Notice

DESCRIPTION WO2025146088A1

A nanomaterial for preventing bone metastasis of tumors, its preparation method and application

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一种用于预防肿瘤骨转移的纳米材料及其制备方法和应用

[0001]

Cross-reference to related applications

相关申请的交叉引用

[0002]

This application claims the rights of Chinese application No. 202410007256.9 filed on January 3, 2024 and Chinese application No. 202410194342.5 filed on February 21, 2024.

本申请要求2024年01月3日提交的中国申请号202410007256.9和2024年02月21日提交的中国申请号202410194342.5的权益。

Applications 202410007256.9 and 202410194342.5 are hereby incorporated herein by reference in their entirety.

所述申请号202410007256.9和202410194342.5据此全文以引用方式并入本文。

Technical Field

技术领域

[0003]

This invention relates to the field of tumor bone metastasis treatment technology, specifically to a nanomaterial for preventing tumor bone metastasis, its preparation method, and its application.

本发明涉及肿瘤骨转移治疗技术领域，具体涉及一种用于预防肿瘤骨转移的纳米材料及其制备方法和应用。

Background Technology

背景技术

[0004]

Among the many organs from which tumors metastasize, bone is the most common preferred site of metastasis for many malignant tumors, especially breast cancer and prostate cancer. Meanwhile, the reprogramming of tumor cells in the bone microenvironment promotes secondary metastasis to other organs. Therefore, preventing bone metastasis is an important part of preventing systemic metastasis of tumors.

在肿瘤转移的许多器官中，骨骼是许多恶性肿瘤最常见的优先转移部位，尤其是乳腺癌和前列腺癌。 同时，骨骼微环境中肿瘤细胞的重编程促进了其他器官的继发转移。 因此，预防骨转移是预防肿瘤全身转移的重要环节。

[0005]

Unlike primary tumors, which are usually treated with local surgery or radiation therapy, metastatic tumors are a sporadic disease that affects the whole body.

与通常通过局部手术或放疗治疗的原发性肿瘤不同，肿瘤转移是一种全身散发性疾病。

Current treatment options mainly include chemotherapy, targeted therapy, and immunotherapy, such as doxorubicin, bisphosphonates, and PD-1 monoclonal antibodies. However, tumor cells are in a non-proliferative state in the long initial stage and evade immune surveillance by forming a tumor microenvironment. Due to the presence of isoproteins, signal bypasses, and tumor heterogeneity, treatments based on single molecules or signaling pathways are prone to drug resistance.

目前的治疗方法主要包括化疗、靶向治疗和免疫疗法，如多柔比星、双膦酸盐和PD-1单抗等。 但是，肿瘤细胞在长期初始阶段处于非增殖状态，并通过肿瘤微环境的形成来逃避免疫监视，由于同工蛋白、信号旁路和肿瘤异质性的存在，基于单分子或信号通路的治疗容易产生耐药性。

[0006]

As early tumor microenvironment construction continues, inducing the conserved behavior of tumor stromal cells may itself be a novel, unnoticed target for inhibiting metastatic lesions in the very early stages.

随着早期肿瘤微环境构建行为的持续，诱导肿瘤基质细胞的保守行为本身可能是一个未被注意到的新靶点，用于在极早期抑制转移灶。

Due to the unique "hard matrix" interface of the bone matrix, "softening" the bone matrix is a prerequisite for initial transfer. Therefore, osteoclasts, the only cells with acid secretion and osteolysis functions, are key to the initial microenvironment of tumor bone metastasis. Osteoclast activation can clearly indicate the activation of bone metastases, therefore, triggering physical killing of bone metastases through osteoclasts is a promising method to avoid drug resistance.

由于骨基质的特殊“硬基”界面，“软化”骨基质是初始转移的先决条件。 因此，唯一具有酸分泌和骨溶解功能的细胞，破骨细胞，是肿瘤骨转移初始微环境中的关键。 破骨细胞的激活可以明确指示骨转移灶的活化，因此通过破骨细胞触发靶向骨转移灶的物理杀伤是一种具有潜力的避免耐药性的方法。

Summary of the Invention

发明内容

[0007]

This invention discovers the spatiotemporal coupling interaction between tumor cells and osteoclasts, and based on the spatiotemporal characteristics of tumor-osteoclast coupling in the initial metastasis process, proposes a behavioral targeting strategy for initial tumor-bone metastasis. This strategy induces physical killing of calcium phosphate crystals through an in situ uncoupling-killing liposome system triggered by osteoclasts. This approach can not only accurately prevent tumor metastasis at the source, but also avoid drug resistance and biochemical resistance.

本发明发现了肿瘤细胞和破骨细胞之间的时空偶联相互作用，并基于肿瘤-破骨细胞偶联在初始转移过程中的时空特征，提出了一种肿瘤-骨初始转移的行为靶向策略，通过破骨细胞触发的原位解偶-杀伤脂质体系统诱导钙磷晶体物理杀伤，不仅可以准确地基于源头预防肿瘤转移，并且可以避免药物耐药和生化耐药。

[0008]

In view of this, the present invention provides a physical killing nanomaterial for targeting tumor-osteoclast coupling, its preparation method and application.

有鉴于此，本发明提供了一种靶向肿瘤-破骨偶联体的物理杀伤纳米材料及其制备方法和应用。

To achieve the above objectives, the present invention provides the following technical solution:

为实现上述目的，本发明提供以下技术方案：

[0009]

In a first aspect, the present invention provides a nanomaterial, wherein the nanomaterial is a nanovesicle modified with bone-targeting groups and loaded with carbonate and phosphate compounds.

在第一方面，本发明提供了一种纳米材料，所述纳米材料为骨靶向基团修饰的包载碳酸盐类化合物和磷酸盐类化合物的纳米囊泡。

[0010]

In one embodiment, the nanovesicles comprise nanoliposomes.

在一个实施方案中，所述纳米囊泡包括纳米脂质体。

[0011]

In one embodiment, the nanomaterial targets the tumor-osteoclast conjugate.

在一个实施方案中，所述纳米材料靶向肿瘤-破骨偶联体。

[0012]

In one embodiment, the nanomaterial is capable of physically killing tumors.

在一个实施方案中，所述纳米材料能够物理杀伤肿瘤。

Preferably, the physical killing is achieved through calcium phosphate crystals formed by phosphate compounds and calcium ions.

优选的，所述物理杀伤通过磷酸盐类化合物与钙离子形成的钙磷晶体实现。

[0013]

In one embodiment, the nanomaterial has an average particle size of 50-1000 nanometers, more preferably 50-200 nanometers, and most preferably less than 100 nanometers.

在一个实施方案中，所述纳米材料平均粒径为50-1000纳米，更优选的，平均粒径为50-200纳米，最优选的，平均粒径为100纳米以下。

[0014]

In one embodiment, the bone-targeting group includes at least one of tetracycline, phosphonate, calcein, and aspartic acid polypeptide sequences, wherein the phosphonate is, for example, alendronate sodium; preferably, the bone-targeting group is tetracycline.

在一个实施方案中，骨靶向基团包括四环素、膦酸盐、钙黄绿素、天冬氨酸类多肽序列中的至少一种，所述膦酸盐例如为阿仑膦酸钠；优选的，骨靶向基团采用四环素。

[0015]

In this invention, the carbonate compound is capable of reacting with acid to produce gas; preferably, it is selected from carbonate or bicarbonate, more preferably, it is selected from at least one of sodium bicarbonate, potassium bicarbonate, ammonium bicarbonate, sodium carbonate, potassium carbonate, and ammonium carbonate, and most preferably, sodium bicarbonate is used.

本发明中，所述碳酸盐类化合物能够与酸反应产生气体；优选的，其选自碳酸根盐或碳酸氢根盐，更优选的，其选自碳酸氢钠、碳酸氢钾、碳酸氢铵、碳酸钠、碳酸钾、碳酸铵中的至少一种，最优选的，采用碳酸氢钠。

[0016]

In this invention, the phosphate compound is capable of forming calcium phosphate crystals with calcium ions; preferably, it is selected from hydrogen phosphate salts or dihydrogen phosphate salts, more preferably, it is selected from at least one of disodium hydrogen phosphate, dipotassium hydrogen phosphate, diammonium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, and ammonium dihydrogen phosphate, and most preferably, disodium hydrogen phosphate is used.

本发明中，所述磷酸盐类化合物能够与钙离子形成钙磷晶体；优选的，其选自磷酸氢根盐或磷酸二氢根盐，更优选的，其选自磷酸氢二钠、磷酸氢二钾、磷酸氢二铵、磷酸二氢钠、磷酸二氢钾、磷酸二氢铵中的至少一种，最优选的，采用磷酸氢二钠。

[0017]

In one embodiment, the molar ratio of carbonate compound to phosphate compound is 1-4:4-1, preferably 1-3:3-1 or 1-2:2-1, and more preferably 1:1.

在一个实施方案中，碳酸盐类化合物和磷酸盐类化合物的摩尔比为1-4:4-1，优选为1-3:3-1或者1-2:2-1，更优选为1:1。

[0018]

In one embodiment, the liposome membrane of the nanoliposome contains bone-targeting phospholipids, other phospholipids besides bone-targeting phospholipids, and cholesterol.

在一个实施方案中，所述纳米脂质体的脂质体膜包含骨靶向磷脂、骨靶向磷脂外的其他磷脂、胆固醇。

[0019]

Preferably, the bone-targeting phospholipid is obtained by covalently binding functionalized polyethylene glycol phospholipid with bone-targeting group molecules.

优选的，骨靶向磷脂由功能化聚乙二醇化磷脂与骨靶向基团分子进行共价结合得到。

More preferably, the bone-targeting phospholipid is a bone-targeting group-polyethylene glycol-modified phospholipid.

更优选的，所述骨靶向磷脂为骨靶向基团-聚乙二醇化磷脂。

[0020]

Preferably, the functionalized polyethylene glycol phospholipid refers to polyethylene glycol phospholipid modified with reactive functional groups.

优选的，所述功能化聚乙二醇化磷脂是指修饰有反应性官能团的聚乙二醇化磷脂。

The reactive functional group can be hydroxyl, carboxyl, amino, maleimide, etc.

所述反应性官能团可以是羟基、羧基、氨基、马来酰亚胺基等。

The molecular weight of polyethylene glycol can be 500-50000, preferably 800-6000.

聚乙二醇的分子量可以是500-50000，优选为800-6000。

[0021]

Preferably, the functionalized polyethylene glycol phospholipid is functionalized DSPE-PEG, and more preferably, the functionalized polyethylene glycol phospholipid is DSPE-PEG-NHS.

优选的，所述功能化聚乙二醇化磷脂采用功能化DSPE-PEG，更优选的，功能化聚乙二醇化磷脂采用DSPE-PEG-NHS。

[0022]

Preferably, the functionalized polyethylene glycol phospholipids are DSPE-PEG2000-NHS and DSPE-PEG5000-NHS.

优选的，功能化聚乙二醇化磷脂采用DSPE-PEG2000-NHS、DSPE-PEG5000-NHS。

[0023]

Therefore, preferably, the bone-targeting phospholipid is DSPE-PEG2000-TC (DSPE-PEG2000-tetracycline) or DSPE-PEG5000-TC (DSPE-PEG5000-tetracycline).

因此，优选的，所述骨靶向磷脂为DSPE-PEG2000-TC(DSPE-PEG2000-四环素)、DSPE-PEG5000-TC(DSPE-PEG5000-四环素)。

[0024]

Preferably, the phospholipid outside the bone-targeting phospholipid is at least one of natural phospholipids, semi-synthetic phospholipids, and fully synthetic phospholipids; preferably, the phospholipid outside the bone-targeting phospholipid is at least one of lecithin, hydrogenated lecithin, and cephalin; more preferably, the phospholipid is lecithin.

优选的，所述骨靶向磷脂外的磷脂为天然磷脂、半合成磷脂和全合成磷脂中的至少一种；优选的，所述骨靶向磷脂外的磷脂为卵磷脂、氢化卵磷脂和脑磷脂中的至少一种，更优选的，所述磷脂为卵磷脂。

[0025]

Therefore, preferably, the liposome membrane comprises bone-targeting phospholipids, lecithin, and cholesterol.

因此，优选的，所述脂质体膜包含骨靶向磷脂、卵磷脂和胆固醇。

[0026]

Preferably, the mass ratio of bone-targeting phospholipids, phospholipids other than bone-targeting phospholipids, and cholesterol is 5-40:100:10-25, more preferably 15-25:100:12-20, and even more preferably 20:100:16.

优选的，骨靶向磷脂、骨靶向磷脂外的磷脂、胆固醇的质量比例为5-40:100:10-25，优选为15-25:100:12-20，更优选为20:100:16。

[0027]

Preferably, the bone-targeting phospholipids account for 10-40% of the mass of the liposome membrane, and more preferably 14-28%.

优选的，骨靶向磷脂的质量占比为脂质体膜的10-40%，优选为14-28%。

[0028]

In a second aspect, the present invention also provides a method for preparing the nanomaterial, comprising the following steps:

在第二方面，本发明还提供一种所述纳米材料的制备方法，包括以下步骤：

[0029]

Bone-targeting phospholipids, phospholipids outside bone-targeting phospholipids, and cholesterol are placed in an organic solvent. After removing the solvent, a solution of carbonate and phosphate compounds is added and ultrasonically hydrated. The mixture is then placed in a dialysis bag for dialysis and extruded and granulated to obtain bone-targeting group-modified nanoliposomes loaded with carbonate and phosphate compounds.

将骨靶向磷脂、骨靶向磷脂外的磷脂、胆固醇置于有机溶剂中，去除溶剂后，加入碳酸盐类化合物和磷酸盐类化合物的溶液后超声水化，置于透析袋中进行透析，并进行挤出整粒，获得骨靶向基团修饰的包载碳酸盐类化合物和磷酸盐类化合物的纳米脂质体。

[0030]

In one embodiment, the organic solvent may be at least one of alcohol solvents, ester solvents, halogenated hydrocarbons, nitrile solvents, and ether solvents; preferably, the organic solvent may be at least one of methanol, ethanol, methyl acetate, ethyl acetate, dichloromethane, chloroform, acetonitrile, and diethyl ether.

在一个实施方案中，所述有机溶剂可以是醇类溶剂、酯类溶剂、卤代烃、腈类溶剂、醚类溶剂中的至少一种；优选的，所述有机溶剂可以是甲醇、乙醇、乙酸甲酯、乙酸乙酯、二氯甲烷、氯仿、乙腈、乙醚中的至少一种。

[0031]

In one embodiment, the molar ratio of carbonate compound to phosphate compound is 1-4:4-1, preferably 1-3:3-1 or 1-2:2-1, and more preferably 1:1.

在一个实施方案中，碳酸盐类化合物和磷酸盐类化合物的摩尔比为1-4:4-1，优选为1-3:3-1或者1-2:2-1，更优选为1:1。

[0032]

In one embodiment, the concentration of the carbonate compound in the solution of the phosphate compound is 30-120 mM and the concentration of the phosphate compound is 20-80 mM; more preferably, the concentration of the carbonate compound is 50-70 mM and the concentration of the phosphate compound is 50-70 mM.

在一个实施方案中，碳酸盐类化合物和磷酸盐类化合物的溶液中，碳酸盐类化合物的浓度为30-120mM，磷酸盐类化合物的浓度为20-80mM；更优选的，碳酸盐类化合物的浓度为50-70mM，磷酸盐类化合物的浓度为50-70mM。

[0033]

In one embodiment, the bone-targeting phospholipid can be prepared by covalently binding functionalized polyethylene glycol phospholipids with bone-targeting group molecules and purifying them to obtain bone-targeting phospholipids.

在一个实施方案中，所述骨靶向磷脂可以通过以下方法制备：将功能化聚乙二醇化磷脂与骨靶向基团分子进行共价结合，纯化获得骨靶向磷脂。

[0034]

Preferably, the reaction conditions for the covalent bonding of functionalized polyethylene glycol phospholipids and bone-targeting group molecules are as follows: the functionalized polyethylene glycol phospholipids and bone-targeting group molecules are placed in an organic solvent, the pH is adjusted to 7.5-9.0 using an organic base, and the reaction is carried out at room temperature with stirring for 6-48 hours.

优选的，功能化聚乙二醇化磷脂与骨靶向基团分子进行共价结合的反应条件为：将功能化聚乙二醇化磷脂和骨靶向基团分子置于有机溶剂中，使用有机碱调节pH至7.5-9.0，室温搅拌反应6-48小时。

The organic solvent may be at least one of alcohol solvents, ester solvents, halogenated hydrocarbons, nitrile solvents, and ether solvents; preferably, the organic solvent may be at least one of methanol, ethanol, methyl acetate, ethyl acetate, dichloromethane, chloroform, acetonitrile, and diethyl ether.

其中，所述有机溶剂可以是醇类溶剂、酯类溶剂、卤代烃、腈类溶剂、醚类溶剂中的至少一种；优选的，所述有机溶剂可以是甲醇、乙醇、乙酸甲酯、乙酸乙酯、二氯甲烷、氯仿、乙腈、乙醚中的至少一种。

[0035]

Preferably, after stirring the reaction, the solvent is replaced with water, and then the bone-targeting phospholipids are purified.

优选的，搅拌反应后，将溶剂置换为水，然后纯化得到骨靶向磷脂。

[0036]

Preferably, the molar ratio of functionalized polyethylene glycol phospholipids to bone-targeting group molecules is 1:1-4, more preferably 1:1-3, and even more preferably 1:1-2.5.

优选的，功能化聚乙二醇化磷脂与骨靶向基团分子的摩尔比为1:1-4，优选为1:1-3，更优选为1:1-2.5。

[0037]

Preferably, adjust the pH to 8.0-8.4 and stir for 18-30 hours.

优选的，调节pH至8.0-8.4；搅拌18-30小时。

[0038]

Preferably, the purification is selected from at least one of dialysis and chromatography.

优选的，所述纯化选自透析、色谱法中的至少一种。

[0039]

Preferably, the average particle size of the nanoliposomes obtained by extrusion granulation is 50-1000 nm, more preferably, the average particle size is 50-200 nm, and most preferably, the average particle size is less than 100 nm.

优选的，通过挤出整粒，得到的纳米脂质体的平均粒径为50-1000纳米，更优选的，平均粒径为50-200纳米，最优选的，平均粒径为100纳米以下。

[0040]

In a third aspect, the present invention also provides an application of the nanomaterial.

在第三方面，本发明还提供一种所述纳米材料的应用。

The present invention describes the intravenous injection of the nanomaterial, which was found to accumulate in bone tissue and be released when tumor activation induces the formation of tumor-osteoclasts, forming calcium phosphate crystals for the physical killing of tumor-osteoclasts in bone metastases.

本发明将所述纳米材料通过静脉注射，发现其可富集在骨组织，并在肿瘤活化诱导肿瘤-破骨偶联体形成时释放，形成钙磷结晶，用于骨转移灶中肿瘤-破骨偶联体的物理杀伤。

[0041]

Therefore, the present invention provides an application of the nanomaterial in the preparation of a drug for killing tumor-osteoclasts in bone metastases.

因此，本发明提供了一种所述纳米材料在制备药物中的应用，所述药物用于在骨转移灶中对肿瘤-破骨偶联体进行杀伤。

Furthermore, the present invention also provides a method for killing tumor-osteoclast couplers in bone metastases, comprising providing the nanomaterial to individuals in need.

此外，本发明还提供了一种在骨转移灶中对肿瘤-破骨偶联体进行杀伤的方法，其包括向有需要的个体提供所述纳米材料。

[0042]

In one implementation, the killing effect is physical.

在一个实施方案中，所述杀伤为物理杀伤。

More preferably, the killing effect is physical damage caused by calcium phosphate crystals.

更优选的，所述杀伤为钙磷晶体导致的物理杀伤。

[0043]

In one embodiment, the bone metastases originate from at least one of breast tumors, prostate tumors, bone tumors (including osteosarcoma), lung tumors, liver tumors, kidney tumors, gastrointestinal tumors (including gastric and intestinal tumors), and pancreatic tumors.

在一个实施方案中，所述骨转移灶来源包括乳腺肿瘤、前列腺肿瘤、骨肿瘤(包括骨肉馏)、肺肿瘤、肝脏肿瘤、肾脏肿瘤、胃肠肿瘤(包括胃肿馏和肠肿瘤)、胰腺肿瘤中的至少一种。

[0044]

In addition, the present invention provides the application of the nanomaterial in the preparation of a drug for the prevention of tumor bone metastasis.

另外，本发明还提供了一种所述纳米材料在制备药物中的应用，所述药物用于预防肿瘤骨转移。

Furthermore, the present invention also provides a method for preventing bone metastasis of tumors, which includes providing the nanomaterial to individuals in need.

此外，本发明还提供了一种预防肿瘤骨转移的方法，其包括向有需要的个体提供所述纳米材料。

[0045]

In one embodiment, the tumor includes at least one of breast tumors, prostate tumors, bone tumors (including osteosarcoma), lung tumors, liver tumors, kidney tumors, gastrointestinal tumors (including gastric and intestinal tumors), and pancreatic tumors.

在一个实施方案中，所述肿瘤包括乳腺肿瘤、前列腺肿瘤、骨肿瘤(包括骨肉馏)、肺肿瘤、肝脏肿瘤、肾脏肿瘤、胃肠肿瘤(包括胃肿馏和肠肿瘤)、胰腺肿瘤中的至少一种。

[0046]

In this invention, when tumor cells are activated, the nanomaterial triggers the production of carbon dioxide gas from carbonate compounds through the acid secretion function of tumor-associated osteoclasts in the tumor-osteoclast coupler, which creates pores on the liposome membrane and promotes the release of phosphate compounds. These phosphate compounds then form calcium phosphate crystals with calcium ions to kill nearby tumor cells, achieving a specific inhibitory effect on very early tumor metastases.

本发明中，所述纳米材料在肿瘤细胞活化时，通过近距离接触的肿瘤-破骨偶联体中的肿瘤相关破骨细胞的泌酸功能触发碳酸盐类化合物产生二氧化碳气体，使得脂质体膜上出现了孔隙，促进了磷酸盐

类化合物释放，其与钙离子形成钙磷结晶以杀伤近距离的肿瘤细胞，达到特异性的极早期肿瘤转移灶抑制效果。

This physical killing method, which targets tumor cell behavior, provides a new approach for targeting early bone metastases and avoiding tumor pharmacological and biochemical tolerance.

这种肿瘤细胞行为靶向的物理杀伤方式为靶向早期骨转移灶，并避免肿瘤药理耐受和生化耐受提供了新思路。

[0047]

This invention can also use other bone-targeting groups and other liposome composition ratios to achieve similar technical effects.

本发明还可以使用其他骨靶向基团、其他脂质体成分配比，均可获得相似的技术效果。

[0048]

Therefore, in a fourth aspect, the present invention also provides a method for preventing tumor bone metastasis, the method targeting tumor cells to induce osteoclast behavior.

因此，在第四方面，本发明的还提供了一种预防肿瘤骨转移的方法，所述方法靶向肿瘤细胞诱导破骨细胞的行为。

[0049]

In one implementation, the method uncouples and kills tumor-osteoclast conjugates.

在一个实施方案中，所述方法对肿瘤-破骨偶联体进行解偶-杀伤。

[0050]

In one implementation, the method is triggered by tumor-associated osteoclasts.

在一个实施方案中，所述方法由肿瘤相关破骨细胞触发。

[0051]

In one implementation, the method produces chemical or physical killing of tumor cells.

在一个实施方案中，所述方法对肿瘤细胞产生化学或物理杀伤。

[0052]

In one embodiment, the method is performed using a bone-targeting drug.

在一个实施方案中，所述方法通过骨靶向药物进行。

[0053]

Preferably, the bone-targeting drug is triggered by tumor-associated osteoclasts.

优选的，所述骨靶向药物由肿瘤相关破骨细胞触发。

[0054]

Preferably, the bone-targeting drug has the ability to chemically or physically kill tumor cells.

优选的，所述骨靶向药物具有对肿瘤细胞进行化学或物理杀伤的能力。

[0055]

Preferably, the bone-targeting drug is the nanomaterial described in this invention.

优选的，所述骨靶向药物为本发明所述的纳米材料。

[0056]

In another aspect, this invention investigated the spatiotemporal characteristics of tumor-induced osteoclast maturation. Since classical osteoclast development involves continuous stimulation by M-CSF and RANKL, in order to explore the key periods of tumor-induced osteoclast formation, we divided the osteoclast growth stage into three key periods: osteoclast precursor (OCP), RANKL-stimulated osteoclast precursor for 1 day (R1-OCP), and RANKL-stimulated osteoclast precursor for 5 days (R5-OCP).

在另一个方面，本发明研究了肿瘤诱导破骨细胞成熟的时空特征，由于经典破骨细胞的发育经历M-CSF和RANKL的连续刺激，为了探究肿瘤诱导破骨细胞形成的关键时期，我们将破骨细胞生长阶段分为三个关键时期：破骨细胞前体(OCP)、RANKL 1天刺激破骨细胞前体(R1-OCP)和RANKL 5天刺激破骨细胞前体(R5-OCP)。

By co-culturing with tumor cells, it was found that R1-OCP can be induced into osteoclasts. By culturing with tumor cells in a Transwell chamber, it was found that R1-OCP can only be induced into osteoclasts under spatial conditions of contact with tumor cells.

通过与肿瘤细胞共同培养，发现R1-OCP能够被诱导成破骨细胞，通过与肿瘤细胞经Transwell小室的分隔培养，发现R1-OCP只有在与肿瘤细胞接触的空间条件下才能被诱导为破骨细胞。

[0057]

Therefore, in a fifth aspect, the present invention also provides a method for culturing tumor-associated osteoclasts, the method comprising co-culturing RANKL-prestimulated osteoclast precursors with tumor cells to obtain tumor-associated osteoclasts.

因此，在第五方面，本发明还提供了一种肿瘤相关破骨细胞的培养方法，所述方法包括将RANKL预刺激的破骨细胞前体与肿瘤细胞共同接触培养，从而得到肿瘤相关破骨细胞。

[0058]

Preferably, the osteoclast precursor pre-stimulated by RANKL is an osteoclast precursor stimulated by RANKL for 1-3 days, preferably an osteoclast precursor stimulated by RANKL for 1 day.

优选的，所述RANKL预刺激的破骨细胞前体为RANKL刺激1-3天的破骨细胞前体，优选RANKL刺激1天的破骨细胞前体。

[0059]

Preferably, the tumor includes at least one of the following: breast tumor, prostate tumor, bone tumor (including osteosarcoma), lung tumor, liver tumor, kidney tumor, gastrointestinal tumor (including gastric and intestinal tumors), and pancreatic tumor.

优选的，所述肿瘤包括乳腺肿瘤、前列腺肿瘤、骨肿瘤(包括骨肉馏)、肺肿瘤、肝脏肿瘤、肾脏肿瘤、胃肠肿瘤(包括胃肿馏和肠肿瘤)、胰腺肿瘤中的至少一种。

[0060]

In this invention, the calcium phosphate crystals refer to crystals formed by the reaction of phosphate compounds with calcium ions, and the main component is calcium carbonate.

本发明中，所述钙磷晶体是指磷酸盐类化合物与钙离子反应形成的晶体，主要成分为碳酸钙。

[0061]

In addition, the present invention also provides the following solutions:

另外，本发明还提供了以下方案：

[0062]

This invention provides a physical killing nanomaterial that targets tumor-osteoclast coupling agents. The nanomaterial is a nanoliposome modified with bone-targeting groups and loaded with sodium bicarbonate and sodium hydrogen phosphate.

本发明提供了一种靶向肿瘤-破骨偶联体的物理杀伤纳米材料，所述纳米材料为骨靶向基团修饰的包载碳酸氢钠和磷酸氢钠的纳米脂质体。

[0063]

In one embodiment, the nanoliposomes have a particle size of 50-1000 nanometers, and preferably, the nanoliposomes have a particle size of 100 nanometers.

在一个实施方案中，所述纳米脂质体粒径为50-1000纳米，作为优选，所述的纳米脂质体粒径为采用100纳米。

[0064]

In one embodiment, the bone-targeting group includes tetracycline, phosphonate, or calcein.

在一个实施方案中，所述的骨靶向基团包括四环素、膦酸盐或钙黄绿素。

[0065]

This invention provides a method for preparing a physical killing nanomaterial of tumor-osteoclast coupling, the method comprising the following steps:

本发明提供了一种肿瘤-破骨偶联体的物理杀伤纳米材料的制备方法，该方法包括以下步骤：

[0066]

Step 1, Preparation of bone-targeting phospholipids: Functionalized polyethylene glycol phospholipids are covalently bonded to bone-targeting group molecules, and bone-targeting phospholipids are obtained by dialysis and chromatography.

步骤1、骨靶向磷脂的制备：将功能化聚乙二醇化磷脂与骨靶向基团分子进行共价结合，通过透析、色谱法获得骨靶向磷脂；

[0067]

Step 2: Preparation of bone-targeting group-modified nanoliposomes loaded with sodium bicarbonate and sodium hydrogen phosphate: bone-targeting phospholipids, lecithin, and cholesterol were dissolved in chloroform and evaporated by suspension distillation. After adding a mixed solution of sodium bicarbonate and sodium hydrogen phosphate, the mixture was ultrasonically hydrated and extruded to obtain bone-targeting group-modified nanoliposomes loaded with sodium bicarbonate and sodium hydrogen phosphate.

步骤2、骨靶向基团修饰的包载碳酸氢钠和磷酸氢钠的纳米脂质体的制备：将骨靶向磷脂、卵磷脂、胆固醇溶于氯仿，并采用悬蒸法旋干，加入碳酸氢钠和磷酸氢钠混合溶液后超声水化，并进行挤出整粒，获得骨靶向基团修饰的包载碳酸氢钠和磷酸氢钠的纳米脂质体。

[0068]

In one embodiment, the functionalized polyethylene glycol phospholipid is a functionalized DSPE-PEG.

在一个实施方案中，所述的功能化聚乙二醇化磷脂采用功能化DSPE-PEG。

[0069]

In one embodiment, the functionalized polyethylene glycol phospholipid is prepared by reacting functionalized polyethylene glycol phospholipid with tetracycline using DSPE-PEG2000-NHS. The reaction conditions are as follows: the pH is adjusted to 8.0-8.4 in chloroform using triethylamine, and the reaction is carried out at room temperature with stirring for 24 hours.

在一个实施方案中，所述的功能化聚乙二醇化磷脂为功能化聚乙二醇化磷脂采用DSPE-PEG2000-NHS与四环素反应，反应条件为在氯仿中使用三乙胺调节pH至8.0-8.4，室温搅拌反应24小时。

[0070]

In one embodiment, the bone-targeting phospholipid accounts for 10-30% of the mass of the liposome membrane, and the liposome membrane includes bone-targeting phospholipid, lecithin, and cholesterol; more preferably, the mass ratio of bone-targeting phospholipid, lecithin, and cholesterol is 5:20:4.

在一个实施方案中，所述的骨靶向磷脂的质量占比为脂质体膜的10-30%，所述的脂质体膜包括骨靶向磷脂、卵磷脂、胆固醇；更优选的，骨靶向磷脂、卵磷脂、胆固醇的质量比例为5:20:4。

[0071]

In one embodiment, the concentration of sodium bicarbonate is 30-120 mM and the concentration of sodium hydrogen phosphate is 20-80 mM. More preferably, 60 mM sodium bicarbonate and 60 mM sodium hydrogen phosphate are used.

在一个实施方案中，所述的碳酸氢钠的浓度为30-120mM，磷酸氢钠的浓度为20-80mM，更优选的，采用碳酸氢钠60mM，磷酸氢钠60mM。

[0072]

This invention also provides an application of a physical killing nanomaterial targeting tumor-osteoclast coupling in the physical killing of tumor-osteoclast coupling in bone metastases.

本发明还提供了一种靶向肿瘤-破骨偶联体的物理杀伤纳米材料在骨转移灶中肿瘤-破骨偶联体的物理杀伤上的应用。

[0073]

In one implementation, the sources of bone metastases include breast tumors, prostate tumors, bone tumors, lung tumors, liver tumors, kidney tumors, and gastrointestinal tumors.

在一个实施方案中，所述的骨转移灶来源包括乳腺肿瘤、前列腺肿瘤、骨肿瘤、肺肿瘤、肝脏肿瘤、肾脏肿瘤、胃肠肿瘤。

[0074]

The beneficial effects of this invention are:

本发明的有益效果：

[0075]

1) This invention uses tumor-associated osteoclasts to trigger "behavioral targeting" of tumor cells, avoiding the non-specific killing of traditional targeting methods.

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1)本发明通过肿瘤相关破骨细胞触发，对肿瘤细胞进行“行为靶向”，避免了传统靶向方式的非特异性杀伤。

[0076]

2) This invention releases a high concentration of hydrogen phosphate ions, which rapidly form calcium phosphate crystals with in-situ calcium ions, thereby physically killing tumor cells and avoiding biochemical drug resistance in tumors.

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2)本发明通过释放高浓度磷酸氢根，与原位钙离子迅速形成钙磷结晶，对肿瘤细胞进行物理杀伤，避免了肿瘤的生化耐药。

[0077]

3) Sodium bicarbonate and disodium hydrogen phosphate used in this invention are both physiological environmental substances and have high biocompatibility in drug metabolism.

---

3)本发明所采用碳酸氢钠、磷酸氢二钠均为生理环境物质，在药物代谢中具有高度生物安全性。

[0078]

4) The method for preventing bone metastasis of tumors provided by this invention targets tumor cells to induce osteoclast behavior, which has a definite effect in the early prevention of bone metastasis, and provides more ideas for the development of drugs for the prevention of bone metastasis of tumors, which has broad significance.

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4)本发明提供的预防肿瘤骨转移的方法靶向肿瘤细胞诱导破骨细胞的行为，在早期预防骨转移中具有确切效果，为肿瘤骨转移预防药物的开发提供更多思路，具有广泛的意义。

[0079]

5) The method for culturing osteoclasts in vitro in this invention can more effectively obtain tumor-induced osteoclasts, which provides a more powerful means for studying the spatiotemporal coupling between tumors and osteoclasts.

---

5)本发明体外培养破骨细胞的方法能够更为有效地获得肿瘤诱导的破骨细胞，这为研究肿瘤-破骨之间的时空偶联提供了更为有力的手段。

Attached Figure Description

附图说明

[0080]

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：

[0081]

Figure 1 shows the fluorescence-TRAP pattern of tumor-osteoclast coupling in in vivo bone metastases of GFP-labeled tumor cells.

图1为GFP标记的肿瘤细胞在体骨转移灶的肿瘤-破骨偶联体的荧光-TRAP图。

GFP fluorescence is used to locate tumor cells, while TRAP signaling is used to locate osteoclasts.

GFP荧光用于定位肿瘤细胞，TRAP信号用于定位破骨细胞。

[0082]

Figure 2 shows the TRAP staining pattern of tumor-osteoclast conjugates obtained from contact culture.

图2为接触培养得到的肿瘤-破骨偶联体的TRAP染色图。

The difference in osteoclast induction shown by OCP and R1-OCP after co-culture of 4T1 tumor cells suggests that RANKL prestimulation is an essential time-series condition for tumor-associated osteoclasts.

其中OCP和R1-OCP在4T1肿瘤细胞共培养后显示的破骨细胞诱导差异提示RANKL预刺激是肿瘤相关破骨细胞必须的时间序列条件。

[0083]

Figure 3 shows the TRAP staining of tumor-osteoclast couples obtained from Transwell culture.

图3为Transwell分隔培养得到的肿瘤-破骨偶联体的TRAP染色图。

The absence of osteoclasts after R1-OCP and 4T1 tumor cell culture suggests that close contact is a necessary spatial condition for tumor-associated osteoclasts.

其中R1-OCP与4T1肿瘤细胞分隔培养后未出现破骨细胞提示近距离接触是肿瘤相关破骨细胞必须的空间位置条件。

[0084]

Figure 4 shows a schematic diagram of regional spatial culture and a TRAP staining diagram.

图4为区域空间培养的示意图和TRAP染色图。

R1-OCP cells that only come into contact with tumor cells are induced to become osteoclasts, while R1-OCP cells that are outside the tumor cell range are not induced to become osteoclasts, thus verifying that close contact with tumor cells is a necessary spatial condition for tumor-associated osteoclasts.

其中仅与肿瘤细胞接触的R1-OCP被诱导为破骨细胞，而肿瘤细胞范围外的R1-OCP则未被诱导为破骨细胞，验证与肿瘤细胞近距离接触是肿瘤相关破骨细胞必要的空间位置条件。

[0085]

Figure 5 is a schematic diagram of the structure of the physical killing nanomaterial for targeting tumor-osteoclast coupling obtained in Example 2.1 of the present invention—tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes (HC&HP@TNL).

图5为根据本发明实施例2.1所得的靶向肿瘤-破骨偶联体的物理杀伤纳米材料——四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体(HC&HP@TNL)的结构示意图。

In the right-hand figure, the corresponding bar chart for each organ shows ICG@NL on the left and ICG@TNL on the right.

其中，右图中每个器官的对应柱状图中，左侧为ICG@NL，右侧为ICG@TNL。

[0086]

Figure 6 is a cryo-electron microscopy image of calcium phosphate crystal formation before and after acid response release of HC&HP@TNL obtained in Example 2.1 of the present invention (scale bar: 100 nm).

图6为根据本发明实施例2.1所得的HC&HP@TNL的酸响应释放前及释放后钙磷结晶产生的冷冻电镜图片(比例尺：100纳米)。

[0087]

Figure 7 is an elemental energy spectrum of calcium phosphorus crystals after acid response release of HC&HP@TNL obtained according to Example 2.1 of the present invention.

图7为根据本发明实施例2.1所得的HC&HP@TNL的酸响应释放后钙磷结晶的元素能谱图。

[0088]

Figure 8 shows an in vivo imaging diagram and quantitative statistics of the bone targeting capability of HC&HP@TNL obtained according to Example 2.1 of the present invention.

图8为根据本发明实施例2.1所得的HC&HP@TNL的骨靶向能力的活体成像图及定量统计。

[0089]

Figure 9 shows the quantitative fluorescence intensity of HC&HP@TNL released at different cell-bone interfaces according to Example 2.1 of the present invention.

图9为根据本发明实施例2.1所得的HC&HP@TNL的在不同细胞与骨片界面的释放的荧光强度定量。

[0090]

Figure 10 is a diagram showing the in vivo targeted release of HC&HP@TNL from bone metastases obtained according to Example 2.1 of the present invention.

图10为根据本发明实施例2.1所得的HC&HP@TNL的在体骨转移灶靶向释放图。

[0091]

Figure 11 is a TRAP staining diagram of the uncoupling-killing ability of HC&HP@TNL obtained according to Example 2.1 of the present invention for tumor cells of different origin and tumor-associated osteoclasts.

图11为根据本发明实施例2.1所得的HC&HP@TNL的对于不同来源肿瘤细胞与肿瘤相关破骨细胞偶联的解偶-杀伤能力的TRAP染色图。

[0092]

Figure 12 shows the tumor cell killing effect of HC&HP@TNL obtained according to Example 2.1 of the present invention, characterized by GFP expression signal.

图12为根据本发明实施例2.1所得的HC&HP@TNL的由GFP表达信号表征的肿瘤细胞杀伤效果。

[0093]

Figure 13 is an electron micrograph of HC&HP@TNL tumor cells obtained according to Example 2.1 of the present invention, showing physical killing by calcium phosphate crystal mineralization.

图13为根据本发明实施例2.1所得的HC&HP@TNL的肿瘤细胞被钙磷结晶矿化物理杀伤的电镜图。

[0094]

Figure 14 shows the inhibitory effect of HC&HP@TNL obtained according to Example 2.1 of the present invention on bone metastases of tumors in an in vitro model compared with the classic antitumor drug DOX.

图14为根据本发明实施例2.1所得的HC&HP@TNL的与经典抗肿瘤药DOX相比对离体模型肿瘤骨转移灶抑制效果。

In Figure d, each group of bars, from left to right, represents Control, DOX, Cl@TNL, HC@TNL, and HC&HP@TNL.

其中，d图中每组柱状图从左到右依次为Control、DOX、Cl@TNL、HC@TNL、HC&HP@TNL。

[0095]

Figure 15 shows the in vivo imaging statistics of the bone metastasis inhibition effect of HC&HP@TNL obtained according to Example 2.1 of the present invention.

图15为根据本发明实施例2.1所得的HC&HP@TNL的骨转移灶抑制作用的活体成像统计。

[0096]

Figure 16 shows the Micro-CT statistics of the inhibitory effect of HC&HP@TNL on metaphyseal bone loss obtained according to Example 2.1 of the present invention.

图16为根据本发明实施例2.1所得的HC&HP@TNL的干骺端骨质流失抑制作用的Micro-CT统计。

In each group of bars in the right figure, the left side represents Cl@TNL and the right side represents HC&HP@TNL.

其中，右图的每一组柱状图中，左侧为Cl@TNL，右侧为HC&HP@TNL。

Detailed Implementation

具体实施方式

[0097]

The following detailed description, in conjunction with embodiments, illustrates a physical killing nanomaterial for targeting tumor-osteoclast couplings provided by the present invention, its preparation method, and its application. However, these descriptions 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靶向肿瘤-破骨偶联体的物理杀伤纳米材料及其制备方法和应用进行详细的说明，但是不能把它们理解为对本发明保护范围的限定。

[0098]

The present invention will be further illustrated with examples below.

下面进一步例举实施例以详细说明本发明。

It should also be understood that the following embodiments are only used to further illustrate the present invention and should not be construed as limiting the scope of protection of the present invention. Any non-essential improvements and adjustments made by those skilled in the art based on the above content of the present invention are within the scope of protection of the present invention.

同样应理解，以下实施例只用于对本发明进一步说明，不能理解为对本发明保护范围的限制，本领域的技术人员根据本发明的上述内容作出的一些非本质的改进和调整均属于本发明的保护范围。

The specific process parameters in the examples below are merely examples within a suitable range. Those skilled in the art can make appropriate selections within the appropriate range based on the descriptions in this document, and are not necessarily limited to the specific values in the examples below.

下述示例具体的工艺参数等也仅是合适范围中的一个示例，即本领域技术人员可以通过本文的说明做合适的范围内选择，而并不一定要限定与下文示例的具体数值。

[0099]

Example 1: Exploring the Spatiotemporal Coupling Relationship between Tumors and Osteoclasts

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实施例1、肿瘤-破骨细胞的时空偶联关系探究

[0100]

First, we observed the relative relationship between tumor metastases and osteoclasts at the in vivo level in a mouse bone metastasis model, which was carried out in the following steps:

首先，我们在小鼠骨转移模型中活体水平观察了肿瘤转移灶与破骨细胞的相对关系，由以下步骤进行：

[0101]

(1) Select 8-10 week old female Balb/c mice and anesthetize them.

---

(1)选取8-10周的Balb/c雌鼠，进行麻醉。

[0102]

(2) Make a 1.5 cm incision between the 4th and 5th nipples in the lower right abdomen.

---

(2)在右下腹的第4个和第5个乳头之间形成1.5厘米的切口。

Separate the muscles to expose the iliac artery.

分开肌肉，露出髂动脉。

[0103]

(3) 5 x 10 NER1/mL of GFP-expressing 4T1 breast tumor cells were injected into the iliac artery via a 31G needle in 0.1 mL.

---

(3)将5x10⁶/mL的表达GFP的4T1乳腺肿瘤细胞通过31G针头注射0.1mL进入髂动脉。

[0104]

(4) Use the tip of a cotton ball to press on the arterial incision area to stop bleeding, and suture the incision.

---

(4)使用棉质尖端按压动脉切口区域止血，缝合切口。

[0105]

(5) Eight days later, the right femur of the mouse was taken for serial tissue sections, and fluorescent and TRAP-stained sections were performed to observe the localization of GFP-positive tumor cells and TRAP-positive osteoclasts. It was found that tumor-associated osteoclasts and metastatic lesions had obvious co-localization and "surrounding phenomenon" (Figure 1).

---

(5)8天后，取小鼠右侧股骨进行连续组织切片，分别进行荧光扫片和TRAP染色扫片，观察GFP阳性的肿瘤细胞和TRAP阳性的破骨细胞的定位情况，发现肿瘤相关破骨细胞与转移灶存在明显的共定位和“环绕现象”(图1)。

[0106]

Secondly, we constructed a cell culture model to explore the spatiotemporal relationship between tumor cells and osteoclasts, which was carried out in the following steps:

其次，我们构建了用于探究肿瘤细胞与破骨细胞时空关系的细胞培养模型，由以下步骤进行：

[0107]

(1) Take mouse femur and wash with culture medium to obtain bone marrow cells, and culture them in α-MEM medium with 20 ng/mL M-CSF for 5 days.

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(1)取小鼠股骨并使用培养基冲洗得到骨髓细胞，在20ng/mL M-CSF的α-MEM培养基中培养5天。

[0108]

(2) Digest the cells and plate them, set to day 0. The cells obtained at this time are osteoclast precursors (OCP). Add 50 ng/mL RANKL to the culture system and culture for 1 day. Then remove RANKL. On day 1, osteoclast precursors (R1-OCP) with RANKL pre-stimulation for 1

day are obtained. If RANKL is not removed and RANKL is used to continue culturing for 4 days, osteoclast precursors (R5-OCP) with RANKL stimulation for 5 days are obtained on day 5. These are also classic osteoclasts (OC) in the conventional culture system.

---

(2)消化细胞并铺板，设置为第0天，此时得到细胞为破骨细胞前体(OCP)，在培养体系中加入50ng/mL RANKL培养1天，然后撤去RANKL，在第1天时得到RANKL预刺激1天的破骨细胞前体(R1-OCP)；如不撤去RANKL，继续使用RANKL培养4天，则在第5天时得到RANKL刺激5天的破骨细胞前体(R5-OCP)，也是常规培养体系中的经典破骨细胞(OC)。

[0109]

(3) On day 1, 4T1 tumor cells were added to the OCP, R1-OCP and R5-OCP groups respectively, and cultured until day 5. It was found that 4T1 cells could induce R1-OCP into osteoclasts, while OCP did not have the ability to be induced into osteoclasts by 4T1 cells (Figure 2). Therefore, RANKL prestimulation is a necessary time-series condition for tumor-associated osteoclasts.

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(3)在第1天时，分别向OCP、R1-OCP、R5-OCP组中加入4T1肿瘤细胞，继续培养至第5天，发现4T1细胞可将R1-OCP诱导为破骨细胞，而OCP不具备被4T1细胞诱导为破骨细胞的能力(图2)，因此，RANKL预刺激是肿瘤相关破骨细胞必要的时间序列条件。

[0110]

(4) Keeping other conditions unchanged, the above 4T1 tumor cells were cultured in a Transwell chamber separated from osteoclast precursors. It was found that R1-OCP could not be induced into osteoclasts under non-contact conditions (Figure 3).

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(4)保持其他条件不变，将上述4T1肿瘤细胞培养于与破骨细胞前体分隔的Transwell小室上，发现在非接触条件下，R1-OCP无法被诱导为破骨细胞(图3)。

To further verify this, we seeded 4T1 cells in the central region of R1-OCP cells and cultured them for another 4 days. We found that R1-OCP cells that were only in contact with tumor cells were induced to become osteoclasts, while R1-OCP cells outside the tumor cell range were not induced to become osteoclasts (Figure 4).

为了进一步验证，我们将4T1接种在R1-OCP细胞的中间区域，并继续培养4天，发现仅与肿瘤细胞接触的R1-OCP被诱导为破骨细胞，而肿瘤细胞范围外的R1-OCP则未被诱导为破骨细胞(图4)。

Therefore, close contact with tumor cells is a necessary spatial condition for tumor-associated osteoclasts.

因此，与肿瘤细胞近距离接触是肿瘤相关破骨细胞必要的空间位置条件。

[0111]

The above results indicate that there is a strict spatiotemporal coupling relationship between tumor cells and tumor-associated osteoclasts, especially a strict spatial colocalization. Because it is difficult to target tumor cells, while the acid-producing characteristics of osteoclasts make them an ideal target, the local precise killing of tumor bone metastases can be achieved by targeting tumor-associated osteoclasts.

上述结果说明，肿瘤细胞与肿瘤相关破骨细胞存在严格的时空偶联关系，尤其是存在严格的空间共定位，因为肿瘤细胞的靶向难以进行，而破骨细胞的产酸特性使得它成为一个理想的靶向目标，所以可以通过靶向肿瘤相关破骨细胞触发实现肿瘤骨转移灶的局部精准杀伤。

[0112]

Based on this, we designed a physical killing nanomaterial that targets tumor-osteoclast coupling.

在此基础上，我们设计了一种靶向肿瘤-破骨偶联体的物理杀伤纳米材料。

[0113]

Example 2: Synthesis of Physical Killing Nanomaterials Targeting Tumor-Osteoclast Couplers

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实施例2、靶向肿瘤-破骨偶联体的物理杀伤纳米材料的合成

[0114]

2.1 Tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes

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2.1、四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体

[0115]

DSPE-PEG2000-NHS and tetracycline were dissolved in 10 mL of chloroform at a ratio of 20 mg: 6.8 mg. 100 μL of triethylamine was added to adjust the pH to 8.4. The mixture was stirred at room temperature for 24 hours. After rotary evaporation, the mixture was dissolved in water. Excess tetracycline was removed by dialysis to obtain tetracycline-modified polyethylene glycol 2000 phospholipids.

将DSPE-PEG2000-NHS与四环素以20mg：6.8mg比例溶于10mL氯仿，加入100μL三乙胺调节pH至8.4，室温搅拌反应24小时，旋蒸后溶于水，通过透析法去除多余四环素，得到四环素修饰的聚乙二醇2000磷脂。

Tetracycline-modified polyethylene glycol 2000 phospholipids, lecithin, and cholesterol were dissolved in 10 mL of chloroform at a ratio of 20 mg: 100 mg: 16 mg. The solution was then evaporated to dryness at 37°C using a suspension method. After adding 10 mL of a mixed solution of 60 mM sodium bicarbonate and 60 mM disodium hydrogen phosphate, the solution was ultrasonically hydrated, placed in a dialysis bag for dialysis, and then extruded and granulated to obtain tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes.

将四环素修饰的聚乙二醇2000磷脂、卵磷脂、胆固醇以20mg：100mg：16mg比例溶于10mL氯仿，并采用悬蒸法37摄氏度旋干，加入10mL的60mM碳酸氢钠和60mM磷酸氢二钠混合溶液后超声水化，置于透析袋中进行透析，并进行挤出整粒，得到四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体。

[0116]

2.2. Sodium bicarbonate & disodium hydrogen phosphate nanoliposomes modified with alendronate sodium

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2.2、阿仑膦酸钠修饰的碳酸氢钠&磷酸氢二钠纳米脂质体

[0117]

DSPE-PEG5000-NHS and sodium alendronate were dissolved in 10 mL of chloroform at a ratio of 20 mg: 4.3 mg. 100 μL of triethylamine was added to adjust the pH to 8.4. The mixture was stirred at room temperature for 24 hours. After rotary evaporation, the mixture was dissolved in water. Excess sodium alendronate was removed by dialysis to obtain sodium alendronate-modified polyethylene glycol 5000 phospholipid.

将DSPE-PEG5000-NHS与阿仑膦酸钠以20mg：4.3mg比例溶于10mL氯仿，加入100μL三乙胺调节pH至8.4，室温搅拌反应24小时，旋蒸后溶于水，通过透析法去除多余阿仑膦酸钠，得到阿仑膦酸钠修饰的聚乙二醇5000磷脂。

Alendronate-modified polyethylene glycol 5000 phospholipids, lecithin, and cholesterol were dissolved in 10 mL of chloroform at a ratio of 30 mg: 100 mg: 16 mg. The solution was then evaporated to dryness at 37°C using a suspension method. After adding 10 mL of a mixed solution of 30 mM sodium bicarbonate and 80 mM disodium hydrogen phosphate, the solution was ultrasonically hydrated, placed in a dialysis bag for dialysis, and then extruded and granulated to obtain sodium bicarbonate & disodium hydrogen phosphate nanoliposomes modified with alendronate.

将阿仑膦酸钠修饰的聚乙二醇5000磷脂、卵磷脂、胆固醇以30mg：100mg：16mg比例溶于10mL氯仿，并采用悬蒸法37摄氏度旋干，加入10mL的30mM碳酸氢钠和80mM磷酸氢二钠混合溶液后超

声水化，置于透析袋中进行透析，并进行挤出整粒，得到阿仑膦酸钠修饰的碳酸氢钠&磷酸氢二钠纳米脂质体。

[0118]

2.3 Tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes

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2.3、四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体

[0119]

DSPE-PEG2000-NHS and tetracycline were dissolved in 10 mL of chloroform at a ratio of 20 mg: 6.8 mg. 100 μL of triethylamine was added to adjust the pH to 8.0. The mixture was stirred at room temperature for 24 hours. After rotary evaporation, the mixture was dissolved in water. Excess tetracycline was removed by dialysis to obtain tetracycline-modified polyethylene glycol 2000 phospholipids.

将DSPE-PEG2000-NHS与四环素以20mg：6.8mg比例溶于10mL氯仿，加入100μL三乙胺调节pH至8.0，室温搅拌反应24小时，旋蒸后溶于水，通过透析法去除多余四环素，得到四环素修饰的聚乙二醇2000磷脂。

Tetracycline-modified polyethylene glycol 2000 phospholipids, lecithin, and cholesterol were dissolved in 10 mL of chloroform at a ratio of 45 mg: 100 mg: 16 mg. The solution was then evaporated to dryness at 37°C using a suspension method. After adding 10 mL of a mixed solution of 50 mM sodium bicarbonate and 20 mM disodium hydrogen phosphate, the solution was ultrasonically hydrated, placed in a dialysis bag for dialysis, and then extruded and granulated to obtain tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes.

将四环素修饰的聚乙二醇2000磷脂、卵磷脂、胆固醇以45mg：100mg：16mg比例溶于10mL氯仿，并采用悬蒸法37摄氏度旋干，加入10mL的50mM碳酸氢钠和20mM磷酸氢二钠混合溶液后超声水化，置于透析袋中进行透析，并进行挤出整粒，得到四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体。

[0120]

2.4 Tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes

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2.4、四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体

[0121]

DSPE-PEG2000-NHS and tetracycline were dissolved in 10 mL of chloroform at a ratio of 20 mg: 6.8 mg. 100 μL of triethylamine was added to adjust the pH to 8.2. The mixture was stirred at room temperature for 24 hours. After rotary evaporation, the mixture was dissolved in water. Excess tetracycline was removed by dialysis to obtain tetracycline-modified polyethylene glycol 2000 phospholipids.

将DSPE-PEG2000-NHS与四环素以20mg：6.8mg比例溶于10mL氯仿，加入100μL三乙胺调节pH至8.2，室温搅拌反应24小时，旋蒸后溶于水，通过透析法去除多余四环素，得到四环素修饰的聚乙二醇2000磷脂。

Tetracycline-modified polyethylene glycol 2000 phospholipids, lecithin, and cholesterol were dissolved in 10 mL of chloroform at a ratio of 30 mg: 100 mg: 16 mg. The solution was then evaporated to dryness at 37°C using a suspension method. After adding 10 mL of a mixed solution of 120 mM sodium bicarbonate and 50 mM disodium hydrogen phosphate, the solution was ultrasonically hydrated, placed in a dialysis bag for dialysis, and then extruded and granulated to obtain tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes.

将四环素修饰的聚乙二醇2000磷脂、卵磷脂、胆固醇以30mg：100mg：16mg比例溶于10mL氯仿，并采用悬蒸法37摄氏度旋干，加入10mL的120mM碳酸氢钠和50mM磷酸氢二钠混合溶液后超声

水化，置于透析袋中进行透析，并进行挤出整粒，得到四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体。

[0122]

Example 3: Application of tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes for physical killing of tumor-osteoclast coupling agents in breast cancer bone metastases.

实施例3、应用实施例四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体用于乳腺癌骨转移中肿瘤-破骨偶联体的物理杀伤

[0123]

Tetracycline-modified sodium bicarbonate & disodium hydrogen phosphate nanoliposomes HC&HP@TNL, prepared in Example 2.1 with an average particle size of approximately 100 nm, were selected. A typical structural diagram is shown in Figure 5. Their physicochemical properties for targeting tumor-osteoclast coupling agents were first tested.

选用平均粒径约100纳米的实施例2.1制备的四环素修饰的碳酸氢钠&磷酸氢二钠纳米脂质体HC&HP@TNL，其典型结构示意如图5所示，并首先测试了其用于靶向肿瘤-破骨偶联体的理化特性：

[0124]

(1) Cryo-transmission electron microscopy:

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(1)冷冻透射电镜：

[0125]

1) Treat the copper mesh with glow discharge for 120s to increase its hydrophilicity, and add 2.5 μL of HC&HP@TNL (control or add hydrochloric acid solution and calcium chloride solution to trigger its release) to the copper mesh.

---

1)将铜网使用辉光放电处理120s增加亲水性，将2.5微升HC&HP@TNL(对照或加入盐酸溶液和氯化钙溶液触发其释放)加到铜网上。

[0126]

2) Set the conditions to pressurize for 5 seconds, relax for 3 seconds to form a liquid thin layer, and then immerse in liquid nitrogen for vitrification.

---

2)设置条件为加压5s，松弛3秒以形成液态薄层，浸入液氮进行玻璃化。

[0127]

3) Observe the sample under a 200kV cryo-transmission electron microscope.

---

3)将样品置于200kV冷冻透射电镜下观察。

[0128]

4) It was observed that the originally intact liposomes were triggered to rupture, and the released sodium hydrogen phosphate combined with calcium ions to produce a large number of crystals (Figure 6).

---

4)观察到原本结构完整的脂质体在触发破裂，释放的磷酸氢钠与钙离子结合产生大量结晶(图6)。

[0129]

(2) Crystallization elemental analysis:

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(2)结晶元素分析：

[0130]

1) The copper mesh is treated with glow discharge for 120s to increase its hydrophilicity. 2.5 μL of HC&HP@TNL, which is triggered by adding hydrochloric acid solution and calcium chloride solution, is added to the copper mesh.

---

1)将铜网使用辉光放电处理120s增加亲水性，将2.5微升加入盐酸溶液和氯化钙溶液触发其释放的HC&HP@TNL加到铜网上。

[0131]

2) Place the sample under a transmission electron microscope and perform EDS energy dispersive spectroscopy in scanning mode to determine the proportions of elements such as Ca, P, and O.

---

2)将样品置于透射电镜下采用扫透模式进行EDS能谱测定，并确定Ca、P、O等元素的比例。

[0132]

3) The crystals formed were verified by measurement and calculation to be calcium phosphate crystals (Figure 7).

---

3)通过测定计算验证形成的结晶为钙磷结晶(图7)。

[0133]

(3) In vivo imaging tracing:

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(3)活体成像示踪：

[0134]

1) 0.5 mg/mL indocyanine green (ICG) was added during the ultrasonic hydration process of HC&HP@TNL for in vivo tracking.

---

1)在HC&HP@TNL的超声水化过程中加入0.5mg/mL吲哚氰绿(ICG)用于在体示踪。

[0135]

2) Tetracycline-modified ICG@TNL and unmodified ICG@NL were injected into Balb/c mice via the tail vein at a dose of 10 mL/kg.

---

2)将四环素修饰的ICG@TNL和无四环素修饰的ICG@NL以10mL/kg剂量通过尾静脉注射至Balb/c小鼠体内。

[0136]

3) The distribution of liposomes in mice was observed by IVIS in vivo imaging at 5 minutes, 3 hours and 6 hours.

---

3)在5分钟、3小时、6小时时分别通过IVIS活体成像观察脂质体在小鼠体内的分布。

[0137]

4) Analysis of in vivo imaging data revealed that tetracycline-modified liposomes can be effectively enriched in bone tissue, with a bone/liver signal ratio of about 1 (Figure 8).

---

4)通过活体成像数据分析发现四环素修饰的脂质体可以有效富集在骨组织，其骨/肝信号比达到1左右(图8)。

[0138]

(4) Cell-specific release experiment:

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(4)细胞特异性释放实验：

[0139]

1) A pH probe Lysotracker was added during the ultrasonic hydration process of HC&HP@TNL for liposome release characterization.

---

1)在HC&HP@TNL的超声水化过程中加入pH探针Lysotracker用于脂质体释放表征。

[0140]

2) Tumor-associated osteoclasts (TAOC), 4T1 tumor cells, osteoblasts, and mesenchymal stem cells were cultured on bone slices and cultured in α-MEM medium at 37 degrees Celsius for 1 day.

---

2)将肿瘤相关破骨细胞(TAOC)、4T1肿瘤细胞、成骨细胞、间充质干细胞分别培养在骨片上，使用α-MEM培养基在37度培养箱培养1天。

[0141]

3) Add liposomes to the culture medium at a volume ratio of 1:10 and use WGA-Rhod to stain and label the cell membrane.

---

3)在培养基中按1：10体积比加入脂质体，并使用WGA-Rhod对细胞膜进行染色标记。

[0142]

4) After inverting the bone slices, place them on a glass slide and use a confocal microscope to observe the Lysotracker release signal at the junction of the cells and the bone slices.

---

4)将骨片倒置后载于玻片，并使用共聚焦显微镜观察细胞与骨片交界区的Lysotracker释放信号。

[0143]

5) Analysis of the image data revealed that liposome release was observed only at the contact surface between TAOC and the bone fragment, indicating that TAOC can effectively trigger the release of HC&HP@TNL for the killing of tumor-osteoclast conjugates (Figure 9).

---

5)对图像数据进行分析，发现仅在TAOC与骨片的接触面观察到脂质体释放，说明TAOC可以有效触发HC&HP@TNL的释放，用于肿瘤-破骨偶联体的杀伤(图9)。

[0144]

(4) In vivo release experiment:

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(4)在体释放实验：

[0145]

1) Select 8-10 week old female Balb/c mice and anesthetize them.

---

1)选取8-10周的Balb/c雌鼠，进行麻醉。

[0146]

2) Make a 1.5 cm incision between the 4th and 5th nipples in the lower right abdomen.

---

2)在右下腹的第4个和第5个乳头之间形成1.5厘米的切口。

Separate the muscles to expose the iliac artery.

分开肌肉，露出髂动脉。

[0147]

3) 5 x 10 NER2/mL of GFP-expressing 4T1 breast tumor cells were injected into the iliac artery in 0.1 mL using a 31G needle.

---

3)将5x10⁶/mL的表达GFP的4T1乳腺肿瘤细胞通过31G针头注射0.1mL进入髂动脉。

[0148]

4) Use the tip of a cotton ball to press on the arterial incision area to stop bleeding, and suture the incision.

---

4)使用棉质尖端按压动脉切口区域止血，缝合切口。

[0149]

5) TRITC fluorescent molecules are added during the ultrasonic hydration process of HC&HP@TNL for fluorescence localization of the release region.

---

5)在HC&HP@TNL的超声水化过程中加入TRITC荧光分子用于释放区域的荧光定位。

[0150]

6) The liposomes were injected into mice via the tail vein at a dose of 10 mL/kg.

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6)以10mL/kg剂量通过尾静脉注射脂质体至小鼠体内。

[0151]

7) Take a section of the mouse femur and perform a fluorescence scan.

---

7)取小鼠股骨进行组织切片，并进行荧光扫片。

[0152]

8) TRITC signal was found in the tumor metastases, indicating that the tumor cells and the liposome release had a high degree of co-localization, suggesting that HC&HP@TNL can achieve precise release from tumor metastases through TAOC triggering (Figure 10).

---

8)发现肿瘤转移灶出现TRITC信号，即肿瘤细胞与脂质体释放影响的范围存在高度共定位，说明HC&HP@TNL可以通过TAOC触发实现肿瘤转移灶的精准释放(图10)。

[0153]

Secondly, we verified the uncoupling-killing effect of HC&HP@TNL on tumor-osteoclast coupling:

其次，我们验证了HC&HP@TNL对于肿瘤-破骨偶联体的解偶-杀伤效果：

[0154]

(1) Tumor-associated osteoclast induction and uncoupling-killing effects in tumors from different sources:

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(1)不同来源肿瘤的肿瘤相关破骨细胞诱导及解偶-杀伤效果：

[0155]

1) Obtain bone marrow cells from mouse femurs and wash them with culture medium. Culture them in α-MEM medium with 20 ng/mL M-CSF for 5 days.

---

1)取小鼠股骨并使用培养基冲洗得到骨髓细胞，在20ng/mL M-CSF的α-MEM培养基中培养5天。

[0156]

2) Digest the cells and plate them, set to day 0. Add 50 ng/mL RANKL to the culture system and culture for 1 day, then remove RANKL to obtain R1-OCP. Add tumor cells from different sources (4T1, 4T1.2, EMT-6: breast cancer cells; RM-1: prostate cancer cells; K7M2: osteosarcoma cells; Hepa1-6: liver cancer cells; LLC: lung cancer cells; Panc02: pancreatic cancer cells; MFC: gastric cancer cells; Renca: kidney cancer cells) to R1-OCP for co-culture.

---

2)消化细胞并铺板，设置为第0天，在培养体系中加入50ng/mL RANKL培养1天，然后撤去RANKL，得到R1-OCP；向R1-OCP中加入不同来源肿瘤细胞(4T1、4T1.2、EMT-6：乳腺癌细胞；RM-1：前列腺癌细胞；K7M2:骨肉瘤细胞；Hepa1-6:肝癌细胞；LLC：肺癌细胞；Panc02:胰腺癌细胞；MFC：胃癌细胞；Renca：肾癌细胞)共培养。

[0157]

3) Set up treatment groups, and add tetracycline-modified nanoliposomes loaded with sodium chloride (Cl@TNL), tetracycline-modified nanoliposomes loaded with sodium bicarbonate (HC@TNL), and tetracycline-modified nanoliposomes loaded with sodium bicarbonate and disodium hydrogen phosphate (HC&HP@TNL) to the culture medium at a volume ratio of 1:10.

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3)设置治疗组，分别以1:10体积比向培养基中加入包载氯化钠的四环素修饰纳米脂质体(Cl@TNL)、包载碳酸氢钠的四环素修饰纳米脂质体(HC@TNL)以及包载碳酸氢钠和磷酸氢二钠的四环素修饰纳米脂质体(HC&HP@TNL)。

[0158]

4) Continue culturing until day 5, fix with 4% paraformaldehyde, and then perform TRAP staining.

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4)继续培养至第5天，使用4%多聚甲醛固定后，进行TRAP染色。

[0159]

5) We found that the phenomenon of tumor-associated osteoclast production is universal in tumor cells from multiple sources. Furthermore, after adding HC&HP@TNL, we found that it has an uncoupling-killing effect on tumor-osteoclast couples of various tumors (Figure 11).

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5)发现肿瘤相关破骨细胞产生现象具有多种来源肿瘤细胞的普遍性，同时在加入HC&HP@TNL后，我们发现其对于各种肿瘤的肿瘤-破骨偶联体均具有解偶-杀伤效果(图11)。

[0160]

(2) Validation of tumor cell killing:

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(2)肿瘤细胞杀伤验证：

[0161]

1) GFP-expressing 4T1 tumor cells were seeded at 1000/well in a 96-well cell culture plate containing 10000/well R1-OCP cells.

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1)将表达GFP的4T1肿瘤细胞以1000/孔铺设于含有10000/孔R1-OCP细胞的96孔细胞培养板。

[0162]

2) Tetracycline-modified nanoliposomes loaded with sodium chloride (Cl@TNL), tetracycline-modified nanoliposomes loaded with sodium bicarbonate (HC@TNL), and tetracycline-modified nanoliposomes loaded with sodium bicarbonate and disodium hydrogen phosphate (HC&HP@TNL) were added to the culture medium at a volume ratio of 1:10.

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2)分别以1:10体积比向培养基中加入包载氯化钠的四环素修饰纳米脂质体(Cl@TNL)、包载碳酸氢钠的四环素修饰纳米脂质体(HC@TNL)以及包载碳酸氢钠和磷酸氢二钠的四环素修饰纳米脂质体(HC&HP@TNL)。

[0163]

3) One day later, place the 96-well cell culture plate under a multi-functional microplate reader to quantify the GFP signal.

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3)1天后，将96孔细胞培养板置于多功能酶标仪下进行GFP信号定量。

[0164]

4) The fluorescence intensity of GFP confirmed that HC&HP@TNL caused tumor cell killing and growth inhibition triggered by tumor-associated osteoclasts (Figure 12).

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4)通过GFP的荧光强度验证了HC&HP@TNL在肿瘤相关破骨细胞的触发下导致肿瘤细胞的杀伤和生长抑制(图12)。

[0165]

5) The cells after killing were fixed with glutaraldehyde and prepared for transmission electron microscopy.

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5)对杀伤后的细胞进行戊二醛固定并进行透射电镜制样。

[0166]

6) After resin fixation, the cell samples were ultra-thinly sectioned.

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6)树脂固定后对细胞样品进行超薄切片。

[0167]

7) Observation under a transmission electron microscope revealed that a large number of calcium phosphate crystals appeared on the surface and inside the TAOC and 4T1 cells, which destroyed the integrity of the tumor cells and achieved direct physical killing of the tumor cells (Figure 13).

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7)置于透射电镜下观察，发现TAOC和4T1细胞表面和细胞内均出现了大量的钙磷结晶，破坏了肿瘤细胞完整性，实现对肿瘤的细胞的直接物理杀伤(图13)。

[0168]

(3) In vitro evaluation of tumor suppression effect:

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(3)肿瘤抑制效果离体评估：

[0169]

1) We selected 8-10 week old Balb/c female mice, isolated the femurs of the mice, and cultured the femurs in vitro in cell culture plates. We constructed a bone-in-culture array (BICA).

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1)选取8-10周的Balb/c雌鼠，分离小鼠股骨，将小鼠股骨进行离体培养于细胞培养板中，我们构建了骨培养阵列(bone-in-culture array,BICA)。

[0170]

2) On day 0, 4T1 tumor cells expressing Luciferase were injected into the femur to construct a bone metastasis model.

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2)在第0天向股骨中注射表达Luciferase的4T1肿瘤细胞，构建骨转移模型。

[0171]

3) On days 1, 3, 5, and 7, a micro-injector was used to administer a low-rate drug perfusion of 10 μL/min to the femur using tetracycline-modified nanoliposomes loaded with sodium chloride (Cl@TNL), tetracycline-modified nanoliposomes loaded with sodium bicarbonate (HC@TNL), tetracycline-modified nanoliposomes loaded with sodium bicarbonate and disodium hydrogen phosphate (HC&HP@TNL), and the classic antitumor drug DOX.

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3)在第1，3，5，7天向股骨中使用微量进液器进行包载氯化钠的四环素修饰纳米脂质体(Cl@TNL)、包载碳酸氢钠的四环素修饰纳米脂质体(HC@TNL)、包载碳酸氢钠和磷酸氢二钠的四环素修饰纳米脂质体(HC&HP@TNL)和经典抗肿瘤药物DOX的10微升/分钟低速药物灌流。

[0172]

4) On day 14, 1.5 mg/mL of fluorescein potassium salt substrate was added to each sample, and the effects were compared by IVIS imaging (Figure 14a).

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4)在第14天向各样品中加入1.5mg/mL的荧光素钾盐底物，通过IVIS成像比较效果(图14a)。

[0173]

5) Comparing the effects of HC&HP@TNL and the traditional chemotherapy drug doxorubicin (DOX) in BICA, HC&HP@TNL showed a more pronounced tumor-suppressive effect and fewer side effects on other cells compared to DOX (Figures 14b-d).

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5)比较HC&HP@TNL与传统化疗药物阿霉素(DOX)在BICA中的作用，与DOX相比，HC&HP@TNL具有更明确的肿瘤抑制作用，对其他细胞的副作用更小(图14b-d)

[0174]

(4) Mouse bone metastasis inhibition experiment:

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(4)小鼠骨转移灶抑制实验：

[0175]

1) Select 8-10 week old female Balb/c mice and anesthetize them.

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1)选取8-10周的Balb/c雌鼠，进行麻醉。

[0176]

2) Make a 1.5 cm incision between the 4th and 5th nipples in the lower right abdomen.

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2)在右下腹的第4个和第5个乳头之间形成1.5厘米的切口。

Separate the muscles to expose the iliac artery.

分开肌肉，露出髂动脉。

[0177]

3) 5 x 10 NER3/mL of GFP- or Luciferase-expressing 4T1 breast tumor cells were injected into the iliac artery in 0.1 mL using a 31G needle.

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3)将5x10⁶/mL的表达GFP或表达Luciferase的4T1乳腺肿瘤细胞通过31G针头注射0.1 mL进入髂动脉。

[0178]

4) Use the tip of a cotton ball to press on the arterial incision area to stop bleeding, suture the incision, and consider it as day 0.

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4)使用棉质尖端按压动脉切口区域止血，缝合切口，视为第0天。

[0179]

5) On days 1, 3, 5, and 7, tetracycline-modified nanoliposomes loaded with sodium chloride (Cl@TNL), tetracycline-modified nanoliposomes loaded with sodium bicarbonate (HC@TNL), and tetracycline-modified nanoliposomes loaded with sodium bicarbonate and disodium hydrogen phosphate (HC&HP@TNL) were injected via tail vein at a dose of 10 ml/kg.

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5)在第1、3、5、7天分别通过尾静脉注射10ml/kg剂量的包载氯化钠的四环素修饰纳米脂质体(Cl@TNL)、包载碳酸氢钠的四环素修饰纳米脂质体(HC@TNL)以及包载碳酸氢钠和磷酸氢二钠的四环素修饰纳米脂质体(HC&HP@TNL)。

[0180]

6) On day 8, the femurs of mice in each group inoculated with GFP-4T1 cells were subjected to Micro-CT scans, and their metaphyseal bone parameters were measured.

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6)在第8天取接种GFP-4T1细胞的各组小鼠股骨进行Micro-CT扫描，并测量其干骺端骨参数。

[0181]

7) On day 28, mice in each group inoculated with Luciferase-4T1 cells were injected with 150 mg/kg of fluorescein potassium salt substrate via tail vein, and the growth of tumor metastases was observed using IVIS imaging.

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7)在第28天向接种Luciferase-4T1细胞的各组小鼠通过尾静脉注射150mg/kg的荧光素钾盐底物，使用IVIS成像观察肿瘤转移灶生长情况。

[0182]

8) Analysis of Micro-CT and IVIS luminescence intensity data verified that HC&HP@TNL effectively inhibited bone metastases through physical killing of tumor-osteoclast coupling agents (Figure 15), and also effectively inhibited metaphyseal bone loss (BMD: 0.24±0.04 g /cm NER4 vs 0.17±0.02 g/cm NER5, p＝0.0007; BV/TV: 15.44±4.44% vs 8.96±1.81%, P<0. 0019; Tb.N: 2.43±0.58mm NER6_ vs 1.25±0.14mm NER7_, P<0.0001; Tb.Sp: 0.23±0.03mm vs 0.38±0.05mm, P<0.0001) (Figure 16).

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8)分析Micro-CT和IVIS发光强度数据，验证了HC&HP@TNL的对肿瘤-破骨偶联体的物理杀伤实现了骨转移灶的高效抑制(图15)，并且有效抑制了干骺端骨质流失(BMD：0.24±0.04g/cm³vs 0.17±0.02g/cm³,p＝0.0007；BV/TV：15.44±4.44%vs 8.96±1.81%,P<0. 0019；Tb.N：2.43±0.58mm⁻¹vs 1.25±0.14mm⁻¹,P<0.0001；Tb. Sp：0.23±0.03mm vs 0.38±0.05mm,P<0.0001)(图16)。

This invention demonstrates that the nanomaterials for preventing tumor bone metastasis can effectively inhibit tumor bone metastasis.

说明本发明的预防肿瘤骨转移的纳米材料可有效抑制肿瘤骨转移。

[0183]

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。