees Celsius.

5、于透析袋中透析24小时，取出后经0.22微米滤头过滤，4度保存。

[0067]

Example 6: Preparation of alendronate-modified nanoliposomes loaded with ammonium bicarbonate

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实施例6、包载碳酸氢铵的阿仑膦酸修饰纳米脂质体的制备

[0068]

1. Dissolve 20.00 mg of DSPE-PEG-NHS and 2.30 mg of sodium alendronate in 10.00 mL of chloroform, add triethylamine to adjust the pH to 8.2, and crosslink at room temperature with magnetic stirring for 48 hours.

1、将20.00mg的DSPE-PEG-NHS与2.30mg阿仑膦酸钠溶于10.00mL氯仿，加入三乙胺调节pH至8.2，室温下磁力搅拌交联48小时。

[0069]

2. Dissolve the product obtained in step 1 with 100.00 mg of lecithin and 20.00 mg of cholesterol in chloroform and thin film in a rotary evaporator.

2、将步骤1得到的产物与100.00mg卵磷脂、20.00mg胆固醇溶于氯仿，在旋蒸仪中薄膜化。

[0070]

3. Add 10 mL of 1 mol/L ammonium bicarbonate solution to the flask and shake to hydrate.

3、在烧瓶中加入10mL的1mol/L碳酸氢铵溶液进行震荡水化。

[0071]

4. Perform ultrasonic emulsification. The ultrasonic emulsification process is as follows: on for 2 seconds, off for 2 seconds, power 70%, time 10 minutes.

4、进行超声乳化，超声乳化流程为开2s，关2s，功率70％，时间10分钟

[0072]

5. Dialyze in a dialysis bag for 72 hours, then remove and filter through a 0.22-micron filter and store at 4 degrees Celsius.

5、于透析袋中透析72小时，取出后经0.22微米滤头过滤，4度保存。

[0073]

Example 7: Evaluation of the synthesis of NaHCO₃-TNLs

实施例7、NaHCO₃-TNLs的合成评估

[0074]

1. Mass spectrometry analysis of DSPE-PEG-NHS and DSPE-PEG-TC using MALDI-TOF showed that the molecular weight distribution of DSPE-PEG-NHS was 2900 and that of DSPE-PEG-TC was 3250, which is consistent with the theoretical molecular weight (d in Figure 1).

1、将DSPE-PEG-NHS与DSPE-PEG-TC分别使用MALDI-TOF进行质谱检测，可得DSPE-PEG-NHS分子量分布于2900，DSPE-PEG-TC分子量分布于3250，与理论分子量一致(图1中d)。

[0075]

2. When FITC and sodium bicarbonate solution are co-encapsulated, the tetracycline fluorescence is observed to be consistent with the liposome membrane localization under laser confocal microscopy (Figure 1e).

2、将FITC与碳酸氢钠溶液共同包载，激光共聚焦显微镜可见四环素荧光与脂质体膜定位一致(图1中e)。

[0076]

3. Fluorescence spectrophotometry showed that the tetracycline-modified material exhibited significantly enhanced fluorescence at 525 nm compared to the unmodified material (Figure 1f).

3、荧光分光光度计显示四环素修饰的材料在525nm处荧光比未修饰的材料明显增强(图1中f)。

[0077]

4. Cryo-transmission electron microscopy revealed that the material particles were all at the nanometer level and had regular shapes (g in Figure 1).

4、冷冻透射电子显微镜显示材料粒径均为纳米级别且形状规则(图1中g)。

[0078]

Example 8: Characteristic Evaluation of NaHCO₃-TNLs

实施例8、NaHCO₃-TNLs的特性评估

[0079]

1. Tetracycline-modified nanoliposomes loaded with sodium bicarbonate (NaHCO₃-TNLs), tetracycline-modified nanoliposomes loaded with sodium chloride (NaCL-TNLs), water, 1 mol/L sodium bicarbonate solution, and 0.02 mol/L sodium bicarbonate solution

were titrated with 1% hydrochloric acid, and the dynamic changes of pH were monitored in real time. The results showed that NaHCO₃-TNLs had significant acid resistance (Figure 2a).

1、将包载碳酸氢钠的四环素修饰的纳米脂质体(NaHCO₃-TNLs)、包载氯化钠的四环素修饰的纳米脂质体(NaCL-TNLs)、水、1mol/L碳酸氢钠溶液、0.02mol/L碳酸氢钠溶液用1%的盐酸进行滴定实验，并实时检测pH的动态变化，结果显示NaHCO₃-TNLs具有显著的抗酸能力(图2中a)。

[0080]

2. The particle size of NaHCO₃-TNLs and NaCL-TNLs was measured in environments with pH values of 7, 6 and 4, respectively. The results showed that the particle size of NaHCO₃-TNLs decreased significantly in an acidic environment with pH = 4, indicating that the contents were released in an acidic environment (Figure 2b).

2、将NaHCO₃-TNLs、NaCL-TNLs分别于pH为7、6、4的环境中测定粒径，结果显示NaHCO₃-TNLs在pH＝4酸性环境中粒径显著减小，提示内容物在酸性环境中释放(图2中b)。

[0081]

3. The mechanical properties of NaHCO₃-TNLs were compared under pH=7 and pH=4 conditions using in-situ liquid atomic force microscopy. The results showed that the particle size was significantly smaller under pH=4 conditions, and the liposome membrane tended to rupture (c and d in Figure 2).

3、将NaHCO₃-TNLs通过原位液体原子力显微镜进行pH＝7和pH＝4环境下力学特性的比较，结果显示在pH＝4条件下粒径显著变小，且脂质体膜倾向破裂(图2中c、d)。

[0082]

4. The morphology of NaHCO₃-TNLs was compared under pH=7 and pH=4 conditions by cryo-transmission electron microscopy. The results showed that the liposome membrane was broken under pH=4 conditions (e in Figure 2).

4、将NaHCO₃-TNLs通过冷冻透射电子显微镜进行pH＝7和pH＝4环境下形态的比较，结果显示pH＝4条件下脂质体膜出现断裂(图2中e)。

[0083]

5. After loading FITC packages into NaHCO₃-TNLs, incubate them with bovine bone slices in 10% serum medium for 1 day, 3 days, and 7 days to observe fluorescence. After 7 days, change the medium environment to pH=4 and observe the liposome fluorescence at 0 minutes, 1 minute, and 3 minutes.

5、将FITC包载入NaHCO₃-TNLs后与牛骨片在10%血清培养基中孵育1天、3天、7天观察荧光，并在7天后将培养基环境换为pH＝4，观察0分钟、1分钟、3分钟的脂质体荧光。

The results showed that NaHCO₃-TNLs could be adsorbed onto the bone surface and remain stable within 7 days, and still had a rapid pH response function after 7 days.

结果显示NaHCO₃-TNLs在7天内可吸附于骨面并保持稳定，且在7天后仍具有对pH的快速响应功能。

(f, g in Figure 2).

(图2中f，g)。

[0084]

6. Tetracycline-modified nanoliposomes loaded with indocyanine green (ICG-TNLs) and non-tetracycline-modified nanoliposomes loaded with indocyanine green (ICG-NLs) were administered via tail vein injection at a dose of 0.025 ml/g.

6、将包载吲哚氰绿的四环素修饰的纳米脂质体(ICG-TNLs)与包载吲哚氰绿的无四环素修饰的纳米脂质体(ICG-NLs)以0.025ml/g剂量进行尾静脉注射。

The results showed that ICG-TNLs had a significant rapid bone tissue enrichment effect compared to ICG-NLs (h in Figure 2).

结果显示ICG-TNLs相对于ICG-NLs具有显著的快速骨组织富集作用(图2中h)。

[0085]

7. NaHCO₃-TNLs and NaCL-TNLs were co-incubated with FITC-coated bovine bone slices, and mature osteoclasts were seeded in each. The results showed that the FITC fluorescence intensity and area on the bone surface of the NaHCO₃-TNLs group were significantly better than those of the NaCL-TNLs group, suggesting that NaHCO₃-TNLs can effectively inhibit the bone erosion of osteoclasts.

7、将NaHCO₃-TNLs、NaCL-TNLs分别与FITC包被的牛骨片共孵育，并分别种植成熟破骨细胞，结果显示NaHCO₃-TNLs组骨面FITC荧光强度及面积显著优于NaCL-TNLs组，提示NaHCO₃-TNLs可有效抑制破骨细胞的骨侵蚀作用。

(i in Figure 2).

(图2中i)。

[0086]

Example 9: Osteoclast Inhibition Effect of NaHCO₃-TNLs

实施例9、NaHCO₃-TNLs的破骨细胞抑制作用

[0087]

1. When NaHCO₃-TNLs were added to the osteoclast induction system and compared with the osteoclast induction system alone, the results showed that the number and area of TRAP-stained osteoclasts in the NaHCO₃-TNLs group were significantly inhibited, suggesting that NaHCO₃-TNLs have a significant inhibitory effect on osteoclasts (Figure 3a).

1、将NaHCO₃-TNLs加入破骨细胞诱导体系并与单纯破骨细胞诱导体系对照，结果显示NaHCO₃-TNLs组TRAP染色破骨细胞数量与面积均显著抑制，提示NaHCO₃-TNLs对破骨细胞具有显著的抑制作用(图3中a)。

[0088]

2. When NaHCO₃-TNLs were added to the osteoclast induction system cultured from bovine bone slices and compared with the osteoclast induction system cultured solely from bovine bone slices, the results showed that the number and area of bone resorption regions were significantly inhibited under scanning electron microscopy in the NaHCO₃-TNLs group, suggesting that NaHCO₃-TNLs had a significant inhibitory effect on osteoclasts (Figure 3b).

2、将NaHCO₃-TNLs加入牛骨片培养的破骨细胞诱导体系并与牛骨片培养的单纯破骨细胞诱导体系对照，结果显示NaHCO₃-TNLs组扫描电镜骨吸收区域数量与面积均显著抑制，提示NaHCO₃-TNLs对破骨细胞具有显著的抑制作用(图3中b)。

[0089]

3. When NaHCO₃-TNLs were added to the osteoclast induction system and compared with the osteoclast induction system alone, Western blot and q-PCR results showed that NaHCO₃-TNLs could inhibit the time-dependent increase of NFATC-

1, c-Fos, and CTSK expression in osteoclasts. Laser confocal microscopy results showed that NaHCO₃-TNLs could inhibit the formation of actin loops in osteoclasts, suggesting that NaHCO₃-TNLs had a significant inhibitory effect on osteoclast bone resorption function (c-e in Figure 3).

3、将NaHCO₃-TNLs加入破骨细胞诱导体系并与单纯破骨细胞诱导体系对照，Western-blot及q-PCR结果显示NaHCO₃-TNLs可抑制破骨细胞NFATc-1、c-Fos、CTSK表达随时间的递增效应，激光共聚焦显微镜结果显示NaHCO₃-TNLs可抑制破骨细胞actin环形成，提示NaHCO₃-TNLs对破骨细胞骨吸收功能具有显著的抑制作用(图3中c-e)。

[0090]

4. Daily RANK expression was assessed in the osteoclast induction system. Western blot and laser confocal microscopy results showed that osteoclast RNAK expression increased with osteoclast maturation (f, g in Figure 3).

4、对破骨细胞诱导体系进行每日的RANK表达评估，Western-blot及激光共聚焦显微镜结果提示破骨细胞RNAK表达量随破骨细胞成熟递增(图3中f，g)。

[0091]

5. NaHCO₃-TNLs were added to the osteoclast induction system and compared with the osteoclast induction system alone. Exosomes were extracted and evaluated. Western blot and exosome flow cytometry results showed that the RANK content of extracellular vesicles in the NaHCO₃-TNLs group was significantly increased. The results were analyzed with those in steps 1-4, suggesting that NaHCO₃-TNLs can promote osteoclast secretion of RANK-rich extracellular vesicles (h, i in Figure 3).

5、将NaHCO₃-TNLs加入破骨细胞诱导体系并与单纯破骨细胞诱导体系对照，提取外泌体进行评估，Western-blot及外泌体流式计数结果显示NaHCO₃-TNLs组细胞外囊泡RANK含量显著增多，与步骤1-4结果进行分析，提示NaHCO₃-TNLs可促进破骨细胞分泌富含RANK的细胞外囊泡(图3中h，i)。

[0092]

6. The extracellular vesicles extracted in step 5 were added to the osteoclast induction system. The results showed that the extracellular vesicles containing NaHCO₃-TNLs significantly inhibited TRAP staining of osteoclasts, suggesting that extracellular vesicles rich in RANK can further inhibit osteoclasts (j, k in Figure 3).

6、将按步骤5中提取的细胞外囊泡分别加入破骨细胞诱导体系，结果显示计入NaHCO₃-TNLs细胞外囊泡组TRAP染色破骨细胞显著抑制，提示富含RANK的细胞外囊泡可进一步抑制破骨细胞(图3中j，k)。

[0093]

Example 10: The therapeutic effect of NaHCO₃-TNLs on osteoporosis in OVX mice

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实施例10、NaHCO₃-TNLs对OVX小鼠骨质疏松的治疗作用

[0094]

1. Animal disease model construction and grouping

1、动物疾病模型构建及分组

[0095]

Grouping method: 11-week-old C57BL/6 female mice were divided into four groups: a) sham surgery and tail vein injection of saline (Sham); b) ovariectomy and tail vein injection of saline (OVX); c) ovariectomy and tail vein injection of NaCL-TNLs (OVX+NaCL-TNLs); d) ovariectomy and tail vein injection of NaHCO₃-TNLs (OVX+NaHCO₃-TNLs).

分组方式：将11周C57BL/6母鼠分为四组：a)施行假手术并尾静脉注射生理盐水(Sham)；b)施行卵巢切除并尾静脉注射生理盐水(OVX)；c)施行卵巢切除并尾静脉注射NaCL-TNLs(OVX+NaCL-TNLs)；d)施行卵巢切除并尾静脉注射NaHCO₃-TNLs(OVX+NaHCO₃-TNLs)。

[0096]

Implementation method: Perform the corresponding surgery on each group of animals. One week later, start the corresponding drug by tail vein injection at a dose of 0.025 ml/g, once every 2 days for 2 weeks.

实施方式：对各组动物进行相应手术，1周后开始以0.025ml/g剂量进行尾静脉注射相应药物，每2天给药一次，持续2周。

Four weeks after the end of drug administration, the spine, femur, tibia and blood of mice in each group were taken for analysis. The analysis methods included: micro-CT, H&E staining, TRAP staining, and serum bone metabolism index ELISA (Figure 4a).

给药结束4周后取出各组小鼠脊柱、股骨、胫骨及血液进行分析，分析方式包括：micro-CT、H&E染色、TRAP染色、血清骨代谢指标ELISA(图4中a)。

[0097]

2. Comparison of micro-CT scans of the spine, femur, and tibia in each experimental group showed that the bone mass, number of trabeculae, and trabecular spacing in the OVX+NaHCO₃-TNLs group were significantly better than those in the OVX group (Figure 4b, c).

2、将各实验组脊柱、股骨、胫骨micro-CT进行比较，结果显示OVX+NaHCO₃-TNLs组骨量、骨小梁数量、骨小梁间隙指标均显著优于OVX组(图4中b，c)。

[0098]

3. H&E staining and TRAP staining of the spine, femur and tibia of each experimental group were compared. The results showed that the bone mass, osteoclast area and osteoclast number of the OVX+NaHCO₃-TNLs group were significantly better than those of the OVX group (d, e in Figure 4).

3、将各实验组脊柱、股骨、胫骨H&E染色、TRAP染色进行比较，结果显示OVX+NaHCO₃-TNLs组骨量、破骨细胞面积、破骨细胞数量指标均显著优于OVX组(图4中d，e)。

[0099]

4. The serum bone metabolism indicators of each experimental group were compared. The results showed that the osteoclast metabolism indicators of the OVX+NaHCO₃-TNLs group were significantly lower than those of the OVX group.

4、将各实验组血清骨代谢指标进行比较，结果显示OVX+NaHCO₃-TNLs组破骨细胞代谢指标显著低于OVX组。

Based on the experimental results in steps 2 and 3, it is suggested that NaHCO₃-TNLs can effectively treat bone loss and osteoclast metabolism in OVX mice, thereby treating osteoporosis (Figure 4f).

结合步骤2，3实验结果，提示NaHCO₃-TNLs可有效治疗OVX小鼠的骨质流失及破骨细胞代谢，从而治疗骨质疏松(图4中f)。

[0100]

The nanomaterials targeting the acidic blocking region of osteoclasts obtained in Examples 2-6 were evaluated for synthesis, properties, osteoclast inhibition, and therapeutic effects on OVX mice with osteoporosis. The results were similar to those of the NaHCO₃-TNLs material in Examples 7-10. This indicates that by adjusting the reagent concentration and treatment time determined after the above optimization, nanomaterials targeting the acidic blocking region of osteoclasts with similar effects can be prepared.

对实施例2-6所得的针对破骨细胞酸性封闭区的纳米材料分别进行合成评估、特性评估、破骨细胞抑制作用评估、对OVX小鼠骨质疏松小鼠的治疗作用评估，结果与实施例7-10中NaHCO₃-TNLs材料结果相似，这表明可通过上述优化后确定的试剂浓度和处理时间的调整，实现效果类似的针对破骨细胞酸性封闭区的纳米材料的制备。

[0101]

As can be seen from the above embodiments, the nanomaterials targeting the acidic blocking region of osteoclasts provided by the present invention target bone tissue and generate gas in the acidic blocking region of osteoclasts in a pH response. While neutralizing the acidification, it destroys the blocking region of osteoclasts, inhibits osteoclast maturation, and promotes osteoclasts to secrete extracellular vesicles rich in RANK, which form ineffective binding with serum RANKL, thereby achieving the effect of long-term treatment of abnormal activation of osteoclasts.

由上述实施例可知，本发明提供的针对破骨细胞酸性封闭区的纳米材料通过靶向骨组织，对破骨细胞酸性封闭区域进行产气的pH响应，中和酸化的同时破坏破骨细胞封闭区域，抑制破骨细胞成熟，并促使破骨细胞分泌富含RANK的细胞外囊泡，与血清RANKL形成无效结合，从而达到远期治疗破骨细胞异常活化的效果。

[0102]

The above description is only a preferred embodiment of the present invention. It should be noted that although the present invention has been described in detail through the above preferred embodiments, those skilled in the art should understand that several improvements and modifications can be made without departing from the principle of the present invention. These improvements and modifications should also be considered as the scope of protection of the present invention and do not depart from the scope defined by the claims of the present invention.

以上所述仅是本发明的优选实施方式，应当指出，尽管通过上述优选实施例已经对本发明进行了详细的描述，但本技术领域的技术人员来应当理解，在不脱离本发明原理的前提下，还可以做出若干改进和润饰，这些改进和润饰也应视为本发明的保护范围，不偏离本发明权利要求书所限定的范围。