Notice

DESCRIPTION CN112618797A

A method for preparing a specific demineralized extracellular matrix scaffold for cross-linked antibiotics

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一种交联抗生素的特定脱矿细胞外基质支架的制备方法

[0001]

Technical Field

技术领域

[n0001]

This invention relates to the field of bone infection treatment technology, and more specifically to a method for preparing a specific demineralized extracellular matrix scaffold for cross-linked antibiotics.

本发明涉及骨感染治疗技术领域，更具体的说是涉及一种交联抗生素的特定脱矿细胞外基质支架的制备方法。

[0003]

Background Technology

背景技术

[n0002]

Despite continuous improvements in medical standards, bone infection remains a significant challenge in clinical practice, with nearly 40% of patients facing recurrence and prolonged illness.

尽管医疗水平不断提高，骨感染在临床工作中依然是一个巨大的难题，将近40%的患者面临着感染的复发与迁延不愈。

In the pathological process of bone infection, bacteria multiply in large numbers, forming abscesses at the site of infection. These abscesses evade the immune system and protect the viable bacteria at their core. The factors and acidic metabolites secreted by the bacteria in the abscess not only promote the differentiation of osteoclasts and reduce the activity of osteoblasts, but also help bacteria adhere to collagen or cell membranes, promoting the formation of biofilms and leading to persistent bone infection.

在骨感染的病理过程中，细菌大量繁殖，在感染局部形成脓肿，躲避免疫系统追杀并保护其核心的活菌繁殖，脓肿里细菌分泌的因子以及酸性代谢产物，不仅促进破骨细胞的分化，降低成骨细胞的活性，同时有助于细菌粘附胶原或细胞膜上，促进生物膜的形成，导致骨感染迁延不愈。

Among the five key factors that make infections difficult to cure and cause them to turn from acute to chronic—abscess, bacterial adhesion and colonization, biofilm formation, dormant bacteria, and materials without antibacterial activity—surgical methods can only address abscesses and some biofilms; a complete cure still requires the implantation of antibacterial materials.

在脓肿、细菌粘附定植、生物膜形成、休眠细菌、及无抗菌活性的材料这5个导致感染难以治愈并由急性转为慢性的关键因素中，外科手段仅仅能解决脓肿及部分生物膜等因素，彻底的治愈仍需要依靠抗菌材料植入。

[n0003]

However, implanted biomaterials are "foreign objects" to the body. Materials with poor biocompatibility will fail to implant due to foreign body reaction (FBR) or bacterial adhesion. The encapsulation and fibrosis formed in the body not only hinder the integration between the implant and the host and facilitate bacterial adhesion, but also consume a large number of neutrophils and reduce the local tissue's resistance to infection. Both of these factors contribute to the progression of infection.

然而，植入的生物材料对于机体来说是一种“外来异物”，生物相容性差的材料在体内将由于异物反应(foreign body reaction，FBR)或细菌粘附导致植入失败，其在体内形成的包裹和纤维化，不仅阻碍植入物与宿主之间的整合，有利于细菌粘附，同时消耗大量的中性粒细胞，降低组织局部的抗感染能力，两者共同促进了感染的进展。

Therefore, improving the biocompatibility of implants while possessing anti-infection capabilities is crucial for antimicrobial materials.

因此，在具备抗感染能力的同时改进植入物的生物相容性，对于抗菌材料来说十分重要。

The following material design may be the best solution: 1) Modify the material to increase biocompatibility while minimizing bacterial adhesion; 2) Design smart antibacterial materials that respond to release and release on demand, so as to achieve sterilization while minimizing tissue and cell toxicity, and release antibacterial agents again as the environment changes to prevent infection recurrence.

如下设计的材料可能是解决的最佳方案：1)材料改性增加生物相容性的同时最大程度减少细菌的粘附能力；2)设计响应释放和按需释放的智能抗菌材料，在达到杀菌的同时最大程度减少组织及细胞的毒性，并且随着环境的改变再次释放抗菌剂以防止感染复发。

[n0004]

Current biodegradable antibacterial materials are mainly composed of inorganic materials such as hydroxyapatite and tricalcium phosphate, or synthetic materials such as polylactic-co-glycolic acid (PLGA), which are combined with different antibiotics, antimicrobial peptides, or metal ions.

目前的可降解抗菌材料，主要是由无机材料如羟基磷灰石、磷酸三钙或者合成材料如聚乳酸-羟基乙酸共聚物(poly-lactic-co-glycolic acid，PLGA)等复合不同的抗生素、抗菌肽或金属离子组成。

Inorganic materials are often modified with organic matter on their surface to improve their biocompatibility. To avoid cross-linking affecting drug activity, antibiotics, antimicrobial

peptides and other biological agents are mostly added to the material in a mixed manner to release them and kill airborne bacteria. This hybrid approach includes electrostatic adsorption drug delivery, nano-encapsulation, gel-based drug delivery, liposome encapsulation, microsphere encapsulation, and so on. Metal ions can be processed into nanoparticles and adsorbed into materials to kill airborne bacteria, or they can be cross-linked on the surface of materials to prevent bacterial adhesion. The application number is 201510882167. Patent X discloses an anti-infective calcium phosphate composite bone cement material and its preparation method, which achieves a broad-spectrum antibacterial effect; Patent application number 201210027387.0 discloses a method for preparing anti-infective medical materials using plasma technology, which can apply a wide range of antibacterial coatings to medical materials. Although the design of such materials has developed rapidly in recent decades and some have achieved ideal antibacterial functions, there are still many defects that need to be overcome. First, due to the difference in surface free energy between inorganic and organic media, this modification is not simple. The unstable coating will inevitably fall off over time and with changes in the internal environment, leading to a decrease in the biocompatibility of the material. Second, with the complete release of antibiotics or antimicrobial peptides, the material itself no longer has antibacterial properties, becomes a target for bacterial adhesion, and cannot prevent reinfection by "latent" bacteria. Finally, metal ions have cytotoxicity, which is not conducive to tissue repair and bone formation.

无机材料多在其表面进行有机物修饰以改进其生物相容性，而为避免交联对药物活性产生影响，抗生素、抗菌肽等生物制剂大多以混合的方式加入材料中以释放出来杀灭浮游细菌。 这种混合方式有静电吸附载药、纳米化封装、凝胶化载药、脂质体封装、微球封装等等。 金属离子既可加工成纳米颗粒吸附在材料中以杀灭浮游细菌，也可以交联在材料表面避免细菌粘附。 申请号为201510882167. X的专利公开了一种抗感染磷酸钙复合骨水泥材料及其制备方法，得到了广谱抗菌效果；申请号为201210027387.0的专利公开了一种采用等离子技术制备抗感染医用材料的方法，对医用材料可进行广泛的抗菌涂层，尽管在近几十年中，这类材料的设计得以快速发展，部分达到了理想抗菌功能，仍有不少缺陷需要克服。 首先，无机物与有机物两种介质之间由于表面自由能差异，这种修饰并不简单，不稳定的涂层随着时间推移和体内环境的变化，势必脱落导致材料的生物相容性下降；其次，随着抗生素或抗菌肽的完全释放，材料本身不再具备抗菌性能，成为细菌粘附的对象，且不能预防“潜伏”细菌的再次感染；最后，金属离子具有细胞毒性，不利于组织修复和成骨。

[n0005]

Numerous studies have shown that naturally derived extracellular matrix (ECM) scaffolds have advantages that artificial materials cannot match.

大量的研究表明，天然来源的细胞外基质支架(extracellular matrix，ECM)具有人工材料无法比拟的优势。

First, its perfect porosity and good biocompatibility, in addition to inducing the growth and differentiation of mesenchymal stem cells, can also induce chemotaxis of various progenitor cells and macrophages, and participate in immune regulation; second, the extracellular matrix scaffold contains a variety of endogenous proteins and factors, which can actively induce macrophage polarization and enhance the antibacterial ability of the immune system; third, these abilities will not weaken with time and environmental changes, unlike inorganic and synthetic materials modified with organic coatings.

首先，其完美的孔隙和良好的生物相容性，除了诱导间充质干细胞的生长分化以外，还可诱导趋化多种前体细胞和巨噬细胞，参与免疫调节；其次，细胞外基质支架含多种内源性蛋白及因子，可主动诱导巨噬细胞的极化，并且增强自身免疫系统的抗菌能力；第三、这些能力并不会如有机涂层改性的无机材料和合成材料一样，随着时间和环境的变化而减弱。

[n0006]

In the field of intelligent antibacterial research, the protonation of carboxylate groups is considered an effective strategy for acid-sensitive release. The decellularized extracellular matrix contains a large number of active carboxyl groups, which can not only adsorb positively charged antibacterial agents through electrostatic interactions, but also crosslink with the free amino groups of other molecules in the form of amide bonds.

在智能抗菌研究领域，羧酸根的质子化被认为是酸敏感释放的一种有效策略，脱细胞化的细胞外基质含大量的活性羧基，不但可以静电作用吸附带正电荷的抗菌剂，而且可与其他分子的游离氨基以酰胺键形式交联。

Many types of antibiotics carry a positive charge due to the presence of amino groups, and can cross-link with the carboxyl groups of various substances without affecting their antibacterial properties.

多种类的抗生素因含有氨基而带正电荷，同时可以与多种物质的羧基交联，不影响其抗菌性能。

[n0007]

Therefore, providing a method for preparing a specific demineralized extracellular matrix scaffold for cross-linked antibiotics is a problem that urgently needs to be solved by those skilled in the art.

因此，提供一种交联抗生素的特定脱矿细胞外基质支架的制备方法是本领域技术人员亟需解决的问题。

[0010]

Summary of the Invention

发明内容

[n0008]

In view of this, the present invention provides a method for preparing a specific demineralized extracellular matrix scaffold for cross-linked antibiotics, which uses a specific demineralized extracellular matrix as a biocompatible scaffold and combines antibiotics in two forms: electrostatic adsorption and chemical cross-linking.

有鉴于此，本发明提供了一种交联抗生素的特定脱矿细胞外基质支架的制备方法，通过特定脱矿细胞外基质作为生物相容性支架，并以静电吸附和化学交联两种形式复合抗生素。

[n0009]

To achieve the above objectives, the present invention adopts the following technical solution:

为了实现上述目的，本发明采用如下技术方案：

[n0010]

Cancellous bone particles are obtained from cancellous bone-rich parts of animals, and decellularization is performed using an optimized decellularization protocol to prepare a specific demineralized cancellous bone extracellular scaffold. Antibiotics are then compounded in two forms: electrostatic adsorption and chemical cross-linking. The antibiotics are released through sustained release and pH response to achieve long-term anti-infection and repair-promoting effects.

从动物富含松质骨部位获取松质骨颗粒，以优化的脱细胞方案进行脱细胞，制备特定脱矿的松质骨细胞外支架，并以静电吸附和化学交联两种形式复合抗生素，通过缓释及pH响应释放抗生素，达到长期的抗感染和促修复作用。

[n0011]

A method for preparing a specific demineralized extracellular matrix scaffold for cross-linked antibiotics, the specific steps of which are as follows:

一种交联抗生素的特定脱矿细胞外基质支架的制备方法，具体步骤如下：

[n0012]

(1) Obtain cancellous bone particles with a diameter of 4-8 mm from the cancellous bone-rich parts of animals;

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(1)从动物富含松质骨部位获取直径为4-8mm的松质骨颗粒；

[n0013]

(2) Decellularization:

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(2)脱细胞：

[n0014]

① Cut the cancellous bone particles obtained in step (1) into cylinders with a thickness of 2-4 mm to obtain cancellous bone blocks;

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①将步骤(1)获取的松质骨颗粒，切成2-4mm厚度的圆柱体，获得松质骨块；

[n0015]

② Rinse the cancellous bone blocks obtained in step ① with tap water for 1 hour, package them in an embedding box, and then soak them in 0.6% (v/v) peracetic acid ultrapure water for 1 hour;

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②将步骤①获得的松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1h；

[n0016]

③ Transfer the embedding cassette to a flask containing 1% (v/v) Triton-X100 solution after filtration and sterilization, and shake at 100 rpm and 4°C for 12-48 hours;

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③将包埋盒转移至过滤除菌后的1%(v/v)Triton-X100溶液的烧瓶中，100rpm，4°C震动12-48h；

[n0017]

④ Rinse the embedding cassette with 500ml of sterile water and stir continuously for 1 hour. Repeat twice.

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④用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；

[n0018]

⑤ Add the cancellous bone block to a 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 12-48 h, wash the embedding cassette, and obtain the cancellous bone ECM;

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⑤将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动12-48h，清洗包埋盒，获得松质骨ECM；

[n0019]

⑥ Soak the cancellous bone ECM in 10% EDTA decalcification solution, then place it in the VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine and decalcify at 4°C for 2-6 hours;

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⑥将松质骨ECM浸泡于10%EDTA脱钙液中，放入VCare(上海颖穆)快速超声脱钙机中，在4°C下脱钙2-6h；

[n0020]

⑦ Rinse the ECM bone blocks with tap water, sterilize them by radiation, and freeze-dry them to obtain the extracellular scaffold of cancellous bone cells.

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⑦将ECM骨块用自来水冲洗，辐射消毒，并冻干保存，获得松质骨细胞外支架；

[n0021]

(3) Extracellular scaffold of cancellous bone combined with antibiotics:

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(3)松质骨细胞外支架复合抗生素：

[n0022]

30 mg of the material powder was added to the antibiotic solution and soaked for 1 hour to ensure thorough mixing. An equal volume of EDC (1-ethyl-3-(3-dimethyl aminopropyl, 1-ethyl-3-(3-dimethylaminopropyl))-NHS (N-hydroxysuccinimide, N-hydroxysuccinimide) mixed solution was added, wherein the EDC concentration in the EDC-NHS mixed solution was 16 mM and the NHS concentration was 4 mM. The reacted material was washed three times with deionized water, centrifuged at 9000 rpm/min for 5 min, the supernatant was discarded, and the material was lyophilized and stored to obtain a specific demineralized extracellular matrix scaffold for cross-linked antibiotics.

将30mg材料粉末加入到抗生素溶液中，浸泡1h，使两者充分混匀；加入等体积的EDC(1-ethyl-3-(3-dimethyl aminopropyl，1-乙基-3-(3-二甲基氨基丙基))-NHS(N-hydroxysuccinimide，N-羟基琥珀酰亚胺)混合溶液，所述EDC-NHS混合溶液中EDC浓度为16mM，NHS浓度为4mM；反应完成的材料用去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存，获得交联抗生素的特定脱矿细胞外基质支架；

[n0023]

The antibiotic solution is a vancomycin solution of 2-20 mg/ml, or a ceftriaxone solution of 2-20 mg/ml, or a cefepime solution of 2-20 mg/ml.

所述抗生素溶液为2-20mg/ml的万古霉素溶液，或2-20mg/ml的头孢曲松溶液，或2-20mg/ml的头孢吡肟溶液。

[n0024]

Furthermore, the animal mentioned in step (1) is a large mammal, including cattle and pigs.

进一步，步骤(1)所述动物为大型哺乳动物，包括牛、猪。

[n0025]

Furthermore, the cancellous bone-rich areas mentioned in step (1) include the spine, ribs, scapula, and pelvis.

进一步，步骤(1)所述富含松质骨部位包括脊柱、肋骨、肩胛、骨盆。

[n0026]

Furthermore, the 1% (v/v) Triton-X100 described in step (2)③ is sterilized by filtration with a 0.22 μm filter head.

进一步，步骤(2)③所述1%(v/v)Triton-X100用0.22μm滤头过滤除菌。

[n0027]

Furthermore, the radiation dose described in step (2) ⑦ is 250 kGy.

进一步，步骤(2)⑦所述辐射剂量为250kGy。

[n0028]

As can be seen from the above technical solution, compared with the prior art, the present invention discloses a method for preparing a specific demineralized extracellular matrix scaffold for cross-linked antibiotics. The antibiotics are compounded in two forms: electrostatic adsorption and chemical cross-linking. The antibiotics are released through sustained release and pH response, providing a new idea and method for long-term anti-infection and repair promotion of bone implants.

经由上述的技术方案可知，与现有技术相比，本发明公开提供了一种交联抗生素的特定脱矿细胞外基质支架的制备方法，以静电吸附和化学交联两种形式复合抗生素，通过缓释及pH响应释放抗生素，为长期的骨植入物抗感染和促修复提供新思路和新方法。

Compared to existing bone implants containing anti-infection and repair-promoting drugs or materials, the advantages of this invention are as follows:

相比现有骨植入物抗感染和促修复的药物或材料，本发明的有益效果在于：

[n0029]

1) It uses naturally derived extracellular matrix materials, which have good biocompatibility and bioactivity;

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1)采用天然来源的细胞外基质材料，具有良好的生物相容性及生物活性；

[n0030]

2) Specific demineralization of cancellous bone decellularized materials enhances bioactivity while preserving porosity and mechanical strength;

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2)对松质骨脱细胞材料进行特定脱矿，增强生物活性同时保留孔隙及机械强度；

[n0031]

3) Antibiotics are combined with electrostatic adsorption and covalent cross-linking methods respectively to achieve acid-sensitive release and long-term degradation-induced release effects;

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3)分别采取静电吸附及共价交联的方式结合抗生素，以达到酸敏感释放和长期的降解引发释放效果；

[n0032]

4) In vitro experiments verified its significant free bactericidal and contact bactericidal effects, and it had a clear inhibitory effect on the number of airborne bacteria, bacterial adhesion, and total bacterial count.

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4)体外实验验证其显著的游离杀菌和接触杀菌效果，对浮游细菌数量、细菌粘附、细菌总量指标均有明确的抑制作用；

[n0033]

5) The in vitro experiments verified significant anti-infection and osteogenic effects, and it has a clear effect on improving bacterial inflammation and infection in bone tissue and bone repair mediated by osteoclast-osteogenetic balance.

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5)在体外实验验证显著的抗感染和促成骨作用，对骨组织细菌引发的炎症及感染情况及破骨-成骨平衡介导的骨修复均有明确的改善作用；

[n0034]

6) As an antibiotic modification material based on the extracellular matrix, the modified extracellular matrix groups and antibiotics or other bioactive molecules can be replaced, and it has sufficient reference value.

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6)作为一种基于细胞外基质的抗生素修饰材料，所修饰的细胞外基质基团及抗生素或其他生物活性分子均可替换，具有充足的可借鉴性。

[n0035]

The cross-linked antibiotic-specific demineralized extracellular matrix scaffold of the present invention can be used to prevent and treat bone infections, promote bone repair, and has a clear therapeutic effect.

本发明交联抗生素的特定脱矿细胞外基质支架可应用于防治骨感染，促进骨修复，具有明确的治疗效果。

[0039]

Attached Figure Description

附图说明

[n0036]

To more clearly illustrate the technical solutions in the embodiments of the present invention or the prior art, the drawings used in the description of the embodiments or the prior art will be briefly introduced below. Obviously, the drawings described below are only embodiments of the present invention. For those skilled in the art, other drawings can be obtained based on the provided drawings without creative effort.

为了更清楚地说明本发明实施例或现有技术中的技术方案，下面将对实施例或现有技术描述中所需要使用的附图作简单地介绍，显而易见地，下面描述中的附图仅仅是本发明的实施例，对于本领域普通技术人员来讲，在不付出创造性劳动的前提下，还可以根据提供的附图获得其他的附图。

[n0037]

Figure 1 is a schematic diagram illustrating the principle of Van-SDECM stent synthesis and release according to the present invention;

图1附图为本发明Van-SDECM支架合成与释放的原理示意图；

[n0038]

Figure 2 shows the zeta potentials of SDECM, Van, ECM and SDECM+Van (SDECM adsorbed with Van) of the present invention.

图2附图为本发明SDECM、Van、ECM和SDECM+Van(SDECM与Van吸附)的Zeta电位；

[n0039]

Figure 3 is a schematic diagram of the electrostatic interaction between the SDECM carboxyl group and vancomycin amino group under different pH conditions.

图3附图为本发明SDECM羧基与万古霉素氨基在不同pH条件下的静电相互作用示意图；

[n0040]

Figure 4 shows the infrared spectrum of Van-SDECM during synthesis (blue arrows indicate NH stretching bands, red arrows indicate C=O stretching bands, and black curves indicate synthesized Van-SDECM) and the infrared spectrum of Van-SDECM after 6 weeks of degradation (Van-SDECM, red curve; original material, black curve).

图4附图为本发明Van-SDECM合成时红外谱图(蓝色箭头表示NH伸缩带，红色箭头表示C＝O伸缩带，黑色曲线指合成的Van-SDECM)和Van-SDECM降解6周后的红外谱图(Van-SDECM，红色曲线；原始材料，黑色曲线)；

[n0041]

Figure 5 shows the amount of vancomycin in SDECM prepared by the present invention using different EDC and NHS concentrations (30 mg SDECM powder, initial vancomycin concentration 5 mg/mL);

图5附图为本发明用不同的EDC和NHS浓度(SDECM粉末30mg，初始万古霉素浓度5mg/mL)制备的SDECM中万古霉素的量；

[n0042]

Figure 6 shows the amount of vancomycin in SDECM prepared by the present invention with different EDC/NHS ratios;

图6附图为本发明以不同EDC/NHS比例制备的SDECM中万古霉素的量；

[n0043]

Figure 7 shows the residual amount of vancomycin in Van-SDECM prepared with different EDC /NHS concentrations and ratios after ultrasonic cleaning according to the present invention; line c represents the approximate amount of covalently loaded vancomycin; line d represents the total amount of vancomycin in the SDECM EDC = 32mM.

图7附图为本发明在超声清洗不同的EDC/NHS浓度和比例制备的Van-SDECM后，材料中万古霉素的残留量；线c表示共价负载的万古霉素的大约量；线d表示SDECM EDC中万古霉素的总量＝32mM；

[n0044]

Figure 8 shows the cumulative amount of vancomycin released from the material prepared by electrostatic adsorption of the present invention; the dashed line represents the initial amount of vancomycin loaded in different materials.

图8附图为本发明单纯静电吸附制备的材料释放的万古霉素的累积量；虚线表示在不同材料中加载的万古霉素的初始量；

[n0045]

Figure 9 shows the cumulative amount of vancomycin released from the material prepared under the conditions of electrostatic adsorption and chemical crosslinking according to the present invention; the dashed line represents the initial amount of vancomycin loaded in different materials;

图9附图为本发明在静电吸附和化学交联条件下制备的材料释放的万古霉素的累积量；虚线表示在不同材料中加载的万古霉素的初始量；

[n0046]

Figure 10 shows the amount of vancomycin released from Van-SDECM when the scaffold is cultured with osteoclasts for 5 days according to the present invention.

图10附图为本发明当将支架与破骨细胞培养5天时，从Van-SDECM释放的万古霉素的量；

[n0047]

Figure 11 shows the inhibition zone test of the Van-SDECM (experimental group) and SDECM (control group) of the present invention against Staphylococcus aureus and Enterococcus.

图11附图为本发明Van-SDECM(试验组)和SDECM(对照组)对金黄色葡萄球菌和肠球菌的抑菌环试验；

[n0048]

Figure 12 shows the bacterial count of Staphylococcus aureus suspension determined by the plate method after co-culturing different materials for 24 hours according to the present invention; scale bar = 10 mm;

图12附图为本发明不同材料共培养24小时后，用平板法测定金黄色葡萄球菌悬液的细菌数；比例尺＝10mm；

[n0049]

Figure 13 shows the quantitative analysis of the number of Staphylococcus aureus bacteria in suspension determined by the plate method after co-culturing different materials for 24 hours according to the present invention.

图13附图为本发明不同材料共培养24小时后，用平板法测定金黄色葡萄球菌悬液细菌数的定量分析；

[n0050]

Figure 14 shows the materials after ultrasonic cleaning of the present invention, co-cultured with Staphylococcus aureus for 24 hours, and the bacteria adhering to the surface of each group of samples observed by scanning electron microscopy; the bacterial outlines were processed with Photoshop for pseudo-color; it can be seen that the cross-linked samples have significantly increased anti-adhesion bacterial ability; scale bar = 5 μm;

图14附图为本发明超声清洗后的材料与金黄色葡萄球菌共培养24小时，扫描电镜观察各组样本表面的粘附细菌；细菌轮廓用photoshop作伪彩处理；可见经过交联的样本抗粘附细菌能力明显增加；比例尺＝5μm；

[n0051]

Figure 15 shows the quantitative analysis of the adhering bacteria on the surface of each group of samples after ultrasonic cleaning of the material of the present invention and co-culture with Staphylococcus aureus for 24 hours.

图15附图为本发明超声清洗后的材料与金黄色葡萄球菌共培养24小时，各组样本表面的粘附细菌的定量分析；

[n0052]

Figure 16 shows a confocal microscope image of the adhesive bacteria of the present invention after live/dead staining; scale bar = 1 mm.

图16附图为本发明粘附细菌经live/dead染色后的共聚焦显微镜图像；比例尺＝1mm；

[n0053]

Figure 17 shows the quantitative analysis of the adhesive bacteria of the present invention after live/dead staining; significant differences are * (P<0.05), ** (P<0.01), and *** (P<0.001).

图17附图为本发明粘附细菌经live/dead染色后的定量分析；显著性差异为*(P<0.05)，**(P<0.01)；***(P<0.001)；

[n0054]

Figure 18 shows the toxicity of different concentrations of vancomycin to MSCs as determined by the CCK-8 assay of this invention;

图18附图为本发明CCK-8检测不同浓度万古霉素对MSCs的毒性；

[n0055]

Figure 19 shows the toxicity of Van-SDECM leachate to MSCs determined by the CCK-8 test of this invention;

图19附图为本发明CCK-8试验测定Van-SDECM浸出液对MSCs的毒性；

[n0056]

Figure 20 shows the live/dead staining results of MSCs after co-culturing Van-SDECM scaffolds prepared with different concentrations of vancomycin (1 mg/mL, 5 mg/mL, 10 mg/mL) with MSCs for 48 h; scale bar = 100 μm;

图20附图为本发明不同浓度万古霉素(1mg/mL、5mg/mL、10mg/mL)制备的Van-SDECM支架与MSCs共培养48h后，MSCs的live/dead染色结果；比例尺＝100μm；

[n0057]

Figure 21 shows the ALP staining results of the Van-SDECM scaffold prepared by the present invention with initial vancomycin = 5 mg, EDC = NHS = 16 mM, and its extract co-cultured with osteoblasts for 4 days; scale bar = 100 μm.

图21附图为本发明以初始万古霉素＝5mg、EDC＝NHS＝16mM制备Van-SDECM支架，获取其浸提液与成骨细胞共培养4天，ALP染色结果；比例尺＝100μm；

[n0058]

Figure 22 shows typical bacterial images of soft tissue homogenized and spread on plates from the defect area of each group at 1 week and 6 weeks after the establishment of the experimental model of infected bone defect according to this invention; Group I: simple defect group; Group II: defect + infection group; Group III: defect + infection + SDECM group; Group IV: defect + infection + vancomycin group; Group V: defect + infection + van-SDECM group; Scale bar = 10 mm;

图22附图为本发明对感染性骨缺损的实验模型建立后1周、6周，分别获取各组别缺损区的软组织进行匀浆，涂布于平板上的细菌典型图片；Group I：单纯缺损组(Defect)；Group II：缺损+感染组(Defect+infection)；Group III：缺损+感染+SDECM组(Defect+infection+SDECM)；Group IV：缺损+感染+万古霉素组(Defect+infection+Van)；Group V：缺损+感染+Van-SDECM组(Defect+infection+Van-SDECM)；比例尺＝10mm；

[n0059]

Figure 23 shows the quantitative statistical analysis of bacteria on plates at 1 week and 6 weeks after the establishment of the experimental model of infectious bone defect of the present invention.

图23附图为本发明感染性骨缺损的实验模型建立后1周、6周，涂布于平板上的细菌定量统计分析；

[n0060]

Figure 24 shows typical images of Micro-CT scans performed on each group at 1 week and 6 weeks after the establishment of the experimental model of infectious bone defect according to this invention; scale bar = 1 mm;

图24附图为本发明对感染性骨缺损的实验模型建立后1周、6周，分别行各组别Micro-CT检查的典型图片；比例尺＝1mm；

[n0061]

Figure 25 shows the semi-quantitative statistical analysis of Micro-CT examinations performed on each group at 1 week and 6 weeks after the establishment of the experimental model of infectious bone defects according to the present invention.

图25附图为本发明对感染性骨缺损的实验模型建立后1周、6周，分别行各组别Micro-CT检查的半定量统计分析；

[n0062]

Figure 26 shows the anti-infection performance of the H&E observation material at 6 weeks post-surgery according to the present invention; scale bar = 1 mm (4X), 250 μm (25X), 100 μm (40X);

图26附图为本发明术后6周H&E观察材料的抗感染性能；比例尺＝1mm(4X)、250μm(25X)、100μm(40X)；

[n0063]

Figure 27 shows the osteogenic properties of the material observed by Masson staining 6 weeks post-surgery; scale bar = 1 mm (4X), 250 μm (25X), 100 μm (40X);

图27附图为本发明术后6周Masson染色观察材料的成骨性能；比例尺＝1mm(4X)、250μm(25X)、100μm(40X)；

[n0064]

Figure 28 shows the flow cytometry results of this invention, which confirmed that more osteoblasts apoptotic at pH 6.0 than at pH 7.4. The horizontal axis represents AV-FITC, and the vertical axis represents PI.

图28附图为本发明流式细胞计数证实在pH 6.0下比在pH 7.4下凋亡的成骨细胞更多，水平轴为AV-FITC，垂直轴为PI；

[n0065]

Figure 29 shows that TRAP staining in this invention indicates that more osteoclasts differentiate and fuse in RANKL+M-CSF medium at pH 6.0 than in medium at pH 7.4;

图29附图为本发明TRAP染色表明破骨细胞在pH 6.0的RANKL+M-CSF培养基中比在pH 7.4分化和融合的更多；

[n0066]

Figure 30 shows the sporadic microenvironment between osteoclasts and Van-SDECM that can be observed in red fluorescence according to the present invention. The outline of osteoclasts can be seen in blue by using the AIE pH probe.

图30附图为本发明在红色荧光中可以观察到破骨细胞和Van-SDECM之间的零星微环境，通过AIE pH探针可以看到破骨细胞的轮廓为蓝色；

[n0067]

Figure 31 shows Giemsa staining at 1 week and 6 weeks post-surgery, indicating that bacterial infiltration (red dots) was significantly increased in groups II and III compared to groups IV and V (red arrows); scale bar = 10 μm.

图31附图为本发明术后1周和6周，Giemsa染色显示，与IV组和V组相比，II组和III组的细菌浸润(红点)显著增加(红色箭头)；比例尺＝10μm；

[n0068]

Figure 32 shows TRAP staining results from one to six weeks post-surgery, indicating that groups II and III had more activated osteoclasts (red dots) than group IV, followed by group V (green arrows); scale bar = 50 μm.

图32附图为本发明手术后一到六周，TRAP染色显示，II组和III组中活化的破骨细胞(红点)比IV组中多，其次是V组(绿色箭头)；比例尺＝50μm；

[n0069]

Figure 33 shows the quantitative analysis of bacterial counts in each field of view according to the present invention;

图33附图为本发明定量分析每个视野中的细菌计数；

[n0070]

Figure 34 shows the quantitative analysis of osteoclast counts in each field of view according to the present invention.

图34附图为本发明定量分析每个视野中的破骨细胞计数；

[n0071]

Figure 35 shows the protein mass spectrum of the present invention;

图35附图为本发明蛋白质质谱；

[n0072]

Figure 36 shows the protein function enrichment analysis performed using the KEGG database in this invention.

图36附图为本发明利用KEGG数据库进行蛋白质功能富集分析；

[n0073]

Figure 37 shows the pathway interaction network of the enriched proteins of this invention.

图37附图为本发明富集蛋白的途径相互作用网络。

[0078]

Detailed Implementation

具体实施方式

[n0074]

The technical solutions of the present invention will be clearly and completely described below with reference to the accompanying drawings of the embodiments of the present invention. Obviously, the described embodiments are only some embodiments of the present invention, and not all embodiments.

下面将结合本发明实施例中的附图，对本发明实施例中的技术方案进行清楚、完整地描述，显然，所描述的实施例仅仅是本发明一部分实施例，而不是全部的实施例。

Based on the embodiments of the present invention, all other embodiments obtained by those skilled in the art without creative effort are within the scope of protection of the present invention.

基于本发明中的实施例，本领域普通技术人员在没有做出创造性劳动前提下所获得的所有其他实施例，都属于本发明保护的范围。

[n0075]

Example 1

实施例1

[n0076]

The preparation method of cross-linked vancomycin-specific demineralized extracellular matrix scaffold (Van-SDECM) is as follows:

交联万古霉素的特定脱矿细胞外基质支架(Van-SDECM)的制备方法，具体步骤如下：

[n0077]

(1) Fresh pig scapulae are used to obtain cancellous bone with a hollow bone drill with a diameter of 6mm, and then cut into cylinders with a thickness of 3mm.

---

(1)将新鲜的猪肩胛骨以直径6mm的空心骨钻获取松质骨，再切成3mm厚度的圆柱体；

[n0078]

(2) Rinse the cancellous bone blocks with tap water for 1 hour, package them in embedding cassettes, and then soak them in 0.6% (v/v) peracetic acid ultrapure water; after sterilization by filtration with a 0.22 μm filter of 1% (v/v) Triton-X100, transfer the embedding cassettes to flasks containing Triton-X100 solution, and shake at 100 rpm and 4°C for 24 hours; wash the embedding cassettes with 500 ml of sterile water and stir continuously for 1 hour, repeating twice; then add the cancellous bone blocks to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 36 hours, and wash the embedding cassettes again;

---

(2)将松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1；1%(v/v)Triton-X100用0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动24h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动36h，再次清洗包埋盒；

[n0079]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 4 hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙4h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0080]

(4) Add 30 mg of material powder to a 10 mg/ml vancomycin solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of vancomycin at this point is 5 mg/ml. React at room temperature for 12 h. After the reaction is complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到10mg/ml的万古霉素溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的万古霉素终浓度为5mg/ml，常温下

反应12h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存。

Figure 1 shows a schematic diagram illustrating the principle of Van-SDECM stent synthesis and release.

Van-SDECM支架合成与释放的原理示意图见图1。

Van and SDECM are combined in two ways: electrostatic adsorption and chemical cross-linking. In an acidic environment, the adsorbed vancomycin can be released rapidly, and the material can still release vancomycin molecules with bactericidal activity when it degrades in vivo.

Van与SDECM之间以静电吸附和化学交联两种形式复合，在酸性环境中，吸附的万古霉素可以快速释放，同时材料在体内降解的时候仍可释放具有杀菌活性的万古霉素分子。

Due to its unique mechanism of action, vancomycin cross-linked on the scaffold does not lose its bactericidal activity.

由于其独特的作用机理，交联在支架上的万古霉素并不丧失其杀菌活性。

[n0081]

Example 2

实施例2

[n0082]

The preparation method of cross-linked vancomycin-specific demineralized extracellular matrix scaffold (Van-SDECM) is as follows:

交联万古霉素的特定脱矿细胞外基质支架(Van-SDECM)的制备方法，具体步骤如下：

[n0083]

(1) Use a hollow bone drill with a diameter of 4mm to obtain cancellous bone from fresh pig scapulae, and then cut it into cylinders with a thickness of 2mm.

---

(1)将新鲜的猪肩胛骨以直径4mm的空心骨钻获取松质骨，再切成2mm厚度的圆柱体；

[n0084]

(2) Rinse the cancellous bone blocks with tap water for 1 hour, package them in embedding cassettes, and then soak them in 0.6% (v/v) peracetic acid ultrapure water; after sterilization

by filtration with 1% (v/v) Triton-X100 using a 0.22 μm filter, transfer the embedding cassettes to a flask containing Triton-X100 solution, and shake at 100 rpm and 4°C for 12 hours; wash the embedding cassettes with 500 ml of sterile water and stir continuously for 1 hour, repeating twice; then add the cancellous bone blocks to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 12 hours, and wash the embedding cassettes again;

---

(2)将松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1；1%(v/v)Triton-X100用0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动12h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动12h，再次清洗包埋盒；

[n0085]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 2 hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙2h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0086]

(4) Add 30 mg of material powder to a 2 mg/ml vancomycin solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of vancomycin at this point is 1 mg/ml. React at room temperature for 18 h. After the reaction is complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到2mg/ml的万古霉素溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的万古霉素终浓度为1mg/ml，常温下反应18h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存。

[n0087]

Example 3

实施例3

[n0088]

The preparation method of cross-linked vancomycin-specific demineralized extracellular matrix scaffold (Van-SDECM) is as follows:

交联万古霉素的特定脱矿细胞外基质支架(Van-SDECM)的制备方法，具体步骤如下：

[n0089]

(1) Fresh pig shoulder blades are drilled with a hollow bone drill with a diameter of 8mm to obtain cancellous bone, and then cut into cylinders with a thickness of 4mm.

---

(1)将新鲜的猪肩胛骨以直径8mm的空心骨钻获取松质骨，再切成4mm厚度的圆柱体；

[n0090]

(2) Rinse the cancellous bone blocks with tap water for 1 hour, package them in embedding cassettes, and then soak them in 0.6% (v/v) peracetic acid ultrapure water; after sterilization by filtration with a 0.22 μm filter of 1% (v/v) Triton-X100, transfer the embedding cassettes to flasks containing Triton-X100 solution, and shake at 100 rpm and 4°C for 48 hours; wash the embedding cassettes with 500 ml of sterile water and stir continuously for 1 hour, repeating twice; then add the cancellous bone blocks to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 48 hours, and wash the embedding cassettes again;

---

(2)将松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1；1%(v/v)Triton-X100用0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动48h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动48h，再次清洗包埋盒；

[n0091]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 6 hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙6h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0092]

(4) Add 30 mg of material powder to a 20 mg/ml vancomycin solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of vancomycin at this point is 10 mg/ml. React at room temperature for 24 h. After the reaction is

complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到20mg/ml的万古霉素溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的万古霉素终浓度为10mg/ml，常温下反应24h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存。

[n0093]

Example 4

实施例4

[n0094]

The specific steps for preparing a cross-linked ceftriaxone-specific demineralized extracellular matrix scaffold are as follows:

交联头孢曲松的特定脱矿细胞外基质支架的制备方法，具体步骤如下：

[n0095]

(1) Fresh pig scapulae are used to obtain cancellous bone with a hollow bone drill with a diameter of 6mm, and then cut into cylinders with a thickness of 3mm.

---

(1)将新鲜的猪肩胛骨以直径6mm的空心骨钻获取松质骨，再切成3mm厚度的圆柱体；

[n0096]

(2) The cancellous bone blocks were rinsed with tap water for 1 hour, packaged in embedding cassettes, and then soaked in 0.6% (v/v) peracetic acid ultrapure water for 1 hour; after sterilization by filtration with 1% (v/v) Triton-X100 through a 0.22 μm filter, the embedding cassettes were transferred to a flask containing Triton-X100 solution and shaken at 100 rpm and 4°C for 24 hours; the embedding cassettes were washed with 500 ml of sterile water and stirred continuously for 1 hour, and this process was repeated twice; then the cancellous bone blocks were added to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution and shaken at 100 rpm and 4°C for 36 hours, and the embedding cassettes were washed again;

---

(2)松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1h；1%(v/v)Triton-X100以0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动24h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动36h，再次清洗包埋盒；

[n0097]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 4 hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙4h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0098]

(4) Add 30 mg of material powder to 5 mg/ml of ceftriaxone and soak for 1 h to ensure thorough mixing. Add the same volume of EDC-NHS mixed solution with EDC concentration of 16 mM and NHS concentration of 4 mM. The final concentration of ceftriaxone at this point is 2.5 mg/ml. React at room temperature for 24 h. After the reaction is complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到5mg/ml的头孢曲松中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的头孢曲松终浓度为2.5mg/ml，常温下反应24h；反应完成的材料以去离子水清洗三遍，9000rpm/mi离心5min，弃去上清液，冻干保存。

[n0099]

Example 5

实施例5

[n0100]

The specific steps for preparing a cross-linked ceftriaxone-specific demineralized extracellular matrix scaffold are as follows:

交联头孢曲松的特定脱矿细胞外基质支架的制备方法，具体步骤如下：

[n0101]

(1) Use a hollow bone drill with a diameter of 4mm to obtain cancellous bone from fresh pig scapulae, and then cut it into cylinders with a thickness of 2mm.

---

(1)将新鲜的猪肩胛骨以直径4mm的空心骨钻获取松质骨，再切成2mm厚度的圆柱体；

[n0102]

(2) Rinse the cancellous bone blocks with tap water for 1 hour, package them in embedding cassettes, and then soak them in 0.6% (v/v) peracetic acid ultrapure water; after sterilization by filtration with 1% (v/v) Triton-X100 using a 0.22 μm filter, transfer the embedding cassettes to a flask containing Triton-X100 solution, and shake at 100 rpm and 4°C for 12 hours; wash the embedding cassettes with 500 ml of sterile water and stir continuously for 1 hour, repeating twice; then add the cancellous bone blocks to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 12 hours, and wash the embedding cassettes again;

---

(2)将松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1；1%(v/v)Triton-X100用0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动12h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动12h，再次清洗包埋盒；

[n0103]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 2

hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙2h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0104]

(4) Add 30 mg of material powder to a 2 mg/ml ceftriaxone solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of ceftriaxone at this point is 1 mg/ml. React at room temperature for 18 h. After the reaction is complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到2mg/ml的头孢曲松溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的头孢曲松终浓度为1mg/ml，常温下反应18h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存。

[n0105]

Example 6

实施例6

[n0106]

The specific steps for preparing a cross-linked ceftriaxone-specific demineralized extracellular matrix scaffold are as follows:

交联头孢曲松的特定脱矿细胞外基质支架的制备方法，具体步骤如下：

[n0107]

(1) Fresh pig shoulder blades are drilled with a hollow bone drill with a diameter of 8mm to obtain cancellous bone, and then cut into cylinders with a thickness of 4mm.

---

(1)将新鲜的猪肩胛骨以直径8mm的空心骨钻获取松质骨，再切成4mm厚度的圆柱体；

[n0108]

(2) Rinse the cancellous bone blocks with tap water for 1 hour, package them in embedding cassettes, and then soak them in 0.6% (v/v) peracetic acid ultrapure water; after sterilization

by filtration with a 0.22 μm filter of 1% (v/v) Triton-X100, transfer the embedding cassettes to flasks containing Triton-X100 solution, and shake at 100 rpm and 4°C for 48 hours; wash the embedding cassettes with 500 ml of sterile water and stir continuously for 1 hour, repeating twice; then add the cancellous bone blocks to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 48 hours, and wash the embedding cassettes again;

---

(2)将松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1；1%(v/v)Triton-X100用0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动48h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动48h，再次清洗包埋盒；

[n0109]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 6 hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙6h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0110]

(4) Add 30 mg of material powder to a 20 mg/ml ceftriaxone solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of ceftriaxone at this point is 10 mg/ml. React at room temperature for 24 h. After the reaction is complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到20mg/ml的头孢曲松溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的头孢曲松终浓度为10mg/ml，常温下反应24h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存。

[n0111]

Example 7

实施例7

[n0112]

The specific steps for preparing a cross-linked cefepime-specific demineralized extracellular matrix scaffold are as follows:

交联头孢吡肟的特定脱矿细胞外基质支架的制备方法，具体步骤如下：

[n0113]

(1) Fresh pig scapulae are used to obtain cancellous bone with a hollow bone drill with a diameter of 6mm, and then cut into cylinders with a thickness of 3mm.

---

(1)将新鲜的猪肩胛骨以直径6mm的空心骨钻获取松质骨，再切成3mm厚度的圆柱体；

[n0114]

(2) The cancellous bone blocks were rinsed with tap water for 1 hour, packaged in embedding cassettes, and then soaked in 0.6% (v/v) peracetic acid ultrapure water for 1 hour; after sterilization by filtration with 1% (v/v) Triton-X100 through a 0.22 μm filter, the embedding cassettes were transferred to a flask containing Triton-X100 solution and shaken at 100 rpm and 4°C for 24 hours; the embedding cassettes were washed with 500 ml of sterile water and stirred continuously for 1 hour, and this process was repeated twice; then the cancellous bone blocks were added to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution and shaken at 100 rpm and 4°C for 36 hours, and the embedding cassettes were washed again;

---

(2)松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1h；1%(v/v)Triton-X100以0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动24h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动36h，再次清洗包埋盒；

[n0115]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 4 hours; thereafter, the ECM bone block was rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙4h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干。

[n0116]

(4) Add 30 mg of material powder to a 4 mg/ml cefepime solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of cefepime at this point is 2 mg/ml. React at room temperature for 12 h. After the reaction is

complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到4mg/ml的头孢吡肟溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的头孢吡肟终浓度为2mg/ml，常温下反应12h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min后弃去上清液，冻干保存。

[n0117]

Example 8

实施例8

[n0118]

The specific steps for preparing a cross-linked cefepime-specific demineralized extracellular matrix scaffold are as follows:

交联头孢吡肟的特定脱矿细胞外基质支架的制备方法，具体步骤如下：

[n0119]

(1) Use a hollow bone drill with a diameter of 4mm to obtain cancellous bone from fresh pig scapulae, and then cut it into cylinders with a thickness of 2mm.

---

(1)将新鲜的猪肩胛骨以直径4mm的空心骨钻获取松质骨，再切成2mm厚度的圆柱体；

[n0120]

(2) Rinse the cancellous bone blocks with tap water for 1 hour, package them in embedding cassettes, and then soak them in 0.6% (v/v) peracetic acid ultrapure water; after sterilization by filtration with 1% (v/v) Triton-X100 using a 0.22 μm filter, transfer the embedding cassettes to a flask containing Triton-X100 solution, and shake at 100 rpm and 4°C for 12 hours; wash the embedding cassettes with 500 ml of sterile water and stir continuously for 1 hour, repeating twice; then add the cancellous bone blocks to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 12 hours, and wash the embedding cassettes again;

---

(2)将松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1；1%(v/v)Triton-X100用0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动12h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动12h，再次清洗包埋盒；

[n0121]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 2 hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙2h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0122]

(4) Add 30 mg of material powder to a 2 mg/ml cefepime solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of cefepime at this point is 1 mg/ml. React at room temperature for 18 h. After the reaction is complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到2mg/ml的头孢吡肟溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的头孢吡肟终浓度为1mg/ml，常温下

反应18h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存。

[n0123]

Example 9

实施例9

[n0124]

The specific steps for preparing a cross-linked cefepime-specific demineralized extracellular matrix scaffold are as follows:

交联头孢吡肟的特定脱矿细胞外基质支架的制备方法，具体步骤如下：

[n0125]

(1) Use a hollow bone drill with a diameter of 8mm to obtain cancellous bone from fresh pig scapulae, and then cut it into cylinders with a thickness of 4mm.

---

(1)将新鲜的猪肩胛骨以直径8mm的空心骨钻获取松质骨，再切成4mm厚度的圆柱体；

[n0126]

(2) Rinse the cancellous bone blocks with tap water for 1 hour, package them in embedding cassettes, and then soak them in 0.6% (v/v) peracetic acid ultrapure water; after sterilization by filtration with a 0.22 μm filter of 1% (v/v) Triton-X100, transfer the embedding cassettes to flasks containing Triton-X100 solution, and shake at 100 rpm and 4°C for 48 hours; wash the embedding cassettes with 500 ml of sterile water and stir continuously for 1 hour, repeating twice; then add the cancellous bone blocks to 1% (w/v) sodium dodecyl sulfate (SDS) aqueous solution, shake at 100 rpm and 4°C for 48 hours, and wash the embedding cassettes again;

---

(2)将松质骨块用自来水冲洗1h，包装在包埋盒中，然后浸泡在0.6%(v/v)过氧乙酸超纯水中1；1%(v/v)Triton-X100用0.22μm滤头过滤除菌后，再将包埋盒转移至Triton-X100溶液的烧瓶中，100rpm，4°C震动48h；用500ml灭菌水清洗包埋盒，并持续搅拌1h，重复两次；随后将松质骨块加入1%(w/v)十二烷基硫酸钠(SDS)水溶液中，100rpm，4°C震动48h，再次清洗包埋盒；

[n0127]

(3) The cancellous bone ECM was soaked in 10% EDTA decalcification solution, placed in VCare (Shanghai Yingmu) rapid ultrasonic decalcification machine, and decalcified at 4°C for 6 hours; thereafter, the ECM bone blocks were rinsed with tap water, irradiated (dose 250kGy), and freeze-dried.

---

(3)将松质骨ECM浸泡于10%EDTA脱钙液中，放入在VCare(上海颖穆)快速超声脱钙机中，并在4°C下脱钙6h；此后，将ECM骨块用自来水冲洗，辐射消毒(剂量250kGy)，并冻干；

[n0128]

(4) Add 30 mg of material powder to a 20 mg/ml cefepime solution and soak for 1 h to ensure thorough mixing. Add the same volume of an EDC-NHS mixed solution with an EDC concentration of 16 mM and an NHS concentration of 4 mM. The final concentration of cefepime at this point is 10 mg/ml. React at room temperature for 24 h. After the reaction is complete, wash the material three times with deionized water, centrifuge at 9000 rpm/min for 5 min, discard the supernatant, and freeze dry for storage.

---

(4)将30mg材料粉末加入到20mg/ml的头孢吡肟溶液中，浸泡1h，使两者充分混匀；加入同体积的EDC浓度16mM，NHS浓度4mM的EDC-NHS混合溶液，此时的头孢吡肟终浓度为10mg/ml，常温下反应24h；反应完成的材料以去离子水清洗三遍，9000rpm/min离心5min，弃去上清液，冻干保存。

[n0129]

The Van-SDECM obtained in Example 1 was evaluated for its synthesis, drug loading and release efficiency, anti-plankton and anti-adhesive bacterial properties, cytotoxicity and

osteogenic properties, anti-infective and osteogenic properties, osteoclast inhibition properties, and protein function.

对实施例1所得的Van-SDECM分别进行合成评估、载药量及释放效率评估、抗浮游和抗粘附细菌特性的评价、细胞毒性和成骨性能评价、抗感染和成骨性能评价、抑制破骨细胞性能评价、蛋白质功能分析

[n0130]

Evaluation of the synthesis of Van-SDECM in Experimental Example 1

试验例1Van-SDECM的合成评估

[n0131]

(1) The charge of the stent and vancomycin was detected by the Zeta potential method, and the results are shown in Figure 2.

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(1)支架和万古霉素的电荷以Zeta电位法检测，结果见图2。

[n0132]

The results in Figure 2 show that the stent (SDECM) exhibits a significant negative charge (-28.17±3.59mV), while vancomycin (Van) shows a weak positive charge (0.54±0.08mV), indicating a strong electrostatic interaction between vancomycin and the stent.

图2结果显示，支架(SDECM)呈现出明显的负电荷(-28.17±3.59mV)，而万古霉素(Van)则表现为微弱的正电荷(0.54±0.08mV)，意味着在万古霉素和支架之间存在强烈的静电作用。

Compared to undemineralized samples (ECM), bone ECM scaffolds (SDECM) that underwent specific demineralization showed a significant increase in negative charge (-28.17±3.59 mV vs. -14.53±1.94 mV, P=0.0003), which decreased with the adsorption of vancomycin (SDECM+Van) (-19.5±1.47 mV, P=0.0078).

相比较于未脱矿的样本(ECM)，经过特定脱矿的骨ECM支架(SDECM)负电荷值明显增加(-28.17±3.59mV VS.-14.53±1.94mV，P＝0.0003)，并且随着万古霉素的吸附(SDECM+Van)而减弱(-19.5±1.47mV，P＝0.0078)。

[n0133]

(2) The interaction mechanism between the carboxyl group in SDECM and the amino group in vancomycin under different pH environments is illustrated in Figure 3.

---

(2)以图示的形式阐述了SDECM中羧基和万古霉素中氨基之间在不同pH环境中的作用机制，见图3。

Since the pKa of the carboxyl and amino groups are approximately ~5 and ~8 respectively, in a physiological environment (pH 7.4), most of the carboxyl groups are in a deprotonated anionic state (-COO-) with a negative charge, while vancomycin is in a positively charged cationic state. The two are bonded together by hydrogen bonds.

由于羧基和氨基的pKa之约为～5和～8，在生理环境中(pH7.4)，大部分的羧基为去质子化的阴离子态(-COO-)，带负电荷，而万古霉素为带正电荷的阳离子态，两者之间以氢键形式结合。

This electrostatic attraction results in a high loading and slow release of the drug within the material.

这种静电吸引的方式，导致了药物在材料中的大量负载和缓慢释放。

When the material is in an acidic environment with a pH of around 6.0, the number of protonated carboxylic acids (-COOH, which are positively charged) on the surface of the SDECM material increases, leading to rapid drug release. This phenomenon was also confirmed in subsequent experiments.

当材料存在于pH6.0左右的酸性环境中，SDECM材料表面质子化的羧酸(-COOH，带正电荷)增加，导致了药物的快速释放，这一现象也在后续实验中得到证实。

[n0134]

(3) The infrared spectral detection results during the synthesis of Van-SDECM are shown in Figure 4. The formation of amide bonds can be confirmed by the appearance of the new NH stretching band (2910 cm NER1) and the relative decrease of the peak of the C=O stretching band (1640 cm NER2) (blue arrows represent NH stretching bands, red arrows represent C=O stretching bands, and black curves indicate the synthesized Van-SDECM).

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(3)Van-SDECM合成时红外光谱检测结果见图4，酰胺键的形成可以通过新的NH伸缩带(2910cm⁻¹)的出现和C＝O伸缩带(1640cm⁻¹)的峰值相对下降来证实(蓝色箭头表示NH伸缩带，红色箭头表示C＝O伸缩带，黑色曲线指合成的Van-SDECM)。

Infrared spectroscopy revealed that the typical C=O and NH stretching bands of the amide bond are located at 1640 cm¹NER and 2910 cm¹NER, respectively.

通过红外光谱检测可以发现，酰胺键典型的C＝O和NH伸缩带分别位于1640cm⁻¹和2910cm⁻¹处。

The appearance of the new NH stretching band and the reduction of the C=O stretching band confirm the formation of amide bonds.

新NH伸缩带的出现和C＝O伸缩带的减少证实了酰胺键的形成。

The infrared spectrum of Van-SDECM after 6 weeks of degradation is shown in Figure 4. As Van-SDECM degrades (red curve), its NH stretching band disappears compared to the original material (black curve), and the relative peak height of the C=O stretching band decreases even further.

Van-SDECM降解6周后的红外谱图见图4，随着Van-SDECM的降解(红色曲线)，相对于原始材料(黑色曲线)，其NH伸缩带消失，C＝O伸缩带相对峰高降得更低。

[n0135]

Evaluation of drug loading and release efficiency of Van-SDECM in Experiment Example 2

试验例2Van-SDECM的载药量及释放效率评估

[n0136]

(1) When the SDECM powder is 30 mg, EDC/NHS = 1:1, and vancomycin is 5 mg, the maximum vancomycin loading content (about 85%) can be obtained when the EDC concentration is 32 mM, as shown in Figure 5; and the ratio between EDC and NHS has little effect on the vancomycin loading, as shown in Figure 6.

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(1)当SDECM粉末均为30mg，EDC/NHS＝1：1，万古霉素为5mg时，EDC浓度在32mM可获得材料的最大载万古霉素含量(约85%)，见图5；而且EDC/NHS之间的比率对于万古霉素载药量影响不是很明显，见图6。

[n0137]

(2) After continuous ultrasonic cleaning, record the amount of vancomycin remaining on the material after different cleaning times (horizontal axis).

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(2)在不断的超声清洗后，记录不同清洗次数(横坐标)后材料上残留的万古霉素含量。

In the group with simple electrostatic adsorption, the amount of vancomycin residue on the material was the least. However, with the increase of EDC concentration, the amount of vancomycin residue on the material gradually increased. After three washes, the vancomycin content in each group stabilized at a near-linear level, as shown in Figure 7. The crosslinking amount was expressed as the difference in vancomycin content between each group and the simple adsorption group, and the crosslinking rate was calculated by dividing c by d. It can be seen that at EDC = 16 mM and 32 mM, the two crosslinking rates were 37.5 ± 5.6% and 65.5 ± 7.8%, respectively (P = 0.0074).

单纯静电吸附的组别中，材料上残留万古霉素最少，而随着EDC浓度的增加，材料上残留的万古霉素逐渐增加，各组别均在3次清洗以后，万古霉素含量稳定于将近直线水平，结果见图7；以各组别与单纯吸附组的万古霉素含量差值表示交联量，并计算其交联率，交联速率计算为c除以d；可以得出在EDC＝16mM和32mM时，两种交联率分别为37.5±5.6%和65.5±7.8%(P＝0.0074)。

[n0138]

(3) After 6 weeks of preparation by simple electrostatic adsorption, the cumulative release of vancomycin in Van-SDECM under acidic environment (pH 6.0) was significantly increased compared with that under physiological state (pH 7.4) (2285.0±105.6μg vs. 686.3±43.6μg, P<0.0001).

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(3)单纯静电吸附制备的材料6周后，与生理状态(pH7.4)比较，酸性环境下(pH6.0)Van-SDECM中万古霉素的累积释放量大大增加，差异具有显著性(2285.0±105.6μgVS.686.3±43.6μg，P<0.0001)。Undemineralized ECM has poor acid-sensitive release capacity, while tricalcium phosphate (TCP) has no acid-sensitive release capacity, as shown in Figure 8.

而未脱矿的ECM酸敏感释放能力较差，磷酸三钙(TCP)则无酸敏感释放能力，见图8。

[n0139]

As the degree of cross-linking increases, the ability of the stent to release this acid-sensitive drug gradually weakens.

随着交联程度的增加，支架中这种酸敏感药物释放的能力逐渐减弱。

The uncrosslinked group exhibited the most pronounced acid sensitivity, while the EDC=32mM group lost this characteristic, and the EDC=16mM group exhibited moderate acid-sensitive release capacity.

未交联组表现出最明显的酸敏感性，而EDC＝32mM组别则丧失了这一特性，EDC＝16mM的组别则表现出中等的酸敏感释放能力。

When the initial vancomycin concentration was 5 mg/mL and the EDC was 16 mM, the total time for vancomycin release by Van-SDECM exceeded 6 weeks, and the final release concentrations reached 101.1 ± 13.8 μg/mL and 72.2 ± 10.3 μg/mL in acidic and alkaline environments, respectively, both exceeding the minimum therapeutic dose of vancomycin (20 μg/mL), as shown in Figure 9.

在初始万古霉素为5mg/mL、EDC为16mM的时候，Van-SDECM释放万古霉素的总时间超过了6周，而且最终释放浓度在酸碱环境中分别达到了101.1±13.8μg/mL和72.2±10.3μg/mL，均超过万古霉素的最低治疗剂量(20μg/mL)，见图9。

[n0140]

(4) After 5 days of culture, the amount of vancomycin released from the osteoclast group (Van-SDECM+OC) was significantly higher than that of the cell-free co-culture control group (Van-SDECM) (4.0±0.5μg/mL VS.2.3±0.5μg/mL, P＝0.0109), as shown in Figure 10, which confirms its good acid-sensitive release ability in the infection simulation environment.

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(4)培养5天以后破骨细胞组(Van-SDECM+OC)中材料释放的万古霉素含量明显高于无细胞共培养的对照组(Van-SDECM)(4.0±0.5μg/mL VS.2.3±0.5μg/mL，P＝0.0109)，见图10，证实了其在感染模拟环境中的良好酸敏感释放能力。

[n0141]

Example 3: Evaluation of the anti-planktonic and anti-adhesive bacterial properties of Van-SDECM

试验例3Van-SDECM抗浮游和抗粘附细菌特性的评价

[n0142]

(1) The inhibition zone test and the material/bacteria co-culture test are used to verify the ability of the scaffold to kill free bacteria.

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(1)抑菌环试验和材料/细菌共培养试验用来验证支架的杀游离细菌能力。

Different samples were placed on MH plates containing Staphylococcus aureus and Enterococcus. After 48 hours, the inhibition zones around Van-SDECM were observed to be 8 mm (Staphylococcus aureus) and 2 mm (Enterococcus), respectively, which were significantly larger than the 2 mm and 0 mm of the control group (Figure 11).

将不同样本放置于生长金黄色葡萄球菌和肠球菌的MH平板上，48小时后可以观察到，Van-SDECM周围的抑菌圈分别为8mm(金黄色葡萄球菌)和2mm(肠球菌)，明显大于对照组的2mm和0mm，见图11。

[n0143]

(2) The residual bacterial content in the supernatant after co-culturing the bacterial suspension with the material was quantitatively determined by the spread-plate method, and the antibacterial rate of each sample against airborne bacteria was calculated.

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(2)细菌悬液与材料共培养后其上清液中残留细菌含量以涂布平板法(spread-plate method)定量测定，同时计算各不同样本对于浮游细菌的抗菌率。

Compared to SDECM, which has almost no bactericidal effect, Van-SDECM exhibits excellent bactericidal performance (over 98%), as shown in Figures 12 and 13.

相比较于几乎无杀菌作用的的SDECM，Van-SDECM表现出优异的杀菌性能(98%以上)，见图12和图13。

EDC = 0, 16, 32 mM indicates that the material was prepared under the conditions of initial vancomycin 5 mg/mL, EDC = NHS = 0, 16, 32 mM; SDECM without vancomycin was selected as the control group.

EDC＝0、16、32mM表示材料在初始万古霉素5mg/mL，EDC＝NHS＝0、16，32mM的条件下制备；选择不含万古霉素的SDECM作为对照组。

[n0144]

(3) Scanning electron microscopy showed that when the initial vancomycin concentration of the prepared material was 5 mg/mL and the EDC was 32 mM (Vanini = 5 mg/mL + EDC = 32 mM), the amount of bacteria on the sample surface was the least, and the anti-adhesion bacterial rate reached almost 98% compared with the control group. However, there seemed to be no significant difference between the sample group with EDC of 16 mM (Vanini = 5 mg /mL + EDC = 16 mM), as shown in Figures 14 and 15.

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(3)扫描电镜显示，当制备材料的初始万古霉素浓度为5mg/mL，EDC为32mM时(Vanini＝5mg/mL+EDC＝32mM)，样本表面的细菌量最少，与对照组相比，几乎达到98%的抗粘附细菌率，但与EDC为16mM时(Vanini＝5mg/mL+EDC＝16mM)组样本差异似乎无明显显著性，见图14和图15。

[n0145]

(4) Laser confocal microscopy reveals the relationship between crosslinking agents and the material's ability to resist bacterial adhesion.

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(4)激光共聚焦显示交联剂与材料抗粘附细菌能力的关系。

The sample prepared with EDC of 32mM showed the highest red fluorescence (dead bacteria) and the lowest green fluorescence (live bacteria) on its surface, indicating that this group of samples had the strongest anti-adhesive bacterial ability. The sample group prepared with EDC of 16mM was the second strongest, while the uncrosslinked sample group had no contact sterilization ability.

EDC为32mM时制备的样本表面可见最高的红色荧光(死细菌)和最低的绿色荧光(活细菌)，提示该组样本拥有最强的抗黏附细菌能力，EDC为16mM时制备的样本组次之，而未交联组样本无接触杀菌能力。

The ratio of live to dead bacteria on the surface of each sample showed that the ratios were 7.8 for the simple material group SDECM, 6.0 for the drug direct mixing group (EDC = 0 mM), 1.8 for the medium concentration EDC crosslinking group (EDC = 16 mM), and 0.3 for the high concentration EDC crosslinking group (EDC = 32 mM). This indicates that the anti-adhesive bacterial ability of the Van-SDECM scaffold increases with the increase of crosslinking agent concentration, as shown in Figures 16 and 17.

各样本表面活/死细菌的比例显示，单纯材料组SDECM、药物直接混合组(EDC＝0mM)，中浓度EDC交联组(EDC＝16mM)、高浓度EDC交联组(EDC＝32mM)的活/死菌比例分别为7.8，6.0，1.8和0.3，表明随着交联剂浓度的增加，Van-SDECM支架的抗粘附细菌能力也随之增强，见图16和图17。

Calculate the ratio between the two using the red and green pixel values in Photoshop.

用photoshop中的红绿像素值计算两者比例。

[n0146]

Example 4: Evaluation of the cytotoxicity and osteogenic properties of Van-SDECM

试验例4Van-SDECM的细胞毒性和成骨性能评价

[n0147]

(1) Toxicity tests of vancomycin solution alone showed that concentrations above 3 mg/ml could produce significant toxicity to bone marrow mesenchymal stem cells (MSCs), as shown in Figure 18.

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(1)单纯万古霉素溶液的毒性检测表明高于3mg/ml可对骨髓间充质干细胞(MSCs)产生较为明显的毒性，见图18。

[n0148]

(2) Van-SDECM materials prepared with initial vancomycin concentrations of 1 mg/ml and 5 mg/ml were immersed in culture medium to obtain 20%, 50%, and 100% extracts for cytotoxicity analysis.

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(2)以初始万古霉素浓度为1mg/ml、5mg/ml制备的Van-SDECM材料浸入培养基中，获取20%、50%、100%的浸提液，作为细胞毒性分析。

Both the CCK-8 test and live/dead staining indicated that different concentrations of extracts from the two materials had no significant effect on MSC proliferation (see Figures 19 and 20).

CCK-8测试和live/dead染色均提示两种材料不同浓度的浸提液，对于MSCs增值无明显影响，见图19和图20。

[n0149]

(3) Take Van = 5 mg/mL + EDC = 16 mM group and obtain its extract to test osteogenic capacity. It can be found that ALP staining indicates that it has no significant adverse effect on osteoblast activity, as shown in Figure 21.

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(3)取Van＝5mg/mL+EDC＝16mM组，获取其浸提液测试成骨能力，可以发现，ALP染色提示其对于成骨细胞活性无明显不利影响，见图21。

[n0150]

Example 5: Evaluation of the in vivo antibacterial and osteogenic properties of Van-SDECM

试验例5Van-SDECM的体内抗菌与成骨性能评价

[n0151]

(1) The plating method at week 1 and week 6 of the skull infection model showed that the number of bacteria in the infected bone defect group (Defect+infection, group II) and the infection+SDECM group (Defect+infection+SDECM, group III) was much higher than that in the blank group (Defect, group I), while the infection+Van-SDECM group (Defect+infection+Van-SDECM, group V) had almost no obvious bacteria at both time points.

---

(1)颅骨感染感染模型第1周和第6周的涂布平板法显示，感染性骨缺损组(Defect+infection，II组)和感染+SDECM组(Defect+infection+SDECM，III组)的细菌数量远高于空白组(Defect，I组)，而感染+Van-SDECM组(Defect+infection+Van-SDECM，V组)几乎在两个时间点都无明显细菌存在。Conversely, the number of residual colonies in the infection+Van group (Defect+infection+Van, IV group) was between that in the SDECM group and the Van-SDECM group, as shown in Figures 22 and 23.

相反，感染+Van组(Defect+infection+Van，IV组)中的残留菌落数量介于SDECM组和Van-SDECM组之间，见图22和图23。

[n0152]

(2) Compared with the simple defect group 1 week after surgery, the infection group, infection + SDECM group and infection + Van group all had a large number of insect bite-like bone erosion areas around the defect, while the degree of erosion in the Van-SDECM group was slightly lower than that in the other groups.

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(2)与术后1周的单纯缺损组相比，感染组、感染+SDECM组和感染+Van组的缺损周围均有大量虫咬样骨侵蚀区，而Van-SDECM组的侵蚀程度略低于其他各组。

After 6 weeks, new bone formation was significantly greater in the Van-SDECM group than in the control group, with a bone regeneration rate (BV/TV) of over 90%, followed by the vancomycin group (approximately 60%).

6周后，Van-SDECM组的新骨形成比对照组明显，骨再生率(BV/TV)达到90%以上，其次是万古霉素组(约60%)。

The osteogenic capacity of the infection + SDECM group was similar to that of the blank group (Group I) (23.0±2.4% vs. 20.5±3.1%, P=0.9055), and both groups were superior to the infection-only group (Group II) (10.9±1.4%), as shown in Figures 24 and 25.

感染+SDECM组的成骨能力与空白组(I组)相似(23.0±2.4%VS.20.5±3.1%，P＝0.9055)，两组均优于单纯感染组(II组)(10.9±1.4%)，见图24和图25。

[n0153]

Example 6: Evaluation of the anti-infection and osteogenic properties of Van-SDECM

试验例6Van-SDECM的抗感染和成骨性能评价

[n0154]

(1) The anti-infection performance of the H&E observation materials at 6 weeks post-operation is shown in Figure 26. A large number of neutrophil infiltrations were observed in groups II, III and IV, while group V showed a significant reduction, similar to group I.

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(1)术后6周H&E观察材料的抗感染性能见图26， II 、III、IV组内均可见大量中性粒细胞浸润，而 V 组则明显减少，与 I 组相似。

[n0155]

(2) Masson staining showed that new bone formation in infected skull defects treated with Van-SDECM was significantly higher than in the four control groups.

---

(2)Masson染色显示在Van-SDECM治疗的感染性颅骨缺损中新骨形成明显高于四个对照组。

In addition, most of the new bone formed after Van-SDECM treatment is mature, fully mineralized cancellous bone (blue staining).

此外，经Van-SDECM处理后形成的新骨大部分为成熟的完全矿化松质骨(蓝色染色)。

No mature bone formation was observed at the defect sites in the infection group and the infection + SDECM group, while only a small amount of blue bone tissue was observed in the infection + van group (see Figure 27).

感染组和感染+SDECM组的缺损处未观察到成熟骨形成，而感染+van组仅观察到少量蓝色骨组织，见图27。

[n0156]

Evaluation of the osteoclast-inhibiting performance of Van-SDECM in Experiment 7

试验例7Van-SDECM的抑制破骨细胞性能评价

[n0157]

(1) TRAP staining showed that osteoclast differentiation and fusion were more obvious in RANKL+M-CSF medium at pH 6.0 (73.7±5.0/well VS. 45.3±5.0/well, P＝0.0162), while flow cytometry analysis confirmed that apoptosis of osteoblast population was significantly increased in this environment (89.9±0.6% VS. 78.6±1.6%, P＝0.0026), as shown in Figures 28 and 29.

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(1)TRAP染色可见pH6.0的RANKL+M-CSF培养基中破骨细胞的分化融合更加明显(73.7±5.0/well VS. 45.3±5.0/well,P＝0.0162)，而流式细胞分析则证实在该环境下，成骨细胞群体的凋亡显著增加(89.9±0.6%VS.78.6±1.6%，P＝0.0026)，见图28和图29。

[n0158]

(2) One week after surgery, Giemsa staining showed a significant increase in bacterial infiltration at the bone defect site in the infection group and the infection + SDECM group.

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(2)术后1周，Giemsa染色显示感染组和感染+SDECM组骨缺损处的细菌浸润明显增加。

Meanwhile, TRAP staining showed a similar trend.

同时，TRAP染色显示出类似的趋势。

However, no bacteria or activated osteoclasts were found in the Van-SDECM group at 1 week and 6 weeks, indicating a linear trend between bacterial infection and osteoclast activity (Figures 30-34).

然而，Van-SDECM组在1周和6周后均未发现细菌和活化的破骨细胞，表明了细菌感染与破骨细胞活性之间的线性变化趋势，见图30-图34。

[n0159]

Protein function analysis of Van-SDECM in Experiment Example 8

试验例8Van-SDECM的蛋白质功能分析

[n0160]

(1) Protein mass spectrometry showed that the scaffold contained a large number of factors that contribute to M2 polarization, such as type I, II, V and XII collagen, coagulation factors II, IX and X, vitamin K-dependent protein C, etc., and also contained factors that synergistically fight bacteria, such as vitronectin, fibronectin I, decorin and tenascin, etc. (See Figure 35).

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(1)蛋白质质谱显示在支架中含有大量有助于M2极化的因子，I型、II型、V型、XII型胶原(collagen I、II、V、XII)、凝血因子II、IX、X(coagulation factor II、IX、X)维生素K依赖性蛋白C(Vatimin K-dependent protein C)等，并且还含有协同抗菌的因子，如玻连蛋白(vitronectin)、纤维连接蛋白I(fibronectin I)、核心蛋白聚糖(decorin)和生腱蛋白(tenascin)等等，见图35。

The red bars on the left represent proteins in the material that have auxiliary antibacterial effects, and the blue bars on the right represent proteins that promote regeneration; the horizontal axis represents the percentage of each type of protein.

左边的红色条表示材料中具有辅助抗菌作用的蛋白质，右边的蓝色条表示促进再生能力的蛋白质；横坐标是各种蛋白质的百分比。

[n0161]

(2) Protein function enrichment analysis was performed using the KEGG database.

---

(2)利用KEGG数据库进行蛋白质功能富集分析。

Downstream analysis was conducted using functional categories with p-values < 0.05.

选择P值<0.05的功能分类进行下游分析。

Gene ratios represent the frequency of a protein detected by mass spectrometry relative to the total number of protein items in a particular functional class.

基因比值表示质谱检测到的蛋白质相对于某一功能类别的总蛋白项目的频率。

Several early-stage inflammation-related pathways are found in the ECM protein library, such as complement and coagulation cascade (corrected P-value 9.27E-17; all subsequent responses use corrected P-value), platelet activation (P-value 1.05E-4), and pathogenic infection response (P-values 1.18E-4 and 5.13E-3), which promote the differentiation of monocytes into M1 immune cells or immunosuppressive macrophages (M2) to defend against microorganisms through the first-level response.

在ECM蛋白质库中有几个炎症初期相关通路，如补体和凝血级联反应(校正P-value为9.27E-17；此后都用校正P-value)、血小板活化(P-value为1.05E-4)，以及致病性感染反应(P-value为1.18E-4和5.13E-3)，通过第一级反应促进单核细胞分化为抵御微生物的免疫细胞M1或免疫抑制型巨噬细胞(M2)。

For example, we found that many clotting factors, including F2, F7B, F9, and F10, were detected in the proteomic dataset and were shown to be stimulators of M2 polarization.

例如，我们发现在蛋白质组数据集中检测到许多凝血因子，包括F2、F7B、F9和F10，它们被证明是M2极化的刺激因素。

This process promotes a cascade of tissue repair and bone regeneration.

这一过程促进了组织修复和骨再生的级联反应。

Functional groups involved in ECM-cell membrane interactions, including the PI3K-Akt signaling pathway (P-value 4.74E-5), ECM-receptor interaction (P-value 1.47E-10), focal adhesion (P-value 1.04E-6), and phagosomes (P-value 0.01), were significantly enriched in the ECM proteome (Figure 36).

涉及ECM与细胞膜相互作用的功能组别，包括PI3K-Akt信号途径(P-value4.74E-5)、ECM-受体相互作用(P-value 1.47E-10)、局灶性粘附(P-value 1.04E-6)和吞噬体(P-value 0.01)，在ECM蛋白质组中显著富集，见图36。

[n0162]

(3) Enriched protein pathway interaction network, orange circles represent functional pathways, labeled with the corresponding classification title in bold; blue to red circles represent protein items stained by log-transfer mass spectrometry intensity and labeled with their corresponding gene symbols.

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(3)富集蛋白的途径相互作用网络，橙色圆圈表示功能通路，由相应的分类标题用粗体字标注；蓝色到红色圆圈表示通过对数转移质谱强度染色的蛋白质项目，并用其相应的基因符号标记。

Gene functional pathway interaction analysis showed that functional clusters were closely clustered together and contained many proteins with strong mass spectrometry signals, suggesting that cytokine matrix factors may trigger intracellular reprogramming processes in macrophages (Figure 37).

基因功能通路互作分析表明，功能簇彼此紧密聚集，包含许多强质谱信号的蛋白质，表明细胞基质因子可能触发巨噬细胞的细胞内重编程过程，见图37。

[n0163]

The cross-linked antibiotic-specific demineralized extracellular matrix scaffolds obtained in Examples 2-9 were evaluated for synthesis, drug loading and release efficiency, anti-plankton

and anti-adhesive bacterial properties, cytotoxicity and osteogenic properties, anti-infection and osteogenic properties, osteoclast inhibition properties, and protein function. The results were similar to those of the Van-SDECM material in Examples 1-8. This indicates that by adjusting the scaffold type, antibiotic, and preparation conditions determined after the above optimization, it is possible to prepare cross-linked antibiotic-specific demineralized extracellular matrix scaffolds with similar effects.

对实施例2-9所得的交联抗生素的特定脱矿细胞外基质支架分别进行合成评估、载药量及释放效率评估、抗浮游和抗粘附细菌特性的评价、细胞毒性和成骨性能评价、抗感染和成骨性能评价、抑制破骨细胞性能评价、蛋白质功能分析，结果与试验例1-8中Van-SDECM材料结果相似，这表明可通过上述优化后确定的支架类型和抗生素及制备条件的调整，实现效果类似的交联抗生素的特定脱矿细胞外基质支架的制备。

[n0164]

The above description of the disclosed embodiments enables those skilled in the art to implement or use the present invention.

对所公开的实施例的上述说明，使本领域专业技术人员能够实现或使用本发明。

Various modifications to these embodiments will be readily apparent to those skilled in the art, and the general principles defined herein may be implemented in other embodiments without departing from the spirit or scope of the invention.

对这些实施例的多种修改对本领域的专业技术人员来说将是显而易见的，本文中所定义的一般原理可以在不脱离本发明的精神或范围的情况下，在其它实施例中实现。

Therefore, the present invention is not to be limited to the embodiments shown herein, but is to be accorded the widest scope consistent with the principles and novel features disclosed herein.

因此，本发明将不会被限制于本文所示的这些实施例，而是要符合与本文所公开的原理和新颖特点相一致的最宽的范围。