Notice

DESCRIPTION CN118662693A

A biomimetic bone material with controllable solidification time, its preparation method and application

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一种凝固时间可控的仿生骨质及其制备方法和应用

[0001]

Technical Field

技术领域

[n0001]

This invention belongs to the field of bone repair biomaterials, specifically relating to a biomimetic bone material with controllable solidification time, its preparation method, and its application.

本发明属于骨修复生物材料领域，具体涉及一种凝固时间可控的仿生骨质及其制备方法和应用。

[0003]

Background Technology

背景技术

[n0002]

The main treatment strategies for fractures and bone defects are reduction, fixation, and functional exercises.

骨折、骨缺损的治疗策略主要是复位、固定、功能锻炼。

Clinically, the fixation and filling of fractures and bone defects currently rely mainly on internal fixation and bone filling materials, including autologous bone, allogeneic bone, and xenogeneic decellularized bone, and bone integration and repair are carried out under the body's self-regenerative capacity.

临床上目前对骨折、骨缺损的固定和填充主要依靠内固定和骨填充材料，包括自体骨、同种异体骨和异种脱细胞骨等，并在机体自我再生能力下进行骨整合和修复。

However, this surgical method requires two subsequent surgeries to remove the internal fixation, and the integration of the bone filling material with the body's bone takes a long time.

但是这种手术方式在后续一是需要二次手术将内固定取出，二是骨填充材料与机体的骨整合需要较长时间。

Therefore, there is an urgent need to develop a new biomedical material for immediate repair of bone tissue structures in clinical fracture and bone defect surgeries.

因此，亟需开发一种用于即刻修复骨组织结构的新型生物医用材料用于临床骨折、骨缺损手术。

[n0003]

There are currently some solutions for immediate bone tissue repair.

对于即刻骨组织修复，目前已有一些解决方案。

First, bone adhesive is considered an effective solution for repairing cortical bone. It can form a strong bond at the fracture ends, restore the physiological position of the bone, and achieve the support and stress strength of the bone. Patent application CN202310767115.2 discloses an injectable adhesive that promotes fracture healing. This invention mimics the ability of carboxyl groups on the side chains of marine barnacle biomaterials to absorb moisture from the interface, thereby breaking the bond between the interface and the water layer and improving the bonding strength between the dynamic network and the interface, thus enhancing the adhesive's adhesion performance. This results in good adhesion performance of the adhesive in solution. However, due to the significant difference in bone composition, there are issues with mechanical strength and biosafety. Patent application CN202211370224.2 discloses a photocurable bone adhesive. This patent incorporates a photoinitiator into the bone adhesive, reducing the curing time. However, the curing process requires light initiation, which reduces the ease of use of the adhesive. Secondly, artificial bone is another solution, which can achieve biomimicry of the structure of natural bone tissue, thereby promoting bone ingrowth and bone integration. Patent application CN200910043405.2 discloses a method for manufacturing a biomimetic bone material with non-uniformly distributed pores. Using HA/Ti or HA/316L composite powder as raw material, the raw powder is separated into

a dense layer/transition layer/loose layer within a mold using a molybdenum sheet. A compact is obtained through conventional pressing, and the compact is then vacuum sintered to obtain the biomimetic bone material. However, its composition and physical properties differ significantly from bone tissue, while achieving immediate integration with bone tissue. Patent application CN202210656568.3 discloses a biomimetic bone material based on icariin-functionalized polylactic acid and its preparation method, which also suffers from significant differences in composition compared to bone tissue.

首先，骨粘合剂被认为是一种修复皮质骨的有效方案，其可以在骨折的断端形成强大的粘合能力，恢复骨骼的生理位置，达到骨骼的支撑及应力强度。 申请号为CN202310767115.2的专利发明了一种促进骨折愈合的可注射粘合剂，该发明仿生海洋藤壶生物物质侧链上的羧基能够吸收界面中的水分以打破界面与水层的结合，提高动态网络与界面的结合强度，从而提高粘合剂的粘附性能，其使得粘合剂在溶液中具有较好的粘连性能，但是相对于骨质成分差异过大，力学强度和生物安全存在问题；申请号为CN202211370224.2的发明专利发明了一种光固化的骨粘合剂，该专利将光引发剂加入骨粘合剂中，降低了骨粘合剂的固化时间，但是在粘合剂的固化过程中需要使用光照进行引发，这降低了该粘和剂的使用便捷性。 其次，人工骨质也是另一种解决方案，其可以实现对天然骨组织结构的仿生，从而促进骨长入和骨整合。 申请号为CN200910043405.2的发明专利公开了一种孔隙非均匀分布仿生骨质材料制造方法，以HA/Ti或HA/316L复合粉为原料，在模具内通过钼片将原料粉分开形成致密层/过渡层/疏松层，常规压制得到压坯，压坯进行真空烧结得到仿生骨质，但是其成分与物理性质与骨组织差异较大，同时实现与骨组织的即刻整合；申请号为CN202210656568.3的发明专利公开

了了一种基于淫羊藿苷功能化聚乳酸仿生骨质材料及其制备方法，同样存在与骨组织成分差异大的问题。

[n0004]

Therefore, there is an urgent need to develop a biomimetic bone material with a composition close to bone and a controllable coagulation time that can achieve immediate bone repair.

因此，迫切需要开发出一种成分接近骨质且可实现即刻骨修复的凝固时间可控的仿生骨质。

[0007]

Summary of the Invention

发明内容

[n0005]

In order to overcome the defects and shortcomings of the existing technology, the present invention provides a biomimetic bone material with controllable coagulation time, its preparation method and application.

为了克服现有技术中存在的缺陷和不足，本发明提供一种凝固时间可控的仿生骨质及其制备方法和应用。

[n0006]

Specifically, the present invention is achieved through the following technical solutions:

具体地，通过以下几个方面的技术方案实现了本发明：

[n0007]

In a first aspect, the present invention provides a biomimetic bone material with controllable coagulation time, wherein the biomimetic bone material with controllable coagulation time is a biomimetic bone material obtained by mixing and reacting calcium phosphate salt, phosphate-based organic matter, and a biomimetic cancellation agent.

在第一方面，本发明提供了一种凝固时间可控的仿生骨质，所述凝固时间可控的仿生骨质为磷酸钙盐、磷酸基有机物、仿生松质化反应剂混合反应得到的仿生骨质。

[n0008]

In some embodiments, the ratio of calcium phosphate salt, phosphate-based organic matter, and biomimetic loosening agent is 0.1g-5g: 0.05-3g: 0.1-1mL.

在一些实施例中，所述磷酸钙盐、磷酸基有机物、仿生松质化反应剂配比为0.1g-5g：0.05-3g：0.1-1mL。

[n0009]

In some embodiments, the ratio of calcium phosphate salt, phosphate-based organic matter, and biomimetic loosening agent is 0.5g-2g:0.2-1g:0.2-0.5mL.

在一些实施例中，所述磷酸钙盐、磷酸基有机物、仿生松质化反应剂配比为0.5g-2g：0.2-1g：0.2-0.5mL。

[n0010]

Preferably, the ratio of calcium phosphate salt, phosphate-based organic matter, and biomimetic loosening agent is 1g:0.5g:0.35mL.

优选的，磷酸钙盐、磷酸基有机物、仿生松质化反应剂配比为1g：0.5g：0.35mL。

[n0011]

In some embodiments, the calcium phosphate salt is one or more of tetracalcium phosphate, calcium phosphate, and hydroxyapatite.

在一些实施例中，所述磷酸钙盐为磷酸四钙、磷酸钙、羟基磷灰石中的一种或多种的组合。

[n0012]

Preferably, the calcium phosphate salt is tetracalcium phosphate.

优选的，磷酸钙盐采用磷酸四钙。

[n0013]

In some embodiments, the phosphate-based organic compound is used to integrate calcium phosphate salts, including one or more combinations of phosphate amino acids, phosphonic acid carboxylic acids, and bisphosphonates.

在一些实施例中，所述磷酸基有机物用于整合磷酸钙盐，包括磷酸氨基酸、膦酸基羧酸、双膦酸盐中的一种或多种的组合。

[n0014]

Preferably, the phosphate-based organic compound is a phosphate amino acid.

优选的，磷酸基有机物采用磷酸氨基酸。

[n0015]

More preferably, the phosphate-based organic compound is phosphoserine.

更优选的，磷酸基有机物采用磷酸丝氨酸。

[n0016]

In some embodiments, the biomimetic fluffing agent is a mixture of a biomimetic fluffing liquid-phase reactant and a biomimetic fluffing solid-phase reactant.

在一些实施例中，所述仿生松质化反应剂由仿生松质化液相反应剂和仿生松质化固相反应剂混合而成。

[n0017]

The liquid-phase reactant contains sodium citrate and/or citric acid, and the liquid-phase reactant also contains water, while the solid-phase reactant is sodium bicarbonate.

液相反应剂包含柠檬酸钠和/或柠檬酸，所述液相反应剂还包含水，固相反应剂为碳酸氢钠。

[n0018]

Alternatively, the biomimetic fluffing agent may be sodium citrate.

或者，可选地，所述仿生松质化反应剂为柠檬酸钠。

[n0019]

Preferably, the molar ratio of sodium bicarbonate to citric acid is 3:1, and the reaction products are sodium citrate and carbon dioxide.

优选的，碳酸氢钠与柠檬酸的摩尔比为3：1，其反应产物为柠檬酸钠和二氧化碳。

[n0020]

Preferably, the ratio of water, sodium citrate, and citric acid is 0.2-0.5 mL: 0-500 mg: 0-400 mg.

优选的，水、柠檬酸钠、柠檬酸的配比为0.2-0.5mL：0-500mg：0-400mg。

[n0021]

More preferably, the ratio of water, sodium citrate, and citric acid is 0.3-0.4 mL: 0-155 mg: 0-115 mg.

更优选的，水、柠檬酸钠、柠檬酸的配比为0.3-0.4mL：0-155mg：0-115mg。

[n0022]

In a second aspect, the present invention provides a method for preparing biomimetic bone with controllable coagulation time as described in the first aspect above, comprising the following steps:

在第二方面，本发明提供了上述第一个方面所述的凝固时间可控的仿生骨质的制备方法，包括以下步骤：

[n0023]

(1) Weigh out calcium phosphate, phosphate-based organic matter and sodium bicarbonate respectively, mix them evenly to prepare a solid phase component, and put it into a threaded disposable syringe for later use.

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(1)分别称取磷酸钙盐、磷酸基有机物、碳酸氢钠，均匀混合后配置成固相组分，装入带螺纹一次性注射器中备用；

[n0024]

(2) Weigh out citric acid and sodium citrate solids respectively, add them to water to prepare a liquid phase component, and put them into another threaded disposable syringe for later use;

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(2)分别称取柠檬酸和柠檬酸钠固体，加入水中配制成液相组分，装入另外一支带螺纹一次性注射器中备用；

[n0025]

(3) After connecting the two threaded disposable syringes containing solid components and liquid components prepared in steps (1) and (1) respectively with disposable connectors, the solid components and liquid components are quickly and uniformly mixed to form a paste-like biomimetic bone fluid.

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(3)将步骤(1)和步骤(1)中制备的分别包含固相组分和液相组分的两只带螺纹一次性注射器使用一次性连接头进行连接后，使所述固相组分和所述液相组分快速均匀混合，制成糊状的仿生骨质液；

[n0026]

(4) Inject the paste-like biomimetic bone fluid prepared in step (3) into the site to be used for solidification.

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(4)将步骤(3)中制备得到的所述糊状的仿生骨质液注射到待用部位进行固化。

[n0027]

In some embodiments, the threaded disposable syringe has a volume of 1-10 mL.

在一些实施例中，所述带螺纹一次性注射器容积为1-10mL。

[n0028]

Preferably, a 5mL threaded disposable syringe is used.

优选的，使用5mL带螺纹一次性注射器。

[n0029]

In some embodiments, the curing time is 0-15 min.

在一些实施例中，所述固化时间为0-15min。

The setting time can be adjusted according to the total sodium citrate content.

凝固时间可随总柠檬酸钠含量调节。

[n0030]

Alternatively, in step (4), the contents of the two syringes can be mixed before being injected into one of the syringes.

作为可选的方式，在步骤(4)中，可以在将两只注射器中的内容物混匀后再推入其中一支注射器中进行注射。

[n0031]

In a third aspect, the present invention provides the application of biomimetic bone with controllable coagulation time as described in the first aspect above, or biomimetic bone with controllable coagulation time prepared by the preparation method described in the second aspect above.

在第三方面，本发明提供了上述第一个方面所述的凝固时间可控的仿生骨质或者采用上述第二个方面所述的制备方法制备得到的凝固时间可控的仿生骨质的应用。

[n0032]

Preferably, the present invention provides the application of a biomimetic bone material with controllable solidification time as described in the first aspect above, or a biomimetic bone

material with controllable solidification time prepared by the preparation method described in the second aspect above, in the preparation of bone repair biomaterials.

优选的，本发明提供了上述第一个方面所述的一种凝固时间可控的仿生骨质或者采用上述第二个方面所述的制备方法制备得到的凝固时间可控的仿生骨质在制备骨修复生物材料中的应用。

[n0033]

In some embodiments, the paste-like biomimetic bone fluid is injected into the intended site using a disposable syringe and then solidified.

在一些实施例中，将糊状的仿生骨质液使用一次性注射器注射到待用部位进行固化。

[n0034]

In some embodiments, the paste-like biomimetic bone material is used for bonding and filling fractures and bone defects. The biomimetic bone material of the present invention has certain compressive strength, tensile strength, and bonding properties, and can effectively control the curing time and degree of cancellation. Different ratios can be selected according to the clinical surgical time requirements and the degree of bone cancellation at the clinical filling site, making it easy to use in clinical practice.

在一些实施例中，所述糊状的仿生骨质应用于骨折、骨缺损粘合填充，本发明所述的仿生骨质具有一定的抗压、抗拉、粘结性能，同时能够有效地控制固化时间和松质化程度，根据临床手术时间需求和临床填充部位骨质松质化情况选择不同配比，易于临床使用。

[n0035]

In some embodiments, the biomimetic bone material has compressive strength, with an index range of 1.0-10.0 MPa.

在一些实施例中，仿生骨质具有抗压缩性能，其指标范围为1.0-10.0MPa。

[n0036]

In some embodiments, the biomimetic bone material has tensile strength, with an index range of 1.0-2.5 MPa.

在一些实施例中，仿生骨质具有抗拉伸性能，其指标范围为1.0-2.5MPa。

[n0037]

In some embodiments, the biomimetic bone material has adhesive properties, with an index range of 0.2-1.0 MPa.

在一些实施例中，仿生骨质具有粘结性能，其指标范围为0.2-1.0MPa。

[n0038]

In some embodiments, the coagulation time of the biomimetic bone can be adjusted, ranging from 10s to 900s.

在一些实施例中，仿生骨质的凝固时间可调节，其时间范围为10s-900s。

[n0039]

In some embodiments, the degree of cancellation of the biomimetic bone can be adjusted, with a trabecular distance of 45 μm-1000 μm, a bone area of 53%-95%, and a bone volume of 38%-96%.

在一些实施例中，仿生骨质的松质化程度可调节，其骨小梁距离为45μm-1000μm，骨质面积范围为53%-95%，骨质体积范围为38%-96%。

[n0040]

Beneficial effects of the present invention

本发明的有益效果

[n0041]

(1) The biomimetic bone of the present invention forms biomimetic bone immediately through reaction, which is beneficial to the immediate integration of biomimetic bone and autologous bone during surgery.

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(1)本发明的仿生骨质通过反应即刻形成仿生骨质，有利于手术中仿生骨质与自体骨质的立即整合。

[n0042]

(2) The biomimetic bone material of the present invention can effectively control the curing time by adjusting the sodium citrate ratio, which is beneficial to select according to the surgical implantation time requirements of different parts of the surgery and avoid curing too early or too late.

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(2)本发明的仿生骨质通过调控柠檬酸钠比例，可有效控制固化时间，有利于根据手术中不同部位手术植入时间需求选择，避免过早或过晚固化。

[n0043]

(3) The bionic bone of the present invention can effectively control the degree of cancellation by adjusting the ratio of bionic cancellation reactant to adapt to the mechanical strength and trabecular density of the surgical implantation site.

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(3)本发明的仿生骨质通过调控仿生松质化反应剂配比，可有效控制松质化程度，以适应手术植入部位的力学强度和骨小梁密度。

[0047]

Attached Figure Description

附图说明

[n0044]

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：

[n0045]

Figure 1 shows the surface of the biomimetic bone as observed under a scanning electron microscope.

图1为仿生骨质在扫描电镜下观察到的骨表面情况图。

[n0046]

Figure 2 shows the trabecular density of biomimetic bone under Micro-CT reconstruction.

图2为仿生骨质在Micro-CT重建下的骨小梁密度情况图。

[n0047]

Figure 3 shows the image of MC3T3 cells cultured with the extract of biomimetic bone and stained with an alkaline phosphatase staining kit after osteogenic induction with osteogenic induction solution.

图3为仿生骨质的浸提液对MC3T3细胞培养并使用成骨诱导液诱导成骨后用碱性磷酸酶染色试剂盒染色后的图片。

[n0048]

Figure 4 shows the effect of biomimetic bone extract on HUVEC cell culture and the promotion of angiogenesis.

图4为仿生骨质的浸提液对HUVEC细胞培养并促进血管成环的情况。

[n0049]

Figure 5 shows the maintenance of bone continuity and bone integration under Micro-CT reconstruction after the use of biomimetic bone in a rabbit comminuted fracture model.

图5为仿生骨质在兔子粉碎性骨折模型中使用后，Micro-CT重建下的骨连续性维持和骨质整合情况。

[n0050]

Figure 6 shows the bone integration under Micro-CT reconstruction after the use of biomimetic bone in a rabbit defect model.

图6为仿生骨质在兔子缺损模型中使用后，Micro-CT重建下的骨质整合情况。

[0055]

Detailed Implementation

具体实施方式

[n0051]

The following detailed description, in conjunction with embodiments, illustrates a biomimetic bone material with controllable coagulation time, its preparation method, and its applications provided by the present invention. However, these descriptions should not be construed as limiting the scope of protection of the present invention.

下面结合实施例对本发明提供的一种凝固时间可控的仿生骨质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。

[n0052]

Example 1

实施例1

[n0053]

Weigh out 1.0g of tetracalcium phosphate and 0.5g of phosphoserine, mix them evenly to prepare a solid phase, and put it into a 5mL threaded disposable syringe for later use. Use 0.35

mL of water as the liquid phase and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸丝氨酸0.5g，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，使用0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0054]

Example 2

实施例2

[n0055]

Weigh out 1.0g of tetracalcium phosphate, 0.5g of phosphoserine, and 76mg of sodium bicarbonate. Mix them evenly to prepare a solid phase component and put it into a 5mL threaded disposable syringe for later use. Add 58mg of citric acid to 0.35mL of water to prepare a liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸丝氨酸0.5g、碳酸氢钠76mg，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将58mg柠檬酸加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0056]

Example 3

实施例3

[n0057]

Weigh out 1.0g of tetracalcium phosphate, 0.5g of phosphoserine, and 38mg of sodium bicarbonate. Mix them evenly to prepare a solid phase component and put it into a 5mL threaded disposable syringe for later use. Add 39mg of sodium citrate and 29mg of citric acid to 0.35mL of water to prepare a liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸丝氨酸0.5g、碳酸氢钠38mg，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将39mg柠檬酸钠、29mg柠檬酸加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0058]

Example 4

实施例4

[n0059]

Weigh out 1.0g of tetracalcium phosphate and 0.5g of phosphoserine, mix them evenly to prepare a solid phase component, and put it into a 5mL threaded disposable syringe for later use. Add 78mg of sodium citrate to 0.35mL of water as the liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸丝氨酸0.5g，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将78mg柠檬酸钠加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0060]

Example 5

实施例5

[n0061]

Weigh out 1.0g of tetracalcium phosphate, 0.5g of phosphoserine, and 151mg of sodium bicarbonate. Mix them evenly to prepare a solid phase component and put it into a 5mL threaded disposable syringe for later use. Add 115mg of sodium citrate to 0.35mL of water to prepare a liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸丝氨酸0.5g、碳酸氢钠151mg，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将115mg柠檬酸钠加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0062]

Example 6

实施例6

[n0063]

Weigh out 1.0g of tetracalcium phosphate, 0.5g of phosphoserine, and 76mg of sodium bicarbonate. Mix them evenly to prepare a solid phase component and put it into a 5mL threaded disposable syringe for later use. Add 78mg of sodium citrate and 58mg of citric acid to 0.35mL of water to prepare a liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸丝氨酸0.5g、碳酸氢钠76mg，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将78mg柠檬酸钠、58mg柠檬酸加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0064]

Example 7

实施例7

[n0065]

Weigh out 1.0g of tetracalcium phosphate and 0.5g of phosphoserine, mix them evenly to prepare a solid phase component, and put it into a 5mL threaded disposable syringe for later use. Add 155mg of sodium citrate to 0.35mL of water as the liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸丝氨酸0.5g，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将155mg柠檬酸钠加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0066]

Example 8

实施例8

[n0067]

Weigh 1.0g of hydroxyapatite, 0.5g of phosphoserine, and 38mg of sodium bicarbonate. Mix them evenly to prepare a solid phase component and fill it into a 5mL threaded disposable syringe for later use. Add 39mg of sodium citrate and 29mg of citric acid to 0.35mL of water to prepare a liquid phase component and fill it into another 5mL threaded disposable syringe for later use.

称取羟基磷灰石1.0g、磷酸丝氨酸0.5g、碳酸氢钠38mg，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将39mg柠檬酸钠、29mg柠檬酸加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0068]

Example 9

实施例9

[n0069]

Weigh out 1.0g of tetracalcium phosphate, 0.5g of phosphotyrosine, and 38mg of sodium bicarbonate. Mix them evenly to prepare a solid phase component and put it into a 5mL

threaded disposable syringe for later use. Add 39mg of sodium citrate and 29mg of citric acid to 0.35mL of water to prepare a liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、磷酸酪氨酸0.5g、碳酸氢钠38mg，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将39mg柠檬酸钠、29mg柠檬酸加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0070]

Example 10

实施例10

[n0071]

Weigh out 1.0g of tetracalcium phosphate, 0.5g of 3-phosphonopropionic acid, and 38mg of sodium bicarbonate. Mix them evenly to prepare a solid phase component and put it into a 5mL threaded disposable syringe for later use. Add 39mg of sodium citrate and 29mg of citric acid to 0.35mL of water to prepare a liquid phase component and put it into another 5mL threaded disposable syringe for later use.

称取磷酸四钙1.0g、3-膦酸基丙酸0.5g、碳酸氢钠38mg，均匀混合后配置成固相组分，装入5mL带螺纹一次性注射器中备用，将39mg柠檬酸钠、29mg柠檬酸加入0.35mL水作为液相组分装入另一支5mL带螺纹一次性注射器中备用。

The two threaded disposable syringes, one containing a solid phase component and the other a liquid phase component, were connected using a disposable connector and then quickly and evenly mixed to produce a paste-like biomimetic bone fluid.

将上述两只分别包含固相组分和液相组分的带螺纹一次性注射器使用一次性连接头进行连接后快速均匀混合，制成糊状的仿生骨质液。

[n0072]

The biomimetic bone materials obtained in Examples 1-10 were subjected to curing time, compressive strength, tensile strength, and adhesion strength tests, respectively.

将实施例1-10得到的仿生骨质分别进行固化时间、抗压强度、拉伸强度、粘连强度测试。

[n0073]

Example 1: Curing time, compressive strength, tensile strength, and adhesive strength tests

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效果例1：固化时间、抗压强度、拉伸强度、粘连强度测试

[n0074]

1.

1.

The paste-like biomimetic bone fluid obtained in Examples 1-10 was squeezed into a mold with a diameter of 6 mm for curing. The curing time was timed. After curing, the obtained biomimetic bone was taken out and subjected to a compressive strength test.

将实施例1-10的到的糊状仿生骨质液挤入直径6mm的模具中进行固化，对固化时间进行计时，固化后将得到的仿生骨质取出，进行抗压测试。

[n0075]

2.

2.

The paste-like biomimetic bone fluid obtained in Examples 1-10 was evenly applied to both ends of the broken pig femur. After the bonding process began, a pressure of 10N was applied to the top of the metal column. After curing, the test object was placed in a fixture, and the tensile strength was measured. The applied force was perpendicular to the surface of the bonded pig bone at a 90° angle. A universal joint or metal wire was used to connect the testing machine and the bonded pig bone. The tensile strength of the bonded pig femur was tested at a speed of 20mm/min.

将实施例1-10得到的糊状仿生骨质液均匀的涂抹在猪股骨的断端两头，从粘接开始后对金属柱顶施加10N压力，固化后被测物放置于夹具中，测量拉伸强度，施加的力与被粘猪骨表面成90°角垂直，试验机和被粘接猪骨之间使用万能关节或金属丝连接，以20mm/min速度测试粘接猪股骨拉伸强度。

[n0076]

3.

3.

The paste-like biomimetic bone fluid obtained in Examples 1-10 was applied to a smooth pig bone piece and pressed with a force of 10N to solidify it. After solidification, the sample was placed at 37 degrees Celsius for 30 minutes and then placed in a fixture to measure the adhesion strength. The applied force was parallel to the pig bone piece being bonded, and the adhesion strength of the bonded pig bone piece was tested at a speed of 20 mm/min.

将实施例1-10得到的糊状仿生骨质液涂抹在光滑的猪骨片上，施加10N的力按压使其固化，固化后37摄氏度放置30分钟后被测物放置于夹具中，测量粘连强度，施加的力与被粘猪骨片平行，以20mm/min速度测试粘接猪骨片粘连强度。

[n0077]

The test results are shown in Table 1.

测试结果见表1。

[n0078]

Table 1

表1

[n0081]

The table shows that as the total sodium citrate content increases, the curing time increases accordingly. Meanwhile, as the proportion of citric acid increases, the compressive and tensile strength of the biomimetic bone decreases, while the adhesion strength shows no significant trend.

从表中可以发现，随着总柠檬酸钠的含量提高，固化时间相应提高，同时，随着柠檬酸比例的提高，仿生骨质的抗压和抗拉强度下降，而粘连强度没有显著趋势。

[n0082]

Example 2: Bone Parameter Testing

效果例2：骨质参数测试

[n0083]

1.

1.

The biomimetic bone obtained in Examples 1-7 was observed using a scanning electron microscope to determine the size and number of pores, and the trabecular distance and percentage of bone area were statistically analyzed.

将实施例1-7得到的仿生骨质使用扫描电镜观察孔径大小及数量，统计骨小梁距离及骨质面积百分比。

[n0084]

2.

2.

The biomimetic bone obtained in Examples 1-7 was scanned using Micro-CT and reconstructed in 3D to determine the percentage of bone volume.

将实施例1-7得到的仿生骨质使用Micro-CT扫描，并进行3D重建，统计骨质体积百分比。

[n0085]

The test results are shown in Figure 1, Figure 2 and Table 2.

测试结果见图1、图2及表2。

[n0086]

Table 2

表2

[n0088]

The table shows that as the proportion of citric acid increases, the distance between bone trabeculae increases, the percentage of bone area decreases, and the percentage of bone volume decreases, indicating that the degree of cancellation of biomimetic bone can be controlled by adjusting the proportion of citric acid.

从表中可以发现，随着柠檬酸比例的提高，骨小梁距离大小相应增大，骨质面积百分比降低，骨质体积百分比降低，说明通过调节柠檬酸比例可以控制仿生骨质的松质化程度。

[n0089]

Example 3: Osteogenesis test, angiogenesis test, and osseointegration test

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效果例3：成骨测试、血管成环测试、骨整合测试

[n0090]

3.1 Osteogenesis Test

3.1成骨测试

[n0091]

Bionic bone extract was used in osteogenic differentiation experiments.

仿生骨质浸出液浸出液用于成骨分化实验。

The specific operation method is as follows: MC3T3 cells (from ATCC) are seeded into 96-well plates, with 0.5 million cells per well.

具体操作方法如下：将MC3T3细胞(来自ATCC)种植到96孔板中，每孔0.5万个细胞。

The leachate obtained in Example 4 (experimental group) or ordinary α-MEM medium (control group) was added to different wells. At the same time, an equal amount of osteogenic induction solution (10 mmol/L sodium β-glycerophosphate, 0.05 mmol/L vitamin C and 100

mmol/L dexamethasone) was added to each well and cultured for 7 days, with the medium being changed every two days.

分别将实施例4得到的浸出液(试验组)或普通α-MEM培养基(对照组)加入不同的孔中，同时向每孔中加入等量的成骨诱导液(10mmol/Lβ-甘油磷酸钠、0.05mmol/L维生素C和100mmol/L地塞米松)培养7天，每两天更换一次培养基。

Seven days later, the cells were fixed with 4% paraformaldehyde for 10 minutes and then stained with alkaline phosphatase (ALP) using an alkaline phosphatase (ALP) staining kit. The effect on osteoblast differentiation was determined based on the ALP staining area.

7天后使用4%多聚甲醛固定10分钟，使用碱性磷酸酶(ALP)染色试剂盒进行ALP染色，根据ALP染色面积判断对诱导成骨细胞分化的影响。

The results of osteogenic differentiation are shown in Figure 3.

成骨分化结果见图3。

As shown in the figure, the biomimetic bone extract group showed significantly higher ALP staining compared to the blank control group, indicating an effective osteogenic promoting effect.

图中可见仿生骨质浸出液组相对于空白对照组ALP染色明显，具有有效的成骨促进作用。

[n0092]

3.2 Angiogenesis Test

3.2成血管测试

[n0093]

50 μL of matrix adhesive was pre-spread at the bottom of the 96-well plate at 4°C to reduce air bubble formation, and then left to spread evenly overnight at 4°C.

在4°C条件下提前在96孔板底部铺上50μL的基质胶，过程减少气泡产生，4°C过夜使其均匀铺开。

After overnight curing, the 96-well plate was placed in a 37°C incubator to allow the matrix adhesive to harden.

将过夜后的96孔板放置在37°C培养箱中使基质胶固化。

HUVEC cells (from ATCC) were digested and resuspended in ordinary DMEM medium (control group) and the extract from Example 4 (experimental group), respectively. The resuspended cells were seeded in 96-well plates covered with matrix gel at a cell density of 30,000 cells /well.

消化HUVEC细胞(来自ATCC)，分别使用普通DMEM培养基(对照组)、实施例4的浸提液(试验组)重悬细胞，将重悬后的细胞种植在覆盖基质胶的96孔板中，细胞密度30000个/孔。

After culturing for 6 hours, observe the cell ring formation under a microscope.

培养6h后在显微镜下观察细胞的成环情况。

The results of the vascular circumduction test are shown in Figure 4.

血管成环测试结果见图4。

As shown in the figure, the biomimetic bone extract group exhibits significantly more vascular ring formation compared to the blank control group, demonstrating an effective angiogenesis-promoting effect.

图中可见仿生骨质浸出液组相对于空白对照组血管成环明显，具有有效的成血管促进作用。

[n0094]

3.3 Osteoporosis Integration Test

3.3密质骨整合测试

[n0095]

Modeling of comminuted fracture of rabbit femur: After anesthetizing the rabbit, the skin of the leg was prepared, disinfected and cut open to expose the femur layer by layer. A rectangular bone defect model was created using a wire saw. The bone fragments were crushed into four pieces. During the operation, the experimental group used the biomimetic bone material of Example 4 to bond the cortical bone fragments, while the control group did not undergo bonding.

对兔股骨进行粉碎性骨折造模：将兔麻醉后，对腿部皮肤进行备皮消毒切开，逐层暴露股骨，利用线锯进行长方形骨缺损造模，将骨片夹碎成四片，手术时试验组使用实施例4的仿生骨质粘合皮质骨碎片，对照组不进行粘合。

Four weeks later, a Micro-CT scan was performed, and bone reconstruction was carried out to observe whether the bionic bone effectively adhered to and integrated with the cortical bone tissue.

4周后进行Micro-CT扫描，并进行骨质重建，观察仿生骨质是否有效粘连整合皮质骨组织。

The Micro-CT reconstruction results are shown in Figure 5.

Micro-CT重建结果见图5。

As shown in the figure, compared with the control group, the femoral cortical bone in the biomimetic bone group showed good adhesion and integration, the cortical bone was continuous, and the biomimetic bone fused with the cortical bone, demonstrating a good compact bone integration effect.

图中可见仿生骨质组相对于对照组，股骨皮质骨得到良好粘连整合，皮质骨连续，仿生骨质与皮质骨融为一体，具有良好的密质骨整合效果。

[n0096]

3.4 Cancellous bone integration test

3.4松质骨整合测试

[n0097]

Modeling a circular defect in the femoral condyle of a rabbit: After anesthetizing the rabbit, the skin of the leg was prepared, disinfected, and cut open to expose the femoral condyle layer by layer. A hole was made using a punch to create a circular bone defect, and the bionic bone material of Example 4 was implanted into the defect site.

对兔股骨髁进行圆形缺损造模：将兔麻醉后，对腿部皮肤进行备皮消毒切开，逐层暴露股骨髁，利用打孔器进行打孔造成圆形骨缺损，将实施例4的仿生骨质植入缺损部位。

Four weeks later, a Micro-CT scan was performed, and bone reconstruction was carried out to observe whether the bionic bone integrated with the bone tissue.

4周后进行Micro-CT扫描，并进行骨质重建，观察仿生骨质是否与骨组织进行整合。

The Micro-CT reconstruction results are shown in Figure 6.

Micro-CT重建结果见图6。

As shown in the image, the biomimetic bone is completely integrated with the cancellous bone of the femoral condyle defect, with no boundary appearing, demonstrating a good cancellous bone integration effect.

图中可见仿生骨质与股骨髁缺损部位松质骨完全整合，未出现分界，具有良好的松质骨整合效果。

[n0098]

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。