# Notice



## DESCRIPTION CN116019779A

Osteoporosis Treatment Delivery System Based on Osteoclast Progenitor Cell-Targeted circBBS9 Knockdown

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基于破骨细胞前体细胞靶向circBBS9敲降的骨质疏松治疗递送系统

### [0001]

Technical Field

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技术领域

## [n0001]

This invention pertains to biomaterials and their applications, specifically relating to an osteoporosis treatment delivery system based on osteoclast precursor cell-targeted circRNA knockdown.

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本发明属于生物材料及其应用，具体涉及一种基于破骨细胞前体细胞靶向circRNA敲降的骨质疏松治疗递送系统。

## [0003]

Background Technology

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背景技术

## [n0002]

Osteoporosis is a common chronic bone disease in clinical practice. Its low bone mass and severe destruction of bone microstructure often increase the fragility and brittleness of bones.

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骨质疏松症是临床常见慢性骨骼疾病，其低骨量和严重的骨微组织结构破坏特点，往往会增加骨骼的脆性和易断裂性。

As unique bone resorbing cells, mature multinucleated osteoclasts secrete a large number of enzymes and acids to perform bone resorption due to their high transcriptional activity.

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作为独特的骨吸收细胞，成熟的多核破骨细胞由于其高转录活性，分泌大量酶和酸发挥骨吸收的功能。

However, excessive multinucleation of osteoclasts can lead to an imbalance in bone homeostasis, which is a major factor contributing to osteoporosis.

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然而，破骨细胞的过度多核化会导致骨稳态失衡，从而是引起骨质疏松症的主要因素。

Currently, first-line treatments for osteolytic diseases, such as bisphosphonates, indiscriminately inhibit osteoclast lineages, leading to apoptosis of all bone resorbing cells and thus disrupting necessary bone turnover. Therefore, there is an urgent need to develop a treatment system that can selectively target osteoclast precursor cells at specific disease-related stages of the osteoclast lineage.

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目前，对溶骨性疾病的一线治疗，如双膦酸盐，其不加选择地抑制破骨细胞谱系，导致所有骨吸收细胞凋亡，从而破坏必要的骨转换。 因此，亟需开发一种治疗体系来选择性靶向破骨细胞谱系中疾病相关特定阶段的破骨细胞前体细胞。

## [n0003]

Circular RNA (circRNA) is a covalently closed circular structure that can be back-spliced by pre-mRNA and expressed during cell development and differentiation.

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环状RNA(circRNA)是一种共价闭环结构，它能够被pre-mRNA反向剪接，在细胞发育和分化过程中表达。

Given that circRNAs are resistant to RNase R, they are more stable in cells and exosomes than their corresponding linear transcripts. circRNAs can be effective endogenous regulators in miRNA-mediated RNA interference (RNAi). Therefore, regulating circRNA can effectively intervene in the cell differentiation process. However, the development of targeted delivery nucleic acid drugs against circRNA has become a challenge.

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鉴于circRNA对RNase R具有抗性，它们在细胞和外泌体中比相应的线性转录本更稳定。 circRNA可以在miRNA介导的RNA干扰(RNAi)中成为有效的内源性调节因子。 因此，调控circRNA可以有效干预细胞的分化过程。 然而，靶向递送针对circRNA的核酸药物开发成为一种难题。

## [n0004]

Cell membrane engineering technology has been widely used in various fields, from drug delivery and imaging to phototherapy.

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细胞膜工程化技术已广泛应用于药物输送、成像到光活化治疗等各种领域。

Biomimetic nanoparticles disguised as cell membranes give them cell-like functions, thereby prolonging their circulation in the blood and allowing them to escape the immune system's clearance. Furthermore, because they inherit the necessary fusion proteins and adhesion molecules from cells, nanoparticles coated with cancer cell membranes can specifically target homologous cells. Therefore, isomorphic targeted delivery of these cell membrane materials is a viable strategy for future disease treatment.

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用细胞膜伪装的仿生纳米粒子赋予了类似细胞的功能，从而延长了纳米颗粒在血液中的循环，使它们能够逃脱免疫系统的清除。 此外，由于它们从细胞中继承了必要的融合蛋白和粘附分子，癌细胞膜包被的纳米颗粒可以特异性地靶向同源细胞。 因此，这些细胞膜材料的同型靶向递送是未来疾病治疗的可用策略。

## [n0005]

However, despite the application of cell membrane engineering technology in various fields, selective targeted delivery of cells at specific stages of cell multinucleation has not yet been achieved.

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然而，尽管细胞膜工程化技术已用于各个领域，但尚未实现在细胞多核化过程中具有特定阶段细胞的选择性靶向递送。

Osteoclast precursor cells are derived from macrophages induced by RANKL for 3 days and contain specific proteins involved in osteoclast circulation, recruitment, intercellular recognition and fusion. Therefore, fused cell membranes with specific stage markers facilitate the selective targeted delivery of osteoclast precursor cells.

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破骨细胞前体细胞源自RANKL诱导3天的巨噬细胞，具有参与破骨细胞循环、募集、细胞间识别和融合的特定蛋白质。 因此，具有特定阶段标记的融合细胞膜有助于破骨细胞前体细胞的选择性靶向递送的作用。

## [n0006]

Therefore, based on the above background, this patent discovers that osteoclast-specific circRNAs and their human homologs are prominently expressed in osteoclast multinucleation and osteoporosis.

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因此，基于上述背景，本专利发现破骨细胞特异性表达的circRNA及其人类同源物，在破骨细胞多核化和骨质疏松过程中表达突出。

Among them, circBBS9 was selected as the verification object. However, how to achieve precise targeted delivery of circRNA at a specific time in vivo is a bottleneck problem that limits the application of circRNA. To address this shutdown issue, we innovatively pioneered the use of osteoclast precursor cell membrane materials to carry siRNA, enabling osteoclast precursor cell membrane microvesicles to target osteoclast precursor cells. Among them, the osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> effectively inhibited osteoclast multinucleation and bone resorption capacity without affecting the differentiation of precursor osteoclasts, and increased bone density in osteoporotic mice in in vivo experiments.

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其中，circBBS9被挑选出作为验证对象。 然而如何在体内实现特定时间精准靶向递送circRNA是限制琪应用的瓶颈问题。 为了解决这一关机问题，我们创新性的率先使用破骨前体细胞膜材料搭载siRNA，破骨细胞前体细胞膜微囊泡能够靶向破骨细胞前体细胞。 其中，搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡有效抑制破骨细胞的多核化和骨吸收能力而不影响前体破骨细胞的分化，并在体内实验中增加骨质疏松小鼠的骨密度。

## [n0007]

In summary, osteoclast precursor cell membrane microvesicles carrying siRNA-circRNA can be used for nucleic acid drug research in clinical osteoporosis.

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综上，搭载siRNA-circRNA的破骨细胞前体细胞膜微囊泡可用于临床上骨质疏松的核酸药物研究。

## [0010]

Summary of the Invention

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发明内容

## [n0008]

The present invention aims to address the technical problem that existing delivery systems lack efficient and specific targeting of cells at specific stages in the osteoclast lineage, and provides an osteoporosis treatment delivery system based on osteoclast precursor cell-targeted circBBS9 knockdown.

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本发明是为了解决现有递送系统不具有高效、特异靶向破骨细胞谱系中特定阶段细胞的技术问题，提供一种基于破骨细胞前体细胞靶向circBBS9敲降的骨质疏松治疗递送系统。

## [n0009]

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

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为实现上述目的，本发明提供了如下方案：

## [n0010]

An osteoporosis treatment delivery system based on osteoclast precursor cell-targeted circBBS9 knockdown includes siRNA and cell membrane microvesicles targeting osteoclast precursor cells; siRNA is delivered to osteoclast precursor cells using cell membrane microvesicles targeting osteoclast precursor cells.

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基于破骨细胞前体细胞靶向circBBS9敲降的骨质疏松治疗递送系统，包括siRNA和靶向破骨细胞前体细胞的细胞膜微囊泡；利用靶向破骨细胞前体细胞的细胞膜微囊泡对siRNA进行破骨细胞前体细胞靶向性递送；

## [n0011]

The siRNA is a specific siRNA for circBBS9, and its nucleotide sequence is shown in SEQ ID NO. 5 and SEQ ID NO.6; the circBBS9 has the nucleotide sequence shown in SEQ ID NO.1.

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所述的siRNA为circBBS9的特异性siRNA，其核苷酸序列为SEQ ID NO.5和SEQ IDNO.6所示；所述circBBS9，其核苷酸序列如SEQ ID NO.1所示；

## [n0012]

The method for preparing cell membrane microvesicles targeting osteoclast precursor cells is as follows:

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所述的一种靶向破骨细胞前体细胞的细胞膜微囊泡的制备方法，具体如下：

## [n0013]

(1) Extract mammalian mononuclear cells, induce macrophages with 15-30 ng/mL M-CSF for 3-5 days, and then induce osteoclast precursor cells with 30-80 ng/mL RANKL for 3-5 days;

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(1)提取哺乳动物单核细胞，15～30ng/mL M-CSF诱导3-5天得到巨噬细胞，再用30～80ng/mL RANKL诱导3-5天，获得破骨前体细胞；

## [n0014]

(2) Washing: After the trypsin digestion, specifically after 3-5 min of trypsin digestion, the digestion is terminated and the cells are centrifuged at 1000 rpm for 5 min. The cells are then resuspended in TM buffer at 4°C. The TM buffer is a mixture of 1 mM MgCl and 10 mM Tris with

water, and the pH is adjusted to 7.4. After resuspending, the cells are squeezed out 30-40 times using a microsyringe to disrupt the cells and obtain a cell homogenate.

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(2)洗涤，所述的胰酶消化后，具体为胰酶消化3～5min后，终止消化并1000rpm，5min离心，用4°C的TM缓冲液重悬，所述TM缓冲液为1mM MgCl<sub>2</sub>和10mM Tris加水混合，并将PH调至7.4；重悬后利用微型注射器挤出30～40次以破坏细胞，得到细胞匀浆；

## [n0015]

(3) Add 1M sucrose and mix with the cell homogenate to reach a final sucrose concentration of 0.25M;

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(3)加入1M蔗糖与细胞匀浆混合，达到0.25M蔗糖终浓度；

## [n0016]

(4) Centrifuge the mixture obtained in step (3) at 4°C and 2000xg for 10 min, collect the supernatant, centrifuge at 4°C and 3000xg for 30-35 min, resuspend the precipitate with 0.25M sucrose solution and wash, centrifuge again at 4°C and 3000xg for 30-35 min to obtain the cell membrane;

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(4)将所述步骤(3)中得到的混合物4°C、2000xg条件下离心10min，收集上清液，4°C、3000xg条件下离心30～35min，用0.25M蔗糖溶液重悬沉淀洗涤，再次4°C、3000xg条件下离心30～35min，得到细胞膜；

## [n0017]

(5) The obtained cell membrane is squeezed through a 400nm and 200nm polycarbonate filter membrane 5 to 10 times in sequence to obtain cell membrane nanovesicles that can target osteoclast precursor cells.

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(5)将得到的细胞膜用挤出器依次挤过400nm及200nm聚碳酸酯滤膜5～10次，获得可靶向破骨前体细胞的细胞膜纳米囊泡。

## [n0018]

Preferably, the mammalian mononuclear cells are tibial and femoral mononuclear cells from 6-8 week old C57BL/6J mice.

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作为优选，所述的哺乳动物单核细胞为6～8周C57BL/6J小鼠胫骨及股骨单核细胞。

## [n0019]

Preferably, the micro-injector is a 1ml insulin injector.

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作为优选，所述的微型注射器为1ml胰岛素注射器。

## [n0020]

Preferably, the M-CSF concentration is 25 ng/mL.

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作为优选，所述的M-CSF浓度为25ng/mL。

## [n0021]

As a preferred option, the RANKL concentration is 50 ng/mL.

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作为优选，RANKL浓度为50ng/mL。

## [n0022]

Preferably, circBBS9 is used as an intervention target for osteoporosis, and miR-423-3p is its downstream target.

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作为优选，所述的circBBS9作为骨质疏松干预靶点，miR-423-3p为其下游靶点。

## [n0023]

Preferably, the expression vector for the siRNA is a biomimetic bone-targeting nanoparticle.

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作为优选，所述的siRNA的表达载体为仿生骨靶向纳米颗粒。

## [n0024]

The beneficial effects of this invention are:

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本发明的有益效果：

## [n0025]

Compared with existing osteoporosis drug treatments, the osteoclast precursor cell membrane microvesicle material of this invention represents a significant advancement in the following aspects:

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本发明的破骨细胞前体细胞膜微囊泡材料相比现有骨质疏松药物治疗，本发明显著的进步在于：

## [n0026]

1) circRNA has good stability and is highly expressed during osteoclast precursor cell multinucleation. Intervention can inhibit osteoclast multinucleation and bone resorption while preserving osteoclast precursor cell differentiation.

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1)circRNA具有较好的稳定性，在破骨细胞前体细胞多核化过程中高度表达，干预其可抑制破骨细胞多核化和骨吸收能力而保留破骨细胞前体细胞的分化。

## [n0027]

2) Using osteoclast precursor cells as raw materials, the extracted osteoclast precursor cell membrane microvesicles have the characteristics of good stability, good biocompatibility and low immunogenicity, providing an effective means for targeting osteoclast precursor cells.

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2)以破骨细胞前体细胞为原材料，提取的破骨细胞前体细胞膜微囊泡具有稳定性好，生物相容性好和免疫原性低的特点，为靶向破骨细胞前体细胞提供了一种有效手段。

## [n0028]

3) Osteoclast precursor cell membrane microvesicles have significant in vitro and in vivo osteoclast precursor cell targeting effects. Combined with the specific expression pattern of

circRNA, they solve the problem of non-selective destruction of osteoclast lineage by traditional drugs, thus showing promise as a highly efficient targeted nanodelivery system for osteoporosis treatment.

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3)破骨细胞前体细胞膜微囊泡材料具有明显的体内外破骨细胞前体细胞靶向作用，结合circRNA的特异性表达模式解决了传统药物无选择性破坏破骨细胞谱系问题，从而具有成为骨质疏松治疗的一种高效靶向纳米递送系统前景。

## [0032]

Attached image description:

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附图说明：

## [n0029]

Figure 1 shows the differential expression of circBBS9 in osteoclast multinucleation.

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图1是circBBS9在破骨细胞多核化中的差异表达。

## [n0030]

Figure 2 shows the expression levels of the human homolog circBBS9 in the lumbar vertebrae of osteoporosis patients and normal individuals.

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图2是circBBS9人类同源物在骨质疏松患者和正常人腰椎骨的表达量。

## [n0031]

Figure 3 shows the expression level of circBBS9 after RNase R digestion.

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图3是circBBS9在Rnase R消化后的表达量图

## [n0032]

Figure 4 shows the knockdown effect of siRNA<sup>circBBS9</sup> on circBBS9.

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图4是siRNA<sup>circBBS9</sup>对circBBS9的敲降效果。

## [n0033]

Figure 5 shows the effect of siRNA<sup>circBBS9</sup> transfection on the expression of marker genes at different differentiation stages of osteoclasts.

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图5是转染siRNA<sup>circBBS9</sup>后对破骨细胞不同分化阶段的标记基因表达的影响。

## [n0034]

Figure 6 shows TRAP staining patterns of siRNA_NER5 after transfection at different stages of osteoclasts.

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图6是siRNA<sup>circBBS9</sup>在破骨细胞不同阶段转染后TRAP染色图

## [n0035]

Figure 7 shows the bone resorption effect of siRNA_NER6 after transfection at different stages of osteoclasts.

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图7是siRNA<sup>circBBS9</sup>在破骨细胞不同阶段转染后骨吸收效果图

## [n0036]

Figure 8 shows the Western Blot results and statistical graph of the inhibitory effect of siRNA_NER7 on osteoclast marker proteins.

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图8是siRNA<sup>circBBS9</sup>对破骨细胞标志蛋白抑制效果的Western Blot结果和统计图。

## [n0037]

Figure 9 shows the cryo-electron microscopy and particle size distribution of the extracted osteoclast precursor cell membrane microvesicles.

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图9是提取的破骨细胞前体细胞细胞膜微囊泡冷冻电镜及粒径分布图。

## [n0038]

Figure 10 is a TRAP staining diagram showing the inhibitory effect of osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> on osteoclast formation.

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图10是搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡对破骨细胞形成抑制效果的TRAP染色图。

## [n0039]

Figure 11 shows the RT-qPCR results of key genes in osteoclast formation via osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup>.

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图11是搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡对破骨细胞形成的关键基因RT-qPCR结果图。

## [n0040]

Figure 12 shows the inhibitory effect of osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> on osteoclast bone resorption capacity.

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图12是搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡对破骨细胞骨吸收能力的抑制效果。

## [n0041]

Figure 13 shows the effect of osteoclast precursor cell membrane microvesicles carrying siRNA on the specific entry of osteoclast precursor cells in vitro.

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图13是搭载siRNA的破骨细胞前体细胞膜微囊泡在体外对破骨细胞前体细胞特异性的进入效果。

## [n0042]

Figure 14 shows the fluorescence distribution and statistical diagram of osteoclast precursor cell membrane microvesicles carrying siRNA in various tissues of a mouse in vivo imaging experiment.

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图14是搭载siRNA的破骨细胞前体细胞膜微囊泡在小鼠活体成像实验各组织荧光分布及统计图。

## [n0043]

Figure 15 shows the fluorescence colocalization of osteoclast precursor cell membrane microvesicles carrying siRNA with osteoclast precursor cell marker proteins in mouse bone sections.

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图15是搭载siRNA的破骨细胞前体细胞膜微囊泡在小鼠骨切片中与破骨细胞前体细胞标志蛋白荧光共定位图。

## [n0044]

Figure 16 shows the therapeutic effect of osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> on bone morphology in osteoporotic mice.

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图16是是搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡对骨质疏松小鼠骨形态的治疗效果。

## [n0045]

Figure 17 shows the inhibitory effect of osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> on the number and morphology of osteoclasts in osteoporotic mice.

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图17是搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡对骨质疏松小鼠破骨细胞数量和形态的抑制效果。

## [0050]

Detailed Implementation

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具体实施方式

## [n0046]

The following description, in conjunction with embodiments, further illustrates a circRNA-based delivery system for selectively inhibiting osteoclast precursors and its preparation method provided by the present invention. However, these embodiments should not be construed as limiting the scope of protection of the present invention.

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下面结合实施例对本发明提供的一种基于circRNA的破骨细胞前体选择性抑制的递送系统及其制备方法进行进一步描述，但是不能把以下实施例视为对本发明保护范围的限定。

## [n0047]

This invention aims to address the problem of non-specific osteoclast lineage targeting in existing drugs by providing a circRNA-based osteoclast precursor selective inhibition delivery system, its preparation method, and its application.

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本发明是为了解决现有药物的非特异性破骨细胞谱系靶向问题，提供了一种一种基于circRNA的破骨细胞前体选择性抑制的递送系统及其制备方法的制备方法和应用。

## [n0048]

Example 1: Analysis of circBBS9 expression in osteoclasts and osteoporosis patients

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实施例1circBBS9在破骨细胞和骨质疏松患者中的表达情况分析

## [n0049]

1. This invention uses RNA-seq to detect differentially expressed circRNAs during osteoclast multinucleation.

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1、本发明采用RNA-seq对破骨细胞多核化过程中差异表达的circRNA进行检测。

## [n0050]

2. Design specific primers based on the obtained circRNA, and use RT-qPCR to verify the expression levels of these primers.

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2、根据得到的circRNA设计特异性引物，使用RT-qPCR分别验证这些引物的表达量。

The results showed that circBBS9 expression level increased significantly after RANKL stimulation.

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结果发现，circBBS9表达量在RANKL刺激后显著上升

## [n0051]

3. Using circBBS9-specific reverse primers, the expression level of circBBS9 in osteoporotic mice and osteoclast differentiation process was analyzed by RT-qPCR. It was found that the expression level was most significantly upregulated on the third day after RANKL stimulation (osteoclast multinucleation stage), as shown in Figure 1.

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3、采用circBBS9特异性的反向引物，通过RT-qPCR对circBBS9在骨质疏松小鼠和破骨细胞分化进程中的表达量予以分析，发现给以RANKL刺激后第三天(破骨细胞多核化阶段)表达量上调趋势最为明显，如图1。

Its sequence is shown in (SEQ ID NO.1).

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其序列如(SEQ ID NO.1)所示。

The primers used are shown in (SEQ ID NO.2) and (SEQ ID NO.3).

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所用引物如(SEQ IDNO.2)和(SEQ ID NO.3)所示。

## [n0052]

mmu_circ_0001757(SEQ ID NO.1)

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mmu_circ_0001757(SEQ ID NO.1)

## [n0053]

GTGGCTGTACTCCAATCCCAGAGTCAGACCTAGAGGAAAGGTCACTAGATGACTCCACAGAGCTGTTTAC

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GTGGCTGTACTCCAATCCCAGAGTCAGACCTAGAGGAAAGGTCACTAGATGACTCCACAGAGCTGTTTAC

## [n0054]

primer-circBBS9-F(SEQ ID NO.2)

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primer-circBBS9-F(SEQ ID NO.2)

## [n0055]

TGGAGTAATGCTAATGAGTTGAGG

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TGGAGTAATGCTAATGAGTTGAGG

## [n0056]

primer-circBBS9-R(SEQ ID NO.3)

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primer-circBBS9-R(SEQ ID NO.3)

## [n0057]

GCTGAGACTTCAGGCATGG

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GCTGAGACTTCAGGCATGG

## [n0058]

4. By searching the circBase database for human homologs of circBBS9, hsa_circ_0134188 was found.

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4、通过circBase数据库查找circBBS9的人类同源物，找到hsa_circ_0134188。

Specific primers were designed to measure its expression in the lumbar spine of healthy individuals and patients with osteoporosis, and significant high expression of hsa_circ_0134188 was found.

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设计其特异性引物，在健康人与骨质疏松患者的腰椎中测量其表达，发现hsa_circ_0134188显著高表达。

Detection of human peripheral blood mononuclear cells and osteoclasts derived from peripheral blood mononuclear cells revealed a significant increase in the expression of hsa_circ_0134188.

---

检测人外周血单核细胞和外周血单核细胞来源的破骨细胞发现hsa_circ_0134188表达显著上升。

The results are shown in Figure 2.

---

结果如图2所示。

Its sequence is shown in (SEQ ID NO.4).

---

其序列如(SEQ ID NO.4)所示。

## [n0059]

hsa_circ_0134188(SEQ ID NO.4)

---

hsa_circ_0134188(SEQ ID NO.4)

## [n0060]

GTGGTTGTACTACAATCCCAGAGTCAGACCTAGAAGAAAGATCAGTAGAACAAGACTCTACAGAACTGTTT

---

GTGGTTGTACTACAATCCCAGAGTCAGACCTAGAAGAAAGATCAGTAGAACAAGACTCTACAGAACTGTTT

## [n0061]

5. After extracting osteoclast lysate, digestion was performed using RNase R, and the expression of circBBS9 was measured by RT-qPCR. It was found that circBBS9 has stronger stability compared to GAPDH and its linear mRNA.

---

5、提取破骨细胞裂解液后，使用Rnase R消化，再通过RT-qPCR测量circBBS9的表达，发现其相较于GAPDH及其线性mRNA，其具有较强的稳定性。

The results are shown in Figure 3.

---

结果如图3所示。

## [n0062]

Example 2: Effects of siRNA<sup>circBBS9</sup> transfection on different differentiation stages of osteoclasts

---

实施例2转染siRNA<sup>circBBS9</sup>后对破骨细胞不同分化阶段的影响

## [n0063]

1. Design circBBS9-specific siRNAs, the sequences of which are shown in (SEQ ID NO.4) and (SEQ ID NO.5).

---

1、设计circBBS9特异性的siRNA，其序列如(SEQ ID NO.4)和(SEQ ID NO.5)所示。

As shown in Figure 4, after transfection with siRNA<sup>circBBS9</sup>, the expression of circBBS9 and its linear transcripts was measured. It was found that circBBS9 was significantly inhibited while its linear transcripts were unaffected.

---

如图4所示将siRNA<sup>circBBS9</sup>转染后测量circBBS9及其线性转录本的表达，发现circBBS9被显著抑制而其线性转录本不受影响。

## [n0064]

siRNA-circBBS9-sense(SEQ ID NO.5):

---

siRNA-circBBS9-sense(SEQ ID NO.5)：

## [n0065]

CUCUGGAGGUGGCUGUACUTT

---

CUCUGGAGGUGGCUGUACUTT

## [n0066]

siRNA-circBBS9-antisense(SEQ ID NO.6):

---

siRNA-circBBS9-antisense(SEQ ID NO.6)：

## [n0067]

AGUACAGCCACCUCCAGAGTT

---

AGUACAGCCACCUCCAGAGTT

## [n0068]

2. After stimulating BMMs with RANKL for three days, siRNA<sup>circBBS9</sup> was transfected into them, and RT-qPCR was used to analyze the marker genes of osteoclasts at different differentiation stages.

---

2、用RANKL刺激BMMs三天后，往其中转染siRNA<sup>circBBS9</sup>，采用RT-qPCR对破骨细胞不同分化阶段的标记基因予以分析。

We found that after transfection with siRNA<sup>circBBS9</sup>, the marker genes of osteoclasts in the multinucleation stage were significantly suppressed, while the marker genes of precursor osteoclasts did not change significantly (see Figure 5).

---

我们发现转染siRNA<sup>circBBS9</sup>后，破骨细胞的在多核化阶段的标记基因被显著抑制，而前体破骨细胞的标记基因没有明显变化，见图5。

## [n0069]

3. Transfection with siRNA<sup>circBBS9</sup> on day 1, day 3, or day 5 after RANKL stimulation, followed by TRAP staining on day 6, revealed that transfection with siRNA<sup>circBBS9</sup> on day 3 significantly inhibited the formation of multinucleated osteoclasts.

---

3、在RANKL刺激后的第1天、第3天或者第5天转染siRNA<sup>circBBS9</sup>，并在第六天进行TRAP染色，发现在第三天转染siRNA<sup>circBBS9</sup>显著抑制多核破骨细胞的形成。

The results are shown in Figure 6.

---

结果如图6所示

## [n0070]

4. Transfecting with siRNA<sup>circBBS9</sup> on day 1, day 3 or day 5 after RANKL stimulation and performing bone resorption capacity test on day 6 revealed that transfecting with siRNA<sup>circBBS9</sup> on day 3 significantly inhibited the area of bone resorption pits in osteoclasts.

---

4、在RANKL刺激后的第1天、第3天或者第5天转染siRNA<sup>circBBS9</sup>，并在第六天进行骨吸收能力检测，发现在第三天转染siRNA<sup>circBBS9</sup>显著抑制破骨细胞的骨吸收坑面积。

## [n0071]

The results are shown in Figure 7.

---

结果如图7所示

## [n0072]

5. On day 3 after RANKL stimulation, siRNA<sup>circBBS9</sup> was transfected, and cell lysate was extracted on day 6. Western blot analysis revealed that siRNA<sup>circBBS9</sup> significantly weakened osteoclast maturation-related proteins.

---

5、在RANKL刺激后的第3天转染siRNA<sup>circBBS9</sup>，并在第六天提取细胞裂解液，通过WB分析发现siRNA<sup>circBBS9</sup>显著削弱破骨细胞成熟的相关蛋白。

The results are shown in Figure 8.

---

结果如图8所示。

## [n0073]

Example 3: Preparation of osteoclast precursor cell membrane microvesicles

---

实施例3破骨细胞前体细胞细胞膜微囊泡的制备

## [n0074]

Collection of osteoclast precursor cells: Femurs and tibias of 6-8 week old C57BL/6J mice were taken and the bone marrow cavity was flushed with α-MEM + 10% FBS + penicillin G and streptomycin (complete α-MEM medium). The flushed cells were cultured in complete α-MEM medium containing 25 ng/mL M-CSF for 5 days, with one medium change during the period, to obtain macrophages.

---

破骨细胞前体细胞的收集：取6-8周C57BL/6J老鼠股骨和胫骨，用α-MEM+10%FBS+青霉素G和链霉素(完全α-MEM培养基)冲洗骨髓腔，将冲洗后的细胞在含25ng/mL M-CSF的完全α-MEM培养基中培养5天，期间换液一次，获得巨噬细胞。

The obtained macrophages were cultured for 3 days in complete α-MEM medium containing 25 ng/mL LM-CSF and 50 ng/mL RANKL to obtain osteoclast precursor cells.

---

将所得巨噬细胞在含25ng/mLM-CSF及50ng/mL RANKL的完全α-MEM培养基中培养3天得到破骨细胞前体细胞。

## [n0075]

Preparation of osteoclast precursor cell membrane: Osteoclast precursor cells were suspended in ice-cold TM buffer solution (pH 7.4; 1 mM MgCl2-NER23- + 10 mM Tris) and extruded at least 30 times using a micro extruder to disrupt the cells.

---

破骨细胞前体细胞膜的制备：将破骨细胞前体细胞悬浮在冰冷的TM缓冲溶液(pH7.4；1mM MgCl<sub>2</sub>+10mM Tris)中，通过微型挤出机挤出至少30次以破坏细胞。

The cell homogenate was then mixed with 1M sucrose to achieve a sucrose concentration of 0.25M, and the mixture was centrifuged at 4°C and 2000xg for 10 minutes.

---

随后将细胞匀浆与1M蔗糖混合，使达到0.25M的蔗糖浓度，并将混合物在4°C，2000xg下离心10分钟。

Collect the supernatant and centrifuge it at 4°C and 3000xg for 30 minutes.

---

收集所得上清液，再以4°C，3000xg进一步离心30分钟。

The resulting precipitate was the collected cell membrane, which was then washed twice with ice-cold 0.25M sucrose for purification.

---

所得沉淀即为收集的细胞膜，并用冰冷的0.25M蔗糖洗涤两次以进行纯化。

## [n0076]

Preparation of osteoclast precursor cell membrane microvesicles: The obtained cell membrane was squeezed through 400nm and 200nm polycarbonate filter membranes in sequence, and squeezed repeatedly for 5-10 times to obtain osteoclast precursor cell membrane microvesicles.

---

破骨细胞前体细胞膜微囊泡的制备：将获得的细胞膜依次挤过400nm和200nm的聚碳酸酯滤膜，反复挤压5-10次得到破骨细胞前体细胞膜微囊泡。

## [n0077]

Example 4: Preparation of osteoclast precursor cell membrane microvesicles

---

实施例4破骨细胞前体细胞细胞膜微囊泡的制备

## [n0078]

Collection of osteoclast precursor cells: Femur and tibia of 6-8 week old C57BL/6J mice were used to flush the bone marrow cavity with α-MEM + 10% FBS + penicillin G and streptomycin (complete α-MEM medium). The flushed cells were cultured in complete α-MEM medium containing 15 ng/mL M-CSF for 5 days, with one medium change during the period, to obtain macrophages.

---

破骨细胞前体细胞的收集：取6-8周C57BL/6J老鼠股骨和胫骨，用α-MEM+10%FBS+青霉素G和链霉素(完全α-MEM培养基)冲洗骨髓腔，将冲洗后的细胞在含15ng/mL M-CSF的完全α-MEM培养基中培养5天，期间换液一次，获得巨噬细胞。

The obtained macrophages were cultured for 3 days in complete α-MEM medium containing 15 ng/mL LM-CSF and 30 ng/mL RANKL to obtain osteoclast precursor cells.

---

将所得巨噬细胞在含15ng/mLM-CSF及30ng/mL RANKL的完全α-MEM培养基中培养3天得到破骨细胞前体细胞。

## [n0079]

Preparation of osteoclast precursor cell membrane: Osteoclast precursor cells were suspended in ice-cold TM buffer solution (pH 7.4; 1 mM MgCl2-NER24- + 10 mM Tris) and extruded at least 30 times using a micro extruder to disrupt the cells.

---

破骨细胞前体细胞膜的制备：将破骨细胞前体细胞悬浮在冰冷的TM缓冲溶液(pH7.4；1mM MgCl<sub>2</sub>+10mM Tris)中，通过微型挤出机挤出至少30次以破坏细胞。

The cell homogenate was then mixed with 1M sucrose to achieve a sucrose concentration of 0.25M, and the mixture was centrifuged at 4°C and 2000xg for 10 minutes.

---

随后将细胞匀浆与1M蔗糖混合，使达到0.25M的蔗糖浓度，并将混合物在4°C，2000xg下离心10分钟。

Collect the supernatant and centrifuge it at 4°C and 3000xg for 30 minutes.

---

收集所得上清液，再以4°C，3000xg进一步离心30分钟。

The resulting precipitate was the collected cell membrane, which was then washed twice with ice-cold 0.25M sucrose for purification.

---

所得沉淀即为收集的细胞膜，并用冰冷的0.25M蔗糖洗涤两次以进行纯化。

## [n0080]

Preparation of osteoclast precursor cell membrane microvesicles: The obtained cell membrane was squeezed through 400nm and 200nm polycarbonate filter membranes in

sequence, and squeezed repeatedly for 5-10 times to obtain osteoclast precursor cell membrane microvesicles.

---

破骨细胞前体细胞膜微囊泡的制备：将获得的细胞膜依次挤过400nm和200nm的聚碳酸酯滤膜，反复挤压5-10次得到破骨细胞前体细胞膜微囊泡。

## [n0081]

Example 5: Preparation of osteoclast precursor cell membrane microvesicles

---

实施例5破骨细胞前体细胞细胞膜微囊泡的制备

## [n0082]

Collection of osteoclast precursor cells: Femur and tibia of 6-8 week old C57BL/6J mice were used to flush the bone marrow cavity with α-MEM + 10% FBS + penicillin G and streptomycin (complete α-MEM medium). The flushed cells were cultured in complete α-MEM medium containing 30 ng/mL M-CSF for 5 days, with one medium change during the period, to obtain macrophages.

---

破骨细胞前体细胞的收集：取6-8周C57BL/6J老鼠股骨和胫骨，用α-MEM+10%FBS+青霉素G和链霉素(完全α-MEM培养基)冲洗骨髓腔，将冲洗后的细胞在含30ng/mL M-CSF的完全α-MEM培养基中培养5天，期间换液一次，获得巨噬细胞。

The obtained macrophages were cultured for 3 days in complete α-MEM medium containing 30 ng/mL LM-CSF and 80 ng/mL RANKL to obtain osteoclast precursor cells.

---

将所得巨噬细胞在含30ng/mLM-CSF及80ng/mL RANKL的完全α-MEM培养基中培养3天得到破骨细胞前体细胞。

## [n0083]

Preparation of osteoclast precursor cell membrane: Osteoclast precursor cells were suspended in ice-cold TM buffer solution (pH 7.4; 1 mM MgCl2-NER25- + 10 mM Tris) and extruded at least 30 times using a micro extruder to disrupt the cells.

---

破骨细胞前体细胞膜的制备：将破骨细胞前体细胞悬浮在冰冷的TM缓冲溶液(pH7.4；1mM MgCl<sub>2</sub>+10mM Tris)中，通过微型挤出机挤出至少30次以破坏细胞。

The cell homogenate was then mixed with 1M sucrose to achieve a sucrose concentration of 0.25M, and the mixture was centrifuged at 4°C and 2000xg for 10 minutes.

---

随后将细胞匀浆与1M蔗糖混合，使达到0.25M的蔗糖浓度，并将混合物在4°C，2000xg下离心10分钟。

Collect the supernatant and centrifuge it at 4°C and 3000xg for 30 minutes.

---

收集所得上清液，再以4°C，3000xg进一步离心30分钟。

The resulting precipitate was the collected cell membrane, which was then washed twice with ice-cold 0.25M sucrose for purification.

---

所得沉淀即为收集的细胞膜，并用冰冷的0.25M蔗糖洗涤两次以进行纯化。

## [n0084]

Preparation of osteoclast precursor cell membrane microvesicles: The obtained cell membrane was squeezed through 400nm and 200nm polycarbonate filter membranes in sequence, and squeezed repeatedly for 5-10 times to obtain osteoclast precursor cell membrane microvesicles.

---

破骨细胞前体细胞膜微囊泡的制备：将获得的细胞膜依次挤过400nm和200nm的聚碳酸酯滤膜，反复挤压5-10次得到破骨细胞前体细胞膜微囊泡。

## [n0085]

As shown in Figure 9, the osteoclast precursor cell membrane microvesicles prepared in this embodiment were examined by cryo-transmission electron microscopy and particle size analysis by laser particle size analyzer, indicating that the osteoclast precursor cell membrane microvesicles are spherical lipid bilayers.

---

如图9所示，将本实施例制备的破骨细胞前体细胞膜微囊泡进行冷冻投射电镜检测，激光粒径仪进行粒径检测，提示破骨细胞前体细胞膜微囊泡为球形结构的脂质双分子层。

Furthermore, the particle size of the osteoclast precursor cell membrane microvesicles is approximately 141.9 nm.

---

且破骨细胞前体细胞膜微囊泡的粒径大约在141.9nm左右。

## [n0086]

Example 1: Evaluation of the ability of osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> to inhibit osteoclast multinucleation and bone resorption.

---

试验例1：搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡抑制破骨细胞多核化和骨吸收能力的评估。

[n0087]

The osteoclast precursor cell membrane obtained in Example 1 was mixed with an equal volume of siRNA<sup>circBBS9</sup>40μg/mL, sonicated for 5 minutes, and then squeezed through a 400nm and 200nm polycarbonate filter membrane 5-10 times.

---

如实施例1所得破骨细胞前体细胞膜1mg/mL，与siRNA<sup>circBBS9</sup>40μg/mL等体积混合，超声5分钟，然后依次挤过400nm和200nm的聚碳酸酯滤膜5-10次。

The siRNA<sup>NC</sup> and macrophage membrane groups served as the control group.

---

siRNA<sup>NC</sup>以及巨噬细胞膜组为对照组。

Osteoclast precursor cell membrane microvesicles carrying siRNA_NER29 were co-cultured with osteoclast precursor cells for 4 hours.

---

将搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡与破骨细胞前体细胞共同培养4小时。

Twenty-four hours later, the number of mature osteoclasts in each group was detected by TRAP staining.

---

24小时后，通过TRAP染色检测各组成熟破骨细胞数量。

As shown in Figure 10, osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> significantly inhibited the formation of multinucleated osteoclasts.

---

如图10所示，搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡显著抑制了多核破骨细胞的形成。

RT-qPCR was used to detect osteoclast formation. As shown in Figure 11, osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> significantly reduced the expression of osteoclast maturation-related genes.

---

使用RT-qPCR检测破骨细胞的形成，如图11所示，搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡显著减少了破骨细胞成熟相关基因的表达。

Bone resorption tablets were used to simulate a bone resorption environment in vitro to assess the bone resorption function of osteoclasts.

---

用骨吸收片在体外模拟骨吸收环境，评估破骨细胞骨吸收功能情况。

As shown in Figure 12, the osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> significantly inhibited the bone resorption capacity of osteoclasts.

---

结果如图12所示，搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡显著抑制了破骨细胞的骨吸收能力。

## [n0088]

Experimental Example 2: Evaluation of the in vivo and in vitro targeting ability of osteoclast precursor cell membrane microvesicles

---

试验例2：破骨细胞前体细胞膜微囊泡体内外靶向能力评估

## [n0089]

In vitro targeting ability of osteoclast precursor cell membrane microvesicles: 1 mg/mL of osteoclast precursor cell membrane obtained in Example 1 was mixed with an equal volume of siRNA<sup>cy5</sup>40 μg/mL, sonicated for 5 minutes, and then squeezed through 400 nm and 200 nm polycarbonate filter membranes 5-10 times in sequence.

---

破骨细胞前体细胞膜微囊泡体外靶向能力检测：如实施例1所得破骨细胞前体细胞膜1mg/mL，与siRNA<sup>cy5</sup>40μg/mL等体积混合，超声5分钟，然后依次挤过400nm和200nm的聚碳酸酯滤膜5-10次。

It was incubated with macrophages, osteoclast precursor cells, and osteoclasts for 12 hours.

---

将其与巨噬细胞、破骨细胞前体细胞、破骨细胞共同孵育12小时。

Intracellular fluorescence intensity was analyzed using confocal microscopy and flow cytometry.

---

使用共聚焦显微镜和流式细胞术分析细胞内荧光强度。

As shown in Figure 13, the osteoclast precursor cell membrane microvesicles carrying siRNA can selectively enter osteoclast precursor cells instead of macrophages or osteoclasts.

---

结果如图13所示，搭载siRNA的破骨细胞前体细胞膜微囊泡可以选择性进入破骨细胞前体细胞而不是巨噬细胞或破骨细胞。

## [n0090]

Establishment of a mouse model of osteoporosis: C57BL/6J mice (female, 11 weeks old) were anesthetized with 4% chloral hydrate, and after skin disinfection, bilateral ovariectomy was performed to induce osteoporosis.

---

建立小鼠骨质疏松动物模型：取C57BL/6J小鼠(雌性，11周)在4%水合氯醛麻醉，皮肤消毒后进行双侧卵巢切除术诱导骨质疏松。

Eight weeks later, a microCT scan showed a decrease in bone mass, indicating successful modeling.

---

8周后microCT检测骨量指标减少提示造模成功。

## [n0091]

In vivo targeting ability of osteoclast precursor cell membrane microvesicles: 1 mg/mL of osteoclast precursor cell membrane obtained in Example 1 was mixed with an equal volume of 2.5 mg/mL indocyanine green (ICG), sonicated for 5 minutes, and then squeezed through 400 nm and 200 nm polycarbonate filter membranes 5-10 times in sequence.

---

破骨细胞前体细胞膜微囊泡体内靶向能力检测：如实施例1所得破骨细胞前体细胞膜1mg/mL，与吲哚菁绿(ICG)2.5mg/mL等体积混合，超声处理5分钟，然后依次挤过400nm和200nm的聚碳酸酯滤膜5-10次。

The ICG-only group and the macrophage membrane group served as the control group.

---

单纯ICG以及巨噬细胞膜组为对照组。

After successful osteoporosis modeling in mice, ICG encapsulated in the cell membrane of osteoclast precursor cells was injected via the tail vein. At 6 hours, 1 day, and 3 days later, the fluorescence intensity of the heart, liver, spleen, kidneys, lower limbs, and spine was detected using a small animal in vivo imaging system with an excitation wavelength of 710 nm and an emission wavelength of 785 nm.

---

骨质疏松小鼠造模成功后，将破骨细胞前体细胞膜包裹的ICG予尾静脉注射，在6小时，1天，3天后，用小动物活体成像仪检测心肝脾肾，双下肢及脊柱的荧光强度，采用激发波长710nm，发射波长为785nm。

As shown in Figure 14, at each time point, the fluorescence intensity of the osteoclast precursor cell membrane group in the mouse's lower limbs and spine was significantly higher than that in the control group, suggesting that osteoclast precursor cell membrane microvesicles have an in vivo targeting effect.

---

结果如图14所示，在各个时间段，小鼠的双下肢和脊柱荧光强度，破骨细胞前体细胞膜组均明显高于对照组，提示破骨细胞前体细胞膜微囊泡具有体内靶向的作用。

## [n0092]

To further verify the in vivo targeting ability, osteoclast precursor cell membrane microvesicles were labeled with DiO using the above method. The DiO-labeled osteoclast precursor cell membrane microvesicles were injected into the tail vein of an osteoporosis mouse model. One day later, femoral tissue was taken, fixed with 4% paraformaldehyde, decalcified with 14% EDTA at 37°C for 12 hours, and then embedded in paraffin.

---

进一步验证体内靶向能力，将破骨细胞前体细胞膜微囊泡用上述方法标记DiO，将DiO标记的破骨细胞前体细胞膜微囊泡予尾静脉注射骨质疏松小鼠模型，1天后取股骨组织，4%多聚甲醛固定，14% EDTA，37°C，脱钙12小时后石蜡包埋。

After sectioning, the slides were incubated overnight at 4°C with DC-STAMP (labeled osteoclast precursor cells) primary antibody, washed 4 times with PBST for 5 minutes each time, stained with Alexa Fluor 594 secondary antibody, washed 4 times with PBST for 5 minutes each time, mounted with anti-fluorescence quencher containing DAPI, and the results were captured by laser confocal microscopy.

---

切片后予DC-STAMP(标记破骨细胞前体细胞)一抗4°C孵育过夜，PBST洗4次，每次5分钟，然后用Alexa Fluor 594二抗染色，PBST洗4次，每次5分钟，含DAPI的抗荧光猝灭剂封片，用激光共聚焦拍摄结果图。

As shown in Figure 15, green spots (DiO) and red spots (DC-STAMP) overlap on the bone surface and in the bone marrow, suggesting osteoclast precursor cell targeting of osteoclast precursor cell membrane microvesicles.

---

如图15所示，在骨表面和骨髓中，绿色斑点(DiO)与红色斑点(DC-STAMP)重叠，提示破骨细胞前体细胞膜微囊泡的破骨细胞前体细胞靶向。

## [n0093]

Experimental Example 3: Evaluation of the therapeutic effect of osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> on osteoporosis

---

试验例3：搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡治疗骨质疏松效果评估

## [n0094]

Establishment of a mouse model of osteoporosis: C57BL/6J mice (female, 11 weeks old) were anesthetized with 4% chloral hydrate, and after skin disinfection, bilateral ovariectomy was performed to induce osteoporosis.

---

建立小鼠骨质疏松动物模型：取C57BL/6J小鼠(雌性，11周)在4%水合氯醛麻醉，皮肤消毒后进行双侧卵巢切除术诱导骨质疏松。

Eight weeks later, a microCT scan showed a decrease in bone mass, indicating successful modeling.

---

8周后microCT检测骨量指标减少提示造模成功。

## [n0095]

The osteoclast precursor cell membrane obtained in Example 1 was mixed with an equal volume of siRNA<sup>circBBS9</sup>40μg/mL, sonicated for 5 minutes, and then squeezed through 400nm and 200nm polycarbonate filter membranes 5-10 times in sequence.

---

如实施例1所得破骨细胞前体细胞膜1mg/mL，与siRNA<sup>circBBS9</sup>40μg/mL等体积混合，超声5分钟，然后依次挤过400nm和200nm的聚碳酸酯滤膜5-10次。

The siRNA<sup>NC</sup> and macrophage membrane groups served as the control group.

---

siRNA<sup>NC</sup>以及巨噬细胞膜组为对照组。

One week after osteoporosis modeling, mice were injected via tail vein every 3 days for a total of 6 times.

---

骨质疏松小鼠造模一周后，每3天尾静脉注射一次，共计6次。

Four weeks after administration, the histological parameters of the mouse femur were analyzed using microCT.

---

完成给药4周后使用microCT分析小鼠股骨骨组织学参数。

As shown in Figure 16, osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> significantly improved the trabecular volume ratio (BV/TV), the number of trabeculae (TB.N), the trabecular thickness (Tb.Th), and the intertrabecular spacing (Tb.Sp).

---

如图16所示搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡显著改善骨小梁体积比(BV/TV)，骨小梁数目(TB.N)，骨小梁厚度(Tb.Th)和骨小梁之间的间隙(Tb.Sp)。

As shown in Figure 17, TRAP staining and HE staining showed that osteoclast precursor cell membrane microvesicles carrying siRNA<sup>circBBS9</sup> significantly inhibited the size and number of osteoclasts.

---

如图17所示，TRAP染色和HE染色显示搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡显著抑制破骨细胞的大小和数量。

## [n0096]

As can be seen from the above embodiments, the method for preparing osteoclast precursor cell membrane microvesicle material carrying siRNA<sup>circBBS9</sup> provided by the present invention has the characteristics of simple operation and low cost.

---

由上述实施例可知，本发明提供的搭载siRNA<sup>circBBS9</sup>的破骨细胞前体细胞膜微囊泡材料制备方法，操作简便，成本低等特点。

This material can actively and specifically target osteoclast precursor cells in the osteoclast lineage, and has the potential for precise targeted drug delivery based on the characteristics of cells at different stages in the osteoclast lineage.

---

该材料可以主动特异性靶向破骨细胞谱系中破骨细胞前体细胞，有针对破骨细胞谱系中不同阶段细胞特点进行精准靶向给药的治疗前景。

It is a promising cell membrane targeted delivery system.

---

是一种很有潜力的细胞膜靶向递送系统。

## [0102]

sequence list

---

序列表

## [0103]

<110> Sir Run Run Shaw Hospital, Affiliated to Zhejiang University School of Medicine

---

<110> 浙江大学医学院附属邵逸夫医院

## [0104]

<120> Osteoporosis Treatment Delivery System Based on Osteoclast Progenitor Cell-Targeted circBBS9 Knockdown

---

<120> 基于破骨细胞前体细胞靶向circBBS9敲降的骨质疏松治疗递送系统

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