Notice

DESCRIPTION CN105879120A

A method for preparing a naturally derived tendon and bone decellularized material

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一种天然组织来源的肌腱联合骨脱细胞材料的制备方法

[0001]

Technical Field

技术领域

[0002]

This invention relates primarily to the biological field for tissue or organ repair and regeneration, specifically a method for preparing tendon-bone composite materials derived from natural tissues.

本发明主要涉及用于组织或器官修复及其再生的生物领域，具体是一种天然组织来源的肌腱联合骨材料的制备方法。

[0003]

Background Technology

背景技术

[0004]

Tendon tears are a common condition that often occurs during high-intensity exercise.

肌腱撕裂是一种常见病，常在高强度的运动中发生。

The patients are mostly young people, especially athletes.

患者多为年轻人，以运动员中尤为常见。

It can cause tendon damage, leading to a decline in athletic ability, and in severe cases, even loss of motor function, causing great inconvenience to daily life.

其会造成肌腱损伤，从而造成运动能力下降，严重的甚至会造成运动功能丧失，对生活带来极大不便。

Tears between bones and tendons are even more severe. There is currently no effective surgical treatment for this type of tear. After conventional suturing, the body's natural repair often results in the degeneration of the fibrocartilage layer between the tendon and bone, and scar tissue with poor mechanical properties is used instead. The chance of recurrence after surgery is high, and patients' normal activities are greatly restricted.

而骨和肌腱间的撕裂则更为严重。 现有的手术治疗对于此类撕裂尚无有效的方案，常规缝合后自然机体修复往往发生肌腱和骨之间的纤维软骨层退化，取之以力学性能较差的瘢痕组织。 术后再断的几率高，患者的正常活动受到较大限制。

[0005]

The emergence of regenerative medicine and tissue engineering technologies has brought new hope for the treatment of tendon and bone tears.

再生医学和组织工程技术的出现给了肌腱和骨间撕裂的治疗带来了新的希望。

However, due to the complex structure between tendons, it is difficult to simulate them using traditional biomaterials, and there are also problems with poor biocompatibility.

然而由于肌腱和腱之间的结构复杂，用传统的生物材料模拟难度高，且存在生物相容性差的问题。

[0006]

The extracellular matrix (ECM) contains various biochemical factors required by normal tissue or organ cells and has a natural macroscopic and ultramicro three-dimensional structure.

细胞外基质(ECM)含有正常组织或器官细胞所需的各种生化因子，并且具有天然宏观及超微三维的立体结构。

ECM can regulate a range of factors required for the development and repair of tissues or organs, including biophysical stimulation, biochemical and molecular signals, thereby achieving the recovery and regeneration of tissues or organs. Therefore, ECM, as a novel natural biomaterial, is being widely used in the repair and regeneration of a range of tissues

and organs, such as heart valves, trachea, muscles, tendons, and cartilage. Currently, research on decellularized materials for tendon-bonded bone is still in its early stages. Existing methods for preparing tendon-bonded bone materials do not completely remove cells, resulting in severe immune reactions and significant damage to the ECM, leading to unsatisfactory mechanical properties.

ECM可调节生物物理刺激、生物化学及分子信号等一系列组织或器官发育及修复所需的各种因素，从而实现组织或器官的恢复和再生。 因此ECM作为一个新型的天然生物材料正被广泛的运用于心脏瓣膜、气管、肌肉、肌腱、软骨等一系列组织或器官的修复及再生。 而目前针对肌腱联合骨的脱细胞材料均处于研究的起步阶段，现有报道中制备肌腱联合骨材料的方法，细胞去除不彻底，材料免疫反应较严重，且对ECM的破坏大，材料力学性能均不理想。

[0007]

Summary of the Invention

发明内容

[0008]

This invention provides a novel method for preparing decellularized tendon-bone composite material to overcome the shortcomings of existing methods. It can be used to prepare tendon-

bone composite extracellular matrix with complete removal of immunogenic substances such as allogeneic cells, intact structure, and well-preserved bioactive components and mechanical properties.

本发明提供一种新型的肌腱连骨脱细胞材料的制备方法，以弥补现有文献中方法的不足，可用以制备异体细胞等免疫原性物质去除完全，结构完整，生物活性成分和力学性能保存完好的肌腱联合骨细胞外基质。

[0009]

Physiological saline containing protease inhibitors, wherein the concentration of protease inhibitors is 10-50 KIU/ml;

含蛋白酶抑制剂的生理盐水，其中蛋白酶抑制剂浓度为10-50KIU/ml；

[0010]

PBS buffer containing protease inhibitors, wherein the concentration of protease inhibitors is 10-50 KIU/ml;

含蛋白酶抑制剂的PBS缓冲液，其中蛋白酶抑制剂浓度为10-50KIU/ml；

[0011]

PBS buffer containing Triton X, specifically Triton X-200 or Triton X-100 PBS buffer with a volume concentration of 1%-12%;

含Triton X的PBS缓冲液，体积浓度为1%-12%的Triton X-200或TritonX-100的PBS缓冲液；

[0012]

PBS buffer containing SDS, with a volume concentration of 0.5%-8% SDS;

含SDS的PBS缓冲液，体积浓度为0.5%-8%的SDS的PBS缓冲液；

[0013]

The concentration of DNase in PBS buffer containing DNase is 0.5-2 mg/ml;

含DNA酶的PBS缓冲液中DNA酶浓度为0.5-2mg/ml；

[0014]

The concentrations of penicillin and streptomycin in the mixed antibacterial solution were 20 KIU/ml and 20 KIU/ml, respectively, with a penicillin to streptomycin ratio of 1:1.

混合抗菌液中青霉素和链霉素的浓度分别为20KIU/ml、20KIU/ml，青霉素和链霉素的比例为1:1。

The volume ratio of the added mixed antibacterial solution to the original solution is 1:1.

加入的混合抗菌液与原溶液体积比为1:1。

[0015]

The preparation method of the above-mentioned natural tissue-derived muscle-bone material includes the following steps:

上述天然组织来源的肌联合骨材料的制备方法，包括以下步骤：

[0016]

(1) Take the Achilles tendon and calcaneus of any mammal slaughtered within 12 hours, remove the fat on the Achilles tendon with scissors, rinse it three times with physiological saline containing protease inhibitors for 20 minutes each time to remove blood, attached fat fragments and other impurities.

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(1)取宰杀12h以内任意哺乳动物的跟腱连跟骨，用剪刀除去跟腱上的脂肪，用含蛋白酶抑制剂的生理盐水漂洗3次，每次20min，去除血液，贴附的脂肪碎片和其他杂质。

[0017]

(2) In PBS buffer containing protease inhibitor, shake at 150 rpm for 24-48 hours at 4°C on a low-temperature shaker;

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(2)在含蛋白酶抑制剂的PBS缓冲液中，低温摇床150rpm震荡24-48小时，温度为4°C；

[0018]

(3) Add the mixed antibacterial solution to the PBS buffer containing Triton X, and shake on a low-temperature shaker at 150 rpm for 24-48 hours at 4°C.

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(3)在含Triton X的PBS缓冲液中，加入混合抗菌液，低温摇床150rpm震荡24-48小时，温度为4°C；

[0019]

(4) In PBS buffer containing SDS, shake at 150 rpm for 24-72 hours at a low temperature of 4°C.

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(4)在含SDS的PBS缓冲液中，低温摇床150rpm震荡24-72小时，温度为4°C；

[0020]

(5) Add the mixed antibacterial solution to the PBS buffer containing Triton X-100 and shake at 150 rpm for 7-16 days at a low temperature of 4°C; (6) Shake at 150 rpm for 2-20 days in the PBS buffer containing SDS at a low temperature of 4°C.

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(5)在含Triton X-100的PBS缓冲液中，加入混合抗菌液，低温摇床150rpm震荡7-16天，温度为4°C；(6)在含SDS的PBS缓冲液中，低温摇床150rpm震荡2-20天，温度为4°C；

[0021]

(7) Add penicillin and streptomycin mixed antibacterial solution to PBS buffer containing DNase, with a concentration of 20 KIU/ml, and shake at 150 rpm for 12 hours at 37°C.

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(7)在含DNA酶的PBS缓冲液中，加入青霉素和链霉素混合抗菌液，浓度分别为20KIU/ml，37°C摇床150rpm震荡12小时；

[0022]

In steps (2)-(6), rinse with saline solution for 5 hours after each step is completed.

步骤(2)-(6)中，每个步骤完成后均用生理盐水冲洗5小时。

[0023]

The above preparation method yields acellular tendon and bone material derived from natural tissue.

上述制备方法得到的天然组织来源的肌腱联合骨脱细胞材料。

[0024]

To address the problems existing in current materials and preparation methods used for tendon and bone repair and regeneration, the inventors have established a method for preparing decellularized tendon and bone material derived from natural tissue. This method involves treating mammalian Achilles tendon and bone tissue of any size with physiological saline containing protease inhibitors, PBS containing protease inhibitors, PBS buffer containing Triton X, PBS buffer containing SDS, and PBS buffer containing DNase to obtain decellularized tendon and bone material.

针对目前用于肌腱联合骨修复和再生的材料及其制备方法存在的问题，发明人建立了一种天然组织来源的肌腱联合骨脱细胞材料的制备方法，该法将哺乳动物任意大小的跟腱联合骨组织经含蛋白酶抑制剂的生理盐水、含蛋白酶抑制剂的PBS、含Triton X的PBS缓冲液、含SDS的PBS缓冲液、含DNA酶的PBS缓冲液处理，获得肌腱联合骨脱细胞材料。

This invention can simultaneously and completely decellularize tendon cells, osteocytes, and chondrocytes at the interface of tendon-bond tissue. The process is mild, does not damage the ECM, and is rapid and stable. The resulting tendon-bond material has advantages such as good biocompatibility, strong plasticity, and high biomechanical strength. It can be used to repair tendon degenerative diseases caused by various etiologies, severe tendon tears, and tendon-bond regeneration and repair disorders.

本发明能同时对肌腱联合骨组织上的肌腱细胞和骨细胞以及交界面上的软骨细胞进行完全去细胞化处理，且条件温和、无ECM损害、快速稳定，所得肌腱联合骨材料具有生物相容性好、可塑性强、生物力学强度高等优点，可用于修复临床上各类病因造成的肌腱退行性疾病，肌腱严重撕裂和肌腱联合骨再生、修复障碍。

[0025]

The significant advancement of this invention compared to existing technologies lies in:

相对于现有技术，本发明的显著进步在于：

[0026]

(1) The tendon-bone material used in this invention is a naturally sourced biological material with good biocompatibility and wide availability.

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(1)本发明所采用的肌腱连骨材料是天然来源的生物材料，具有很好的生物相容性和取材广泛性；

[0027]

(2) The tendon-bone obtained by decellularization technology does not contain cells or other antigens, which can minimize the immune rejection reaction of the recipient. At the same time, it does not contain harmful components such as bacteria and viruses, and has high biological safety.

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(2)采用脱细胞技术获得的肌腱连骨不含细胞等抗原物质，可使受体的免疫排斥反应降到最低限度，同时可不含细菌病毒等有害成分生物安全性高；

[0028]

(3) This novel decellularization technology can retain the integrity of the original ECM while removing xenogeneic cells. It has a good extracellular microenvironment, biochemical factors and biomechanical properties, and can simulate the normal tendon and bone components and structure to the greatest extent.

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(3)本新型脱细胞技术在去除异种细胞的同时，可保留原先ECM的完整性，具有良好的细胞外微环境、生化因子和生物力学性质等，可以最大限度的模拟正常肌腱连骨成分和结构；

[0029]

(4) The material of the present invention can be customized according to the size and shape of the patient's tendon and bone, and can be directly used to replace the broken tendon.

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(4)本发明材料可以根据病人的肌腱、骨形态大小定制相应的脱细胞肌腱连骨材料，可以直接用来替换人断裂的肌腱。

[0030]

Attached Figure Description

附图说明

[0031]

Figure 1 is a general appearance diagram of the decellularized tendon-bone material of the present invention.

图1是本发明的肌腱连骨脱细胞材料的大体外观图；

[0032]

Figure 2 is a HE-stained tissue section of the tendon-bone connection, showing no cells and no residual cell nuclear components.

图2是肌腱连骨处HE染色组织切片图显示无细胞及无细胞核成分残留；

[0033]

Figure 3 is a section of tendon stained with HE, showing no cells and no residual cell nuclear components;

图3是肌腱HE染色组织切片图显示无细胞及无细胞核成分残留；

[0034]

Figure 4 is a section of bone stained with HE, showing no cells and no residual nuclear components;

图4是骨HE染色组织切片图显示无细胞及无细胞核成分残留；

[0035]

Figure 5 shows that DNA testing data of the tendon indicates that there is no DNA residue in the material;

图5是肌腱的DNA检测数据显示材料无DNA残留；

[0036]

Figure 6 shows that DNA testing data of the bone indicates that there is no DNA residue in the material;

图6是骨的DNA检测数据显示材料无DNA残留；

[0037]

Figure 7 shows the ultimate tensile strength (UTS) of the tendon connected to the bone, indicating that the material has good mechanical strength.

图7是肌腱连骨的极限抗张强度(UTS)，显示材料力学强度较好；

[0038]

Figure 8 shows the Young's modulus (EM) of the tendon-bone connection, indicating that the material has good mechanical strength.

图8是肌腱连骨的杨氏模量(EM)，显示材料力学强度较好；

[0039]

Figure 9 shows the maximum elongation (E) of the tendon connected to the bone, indicating that the material has good extensibility.

图9是肌腱连骨的最大伸长率(E)，显示材料延伸性较好。

[0040]

Detailed Implementation Plan

具体实施方案

[0041]

The present invention will now be described in detail with reference to the accompanying drawings and embodiments, but the implementation of the present invention is not limited thereto.

下面结合附图及实施例对本发明进行详细描述，但本发明的实施不仅限于此。

[0042]

Example 1: Preparation and Study of Decellularized Tendon Combined with Bone Material

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实施例1脱细胞肌腱联合骨材料的制备和研究

[0043]

1. Preparation of decellularized matrix for tendon-bone connection

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1、制备肌腱连骨脱细胞基质

[0044]

(1) Material selection: Take the Achilles tendon and bone of a mature domestic pig, mechanically remove the fat attached to the Achilles tendon, and then cut the Achilles tendon

into 20mm (length) x 5mm (width) x 3mm (thickness), and the size of the calcaneus bone is 3mm (thickness) x 7mm (diameter).

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(1)取材：取成熟家猪的跟腱连骨，机械剪除跟腱上附着的脂肪，然后把跟腱剪裁成20mm(长)X5mm(宽)X3mm(厚)，跟骨大小为3mm(厚)X7mm(直径)

[0045]

(2) Rinse three times in physiological saline containing 10 KIU/ml protease inhibitor, 20 min each time;

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(2)在含10KIU/ml蛋白酶抑制剂的生理盐水中漂洗3次，每次20min；

[0046]

(3) In PBS buffer containing 10 KIU/ml protease inhibitor, shake at 150 rpm for 24 hours at low temperature (4°C);

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(3)在含10KIU/ml蛋白酶抑制剂的PBS缓冲液中，低温(4°C)摇床150rpm震荡24小时；

[0047]

(4) Add penicillin and streptomycin mixed antibacterial solution at a concentration of 20 KIU /ml to 4% Triton X-100 PBS buffer at a ratio of 1:1, and shake at 150 rpm for 24 hours at low temperature (4°C).

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(4)在浓度为4%的含Triton X-100的PBS缓冲液中，1:1加入青霉素和链霉素混合抗菌液，浓度为20KIU/ml，低温(4°C)摇床150rpm震荡24小时；

[0048]

(5) In PBS buffer containing 0.5% SDS, shake at 150 rpm for 24 hours at low temperature (4°C);

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(5)在浓度为0.5%的含SDS的PBS缓冲液中，低温(4°C)摇床150rpm震荡24小时；

[0049]

(6) Add penicillin and streptomycin (20 KIU/ml, 20 g/ml) mixed antibacterial solution in a 1:1 ratio to a 6% Triton X-100 PBS buffer and shake at 150 rpm for 7 days at low temperature (4°C);

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(6)在浓度为6%的含Triton X-100的PBS缓冲液中，1：1加入青霉素和链霉素(20KIU/ml，20g/ml)混合抗菌液，低温(4°C)摇床150rpm震荡7天；

[0050]

(7) In PBS buffer containing 2% SDS, shake at 150 rpm on a low temperature (4°C) for 2 days;

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(7)在浓度为2%的含SDS的PBS缓冲液中，低温(4°C)摇床150rpm震荡2天；

[0051]

(8) Add penicillin and streptomycin (20 KIU/ml, 20 g/ml) mixed antibacterial solution in PBS buffer containing DNase at a concentration of 0.5 mg/ml, and shake at 150 rpm for 12 hours at 37°C to obtain decellularized tendon-bone material (Figure 1).

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(8)在浓度为0.5mg/ml的含DNA酶的PBS缓冲液中，1:1加入青霉素和链霉素(20KIU/ml，20g/ml)混合抗菌液，37°C摇床150rpm震荡12小时，即可得到脱细胞的肌腱连骨材料(图1)；

[0052]

Note: Rinse with saline solution for 5 hours after each step.

注：完成每一步骤后均用生理盐水冲洗5小时。

[0053]

(3) Histological evaluation of the decellularized tendon-bone scaffold

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(3)肌腱连骨脱细胞支架的组织学评价

[0054]

Figures 2, 3, and 4 are respectively images of the tendon-bone decellularized scaffold of the present invention, showing the tendon-bone portion, tendon portion, and bone portion after HE staining at 400x magnification, revealing the absence of cells and residual cell nuclear components.

图2、图3、图4分别是本发明肌腱连骨脱细胞支架肌腱连骨部分，肌腱部分，骨部分HE染色放大400倍无细胞及无细胞核成分残留图

[0055]

(4) Quantitative detection of antigen components in tendon-bone decellularized scaffold

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(4)肌腱连骨脱细胞支架的抗原成分定量检测

[0056]

Figures 5 and 6 show that the DNA quantitative detection of the tendon and bone portions of the decellularized tendon-bone scaffold of the present invention contains almost no DNA components.

图5和图6分别是本发明肌腱连骨脱细胞支架肌腱部分和骨部分DNA定量检测几乎不含有DNA成分图。

[0057]

(5) Mechanical property testing of tendon-bone decellularized scaffold

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(5)肌腱连骨脱细胞支架的力学性能测验

[0058]

Figures 7, 8, and 9 show the test results of three important indicators of the mechanical properties of the tendon-bone decellularized scaffold: ultimate tensile strength (UTS), Young's modulus (EM), and maximum elongation (E). The results show no significant mechanical difference compared to the pre-decellularized state.

图7，图8，图9分别是肌腱连骨脱细胞支架力学性能三个重要衡量指标极限抗张强度(UTS)，杨氏模量(EM)，最大伸长率(E)测验结果，显示和未脱细胞之前没有明显力学差异。

[0059]

Example 2: Preparation and Study of Decellularized Tendon Combined with Bone Material

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实施例2脱细胞肌腱联合骨材料的制备和研究

[0060]

(1) Material selection: Take the Achilles tendon and bone of a mature domestic pig, mechanically remove the fat attached to the Achilles tendon, and then cut the Achilles tendon into 40mm (length) x 20mm (width) x 5mm (thickness), and the size of the calcaneus bone is 5mm (thickness) x 25mm (diameter).

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(1)取材：取成熟家猪的跟腱连骨，机械剪除跟腱上附着的脂肪，然后把跟腱剪裁成40mm(长)X20mm(宽)X5mm(厚)，跟骨大小为5mm(厚)X25mm(直径)

[0061]

(2) Rinse three times in physiological saline containing 30 KIU/ml protease inhibitor, 20 min each time;

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(2)在含30KIU/ml蛋白酶抑制剂的生理盐水中漂洗3次，每次20min；

[0062]

(3) In PBS buffer containing 30 KIU/ml protease inhibitor, shake at 150 rpm on a low temperature (4°C) for 48 hours;

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(3)在含30KIU/ml蛋白酶抑制剂的PBS缓冲液中，低温(4°C)摇床150rpm震荡48小时；

[0063]

(4) Add penicillin and streptomycin (20 KIU/ml, 20 g/ml) mixed antibacterial solution in 1:1 to 8% Triton X-100 PBS buffer and shake at 150 rpm for 30 hours at low temperature (4°C);

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(4)在浓度为8%的含Triton X-100的PBS缓冲液中，1:1加入青霉素和链霉素(20KIU/ml，20g/ml)混合抗菌液，低温(4°C)摇床150rpm震荡30小时；

[0064]

(5) In 8% SDS-containing PBS buffer, shake at 150 rpm for 36 hours at low temperature (4°C);

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(5)在浓度为8%的含SDS的PBS缓冲液中，低温(4°C)摇床150rpm震荡36小时；

[0065]

(6) Add penicillin and streptomycin (20 KIU/ml, 20 g/ml) mixed antibacterial solution in a 1:1 ratio to a 12% Triton X-100 PBS buffer and shake at 150 rpm for 16 days at low temperature (4°C);

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(6)在浓度为12%的含Triton X-100的PBS缓冲液中，1：1加入青霉素和链霉素(20KIU/ml，20g/ml)混合抗菌液，低温(4°C)摇床150rpm震荡16天；

[0066]

(7) In 6% SDS-containing PBS buffer, shake at 150 rpm on a low temperature (4°C) for 20 days;

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(7)在浓度为6%的含SDS的PBS缓冲液中，低温(4°C)摇床150rpm震荡20天；

[0067]

(8) Add a 1:1 mixture of penicillin and streptomycin antibacterial solution to a 1 mg/ml PBS buffer containing DNase, to a concentration of 20 KIU/ml, and shake at 150 rpm for 12 hours at 37°C.

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(8)在浓度为1mg/ml的含DNA酶的PBS缓冲液中，1:1加入青霉素和链霉素混合抗菌液，浓度为20KIU/ml，37℃摇床150rpm震荡12小时

[0068]

The remaining steps were performed according to the method in Example 1 to obtain decellularized tendon-bone material.

其余参考实施例1的方法进行，得到脱细胞肌腱联合骨材料。

[0069]

Example 3: Preparation and Study of Decellularized Tendon Combined with Bone Material

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实施例3脱细胞肌腱联合骨材料的制备和研究

[0070]

(1) Material selection: Take the Achilles tendon and bone of a mature domestic pig, mechanically remove the fat attached to the Achilles tendon, and then cut the Achilles tendon into 40mm (length) x 20mm (width) x 3mm (thickness), and the size of the calcaneus bone is 4mm (thickness) x 15mm (diameter).

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(1)取材：取成熟家猪的跟腱连骨，机械剪除跟腱上附着的脂肪，然后把跟腱剪裁成40mm(长)X20mm(宽)X3mm(厚)，跟骨大小为4mm(厚)X15mm(直径)

[0071]

(2) Rinse three times in physiological saline containing 50 KIU/ml protease inhibitor, 20 min each time;

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(2)在含50KIU/ml蛋白酶抑制剂的生理盐水中漂洗3次，每次20min；

[0072]

(3) In PBS buffer containing 50 KIU/ml protease inhibitor, shake at 150 rpm for 36 hours at low temperature (4°C);

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(3)在含50KIU/ml蛋白酶抑制剂的PBS缓冲液中，低温(4°C)摇床150rpm震荡36小时；

[0073]

(4) Add penicillin and streptomycin (20 KIU/ml, 20 g/ml) mixed antibacterial solution in a 1:1 ratio to a 4% Triton X-100 PBS buffer and shake at 150 rpm for 48 hours at low temperature (4°C).

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(4)在浓度为4%的含Triton X-100的PBS缓冲液中，1:1加入青霉素和链霉素(20KIU/ml，20g/ml)混合抗菌液，低温(4°C)摇床150rpm震荡48小时；

[0074]

(5) In PBS buffer containing 0.5% SDS, shake at 150 rpm for 72 hours at low temperature (4°C);

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(5)在浓度为0.5%的含SDS的PBS缓冲液中，低温(4°C)摇床150rpm震荡72小时；

[0075]

(6) Add penicillin and streptomycin (20 KIU/ml, 20 g/ml) mixed antibacterial solution in PBS buffer containing Triton X-200 at a concentration of 612% at a ratio of 1:1, and shake at 150 rpm on a low temperature (4°C) for 10 days.

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(6)在浓度为612%的含Triton X-200的PBS缓冲液中，1：1加入青霉素和链霉素(20KIU/ml，20g/ml)混合抗菌液，低温(4°C)摇床150rpm震荡10天；

[0076]

(7) In 6% SDS-containing PBS buffer, shake at 150 rpm on a low temperature (4°C) for 14 days;

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(7)在浓度为6%的含SDS的PBS缓冲液中，低温(4°C)摇床150rpm震荡14天；

[0077]

(8) Add a 1:1 mixture of penicillin and streptomycin antibacterial solution to a 2 mg/ml PBS buffer containing DNase, to a concentration of 20 KIU/ml, and shake at 150 rpm for 12 hours at 37°C.

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(8)在浓度为2mg/ml的含DNA酶的PBS缓冲液中，1:1加入青霉素和链霉素混合抗菌液，浓度为20KIU/ml，37°C摇床150rpm震荡12小时

[0078]

The remaining steps were performed according to the method in Example 1 to obtain decellularized tendon-bone material.

其余参考实施例1的方法进行，得到脱细胞肌腱联合骨材料。

[0079]

Example 4: Preparation and Study of Decellularized Tendon Combined with Bone Material

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实施例4脱细胞肌腱联合骨材料的制备和研究

[0080]

Take the Achilles tendon bone from the horse. In step (6), add penicillin and streptomycin mixed antibacterial solution at a concentration of 20 KIU/ml to 10% PBS buffer containing Triton X-100 at a ratio of 1:1. Shake at 150 rpm for 7 days at low temperature (4°C).

取马跟腱联合骨，步骤(6)在浓度为10%的含Triton X-100的PBS缓冲液中，1：1加入青霉素和链霉素混合抗菌液，浓度为20KIU/ml，低温(4°C)摇床150rpm震荡7天。

The remaining steps were performed according to the method in Example 1 to obtain decellularized Achilles tendon combined bone material.

其余参考实施例1的方法进行，得到脱细胞跟腱联合骨材料。

[0081]

Example 5: Preparation and Study of Decellularized Tendon Combined with Bone Material

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实施例5脱细胞肌腱联合骨材料的制备和研究

[0082]

Take the Achilles tendon synapse bone from the dog. In step (6), add penicillin and streptomycin mixed antibacterial solution at a concentration of 20 KIU/ml to 8% PBS buffer containing Triton X-100 at a ratio of 1:1. Shake at 150 rpm for 7 days at low temperature (4°C).

取狗跟腱联合骨，步骤(6)步骤(6)在浓度为8%的含Triton X-100的PBS缓冲液中，1：1加入青霉素和链霉素混合抗菌液，浓度为20KIU/ml，低温(4°C)摇床150rpm震荡7天。

The remaining steps were performed according to the method in Example 1 to obtain decellularized tendon-bone material.

其余参考实施例1的方法进行，得到脱细胞肌腱联合骨材料。

[0083]

The decellularized tendon-bone materials obtained in Examples 2-5 were subjected to histological evaluation, quantitative detection of antigen components, and mechanical testing, respectively. The results were similar to those in Example 1. This indicates that decellularized tendon-bone materials of various tissue sources and sizes can be prepared by the above methods. Furthermore, histological evaluation, quantitative detection of antigen components, and cartilage repair capacity testing of the decellularized tendon-bone

materials all indicate that the materials have completely removed cellular components and have no obvious antigen component residues.

对实施例2-5所得脱细胞肌腱联合骨材料分别进行组织学评价、抗原成分定量检测和力学检测，结果与实施例1类似，这表明可通过上述多种方法制得多种组织来源，多种组织大小的脱细胞肌腱联合骨材料，并且对脱细胞肌腱联合骨材料进行组织学评价、抗原成分定量检测和软骨修复能力检测均说明材料已经完全去除细胞成分，无明显抗原成分残留。

Decellularized tendon-bone composite material can serve as a safe, reliable, effective, and rapid alternative material for patients with severe tendon tears, degeneration, and tendon-bone junction injuries.

脱细胞肌腱联合骨材料可以作为临床上肌腱严重撕裂，退化以及肌腱-骨联合部位损伤等病人的安全可靠、有效、快速的替代材料。