Notice

DESCRIPTION CN105664255A

A method for preparing a naturally derived cartilage and bone decellularization material

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一种天然组织来源的软骨联合骨脱细胞材料的制备方法

[0001]

Technical Field

技术领域

[0002]

This invention belongs to the field of osteochondral tissue repair and regeneration technology, and particularly relates to a method for preparing a decellularized cartilage combined with bone material derived from natural tissue.

本发明属于骨软骨组织修复及其再生技术领域，尤其涉及一种天然组织来源的软骨联合骨脱细胞材料的制备方法。

[0003]

Background Technology

背景技术

[0004]

Currently, diseases such as osteoarthritis and cartilage defects pose a serious threat to human health, and articular cartilage repair remains a major clinical challenge.

当前，骨关节炎、软骨缺损等疾病是严重威胁着人类健康，而关节软骨修复依然是临床的一大难题。

Therefore, artificial biomaterials are being gradually applied to the repair of patients' cartilage tissue.

为此，人工生物材料正逐步运用于病人的软骨组织修复。

However, due to shortcomings such as biocompatibility, degradability, and the difficulty in regenerating hyaline cartilage, there is still no suitable material that can truly replace healthy cartilage tissue and fulfill its original physiological function requirements.

但是，由于生物相容性、降解性、透明软骨难以再生等不足，使得目前仍然没有合适的材料能够真正替代健康的软骨组织，实现其原有的生理功能需求。

In addition, damage to cartilage often leads to damage and degeneration of subchondral bone. Osteochondrial lesions are very common in clinical practice, restricting joint movement and causing unbearable pain to patients. Currently, there are virtually no reports on biomaterials that can simultaneously repair chondroosteal fusion.

此外，软骨的损伤往往引起软骨下骨的损伤及退变，骨软骨病变在临床上十分常见，限制了关节的活动并且带给病人难以忍受的痛苦。 而目前对于同时修复软骨联合骨的生物材料还基本无报道

[0005]

Natural tissue-derived extracellular matrix (ECM) contains various biochemical factors required by normal tissue or organ cells, has a natural macroscopic and ultramicro three-dimensional structure, and possesses the biomechanical properties of natural tissues.

天然组织来源的细胞外基质(ECM)含有正常组织或器官细胞所需的各种生化因子，具有天然宏观及超微三维的立体结构，并且拥有天然组织的生物力学性能。

Currently, there are some research reports on the use of decellularized cartilage derived from animals (cattle, pigs) and allogeneic humans for repairing fractured bone defects in animal joints. However, no one has yet reported using decellularized material of bone-associated cartilage to repair cartilage or bone-associated cartilage lesions.

目前，运用动物(牛、猪)和异体人来源的脱细胞软骨用于修复动物关节断骨缺损有一定的研究报道。然而，目前还未有人报道采用骨联合软骨的脱细胞材料来修复软骨或软骨联合骨的病变。

[0006]

Summary of the Invention

发明内容

[0007]

To address the shortcomings of existing technologies, this invention proposes a method for preparing decellularized cartilage and bone materials derived from natural tissues.

本发明针对现有技术的不足，提出了一种天然组织来源的软骨联合骨脱细胞材料的制备方法。

This invention simultaneously performs complete decellularization of chondrocytes and osteocytes on the cartilage-bone tissue under mild conditions, without ECM damage, and with rapid and stable results.

本发明同时对软骨联合骨组织上的软骨细胞和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。

[0008]

To solve the above problems, the present invention adopts the following technical solution: a method for preparing decellularized cartilage-bone material from natural tissue, wherein arbitrary cartilage-bone tissue from mammals is treated with physiological saline buffer containing protease inhibitors, organic solvent solution, PBS buffer containing Triton X, PBS buffer containing SDS and PBS buffer containing DNase to obtain decellularized cartilage-bone material.

为解决上述问题，本发明采用以下技术方案：天然组织来源的脱细胞软骨联合骨材料的制备方法，将哺乳动物任意软骨联合骨组织经含蛋白酶抑制剂的生理盐水缓冲液、有机溶剂溶液、含TritonX的PBS缓冲液、含SDS的PBS缓冲液和含DNA酶的PBS缓冲液处理，获得脱细胞软骨联合骨材料。

[0009]

In mammals, any cartilaginous symphysis is the articular cartilaginous symphysis of the long bones of the limbs.

哺乳动物任意软骨联合骨组织为四肢长骨关节软骨联合骨组织。

[0010]

The molar concentration of the saline buffer containing protease inhibitors is 1%-5%, and the protease inhibitor content is 10 KIU/ml;

含蛋白酶抑制剂的生理盐水缓冲液摩尔浓度为1%-5%，蛋白酶抑制剂含量为10KIU/ml；

[0011]

The organic solvent solution is an equal volume ratio of chloroform and methanol, an acetone solution with a molar concentration of 10%-100%, or an ethanol solution with a molar concentration of 30%-70%.

有机溶剂溶液为等体积比的氯仿和甲醇溶液、摩尔浓度10%-100%的丙酮溶液或摩尔浓度30%-70%的乙醇溶液；

[0012]

The PBS buffer containing Triton X is a PBS buffer containing 1%-10% Triton X-200 or Triton X-100.

含TritonX的PBS缓冲液为浓度1%-10%的TritonX-200或TritonX-100的PBS缓冲液；

[0013]

The PBS buffer containing SDS is a PBS buffer with a molar concentration of 0.5%-10% SDS, mixed with 1-20 mmol/L Tris;

含SDS的PBS缓冲液为摩尔浓度0.5%-10%的SDS的PBS缓冲液，其中混合1-20mmol/L的Tris；

[0014]

The concentration of DNase in PBS buffer containing DNase is 0.01-0.5 mg/ml;

含DNA酶的PBS缓冲液中DNA酶浓度为0.01-0.5mg/ml；

[0015]

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

上述天然组织来源的软骨联合骨材料的制备方法，包括以下步骤：

[0016]

(1) Take any cartilage and bone tissue from the whole body of mammals and rinse it three times with sterile physiological saline for 20 minutes each time to remove blood, residual muscle tissue, hair and ligaments;

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(1)取哺乳动物全身任意软骨联合骨组织用无菌生理盐水漂洗3次、20分钟/次，去除血液、残余肌肉组织、毛发和韧带；

[0017]

(2) In physiological saline buffer containing protease inhibitor, shake at 150 rpm for 8-24 hours at a constant temperature of 45°C;

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(2)在含蛋白酶抑制剂的生理盐水缓冲液中，恒温45°C摇床150rpm震荡8-24小时；

[0018]

(3) Degrease in an organic solvent solution at a constant temperature of 45°C and a shaking speed of 150rpm for 2-12 hours;

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(3)在有机溶剂溶液中，恒温45°C摇床150rpm震荡脱脂2-12小时；

[0019]

(4) Add penicillin and streptomycin mixed antibacterial solution to PBS buffer containing TritonX, and shake at 150 rpm for 3-72 hours at a constant temperature of 45°C.

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(4)在含TritonX的PBS缓冲液中，加入青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡3-72小时；

[0020]

(5) Add penicillin and streptomycin mixed antibacterial solution to PBS buffer containing SDS, and shake at 150 rpm for 2-96 hours at a constant temperature of 45°C.

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(5)在含SDS的PBS缓冲液中，加入青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡2-96小时；

[0021]

(6) Add penicillin and streptomycin mixed antibacterial solution to PBS buffer containing DNase, and shake at 150 rpm for 1-12 hours at 37°C.

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(6)在含DNA酶的PBS缓冲液中，加入青霉素和链霉素混合抗菌液，37°C摇床150rpm震荡1-12小时；

[0022]

(7) The cartilage and bone decellularization material derived from natural tissue was obtained by shaking the cartilage in sterile saline at 37°C and 150 rpm for 72 hours.

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(7)在无菌生理盐水中，37°C摇床150rpm震荡72小时，即得天然组织来源的软骨联合骨脱细胞材料。

[0023]

The concentrations of penicillin and streptomycin in the mixed antibacterial solution are 10 KIU/ml and 10 KIU/ml, respectively, and the ratio of penicillin to streptomycin is 1:1; the

volume ratios of PBS buffer and mixed antibacterial solution in steps (4)-(6) are 10:1, 10:1, and 5:1, respectively.

混合抗菌液中青霉素和链霉素的浓度分别为10KIU/ml、10KIU/ml，青霉素和链霉素的比例为1:1；步骤(4)-(6)中PBS缓冲液和混合抗菌液体积比分别为10:1、10:1、5:1。

[0024]

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。

[0025]

The above preparation method yields decellularized cartilage and bone material derived from natural tissue.

上述制备方法得到的天然组织来源的软骨联合骨脱细胞材料。

[0026]

To address the problems existing in current bone materials and preparation methods used for cartilage and cartilage-bone repair and regeneration, the inventors have established a method for preparing decellularized cartilage-bone material from natural tissue sources. This method involves treating arbitrary cartilage-bone tissue from mammals with physiological saline buffer containing protease inhibitors, organic solvent solutions, PBS buffer containing Triton X, PBS buffer containing SDS, and PBS buffer containing DNase to obtain decellularized cartilage-bone material.

针对目前用于软骨和软骨联合骨修复和再生的骨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软骨联合骨脱细胞材料的制备方法，该法将哺乳动物任意软骨联合骨组织经含蛋白酶抑制剂的生理盐水缓冲液、有机溶剂溶液、含TritonX的PBS缓冲液、含SDS的PBS缓冲液、含DNA酶的PBS缓冲液处理，获得软骨联合骨脱细胞材料。

This invention can simultaneously and completely decellularize chondrocytes and osteocytes on cartilage-bone tissue under mild conditions, without ECM damage, and with rapid and stable results. The resulting cartilage-bone material has advantages such as good biocompatibility, strong plasticity, and high biomechanical strength. It can be used to repair cartilage defects and cartilage-bone regeneration and repair disorders caused by various clinical etiologies, such as osteoarthritis.

本发明能同时对软骨联合骨组织上的软骨细胞和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，所得软骨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软骨缺损和骨关节炎等软骨和软骨联合骨再生、修复障碍。

[0027]

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：

[0028]

(1) The present invention uses a decellularization scheme to simultaneously achieve thorough decellularization of any cartilage and bone tissue in the whole body, and obtains highly uniform decellularized cartilage and bone materials more efficiently and conveniently.

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(1)本发明运用一种脱细胞方案即可同时实现对全身任意软骨联合骨组织进行彻底去细胞化处理，更加高效便捷的获得均一性高的脱细胞软骨联合骨材料。

[0029]

(2) While removing immunogenic allogeneic or xenogeneic cells, the present invention can preserve the integrity of the original ECM, and has good extracellular microenvironment,

biochemical factors and biomechanical properties, which can simulate the composition and structure of normal cartilage and bone tissue to the greatest extent.

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(2)本发明在去除具有免疫原性的异体或异种细胞的同时，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软骨和骨组织的成分和结构。

[0030]

(3) This invention can customize individualized, transplantable decellularized cartilage and cartilage-associated bone materials for patients, and can efficiently prepare decellularized materials to meet the complex and diverse clinical needs for cartilage and cartilage-associated bone repair and filling. It can also grind them into powder to dissolve the biochemical factors contained in normal autologous cartilage and cartilage-associated bone tissue for use in orthopedic diseases in any part of the body.

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(3)本发明可以为病人订制具有个体化、可供移植的软骨及软骨联合骨脱细胞材料，并且高效制备脱细胞材料可满足临床上复杂多样的软骨和软骨联合骨修复填补需求，也可以将其研磨制成粉末，将正常自身软骨和软骨联合骨组织中所含有的生化因子溶解，用于全身任意部位的骨科疾病。

[0031]

Attached Figure Description

附图说明

[0032]

Figure 1 is a general appearance diagram of the cartilage-bone composite material of the present invention;

图1是本发明的软骨联合骨材料大体外观图；

[0033]

Figure 2 shows HE staining of decellularized cartilage and bone material, revealing no cells and no residual cell nuclear components.

图2是软骨联合骨脱细胞材料的HE染色无细胞及无细胞核成分残留图；

[0034]

Figure 3 shows the DAPI staining of decellularized cartilage and bone materials, revealing no cells and no residual cell nuclear components.

图3是软骨联合骨脱细胞材料的DAPI染色无细胞及无细胞核成分残留图；

[0035]

Figure 4 shows that the DNA quantitative detection of decellularized cartilage and bone materials showed almost no DNA component.

图4是软骨联合骨脱细胞材料DNA定量检测几乎不含有DNA成分图；

[0036]

Figure 5 shows the quantitative detection of collagen in decellularized cartilage and bone materials, which retains a large amount of collagen components.

图5是软骨联合骨脱细胞材料的胶原定量检测保留大量胶原成分图；

[0037]

Figure 6 shows the Sirius red staining qualitative detection of collagen in decellularized cartilage and bone materials, which showed a large amount of collagen components.

图6是软骨联合骨脱细胞材料的天狼星红染色胶原定性检测保留大量胶原成分图；

[0038]

Figure 7 shows the scanning electron microscopy analysis of the cartilage and bone acellular material, revealing the complete preservation of the extracellular matrix arrangement and three-dimensional spatial structure of cartilage and bone cells.

图7是软骨联合骨脱细胞材料扫描电镜检测软骨和骨细胞外基质排布和空间立体结构完整保留图；

[0039]

Figure 8 shows that the CCK-8 assay showed no cytotoxicity in the decellularized cartilage and bone composite material.

图8是CCK-8检测软骨联合骨脱细胞材料无细胞毒性；

[0040]

Figure 9 shows the repair effect of cartilage and bone acellular material repairing cartilage and bone defects in rabbit joints after 12 weeks (H&E staining);

图9是软骨联合骨脱细胞材料修复兔子关节软骨联合骨缺损的12周修复效果图(H&E染色)；

[0041]

Figure 10. Repair effect of cartilage-bone fusion bone decellularized material in rabbit articular cartilage-bone fusion bone defect after 12 weeks (Safranin-Fix Green staining).

图10软骨联合骨脱细胞材料修复兔子关节软骨联合骨缺损的12周修复效果图(番红固绿染色)。

[0042]

Detailed Implementation

具体实施方式

[0043]

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

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实施例1脱细胞松软骨联合骨材料的制备和研究

[0044]

(1) Take the femoral head cartilage fusion bone from pigs, transfer it to a 4°C environment, and rinse it three times with sterile saline for 20 minutes each time to remove blood, residual muscle tissue, hair and ligaments. The size of the material taken depends on the actual needs of the clinical surgery, usually 1cm*0.5cm*1cm.

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(1)取猪股骨头软骨联合骨，转移至4°C环境中，用无菌生理盐水漂洗3次、20分钟/次，去除血液、残余肌肉组织、毛发和韧带等组织；取材大小视临床实际手术需要而定,通常大小为1cm*0.5cm*1cm。

[0045]

(2) In 1000 ml of physiological saline buffer with a molar concentration of 1% containing protease inhibitor (protease inhibitor content of 10 KIU/ml), shake at 150 rpm for 24 hours at a constant temperature of 45°C, and then rinse with physiological saline for 5 hours.

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(2)在1000ml摩尔浓度为1%含蛋白酶抑制剂的生理盐水缓冲液(蛋白酶抑制剂含量为10KIU/ml)中，恒温45°C摇床150rpm震荡24小时，并用生理盐水冲洗5小时；

[0046]

(3) In 1000 ml of organic solvent solution (chloroform and methanol solution in equal volume ratio), degrease in a shaker at 150 rpm for 12 hours at a constant temperature of 45°C, and rinse with physiological saline for 5 hours.

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(3)在1000ml有机溶剂溶液(等体积比的氯仿和甲醇溶液)中，恒温45°C摇床150rpm震荡脱脂12小时，并用生理盐水冲洗5小时；

[0047]

(4) Add 100 ml of penicillin and streptomycin mixed antibacterial solution to 1000 ml of PBS buffer containing Triton X (1% Triton X-100), shake at 150 rpm for 72 hours at a constant temperature of 45°C, and rinse with physiological saline for 5 hours.

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(4)在1000ml含TritonX的PBS缓冲液(浓度1%的TritonX-100)中，加入100ml青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡72小时，并用生理盐水冲洗5小时；

[0048]

(5) Add 100 ml of penicillin and streptomycin mixed antibacterial solution to 1000 ml of PBS buffer containing SDS (SDS concentration is 0.5%, mixed with 1 mmol of Tris), shake at 150 rpm for 96 hours at a constant temperature of 45°C, and rinse with physiological saline for 5 hours.

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(5)在1000ml含SDS的PBS缓冲液(SDS浓度为0.5%，混合1mmol的Tris)中，加入100ml青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡96小时，并用生理盐水冲洗5小时；

[0049]

(6) Add 60 ml of penicillin and streptomycin mixed antibacterial solution to 300 ml of PBS buffer containing DNase (DNase concentration is 0.01 mg/ml), shake at 150 rpm for 12 hours at 37°C, and rinse with physiological saline for 5 hours.

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(6)在300ml含DNA酶的PBS缓冲液中(DNA酶浓度为0.01mg/ml)，加入60ml青霉素和链霉素混合抗菌液，37°C摇床150rpm震荡12小时，并用生理盐水冲洗5小时；

[0050]

(7) The cartilage and bone decellularization material derived from natural tissue was obtained by shaking in sterile saline at 37°C and 150 rpm for 72 hours. It was then stored in liquid nitrogen and removed during surgery.

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(7)在无菌生理盐水中，37°C摇床150rpm震荡72小时，即得天然组织来源的软骨联合骨脱细胞材料，液氮中保存，待手术时取出。

[0051]

The concentrations of penicillin and streptomycin in the mixed antibacterial solution were 10 KIU/ml and 10 KIU/ml, respectively, with a ratio of 1:1.

其中，混合抗菌液中青霉素和链霉素的浓度分别为10KIU/ml、10KIU/ml，青霉素和链霉素的比例为1:1。

[0052]

Histological evaluation, quantitative detection of antigen components, and osteogenic repair capacity testing were performed on the acellular cartilage and bone material obtained from natural tissue sources in this case. The results are shown in Figure 1-10.

对本例所得天然组织来源的软骨联合骨脱细胞材料进行组织学评价、抗原成分定量检测和成骨修复能力检测，结果如图1-10。

Figures 1 and 2 show that the gross structure of the completely decellularized cartilage-bone material is well preserved, the extracellular matrix is intact, and the nuclear components are completely removed, with no cells or debris remaining. Figure 3 shows that DAPI staining further indicates that the nuclear components in the material are negative, and the antigenicity is completely removed. Figure 4 shows that the quantitative detection of DNA

antigen components shows that the DNA removal rate through decellularization can reach more than 95%. Figures 5 and 6 show that the quantitative and qualitative detection of collagen reveals that the main collagen components of the extracellular matrix are well preserved. Figure 7 shows that the microstructure of the cartilage-bone material is well preserved. Figure 8 shows that the CCK-8 cytotoxicity test indicates that the decellularized cartilage-bone material is non-cytotoxic and has high biocompatibility. Figures 9 and 10 show that the 12-week repair experiment of rabbit articular cartilage-bone defects can significantly repair articular cartilage and achieve cartilage regeneration.

通过图1和2显示完全脱细胞软骨联合骨材料大体结构保留完好，细胞外基质保留完整，细胞核成分完全去除，无细胞及其碎片残留；图3DAPI染色进一步提示材料中细胞核成分呈阴性，抗原性得到完全去除；图4的DNA抗原成分定量检测说明通过去细胞DNA去除率可以达到95%以上；图5和图6的胶原定量和定性检测发现细胞外基质主要胶原成分得到很好保留；图7的扫描电镜先是软骨联合骨微观结构保留完整；图8的CCK-8细胞毒性检测表明软骨联合骨脱细胞材料无细胞毒性，生物相容性高；图9和图10的兔子关节软骨联合骨缺损的12周修复实验表明该材料可以显著的修复关节软骨，实现软骨再生。

[0053]

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

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实施例2脱细胞软骨联合骨材料的制备和研究

[0054]

Take the femoral head cartilage-bone composite bone from pigs. In step (2), place it in 1000 ml of physiological saline buffer containing protease inhibitor (concentration of 5%, protease inhibitor content of 10 KIU/ml) and shake it at 150 rpm for 8 hours at a constant temperature of 45°C. The rest of the process is carried out according to the method in Example 1 to obtain decellularized cartilage-bone composite bone material.

取猪股骨头软骨联合骨，步骤(2)在1000ml含蛋白酶抑制剂的生理盐水缓冲液(浓度为5%，蛋白酶抑制剂含量为10KIU/ml)中，恒温45°C摇床150rpm震荡8小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0055]

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

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实施例3脱细胞软骨联合骨材料的制备和研究

[0056]

Take the femoral head cartilage-bone composite bone from pigs. In step (2), place it in 1000 ml of physiological saline buffer containing protease inhibitor (concentration of 3%, protease inhibitor content of 10 KIU/ml) and shake it at 150 rpm for 12 hours at a constant temperature of 45°C. The rest of the process is carried out according to the method in Example 1 to obtain decellularized cartilage-bone composite bone material.

取猪股骨头软骨联合骨，步骤(2)在1000ml含蛋白酶抑制剂的生理盐水缓冲液(浓度为3%，蛋白酶抑制剂含量为10KIU/ml)中，恒温45°C摇床150rpm震荡12小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0057]

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

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实施例4脱细胞软骨联合骨材料的制备和研究

[0058]

Take the femoral head cartilage-bone combination bone from pigs. In step (4), add 100 ml of penicillin and streptomycin mixed antibacterial solution to 1000 ml of PBS buffer containing

TritonX (5% TritonX-200). Shake at 150 rpm for 3 hours at a constant temperature of 45°C. The rest of the process is carried out according to the method in Example 1 to obtain decellularized cartilage-bone combination bone material.

取猪股骨头软骨联合骨，步骤(4)在1000ml含TritonX的PBS缓冲液(浓度5%的TritonX-200)中，加入100ml青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡3小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0059]

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

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实施例5脱细胞软骨联合骨材料的制备和研究

[0060]

Take the femoral head cartilage-bone combination of pigs. In step (4), add 100 ml of penicillin and streptomycin mixed antibacterial solution to 1000 ml of PBS buffer containing TritonX (10% TritonX-100). Shake at 150 rpm for 50 hours at a constant temperature of 45°C. The rest is carried out according to the method in Example 1 to obtain decellularized cartilage-bone combination material.

取猪股骨头软骨联合骨，步骤(4)在1000ml含TritonX的PBS缓冲液(浓度10%的TritonX-100)中，加入100ml青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡50小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0061]

Example 6: Preparation and Study of Decellularized Cartilage Combined with Bone Material

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实施例6脱细胞软骨联合骨材料的制备和研究

[0062]

Take the cartilage-bone combination of pig knee joints. In step (5), add 100 ml of penicillin and streptomycin mixed antibacterial solution to 1000 ml of PBS buffer containing SDS (SDS concentration is 10%, mixed with 20 mmol of Tris), and shake at 150 rpm for 2 hours at a constant temperature of 45°C. The rest is carried out according to the method of Example 1 to obtain decellularized cartilage-bone combination material.

取猪膝关节软骨联合骨，步骤(5)在1000ml含SDS的PBS缓冲液(SDS浓度为10%，混合20mmol的Tris)中，加入100ml青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡2小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0063]

Example 7: Preparation and Study of Decellularized Cartilage Combined with Bone Material

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实施例7脱细胞软骨联合骨材料的制备和研究

[0064]

Take the cartilage-bone combination of pig knee joints. In step (5), add 100 ml of penicillin and streptomycin mixed antibacterial solution to 1000 ml of PBS buffer containing SDS (SDS concentration of 6%, mixed with 8 mmol of Tris), and shake at 150 rpm for 30 hours at a constant temperature of 45°C. The rest is carried out according to the method of Example 1 to obtain decellularized cartilage-bone combination material.

取猪膝关节软骨联合骨，步骤(5)在1000ml含SDS的PBS缓冲液(SDS浓度为6%，混合8mmol的Tris)中，加入100ml青霉素和链霉素混合抗菌液，恒温45°C摇床150rpm震荡30小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0065]

Example 8: Preparation and Study of Decellularized Cartilage Combined with Bone Material

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实施例8脱细胞软骨联合骨材料的制备和研究

[0066]

Take the cartilage-bone combination of pig knee joints. In step (6), add 60 ml of penicillin and streptomycin mixed antibacterial solution to 300 ml of PBS buffer containing DNase (DNase concentration is 0.5 mg/ml), and shake at 150 rpm for 1 hour at 37°C. The rest is carried out according to the method of Example 1 to obtain decellularized cartilage-bone combination material.

取猪膝关节软骨联合骨，步骤(6)在300ml含DNA酶的PBS缓冲液中(DNA酶浓度为0.5mg/ml)，加入60ml青霉素和链霉素混合抗菌液，37°C摇床150rpm震荡1小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0067]

Example 9: Preparation and Study of Decellularized Cartilage Combined with Bone Material

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实施例9脱细胞软骨联合骨材料的制备和研究

[0068]

Take the cartilage-bone combination of pig knee joints. In step (6), add 60 ml of penicillin and streptomycin mixed antibacterial solution to 300 ml of PBS buffer containing DNase (DNase concentration is 0.3 mg/ml), and shake at 150 rpm for 8 hours at 37°C. The rest is carried out according to the method of Example 1 to obtain decellularized cartilage-bone combination material.

取猪膝关节软骨联合骨，步骤(6)在300ml含DNA酶的PBS缓冲液中(DNA酶浓度为0.3mg/ml)，加入60ml青霉素和链霉素混合抗菌液，37°C摇床150rpm震荡8小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0069]

Example 10: Preparation and Study of Decellularized Cartilage Combined with Bone Material

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实施例10脱细胞软骨联合骨材料的制备和研究

[0070]

Take the cartilage-bone combination of pig knee joints. In step (3), degrease the cartilage in 1000ml of organic solvent solution (70% ethanol solution) at a constant temperature of 45°C and shake at 150rpm for 2 hours. The rest is carried out according to the method of Example 1 to obtain decellularized cartilage-bone combination material.

取猪膝关节软骨联合骨，步骤(3)在1000ml有机溶剂溶液(摩尔浓度70%的乙醇溶液)中，恒温45°C摇床150rpm震荡脱脂2小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0071]

Example 11: Preparation and Study of Decellularized Cartilage Combined with Bone Material

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实施例11脱细胞软骨联合骨材料的制备和研究

[0072]

Take the cartilage-bone combination of pig knee joints. In step (3), degrease the cartilage in 1000ml of organic solvent solution (acetone solution with a molar concentration of 10%) at a constant temperature of 45°C and shake at 150rpm for 2 hours. The rest is carried out according to the method of Example 1 to obtain decellularized cartilage-bone combination material.

取猪膝关节软骨联合骨，步骤(3)在1000ml有机溶剂溶液(摩尔浓度10%的丙酮溶液)中，恒温45°C摇床150rpm震荡脱脂2小时；其余参考实施例1的方法进行，得到脱细胞软骨联合骨材料。

[0073]

The decellularized cartilage combined with bone materials obtained in Examples 2-11 were subjected to histological evaluation, quantitative detection of antigen components, and cartilage repair capacity testing, respectively. The results were similar to those in Example 1, indicating that the decellularized cartilage combined with bone materials can be prepared by the above-mentioned methods. Furthermore, histological evaluation, quantitative detection of antigen components, and cartilage repair capacity testing all showed that the material had completely removed cellular components and had no obvious antigen component residues. When applied to the repair of cartilage defects in large animal models such as rabbits, it can achieve a very good cartilage repair and regeneration effect.

对实施例2-11所得脱细胞软骨联合骨材料分别进行组织学评价、抗原成分定量检测和软骨修复能力检测，结果与实施例1类似，这表明脱细胞软骨联合骨材料可通过上述多种方法制得，并且对其进行组织学评价、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；将其运用于兔子一类的大动物软骨缺损模型修复中可以达到很好的软骨修复再生效果。Decellularized cartilage combined with bone material can serve as a safe, reliable, effective, and rapid alternative material for patients with cartilage and cartilage combined bone defects in clinical practice.

脱细胞软骨联合骨材料可以作为临床上软骨和软骨联合骨缺损移植等病人的安全可靠、有效、快速的替代材料。