Notice

DESCRIPTION CN116328039A

A natural bone repair material with specific mineralization that can regulate inflammatory metabolism, its preparation method and application

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一种可调控炎症代谢的特定矿化度天然骨修复材料及其制备方法和应用

[0001]

Technical Field

技术领域

[n0001]

This invention relates to a biomaterial, specifically to a natural bone repair material with a specific degree of mineralization that can regulate inflammatory metabolism, its preparation method, and its application.

本发明涉及一种生物材料，具体涉及一种可调控炎症代谢的特定矿化度天然骨修复材料及其制备方法和应用。

[0003]

Background Technology

背景技术

[n0002]

Repairing bone defects caused by fractures, diseases, or congenital defects remains an important medical problem to be solved. With the acceleration of population aging, the number of patients with bone defects caused by frequent orthopedic diseases is increasing.

骨折、疾病或先天性缺陷引起的骨缺损的修复仍然是待解决的医学上重要的问题，随着人口老龄化的加快，骨科疾病频发引发的骨缺损患者人数不断增加。

According to statistics, there are 3 million cases of bone defects in my country every year, and 2 million patients need bone repair materials every year. The actual usage of bone repair materials is about 500,000 cases per year. The average price of bone repair products in the Chinese market is about 2,000 yuan per person. Based on this, it is estimated that the actual market for bone repair materials in China is about 1 billion yuan per year, and the potential market size is about 4 billion yuan per year.

根据统计，我国骨缺损的患者病例为300万例/年，骨修复材料的需求患者是200万例/年，且目前的患者实际用量约为50万例/年，在中国市场的骨修复产品的平均价格约为2000元/人，据此测算国内骨修复材料实际市场约达到10亿元/年，潜在市场规模约为40亿元/年。

Meanwhile, large-area bone tissue defects in clinical practice are often caused by various reasons such as bone trauma and bone tumor resection. This not only causes physical deformities and various bodily dysfunctions in patients, but also has an adverse effect on their mental health.

同时，临床上大面积骨组织缺损多由于骨创伤、骨肿瘤的切除等各种原因造成，不仅造成患者身体的畸形和并引起各种机体功能障碍，而且对患者心理方面的也产生不良影响。

Therefore, bone defects cause a huge socioeconomic and psychological burden, and there is an urgent need for a domestically produced innovative bone repair product with biosafety and therapeutic effectiveness to be launched on the market.

因此，骨缺损问题造成了极大的社会经济负担和精神负担，亟须一款具有生物安全性和治疗有效性的国产创新骨修复产品上市。

[n0003]

For bone defect filling, the gold standard for bone defect repair materials currently used in clinical practice is autologous bone grafting, which involves removing the iliac bone, fibula, or ribs from the patient and transplanting them to the defect site. However, its widespread application is limited by its disadvantages, such as limited bone sources, the need for secondary surgery which increases the risk of postoperative infection and possible infectious diseases, and increased economic burden on patients.

对于骨缺损填充，目前临床上常用的骨缺损修复材料黄金标准为自体骨移植，将来源于患者本身的髂骨、腓骨或肋骨切除，并移植到缺损部位，因其存在骨来源有限、需要二次手术并增加了术后感染和可能传染疾病的风险、增加患者的经济负担等缺点，限制了其的广泛应用。

However, the most commonly used allogeneic bone in clinical practice also suffers from limited availability and carries the risk of immune rejection and transmission of potential

diseases. Meanwhile, recent cutting-edge research has found that in organ and tissue injuries, especially those of the musculoskeletal system, excessive inflammation in the early stages of injury may lead to delayed tissue repair or even failure to heal. Therefore, based on the filling of bone defect repair materials, it is necessary to regulate the homeostasis of early inflammatory cells to ensure long-term healing. Therefore, developing artificial bone with properties similar to human autologous bone materials and the ability to regulate inflammatory cells is the future development direction of the bone repair material industry.

而目前临床使用中最多的同种异体骨同样存在来源有限的问题，还具有免疫排斥反应和传播潜在疾病的风险。 同时，近年来的前沿研究成果发现器官组织损伤，尤其是骨肌系统损伤中，损伤早期的过度炎症可能导致组织修复延迟甚至不愈合，因此，在骨缺损修复材料填充的基础上，应当调控早期炎症细胞稳态以保证远期愈合。 因此，研发具有与人自体来源骨材料相似特性并具有炎症细胞调控能力的人工骨是目前骨修复材料产业化未来的发展方向。

[n0004]

Tissue-engineered bone, due to its wide availability and natural structure, can restore biological functions to the greatest extent and avoid surgical failure caused by allogeneic rejection and immune reactions. It has now become a regenerative medicine material for tissue engineering research and clinical applications, attracting the attention and importance of researchers. After more than 20 years of development, many revolutionary innovative

studies and products have emerged, making the complete repair and regeneration of musculoskeletal system tissues possible.

组织工程骨因其广泛来源和天然结构，能够最大程度还原生物性功能，并避免异体排斥免疫反应导致的手术失败，现已成为组织工程学研究及临床应用的再生医学材料，被广大研究人员所关注和重视，经过20余年发展，逐渐涌现出许多革命性的创新研究和产品，他们将骨骼肌肉系统组织的完全修复再生变成可能。

Decellularization, as a representative technique in tissue engineering, uses physical, chemical, and biological enzyme methods to specifically and precisely remove immunogenic components from foreign tissues or organs while preserving highly bioactive and functional tissue structures, namely the extracellular matrix (ECM). Over the past decade, ECM materials have demonstrated excellent organ/tissue regeneration and repair effects in complex organ construction research (e.g., heart, lung, liver, kidney, etc.) and clinical product applications. In recent years, xenograft bone implant products have been launched and used in clinical practice. These products aim to reduce immunogenicity and promote bone repair by removing immunogenic components from the natural bone matrix while retaining its three-dimensional structure. Among the aforementioned inventions, patent application number CN201210060651.0 discloses a method for preparing decellularized bone, which involves preparing animal bone sheets, virus inactivation, defatting, decellularization, freeze drying, cutting, shaping, packaging, and irradiation sterilization processes to obtain decellularized

bone. Patent application number CN201910244264.4 discloses a homologous demineralized bone material for nasal augmentation and its preparation method, which uses cortical bone as raw material and performs degreasing and demineralization treatment to obtain the demineralized bone material.

脱细胞作为组织工程代表性技术，是应用物理、化学、生物酶等方法，可以特异、精确的清除异种组织或器官内具有免疫原性的组分，同时保留具有高度生物活性和功能的组织结构，即细胞外基质(ECM，Extracellular Matrix)。 在过去十年里，ECM材料在复杂器官构建研究(例如：心脏、肺、肝脏、肾脏等)和临床产品应用方面均表现出很好的器官/组织再生修复效果。 近年来，已有异种骨植入产品上市，并运用于临床。 该类产品通过去除天然骨基质的免疫原性组分并保留天然骨基质三维立体结构，以达到降低免疫原性而促进骨修复的目的。 在上述涉及发明中，申请号为CN201210060651.0的专利公布了一种制备脱细胞骨的方法，将动物骨通过制备骨片、病毒灭活、脱脂、脱细胞、冻干、裁剪、成型、包装和辐照消毒工艺后得到脱细胞骨。 申请号为CN201910244264.4的专利公布了一种用于鼻部整形填充的同种脱矿骨材料及其制备方法，以皮质骨作为原材料，进行脱脂脱矿处理，得到脱矿骨材料。

[n0005]

However, the inventions mentioned above are either fully mineralized bone repair materials with dense surface microstructures and weak cell adhesion, or completely demineralized bone repair materials with poor mechanical properties and inability to form effective support.

Furthermore, the inventions mentioned above cannot reasonably regulate the state of inflammatory cells in the early stages of bone injury to achieve a more ideal bone repair effect.

但诸如上述的发明或为完全矿化骨修复材料，表面微结构致密，细胞粘附能力弱，或为完全脱矿骨修复材料，力学性能差，无法形成有效支撑，此外，诸如上述的发明更无法合理调控骨损伤早期炎症细胞状态，以达到更加理想的骨修复效果。

Therefore, it is necessary to prepare a natural bone repair material with a specific mineralization that can regulate inflammatory metabolism, simulate the specific microenvironment required for natural bone repair, and help suppress inflammation and promote bone tissue repair.

因此需要制备一种可调控炎症代谢的特定矿化度天然骨修复材料，模拟天然骨修复所需要的特定微环境，有助于炎症抑制，促进骨组织修复。

[0008]

Summary of the Invention

发明内容

[n0006]

This invention addresses the shortcomings of existing technologies by providing a natural bone repair material with a specific degree of mineralization that can regulate inflammatory metabolism, along with its preparation method and applications.

本发明针对现有技术的不足，提供一种可调控炎症代谢的特定矿化度天然骨修复材料及其制备方法和应用。

The material is an extracellular matrix with a specific mineralization formed after ultrasonic decellularization and demineralization of bone tissue. It simulates the specific microenvironment required for natural bone repair, which helps to suppress inflammation and promote bone tissue repair.

所述材料为骨组织超声脱细胞脱矿后形成的特定矿化度细胞外基质，模拟天然骨修复所需要的特定微环境，有助于炎症抑制，促进骨组织修复。

[n0007]

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：

[n0008]

This invention provides a natural bone repair material with a specific mineralization that can regulate inflammatory metabolism. This material is a mineralized extracellular matrix formed after ultrasonic decellularization and demineralization of bone tissue; the mineralization refers to a calcium mass content of 10-20%.

本发明提供一种可调控炎症代谢的特定矿化度天然骨修复材料，该材料为骨组织超声脱细胞脱矿后形成的矿化度细胞外基质；所述的矿化度为钙的质量含量为10-20%。

[n0009]

Preferably, the bone tissue source is human-derived allogeneic bone tissue or xenogeneic bone tissue from pigs, cattle, sheep, or horses; more preferably, xenogeneic bone tissue from pigs is used.

作为优选，所述的骨组织来源采用人来源的同种骨组织或猪、牛、羊、马来源的异种骨组织，更优选的，采用猪来源的异种骨组织。

[n0010]

Preferably, the bone tissue types include cortical bone, cancellous bone, cartilage, and composite bone of the above components; more preferably, epiphyseal bone tissue is used.

作为优选，所述的骨组织类型包括皮质骨、松质骨、软骨及上述成分的复合骨，优选的，采用骨骺骨组织。

[n0011]

Preferably, bone tissue specifications include bone granules, bone blocks, bone strips, and bone powder, with bone granules being the preferred choice.

作为优选，骨组织规格包括骨粒、骨块、骨条和骨粉，优选的，采用骨粒。

[n0012]

A method for preparing a natural bone repair material with specific mineralization that can regulate inflammatory metabolism includes the following steps:

一种可调控炎症代谢的特定矿化度天然骨修复材料的制备方法，包括以下步骤：

[n0013]

Natural bone is cut into bone slices and cleaned. The bone slices are processed into different specifications and cleaned. They are then disinfected, degreased, decellularized, and specifically demineralized until the calcium content is 10-20%. Finally, they are cleaned and tested for residues, freeze-dried, packaged, and sterilized.

取天然骨切割成骨片并清洗，将骨片加工成不同的规格并清洗，进行消毒处理、脱脂处理、脱细胞处理、特定脱矿处理至钙的质量含量为10-20%，进行末道清洗并检测残留，进行冻干和包装，进行灭菌。

[n0014]

Preferably, the process of taking natural bone, cutting it into bone pieces, and cleaning it involves taking fresh natural bone, removing most of the attached bone and flesh, cutting the epiphysis, cortical bone, or cancellous bone into bone pieces of appropriate size, and cleaning the bone pieces with a cleaning solution after cutting to remove most of the blood and bone marrow from the bone pieces.

作为优选，所述的取天然骨切割成骨片并清洗，具体为：取新鲜的天然骨，去除其上所附的大部分骨肉，将骨骺、皮质骨或松质骨切割成合适规格的骨片，切割完成后用清洗液清洗骨片，去除骨片上大部分的血液和骨髓。

[n0015]

Preferably, the natural bone is human-derived allogeneic bone tissue or xenogeneic bone tissue derived from pigs, cattle, sheep, or horses; more preferably, xenogeneic bone tissue derived from pigs is used.

作为优选，所述的天然骨采用人来源的同种骨组织或猪、牛、羊、马来源的异种骨组织，更优选的，采用猪来源的异种骨组织。

[n0016]

Preferably, the above-mentioned cutting methods include bone sawing machines, oscillating saws, semi-automatic cutting machines, CNC machine tools, high-pressure water jet cutting machines, etc., with bone sawing machines being the preferred choice.

作为优选，上述切割方式包括锯骨机、摆锯、半自动切割机、CNC数控机床、高压水刀等切割方式，优选的，采用锯骨机。

[n0017]

Preferably, the above cleaning methods include high-pressure rinsing, vibration cleaning, spray cleaning or ultrasonic cleaning, etc., with high-pressure rinsing being the preferred method.

作为优选，上述清洗方式包括高压冲洗、振荡清洗、喷淋清洗或超声清洗等，优选的，采用高压冲洗。

[n0018]

Preferably, the cleaning solution includes purified water, ultrapure water, water for injection, physiological saline, phosphate buffer, etc., with purified water being the preferred choice.

作为优选，上述清洗液包括纯化水、超纯水、注射用水、生理盐水、磷酸缓冲液等，优选的，采用纯化水。

[n0019]

Preferably, the process of processing bone fragments into different specifications and cleaning them specifically includes:

作为优选，所述的将骨片加工成不同的规格并清洗，具体为：

[n0020]

Bone fragments are processed into bone granules, bone blocks, bone strips, or bone powder. Preferably, bone granules are used. After processing, the surface of the processed product is cleaned with a solution to remove bone debris and residual blood and bone marrow.

将骨片加工成骨粒、骨块、骨条或骨粉，优选的，采用骨粒，加工完成后用溶液清洗加工成型产品表面的骨渣及残存的血液、骨髓。

[n0021]

Preferably, the above processing methods include cutting, stamping, crushing, etc.

作为优选，上述加工方式包括切割、冲压、粉碎等方式。

For bone strips, bone blocks, and bone granules, preferably, a cutting process or a stamping process is selected. Cutting equipment can include bone saws, oscillating saws, semi-automatic cutting machines, CNC machine tools, high-pressure water jets, etc., while stamping equipment can include stamping machines. More preferably, a bone saw is used. For bone powder, preferably, a pulverizing process is selected. Depending on the different particle sizes of the bone powder, jaw pulverizers, rotary grinders, grinding machines during cutting, disc grinders, etc. can be used. More preferably, a rotary grinder is used.

其中对于骨条、骨块、骨粒，优选的，选择切割工艺或冲压工艺，切割设备可采用锯骨机、摆锯、半自动切割机、CNC数控机床、高压水刀等，冲压设备采用冲压机，更优选的，采用锯骨机；对于骨粉，优选的，选择粉碎工艺，根据不同的骨粉粒径，可采用颚式粉碎仪、旋转式研磨仪、切割时研磨仪、盘式研磨仪等，更优选的，采用旋转式研磨仪。

[n0022]

Preferably, the above cleaning methods include high-pressure rinsing, vibration cleaning, spray cleaning or ultrasonic cleaning, etc., with high-pressure rinsing being the preferred method.

作为优选，上述清洗方式包括高压冲洗、振荡清洗、喷淋清洗或超声清洗等，优选的，采用高压冲洗。

[n0023]

Preferably, the cleaning solution includes purified water, ultrapure water, water for injection, physiological saline, phosphate buffer, etc., with purified water being the preferred choice.

作为优选，上述清洗液包括纯化水、超纯水、注射用水、生理盐水、磷酸缓冲液等，优选的，采用纯化水。

[n0024]

Preferably, the disinfection treatment, degreasing treatment, decellularization treatment, and specific demineralization treatment are as follows:

作为优选，所述的消毒处理、脱脂处理、脱细胞处理、特定脱矿处理，具体为：

[n0025]

The processed product is disinfected with disinfectant solution, then cleaned with cleaning solution. The disinfected product is degreased with degreasing solution, then cleaned with cleaning solution. The degreased product is then decellularized, then cleaned with cleaning solution. The decellularized product is then decalcified to reduce the calcium content to 10-20% by mass, and finally cleaned with cleaning solution.

用消毒液将加工成型的产品进行消毒处理，消毒处理完成后用清洗液清洗，用脱脂液将消毒后的产品进行脱脂处理，脱脂处理完成后用清洗液清洗，脱脂后的产品进行脱细胞处理，脱细胞处理完成后用清洗溶液清洗，将脱细胞处理后的产品进行脱钙处理，使产品的含钙量降低到钙的质量含量为10-20%，脱钙处理完成后用清洗液清洗。

[n0026]

Preferably, the disinfectant solution includes 75% ethanol, isopropanol, hydrogen peroxide, and potassium permanganate solution; more preferably, 75% ethanol is used.

作为优选，所述的消毒液包括75%乙醇、异丙醇、过氧化氢、高锰酸钾溶液，优选的，采用75%乙醇。

[n0027]

Preferably, the disinfection treatment method includes mechanical stirring, constant temperature shaking, and ultrasonic high-frequency oscillation, with ultrasonic high-frequency oscillation being the preferred method; the disinfection conditions are a temperature not higher than 37°C and disinfection for 1-24 hours, with disinfection for 4-8 hours being the preferred method.

作为优选，所述的消毒处理方式包括机械搅拌、恒温摇床振荡、超声高频振荡，优选的，采用超声高频振荡；所述的消毒处理条件为温度不高于37℃，消毒1-24小时，优选的，消毒4-8小时。

[n0028]

Preferably, the degreasing solution includes one or more of ethanol, sodium hydroxide, hydrogen peroxide, acetone, methanol, chloroform, petroleum ether, and n-hexane, with ethanol being the preferred choice.

作为优选，所述的脱脂液包括乙醇、氢氧化钠、过氧化氢、丙酮、甲醇、氯仿、石油醚、正己烷中的一种或多种，优选的，采用乙醇。

[n0029]

Preferably, the degreasing treatment method includes mechanical stirring, constant temperature shaking, ultrasonic high-frequency oscillation, etc., and preferably, ultrasonic high-frequency oscillation is used; the degreasing treatment conditions are a temperature not higher than 37°C and degreasing for 2-72 hours, preferably degreasing for 6-24 hours.

作为优选，所述的脱脂处理方式包括机械搅拌、恒温摇床振荡、超声高频振荡等，优选的，采用超声高频振荡；所述的脱脂处理条件为温度不高于37°C，脱脂2-72小时，优选的，脱脂6-24小时。

[n0030]

Preferably, the decellularization solution includes one or more of Tritium-X100, sodium deoxycholate, sodium dodecyl sulfate, sodium dodecylbenzene sulfonate, and sodium fatty alcohol polyoxyethylene ether sulfate. Preferably, a combination of Tritium-X100 and sodium dodecyl sulfate is used, and the mass concentration of the decellularization solution is 0.5%

-2.5%. More preferably, the mass concentration is 1.0%-2.0%. The decellularization treatment method includes mechanical stirring, constant temperature shaking, and ultrasonic high-frequency oscillation. Preferably, ultrasonic high-frequency oscillation is used.

作为优选，所述的脱细胞液包括Trition-X100、脱氧胆酸钠、十二烷基硫酸钠、十二烷基苯磺酸钠、脂肪醇聚氧乙烯醚硫酸钠中的一种或多种，，优选的，采用Trition-X100、十二烷基硫酸钠组合，脱细胞液的质量浓度为0.5%-2.5%，更优选的，采用质量浓度为1.0%-2.0%；所述的脱细胞处理方式包括机械搅拌、恒温摇床振荡、超声高频振荡，优选的，采用超声高频振荡。

[n0031]

Preferably, the decellularization treatment conditions are a temperature not exceeding 37°C and a decellularization time of 2-72 hours, with 24-36 hours being the most preferred.

作为优选，所述的脱细胞处理条件为温度不高于37°C，脱细胞2-72小时，优选的，采用24-36小时。

[n0032]

Preferably, the specific demineralization treatment uses a decalcification solution, which includes ethylenediaminetetraacetic acid (EDTA) or similar chelating agents, calcium chelating agents such as citric acid, succinic acid, and heparin, as well as formic acid, acetic acid, citric acid, propionic acid, hydrochloric acid, phosphoric acid, nitric acid, and sulfuric

acid. The decalcification solution can be used alone or in combination. Preferably, EDTA is used with a mass concentration of 2-20%, more preferably with a mass concentration of 8-15%. 17. According to claim 9, a method for preparing a natural bone repair material with specific mineralization that can regulate inflammatory metabolism is characterized in that: the demineralization treatment includes mechanical stirring, constant temperature shaking, and ultrasonic high-frequency oscillation, preferably ultrasonic high-frequency oscillation; the demineralization treatment conditions are a temperature not higher than 37°C and decalcification for 0.1-24 hours, preferably 10-60 minutes.

作为优选，所述的特定脱矿处理，采用脱钙液，脱钙液包括乙二胺四乙酸或这种螯合剂的类似物、柠檬酸、琥珀酸、肝素等钙螯合剂及甲酸、乙酸、柠檬酸、丙酸、盐酸、磷酸、硝酸、硫酸，所述脱钙液单一使用或组合使用；优选的，采用乙二胺四乙酸，质量浓度为2-20%，更优选的，采用质量浓度为8-15%17、根据权利要求9所述的一种可调控炎症代谢的特定矿化度天然骨修复材料的制备方法，其特征在于：所述的脱矿处理方式包括机械搅拌、恒温摇床振荡、超声高频振荡，优选的，采用超声高频振荡；脱矿处理条件为温度不高于37°C，脱钙0.1-24小时，优选的，采用10-60分钟。

[n0033]

Preferably, the cleaning method using the cleaning solution includes immersion cleaning, agitation cleaning, running water rinsing, spray cleaning, ultrasonic cleaning, or high-pressure water gun rinsing. Preferably, ultrasonic cleaning is used.

作为优选，所述的用清洗液清洗的方式包括浸泡清洗、振荡清洗、流水冲洗、喷淋清洗、超声清洗或者高压水枪冲洗，优选的，采用超声清洗。

[n0034]

Preferably, the cleaning solution used for cleaning includes purified water, ultrapure water, water for injection, physiological saline, and phosphate buffer. The cleaning solution can be used alone or in combination; preferably, purified water is used. The cleaning time before decalcification is 1-72 hours, preferably 6-12 hours; the cleaning time after decalcification is 6-72 hours, preferably 24-60 hours.

作为优选，所述的用清洗液清洗的清洗液包括纯化水、超纯水、注射用水、生理盐水、磷酸缓冲液，所述清洗液可以单一使用也可组合使用，优选的，采用纯化水；脱钙处理前清洗时间为1-72小时，优选的，采用6-12小时，脱钙处理完成后清洗时间为6-72小时，优选的，采用24-60小时。。

[n0035]

Preferably, the process involves freeze-drying, packaging, and sterilization, specifically:

作为优选，进行冻干和包装，进行灭菌，具体为：

[n0036]

After decalcification and cleaning with a cleaning solution, the product is placed in a freeze-drying mold and freeze-dried. The freeze-dried product is then packaged and sterilized.

将脱钙处理完成并用清洗液清洗后的产品放入冻干模具中，进行冷冻干燥操作，将冻干后的产品进行包装和灭菌。

[n0037]

Preferably, the material of the freeze-drying mold should be heat-conducting and not affect the product characteristics, including 316L stainless steel, alloy steel, medical glass, etc., with 316L stainless steel being the preferred material.

作为优选，上述冻干模具的材质应易导热、不影响产品特性，包括316L不锈钢、合金钢、医用玻璃等材质，优选的，采用316L不锈钢。

[n0038]

Preferably, the above-mentioned packaging should be non-toxic, not affect product performance, and meet sterilization requirements, including materials such as paper-plastic bags, blister packs, Tyvek paper, plastic bags, plastic bottles, and glass bottles. The above

packaging materials can be used alone or in combination. Preferably, paper-plastic bags and blister packs/Tyvek paper are used in combination, with the paper-plastic bag as the inner packaging material and the blister pack/Tyvek as the protective packaging.

作为优选，上述包装应无毒性、不影响产品性能且满足灭菌的要求，包括纸塑袋、吸塑盒、特卫强纸、塑料袋、塑料瓶、玻璃瓶等材料，以上包装材料可单独使用或组合使用，优选的，采用纸塑袋和吸塑盒/特卫强纸组合使用，纸塑袋作为内包材，吸塑盒/特卫强作为保护性包装。

[n0039]

Preferably, the sterilization method includes moist heat sterilization, ethylene oxide sterilization, or irradiation sterilization, etc., preferably, ethylene oxide sterilization is used.

作为优选，所述的灭菌方式包括湿热灭菌、环氧乙烷(灭菌或者辐照灭菌等，优选的，采用环氧乙烷灭菌。

[n0040]

Application of a natural bone repair material with specific mineralization that can regulate inflammatory metabolism in mice and human bones

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一种可调控炎症代谢的特定矿化度天然骨修复材料在小鼠及人体骨上的应用

[n0041]

This product was used to treat bone defects in mice and humans, and its ability to regulate inflammatory metabolism and promote bone repair was verified.

将本产品用于小鼠及人体骨缺损治疗，并验证其调控炎症代谢和促骨修复的能力。

[n0042]

The above verification includes material performance characterization, verification of selective permeation principle, transcriptomics and metabolomics analysis, and verification of the therapeutic effect of in vivo regulation of inflammatory metabolism.

上述验证内容包括材料性能表征、选择性渗透原理验证、转录及代谢组分析、在体调控炎症代谢治疗效果验证。

[n0043]

The beneficial effects of this invention are:

本发明的有益效果：

[n0044]

1) The present invention provides a natural bone repair material with specific mineralization that can regulate inflammatory metabolism. It has surface charged groups that can achieve selective penetration of metabolites, thereby regulating inflammation.

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1)本发明提供的一种可调控炎症代谢的特定矿化度天然骨修复材料，其具有表面带电基团，可实现代谢物的选择性渗透，从而调控炎症。

[n0045]

2) The present invention provides a natural bone repair material with specific mineralization that can regulate inflammatory metabolism, which has surface collagen residues that can promote cell adhesion.

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2)本发明提供的一种可调控炎症代谢的特定矿化度天然骨修复材料，其具有表面胶原残基，可促进细胞粘附。

[n0046]

3) The present invention provides a natural bone repair material with a specific mineralization that can regulate inflammatory metabolism, which has a significant bone repair promoting ability.

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3)本发明提供的一种可调控炎症代谢的特定矿化度天然骨修复材料，其具有显著的促骨修复能力。

[0050]

Attached Figure Description

附图说明

[n0047]

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：

[n0048]

Figure 1 shows the histological, fluorescence staining, DNA, collagen quantification, electron microscopy, and CT characterization of the natural bone repair material with specific mineralization that can regulate inflammatory metabolism in Example 1 before and after processing. The results show that the DNA content is extremely low after processing, the collagen content is almost unchanged, and the structure is well preserved, indicating that the processing process fully removes immunogenic substances and retains the target components.

图1为实施例1的可调控炎症代谢的特定矿化度天然骨修复材料在加工前后的组织学、荧光染色、DNA、胶原定量及电镜、CT表征，显示加工后DNA含量极低，胶原含量几乎不变、结构保存完好，说明加工过程充分去除免疫原性物质，并保留了目标成分。

[n0049]

Figure 2 shows the cytotoxicity test and selective permeability verification of the specific mineralization natural bone repair material with adjustable inflammatory metabolism in Example 1. It shows that cell proliferation is almost unaffected, inflammation is suppressed, and the permeation of substances such as glucose and lactic acid is selectively restricted, indicating that the product has low cytotoxicity and selective permeability of energy substances such as glucose and lactic acid as well as anti-inflammatory ability.

图2为实施例1的可调控炎症代谢的特定矿化度天然骨修复材料的细胞毒性试验及选择性透过能力验证，显示细胞增殖几乎不受影响，炎症得到抑制，同时葡萄糖、乳酸等物质的透过被选择性限制，说明产品细胞毒性低，并具有葡萄糖、乳酸等能量物质的选择性渗透能力和炎症抑制能力。

[n0050]

Figure 3 shows the metabolomics and infrared analysis of the selective permeation of the natural bone repair material with specific mineralization that can regulate inflammatory metabolism in Example 1. It shows that amide-type pro-inflammatory substances are not easily permeable, while carnitine/glycerophospholipid anti-inflammatory substances are easily permeable. The selective permeation ability of this material is concentrated on restricting the passage of positively charged substances. This ability is related to the carboxyl negatively charged groups exposed after decellularization and demineralization.

图3为实施例1的可调控炎症代谢的特定矿化度天然骨修复材料的选择性渗透的代谢组分析与红外分析，显示酰胺类促炎物质不易透过，肉碱/甘油磷脂类抗炎物易透过，本材料的选择性渗透能力集中于限制带正电物质通过，这种能力与脱细胞脱矿后暴露的羧基负电基团相关。

[n0051]

Figure 4 shows the transcriptomic-metabolic analysis of the ability of the natural bone repair material with specific mineralization that can regulate inflammatory metabolism in Example 1

to regulate cellular inflammation. It shows that inflammatory cells have metabolic characteristics of high pentose phosphate pathway, low tricarboxylic acid cycle, and low fatty acid oxidation. After the inflammatory metabolism regulation of this material based on selective permeation, the pentose phosphate pathway, tricarboxylic acid cycle, and fatty acid oxidation metabolism levels of inflammatory cells returned to normal, indicating that this material has a significant ability to regulate inflammatory metabolism.

图4为实施例1的可调控炎症代谢的特定矿化度天然骨修复材料对于细胞炎症调控能力的转录-代谢组联合分析，显示炎症细胞具有高磷酸戊糖途径、低三羧酸循环、低脂肪酸氧化的代谢特征，而经过本材料基于选择性渗透的炎症代谢调控后，炎症细胞的磷酸戊糖途径、三羧酸循环、脂肪酸氧化代谢水平恢复正常，说明本材料具有显著的炎症代谢调控能力。

[n0052]

Figure 5 shows the regulation of the transcriptional levels of inflammation-related proteins in mouse bone repair by the specific mineralization degree of the natural bone repair material with adjustable inflammatory metabolism in Example 1. It shows that the expression levels of Il-6, Nos2, Tnfa, Il1b, and Cd86 genes were significantly downregulated compared with the bone injury control group, indicating that this material has the ability to regulate bone repair inflammation at the level of animal experiments.

图5为实施例1的可调控炎症代谢的特定矿化度天然骨修复材料对小鼠骨修复的炎症相关蛋白转录水平的调控，显示Il-6、Nos2、Tnfa、Il1b、Cd86基因表达水平相对于骨损伤对照组显著下调，说明本材料具有动物试验水平的骨修复炎症调控能力。

[n0053]

Figure 6 shows the regulation of inflammation-related indicators in human bone repair by the specific mineralization degree natural bone repair material with adjustable inflammatory metabolism in Example 1. It shows that the levels of white blood cells, neutrophils and C-reactive protein at 1 week and 12 weeks after surgery were significantly lower than those of the control group medical device, indicating that the material has the ability to regulate bone repair inflammation at the human trial level.

图6为实施例1的可调控炎症代谢的特定矿化度天然骨修复材料对人体骨修复的炎症相关指标的调控，显示术后1周、12周的白细胞、中性粒细胞和C反应蛋白水平相对于对照组医疗器械均显著下降，说明本材料具有人体试验水平的骨修复炎症调控能力。

[n0054]

Figure 7 shows the bone repair promoting effect of the natural bone repair material with specific mineralization that can regulate inflammatory metabolism in Example 1 on human bone repair. It shows that the bone implant degradation rate and new bone formation rate of

the control group medical device increased significantly 12 weeks after the operation, indicating that the material has the bone repair promoting ability at the human trial level.

图7为实施例1的可调控炎症代谢的特定矿化度天然骨修复材料对人体骨修复的骨修复促进效果的分析，显示术后12周相对于对照组医疗器械的骨植入物降解率和新骨形成率显著上升，说明本材料具有人体试验水平的促骨修复能力。

[0059]

Detailed Implementation

具体实施方式

[n0055]

The following detailed description, in conjunction with embodiments, illustrates a natural bone repair material with a specific degree of mineralization that can regulate inflammatory metabolism, its preparation method, and its application, but these descriptions should not be construed as limiting the scope of protection of this invention.

下面结合实施例对本发明提供的一种可调控炎症代谢的特定矿化度天然骨修复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。

[n0056]

Example 1: Preparation of bone granules with specific mineralization that can regulate inflammatory metabolism from porcine epiphyseal bone.

实施例1、猪来源骨骺骨制备可调控炎症代谢的特定矿化度骨粒

[n0057]

Step 1: Take natural bone, cut it into bone pieces of suitable size, and clean it as follows:

步骤1、取天然骨切割成合适规格的骨片并清洗，由以下方式进行：

[n0058]

Take fresh natural pork bones, remove most of the attached bone and meat, cut the epiphysis into appropriately sized bone pieces, and clean the bone pieces with a cleaning solution to remove most of the blood and bone marrow.

取新鲜的天然猪骨，去除其上所附的大部分骨肉，将骨骺切割成合适规格的骨片，切割完成后用清洗液清洗骨片，去除骨片上大部分的血液和骨髓。

The above cutting method uses a bone saw, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述切割方式采用锯骨机，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0059]

Step 2: Process the bone fragments into different sizes and clean them, as follows:

步骤2、将骨片加工成不同的规格并清洗，由以下方式进行：

[n0060]

Bone fragments are processed into bone particles of different sizes. After processing, the bone debris, residual blood, and bone marrow on the surface of the processed product are cleaned with a solution.

将骨片加工成不同规格骨粒，加工完成后用溶液清洗加工成型产品表面的骨渣及残存的血液、骨髓。

The above processing method uses a bone saw, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述加工方式采用锯骨机，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0061]

Step 3, disinfection, degreasing, decellularization, and specific demineralization, shall be carried out in the following manner:

步骤3、消毒处理、脱脂处理、脱细胞处理、特定脱矿处理，由以下方式进行：

[n0062]

The processed product is disinfected with disinfectant solution. After disinfection, it is washed with solution. The disinfected product is degreased with degreasing solution. After degreasing, it is washed with solution. The degreased product is decellularized. After decellularization, it is washed with cleaning solution. The decellularized product is then decalcified to reduce the calcium content to the required level. After decalcification, it is washed with cleaning solution.

用消毒液将加工成型的产品进行消毒处理，消毒处理完成后用溶液清洗，用脱脂液将消毒后的产品进行脱脂处理，脱脂处理完成后用溶液清洗，脱脂后的产品进行脱细胞处理，脱细胞处理完成后用清洗溶液清洗，将脱细胞处理后的产品进行脱钙处理，使产品的含钙量降低到需要的水平，脱钙处理完成后用清洗溶液清洗。

The above disinfectant uses 75% ethanol, and the disinfection method is ultrasonic high-frequency oscillation. The disinfection conditions are a temperature of 35°C and a disinfection time of 4 hours.

上述消毒液采用75%乙醇，消毒处理方式采用超声高频振荡，消毒处理条件为温度为35℃，消毒4小时。

The above degreasing solution uses ethanol, and the degreasing treatment method is ultrasonic high-frequency oscillation. The degreasing treatment conditions are a temperature of 32°C and a degreasing time of 5 hours.

上述脱脂液采用乙醇，脱脂处理方式采用超声高频振荡，脱脂处理条件为温度为32°C，脱脂5小时。

The above decellularization solution uses a combination of Tritium-X100 and sodium dodecyl sulfate, with a concentration of 1.0%. The decellularization process employs high-frequency ultrasonic oscillation, and the decellularization conditions are a temperature of 33°C and a duration of 24 hours.

上述脱细胞液采用Trition-X100、十二烷基硫酸钠组合，脱细胞液的浓度为1.0%，脱细胞处理方式采用超声高频振荡，脱细胞处理条件为温度为33°C，脱细胞24小时。

The above decalcification solution uses ethylenediaminetetraacetic acid (EDTA) at a concentration of 2%. The decalcification treatment method is ultrasonic high-frequency oscillation. The decalcification treatment conditions are a temperature of 36°C and a decalcification time of 10 minutes. The decalcification treatment target is a calcium content of 15%.

上述脱钙液采用乙二胺四乙酸(EDTA)，浓度为2%，，脱钙处理方式采用超声高频振荡，脱钙处理条件为温度为36°C，脱钙10分钟，脱钙处理目标为钙含量15%。

The above cleaning method uses ultrasonic cleaning, purified water as the cleaning solution, and the cleaning time is 6 hours.

上述清洗方式采用超声清洗，清洗液采用纯化水，清洗时间为采用6小时。

[n0063]

Step 4, final cleaning, is performed as follows:

步骤4、末道清洗，由以下方式进行：

[n0064]

The decalcified product is then rinsed with a solution until all reagent residues reach the specified limits.

脱钙处理后的产品用溶液进行末道清洗，直至所有试剂的残留达到规定的限量。

The above cleaning method uses ultrasonic cleaning, the cleaning solution uses purified water, and the cleaning time is 24 hours.

上述清洗方式采用超声清洗，上述清洗液采用纯化水，清洗时间采用24小时。

[n0065]

Step 5: Perform freeze-drying and packaging, followed by sterilization, using the following methods:

步骤5、进行冻干和包装，进行灭菌，由以下方式进行：

[n0066]

The cleaned product is placed in a freeze-drying mold and freeze-dried. The freeze-dried product is then packaged and sterilized.

将清洗后的产品放入冻干模具中，进行冷冻干燥操作，将冻干后的产品进行包装和灭菌。

The freeze-drying molds mentioned above are made of 316L stainless steel.

上述冻干模具采用316L不锈钢。

The above packaging uses a combination of paper-plastic bags and blister packs/Tyvem paper, with the paper-plastic bags serving as inner packaging and the blister packs/Tyvem as protective packaging.

上述包装采用纸塑袋和吸塑盒/特卫强纸组合使用，纸塑袋作为内包材，吸塑盒/特卫强作为保护性包装。

The above sterilization methods include sterilization with ethylene oxide.

上述灭菌方式包括采用环氧乙烷灭菌。

[n0067]

Example 2: Preparation of specific mineralization bone filaments from porcine cortical bone that can regulate inflammatory metabolism

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实施例2、猪来源骨皮质骨制备可调控炎症代谢的特定矿化度骨丝

[n0068]

Step 1: Take natural bone, cut it into bone pieces of suitable size, and clean it as follows:

步骤1、取天然骨切割成合适规格的骨片并清洗，由以下方式进行：

[n0069]

Take fresh natural pork bones, remove most of the attached bone and meat, cut the skin bone into bone pieces of appropriate size, and clean the bone pieces with cleaning solution to remove most of the blood and bone marrow.

取新鲜的天然猪骨，去除其上所附的大部分骨肉，将皮质骨切割成合适规格的骨片，切割完成后用清洗液清洗骨片，去除骨片上大部分的血液和骨髓。

The above cutting method uses a bone saw, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述切割方式采用锯骨机，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0070]

Step 2: Process the bone fragments into different sizes and clean them, as follows:

步骤2、将骨片加工成不同的规格并清洗，由以下方式进行：

[n0071]

Bone fragments are processed into bone filaments of different specifications, with a length of about 1 mm to about 100 mm, a width of about 0.1 mm to about 10 mm, and a thickness of about 0.05 mm to about 1.5 mm. After processing, bone debris and residual blood and bone marrow are removed from the surface of the processed product by cleaning with a solution.

将骨片加工成不同规格骨丝，长度约1mm至约100mm、宽度约0.1mm至约10mm，厚度约0.05mm至约1.5mm，加工完成后用溶液清洗加工成型产品表面的骨渣及残存的血液、骨髓。

The above processing method uses a bone saw, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述加工方式采用锯骨机，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0072]

Step 3, disinfection, degreasing, and specific demineralization treatment, shall be carried out in the following manner:

步骤3、消毒处理、脱脂处理、特定脱矿处理，由以下方式进行：

[n0073]

The processed product is disinfected with disinfectant solution. After disinfection, it is cleaned with solution. The disinfected product is then degreased with degreasing solution. After degreasing, it is cleaned with solution. The degreased product is then decalcified to reduce the calcium content to the required level. After decalcification, it is cleaned with cleaning solution.

用消毒液将加工成型的产品进行消毒处理，消毒处理完成后用溶液清洗，用脱脂液将消毒后的产品进行脱脂处理，脱脂处理完成后用溶液清洗，脱脂后的产品进行脱钙处理，使产品的含钙量降低到需要的水平，脱钙处理完成后用清洗溶液清洗。

The above disinfectant uses 75% ethanol, and the disinfection method is ultrasonic high-frequency oscillation. The disinfection conditions are a temperature not higher than 37°C and a disinfection time of 8 hours.

上述消毒液采用75%乙醇，消毒处理方式采用超声高频振荡，消毒处理条件为温度不高于37°C，消毒8小时。

The above degreasing solution uses ethanol and sodium hydroxide, and the degreasing treatment method is ultrasonic high-frequency oscillation. The degreasing treatment conditions are a temperature of 30°C and a degreasing time of 24 hours. The above decalcifying solution uses hydrochloric acid, and the decalcification treatment method is ultrasonic high-frequency oscillation. The decalcification treatment conditions are a temperature of 35°C and a decalcification time of 50 minutes. The decalcification treatment target is a calcium content of 16%.

上述脱脂液采用乙醇和氢氧化钠，脱脂处理方式采用超声高频振荡，脱脂处理条件为温度为30°C，脱脂24小时；上述脱钙液采用盐酸，脱钙处理方式采用超声高频振荡，脱钙处理条件为温度为35°C，脱钙50分钟，脱钙处理目标为钙含量16%。

The above cleaning method uses ultrasonic cleaning, purified water as the cleaning solution, and the cleaning time is 12 hours.

上述清洗方式采用超声清洗，清洗液采用纯化水，清洗时间为采用12小时。

[n0074]

Step 4, final cleaning, is performed as follows:

步骤4、末道清洗，由以下方式进行：

[n0075]

The decalcified product is then rinsed with a solution until all reagent residues reach the specified limits.

脱钙处理后的产品用溶液进行末道清洗，直至所有试剂的残留达到规定的限量。

The above cleaning method uses ultrasonic cleaning, the cleaning solution uses purified water, and the cleaning time is 60 hours.

上述清洗方式采用超声清洗，上述清洗液采用纯化水，清洗时间采用60小时。

[n0076]

Step 5: Perform freeze-drying and packaging, followed by sterilization, using the following methods:

步骤5、进行冻干和包装，进行灭菌，由以下方式进行：

[n0077]

The cleaned product is placed in a freeze-drying mold and freeze-dried. The freeze-dried product is then packaged and sterilized.

将清洗后的产品放入冻干模具中，进行冷冻干燥操作，将冻干后的产品进行包装和灭菌。

The freeze-drying molds mentioned above are made of 316L stainless steel.

上述冻干模具采用316L不锈钢。

The above packaging uses medical packaging bottles.

上述包装采用使用医用包装瓶。

[n0078]

Example 3: Preparation of bone powder with specific mineralization that can regulate inflammatory metabolism from human-derived cortical bone.

实施例3、人来源骨皮质骨制备可调控炎症代谢的特定矿化度骨粉

[n0079]

Step 1: Take natural bone, cut it into bone pieces of suitable size, and clean it as follows:

步骤1、取天然骨切割成合适规格的骨片并清洗，由以下方式进行：

[n0080]

Take fresh, natural human bones, remove most of the attached flesh and bone, cut the bone into appropriately sized pieces, and clean the bone pieces with a cleaning solution to remove most of the blood and bone marrow.

取新鲜的天然人骨，去除其上所附的大部分骨肉，将皮质骨切割成合适规格的骨片，切割完成后用清洗液清洗骨片，去除骨片上大部分的血液和骨髓。

The above cutting method uses a bone saw, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述切割方式采用锯骨机，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0081]

Step 2: Process the bone fragments into different sizes and clean them, as follows:

步骤2、将骨片加工成不同的规格并清洗，由以下方式进行：

[n0082]

Bone fragments are processed into bone powder of different specifications, with a particle size of 1um-1mm. After processing, the bone debris, residual blood, and bone marrow on the surface of the processed product are cleaned with a solution.

将骨片加工成不同规格骨粉，粒径1um-1mm，加工完成后用溶液清洗加工成型产品表面的骨渣及残存的血液、骨髓。

The above processing method uses a rotary grinder, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述加工方式采用旋转研磨仪，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0083]

Step 3, disinfection, degreasing, and specific demineralization treatment, shall be carried out in the following manner:

步骤3、消毒处理、脱脂处理、特定脱矿处理，由以下方式进行：

[n0084]

The processed product is disinfected with disinfectant solution. After disinfection, it is cleaned with solution. The disinfected product is then degreased with degreasing solution. After degreasing, it is cleaned with solution. The degreased product is then decalcified to reduce the calcium content to the required level. After decalcification, it is cleaned with cleaning solution.

用消毒液将加工成型的产品进行消毒处理，消毒处理完成后用溶液清洗，用脱脂液将消毒后的产品进行脱脂处理，脱脂处理完成后用溶液清洗，脱脂后的产品进行脱钙处理，使产品的含钙量降低到需要的水平，脱钙处理完成后用清洗溶液清洗。

The above disinfectant uses 75% ethanol, and the disinfection method is ultrasonic high-frequency oscillation. The disinfection conditions are a temperature of 37°C and a disinfection time of 6 hours.

上述消毒液采用75%乙醇，消毒处理方式采用超声高频振荡，消毒处理条件为温度为37℃，消毒6小时。

The above degreasing solution uses ethanol, and the degreasing treatment method is ultrasonic high-frequency oscillation. The degreasing treatment conditions are a temperature of 36℃ and a degreasing time of 12 hours.

上述脱脂液采用乙醇，脱脂处理方式采用超声高频振荡，脱脂处理条件为温度为36℃，脱脂12小时。

The above decalcification solution uses hydrochloric acid, the decalcification treatment method uses ultrasonic high-frequency oscillation, the decalcification treatment conditions are a temperature of 36℃, decalcification time of 60 minutes, and the decalcification treatment target is a calcium content of 18%.

上述脱钙液采用盐酸，脱钙处理方式采用超声高频振荡，脱钙处理条件为温度为36℃，脱钙60分钟，脱钙处理目标为钙含量18%。

The above cleaning method uses ultrasonic cleaning, purified water as the cleaning solution, and the cleaning time is 8 hours.

上述清洗方式采用超声清洗，清洗液采用纯化水，清洗时间为采用8小时。

[n0085]

Step 4, final cleaning, is performed as follows:

步骤4、末道清洗，由以下方式进行：

[n0086]

The decalcified product is then rinsed with a solution until all reagent residues reach the specified limits.

脱钙处理后的产品用溶液进行末道清洗，直至所有试剂的残留达到规定的限量。

The above cleaning method uses ultrasonic cleaning, the cleaning solution uses purified water, and the cleaning time is 30 hours.

上述清洗方式采用超声清洗，上述清洗液采用纯化水，清洗时间采用30小时。

[n0087]

Step 5: Perform freeze-drying and packaging, followed by sterilization, using the following methods:

步骤5、进行冻干和包装，进行灭菌，由以下方式进行：

[n0088]

The cleaned product is placed in a freeze-drying mold and freeze-dried. The freeze-dried product is then packaged and sterilized.

将清洗后的产品放入冻干模具中，进行冷冻干燥操作，将冻干后的产品进行包装和灭菌。

The freeze-drying molds mentioned above are made of 316L stainless steel.

上述冻干模具采用316L不锈钢。

The above packaging uses medical packaging bottles.

上述包装采用使用医用包装瓶。

[n0089]

Example 4: Preparation of bone granules with specific mineralization from porcine epiphyseal bone that can regulate inflammatory metabolism

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实施例4、猪来源骨骺骨制备可调控炎症代谢的特定矿化度骨粒

[n0090]

Step 1: Take natural bone, cut it into bone pieces of suitable size, and clean it as follows:

步骤1、取天然骨切割成合适规格的骨片并清洗，由以下方式进行：

[n0091]

Take fresh natural pork bones, remove most of the attached bone and meat, cut the epiphysis into appropriately sized bone pieces, and clean the bone pieces with a cleaning solution to remove most of the blood and bone marrow.

取新鲜的天然猪骨，去除其上所附的大部分骨肉，将骨骺切割成合适规格的骨片，切割完成后用清洗液清洗骨片，去除骨片上大部分的血液和骨髓。

The above cutting method uses a bone saw, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述切割方式采用锯骨机，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0092]

Step 2: Process the bone fragments into different sizes and clean them, as follows:

步骤2、将骨片加工成不同的规格并清洗，由以下方式进行：

[n0093]

Bone fragments are processed into bone particles of different sizes. After processing, the bone debris, residual blood, and bone marrow on the surface of the processed product are cleaned with a solution.

将骨片加工成不同规格骨粒，加工完成后用溶液清洗加工成型产品表面的骨渣及残存的血液、骨髓。

The above processing method uses a bone saw, the cleaning method uses high-pressure rinsing, and the cleaning solution uses purified water.

上述加工方式采用锯骨机，清洗方式采用高压冲洗，清洗液采用纯化水。

[n0094]

Step 3, disinfection, degreasing, decellularization, and specific demineralization, shall be carried out in the following manner:

步骤3、消毒处理、脱脂处理、脱细胞处理、特定脱矿处理，由以下方式进行：

[n0095]

The processed product is disinfected with disinfectant solution. After disinfection, it is washed with solution. The disinfected product is degreased with degreasing solution. After degreasing, it is washed with solution. The degreased product is decellularized. After decellularization, it is washed with cleaning solution. The decellularized product is then decalcified to reduce the calcium content to the required level. After decalcification, it is washed with cleaning solution.

用消毒液将加工成型的产品进行消毒处理，消毒处理完成后用溶液清洗，用脱脂液将消毒后的产品进行脱脂处理，脱脂处理完成后用溶液清洗，脱脂后的产品进行脱细胞处理，脱细胞处理完成后用清洗溶液清洗，将脱细胞处理后的产品进行脱钙处理，使产品的含钙量降低到需要的水平，脱钙处理完成后用清洗溶液清洗。

The above disinfectant uses isopropanol, and the disinfection method is constant temperature shaking. The disinfection conditions are a temperature of 35°C and a disinfection time of 24 hours.

上述消毒液采用异丙醇，消毒处理方式采用恒温摇床振荡，消毒处理条件为温度为35°C，消毒24小时。

The above degreasing solution uses ethanol and sodium hydroxide. The degreasing treatment method is mechanical stirring. The degreasing treatment conditions are a temperature of 35°C and a degreasing time of 70 hours.

上述脱脂液采用乙醇和氢氧化钠，脱脂处理方式采用机械搅拌，脱脂处理条件为温度为35°C，脱脂70小时。

The decellularization solution used was sodium dodecylbenzenesulfonate with a concentration of 2.5%. The decellularization process employed high-frequency ultrasonic oscillation at a temperature of 35°C for 72 hours.

上述脱细胞液采用十二烷基苯磺酸钠，脱细胞液的浓度为2.5%，脱细胞处理方式采用超声高频振荡，脱细胞处理条件为温度为35°C，脱细胞72小时。

The above decalcification solution uses ethylenediaminetetraacetic acid (EDTA) at a concentration of 20%. The decalcification treatment method is ultrasonic high-frequency oscillation. The decalcification treatment conditions are a temperature of 36°C and a decalcification time of 20 hours. The decalcification treatment target is a calcium content of 10%.

上述脱钙液采用乙二胺四乙酸(EDTA)，浓度为20%，，脱钙处理方式采用超声高频振荡，脱钙处理条件为温度为36°C，脱钙20小时，脱钙处理目标为钙含量10%。

The above cleaning method uses ultrasonic cleaning, purified water as the cleaning solution, and the cleaning time is 8 hours.

上述清洗方式采用超声清洗，清洗液采用纯化水，清洗时间为采用8小时。

[n0096]

Step 4, final cleaning, is performed as follows:

步骤4、末道清洗，由以下方式进行：

[n0097]

The decalcified product is then rinsed with a solution until all reagent residues reach the specified limits.

脱钙处理后的产品用溶液进行末道清洗，直至所有试剂的残留达到规定的限量。

The above cleaning method uses ultrasonic cleaning, the cleaning solution uses purified water, and the cleaning time is 60 hours.

上述清洗方式采用超声清洗，上述清洗液采用纯化水，清洗时间采用60小时。

[n0098]

Step 5: Perform freeze-drying and packaging, followed by sterilization, using the following methods:

步骤5、进行冻干和包装，进行灭菌，由以下方式进行：

[n0099]

The cleaned product is placed in a freeze-drying mold and freeze-dried. The freeze-dried product is then packaged and sterilized.

将清洗后的产品放入冻干模具中，进行冷冻干燥操作，将冻干后的产品进行包装和灭菌。

The freeze-drying molds mentioned above are made of 316L stainless steel.

上述冻干模具采用316L不锈钢。

The above packaging uses a combination of paper-plastic bags and blister packs/Tyvem paper, with the paper-plastic bags serving as inner packaging and the blister packs/Tyvem as protective packaging.

上述包装采用纸塑袋和吸塑盒/特卫强纸组合使用，纸塑袋作为内包材，吸塑盒/特卫强作为保护性包装。

The above sterilization methods include sterilization with ethylene oxide.

上述灭菌方式包括采用环氧乙烷灭菌。

[n0100]

Histological, fluorescence staining, DNA, collagen quantification, electron microscopy, and CT characterization of the natural bone repair material with specific mineralization that can regulate inflammatory metabolism before and after processing in Example 1.

实施例1中的可调控炎症代谢的特定矿化度天然骨修复材料在加工前后的组织学、荧光染色、DNA、胶原定量及电镜、CT表征

[n0101]

1. Perform tissue sections on the materials before and after processing, and stain them with H&E, DAPI, Masson, and collagen I respectively.

1、将加工前后的材料进行组织切片，分别进行H&E染色、DAPI染色、Masson染色、胶原I染色。

[n0102]

2. The DNA content and collagen I content of the materials before and after processing were measured respectively.

2、将加工前后的材料分别测定DNA含量及胶原I含量

[n0103]

3. Perform scanning electron microscopy and Micro-CT scans on the materials before and after processing, respectively.

3、将加工前后的材料分别进行扫描电镜拍摄及Micro-CT检测。

[n0104]

4. The results showed that the DNA content of the material was extremely low after processing, the collagen content remained almost unchanged, and the structure was well preserved, indicating that the processing effectively removed immunogenic substances and retained the target components.

4、结果显示材料加工后DNA含量极低，胶原含量几乎不变、结构保存完好，说明加工过程充分去除免疫原性物质，并保留了目标成分。

As shown in Figure 1.

如图1。

[n0105]

Cytotoxicity test and selective permeability verification of the natural bone repair material with specific mineralization that can regulate inflammatory metabolism in Example 1

实施例1中的可调控炎症代谢的特定矿化度天然骨修复材料的细胞毒性试验及选择性透过能力验证

[n0106]

1. Add the material extract to the macrophage cell culture medium, measure the cell proliferation within one week and observe it under a fluorescence microscope, and measure the glucose and lactic acid content in the supernatant.

1、将材料浸提液加入巨噬细胞细胞培养基，测定一周内细胞增殖情况并进行荧光显微镜观察，测定上清葡萄糖及乳酸含量。

[n0107]

2. Place the materials on the Transwell chamber and measure the upper chamber concentrations of GSH, Glucose, Spemidine, and Lactate, respectively.

2、将材料铺设于Transwell小室上，分别测定GSH、Glucose、Spemidine、Lactate的上室浓度。

[n0108]

3. The results showed that cell proliferation was almost unaffected, inflammation was suppressed, and the permeation of substances such as glucose and lactic acid was selectively restricted, indicating that the product has low cytotoxicity and selective permeation ability of energy substances such as glucose and lactic acid, as well as anti-inflammatory ability.

3、结果显示细胞增殖几乎不受影响，炎症得到抑制，同时葡萄糖、乳酸等物质的透过被选择性限制，说明产品细胞毒性低，并具有葡萄糖、乳酸等能量物质的选择性渗透能力和炎症抑制能力。As shown in Figure 2.

如图2。

[n0109]

Metabolomics and Infrared Analysis of Selective Permeation of Natural Bone Repair Material with Specific Mineralization that Can Regulate Inflammatory Metabolism in Example 1

实施例1中的可调控炎症代谢的特定矿化度天然骨修复材料的选择性渗透的代谢组分析与红外分析

[n0110]

1. Metabolite analysis was performed on the supernatant of the material, and metabolites with different properties were clustered.

1、将透过材料的上清进行代谢物分析，对不同性质代谢物进行聚类。

[n0111]

2. Analyze the ionization ability, charge properties, molecular weight, and other indicators of each cluster.

2、分析各个簇的电离能力、电荷性质、分子量大小等指标。

[n0112]

3. Perform infrared spectroscopy on the material to analyze the main functional groups.

3、对材料进行红外光谱检测，分析主要作用基团。

[n0113]

4. The results show that amide-based pro-inflammatory substances are not easily permeable, while carnitine/glycerophospholipid-based anti-inflammatory substances are easily permeable. The selective permeability of this material is concentrated on restricting the passage of positively charged substances. This ability is related to the carboxyl negatively charged groups exposed after decellularization and demineralization.

4、显示酰胺类促炎物质不易透过，肉碱/甘油磷脂类抗炎物易透过，本材料的选择性渗透能力集中于限制带正电物质通过，这种能力与脱细胞脱矿后暴露的羧基负电基团相关。

As shown in Figure 3.

如图3。

[n0114]

Transcriptional-metabolic analysis of the ability of the specific mineralization-based natural bone repair material with modulatory inflammatory metabolism in Example 1 to regulate cellular inflammation.

实施例1中的可调控炎症代谢的特定矿化度天然骨修复材料对于细胞炎症调控能力的转录-代谢组联合分析

[n0115]

1. RNA was extracted from inflammatory cells exposed to the material, and their transcriptome changes were analyzed.

1、将材料作用下的炎症细胞进行RNA抽提，分析其转录组变化。

[n0116]

2. Metabolic mass spectrometry was performed on inflammatory cells under the influence of the material to analyze their metabolomic changes.

2、将材料作用下的炎症细胞进行代谢物质谱检测，分析其代谢组变化。

[n0117]

3. Combine transcriptomics and metabolomics analysis to describe changes in overall cellular metabolic pathways.

3、将转录组与代谢组进行联合分析，描述细胞整体代谢通路变化。

[n0118]

4. The study showed that inflammatory cells exhibited metabolic characteristics of high pentose phosphate pathway, low tricarboxylic acid cycle, and low fatty acid oxidation. However, after the inflammatory metabolism was regulated by this material based on selective osmosis, the levels of pentose phosphate pathway, tricarboxylic acid cycle, and fatty acid oxidation in inflammatory cells returned to normal, indicating that this material has a significant ability to regulate inflammatory metabolism.

4、显示炎症细胞具有高磷酸戊糖途径、低三羧酸循环、低脂肪酸氧化的代谢特征，而经过本材料基于选择性渗透的炎症代谢调控后，炎症细胞的磷酸戊糖途径、三羧酸循环、脂肪酸氧化代谢水平恢复正常，说明本材料具有显著的炎症代谢调控能力。

As shown in Figure 4.

如图4。

[n0119]

The regulation of the transcriptional level of inflammation-related proteins in mouse bone repair by a specific mineralization-based natural bone repair material capable of modulating inflammatory metabolism in Example 1.

实施例1中的可调控炎症代谢的特定矿化度天然骨修复材料对小鼠骨修复的炎症相关蛋白转录水平的调控

[n0120]

1. Construct a mouse bone defect model and implant the materials and control instruments into the mouse bone defects.

1、构建小鼠骨缺损模型，将材料及对照器械材料植入小鼠骨缺损。

[n0121]

2. Cells were extracted from bone defect sites in mice, RNA was extracted, and qPCR detection of inflammation-related genes was performed.

2、提取小鼠骨缺损部位细胞，提取RNA并进行炎症相关基因qPCR检测。

[n0122]

3. The expression levels of Il-6, Nos2, Tnfa, Il1b, and Cd86 genes were significantly downregulated compared to the bone injury control group, indicating that this material has the ability to regulate bone repair and inflammation at the level of animal experiments.

3、显示Il-6、Nos2、Tnfa、Il1b、Cd86基因表达水平相对于骨损伤对照组显著下调，说明本材料具有动物试验水平的骨修复炎症调控能力。

Figure 5.

如图5。

[n0123]

The regulation of inflammation-related indicators in human bone repair by a specific mineralization-based natural bone repair material with modifiable inflammatory metabolism in Example 1.

实施例1中的可调控炎症代谢的特定矿化度天然骨修复材料对人体骨修复的炎症相关指标的调控

[n0124]

1. Conduct clinical trials, implanting the material and control device material into the bone defect site of the patient.

1、进行临床试验，将材料及对照器械材料植入患者骨缺损部位。

[n0125]

2. Continuously monitor the patient's peripheral blood leukocytes, neutrophils, CRP and other inflammation-related indicators.

2、持续监测患者外周血白细胞、中性粒细胞、CRP等炎症相关指标。

[n0126]

3. The results showed that the levels of white blood cells, neutrophils, and C-reactive protein were significantly lower at 1 week and 12 weeks post-operation compared to the control group of medical devices, indicating that this material has the ability to regulate bone repair and inflammation at the level of human trials.

3、显示术后1周、12周的白细胞、中性粒细胞和C反应蛋白水平相对于对照组医疗器械均显著下降，说明本材料具有人体试验水平的骨修复炎症调控能力。

Figure 6.

如图6。

[n0127]

Analysis of the bone repair promoting effect of the specific mineralization degree natural bone repair material with modifiable inflammatory metabolism in Example 1 on human bone repair.

实施例1中的可调控炎症代谢的特定矿化度天然骨修复材料对人体骨修复的骨修复促进效果的分析

[n0128]

1. Conduct clinical trials, implanting the material and control device material into the bone defect site of the patient.

1、进行临床试验，将材料及对照器械材料植入患者骨缺损部位。

[n0129]

2. CT data of patients will be analyzed 12 weeks after surgery to detect bone repair.

2、术后12周对患者CT数据进行分析，检测骨修复情况。

[n0130]

3. The results showed that 12 weeks post-operation, the bone implant degradation rate and new bone formation rate of the medical devices in the control group were significantly higher, indicating that the material has the bone repair promotion capacity at the human trial level.

3、显示术后12周相对于对照组医疗器械的骨植入物降解率和新骨形成率显著上升，说明本材料具有人体试验水平的促骨修复能力。

Figure 7.

如图7。

[n0131]

The specific mineralization natural bone repair materials with modifiable inflammatory metabolism obtained in Examples 2 and 3 were subjected to histological, fluorescence staining, DNA, collagen quantification and electron microscopy, CT characterization, cytotoxicity test and selective permeability verification, selective permeation metabolomics analysis and infrared analysis, transcriptomic-metabolic combined analysis of the ability to regulate cellular inflammation, and detection of the regulation of the transcriptional level of inflammation-related proteins in mouse bone repair. They have similar technical and biological effects to the specific mineralization bone particles with modifiable inflammatory metabolism prepared from porcine epiphyseal bone in Example 1. This indicates that specific mineralization natural bone repair materials with modifiable inflammatory metabolism can be prepared and applied through other bone tissue sources, types, specifications and other processing methods.

对实施例2、3所得的可调控炎症代谢的特定矿化度天然骨修复材料分别进行组织学、荧光染色、DNA、胶原定量及电镜、CT表征、细胞毒性试验及选择性透过能力验证、选择性渗透的代谢组分析与红外分析、对于细胞炎症调控能力的转录-代谢组联合分析、对小鼠骨修复的炎症相关蛋白转录水平的调控检测，与实施例1中猪来源骨骺骨制备可调控炎症代谢的特定矿化度骨粒具有相似的技术效

果和生物效果，这表明可通过上述其他骨组织来源、类型、规格及其他加工方式，可调控炎症代谢的特定矿化度天然骨修复材料的制备和应用。

[n0132]

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。