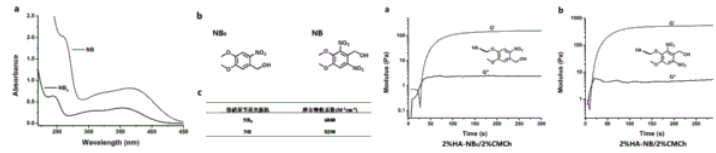
Back to results (polymer); (C08G65/33337);

Preparation method, raw materials, product and application of photo-coupled cross-linked hydrogel material

Abstract

The invention relates to a preparation method, raw materials, a product and application of a photo-coupled cross-linked hydrogel material. Dissolving the component A-o-nitrobenzyl type photo-trigger modified macromolecule derivative in a biocompatible medium to obtain a solution A; dissolving the component B-primary amine, diamine, hydrazide and hydroxylamine high-molecular derivatives in a biocompatible medium to obtain a solution B; uniformly mixing the solution A and the solution B to obtain a hydrogel precursor solution; under the irradiation of a light source, the hydrogel precursor solution is crosslinked with primary amine, diamine, hydrazide and hydroxylamine groups in the component B in the form of Schiff base to form hydrogel. The invention also provides a kit for preparing the hydrogel and application of the hydrogel in tissue engineering and regenerative medicine, 3D printing and as a cell, protein or drug carrier. The hydrogel precursor solution can be sprayed or smeared on the surface of a tissue to realize in-situ gelation under illumination, and is particularly suitable for wound surface sealing after operation and tissue fluid leakage plugging.

Images (8)



Classifications

C08B37/0072 Hyaluronic acid, i.e. HA or hyaluronan; Derivatives thereof, e.g. crosslinked hyaluronic acid (hylan) or hyaluronates

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CN107964056B
China

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Other languages: Chinese

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Current Assignee : Zhongshan Guanghe Medical Technology Co., Ltd.

Worldwide applications

2017 CN

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2018-04-27	Publication of CN107964056A
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Status	Active
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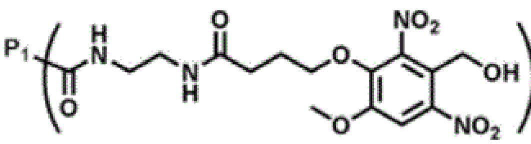
Info: Patent citations (3), Non-patent citations (6), Cited by (19), Legal events, Similar documents, Priority and Related Applications

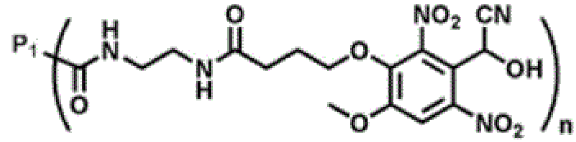
External links: Espacenet, Global Dossier, Discuss

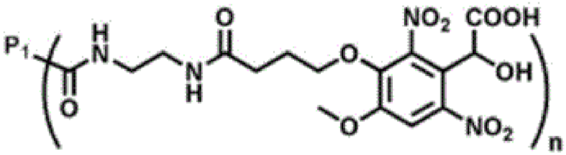
Claims (12)

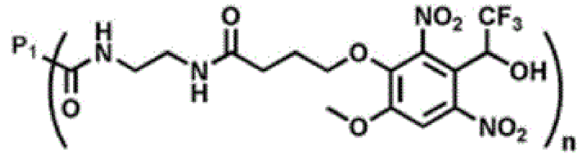
Hide Dependent

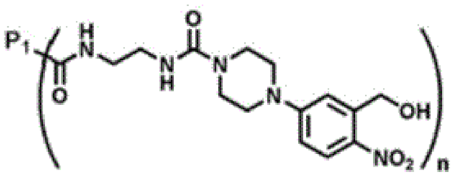
1. The o-nitrobenzyl light trigger modified macromolecule derivative is selected from the structures in the following components:

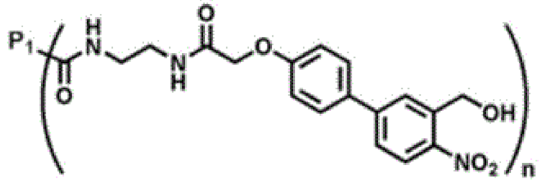


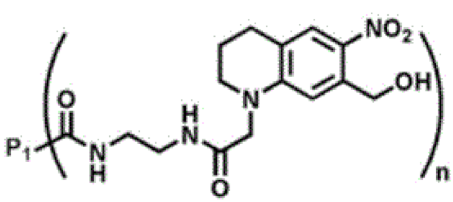


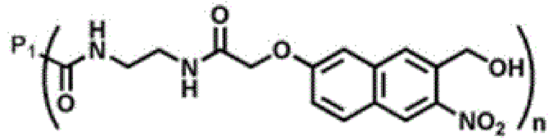


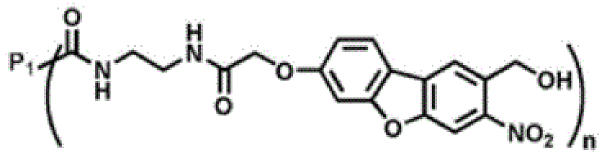


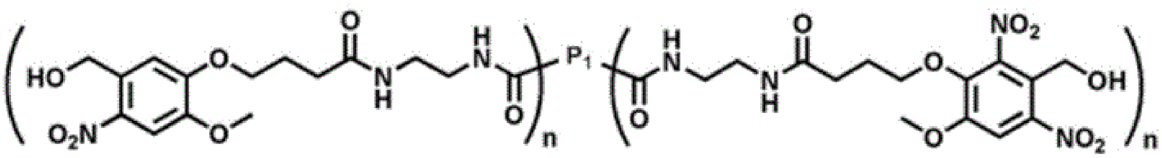


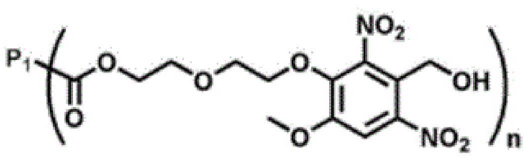


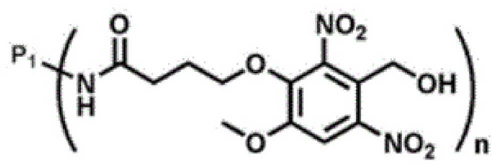


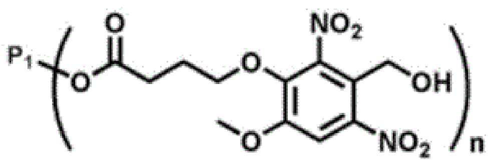


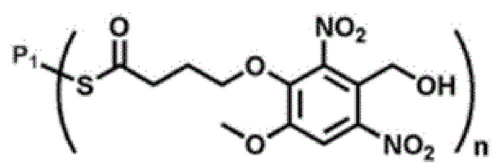


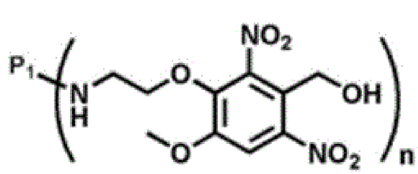


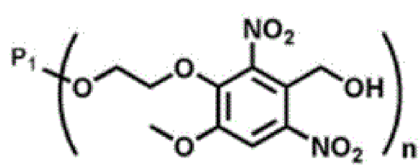


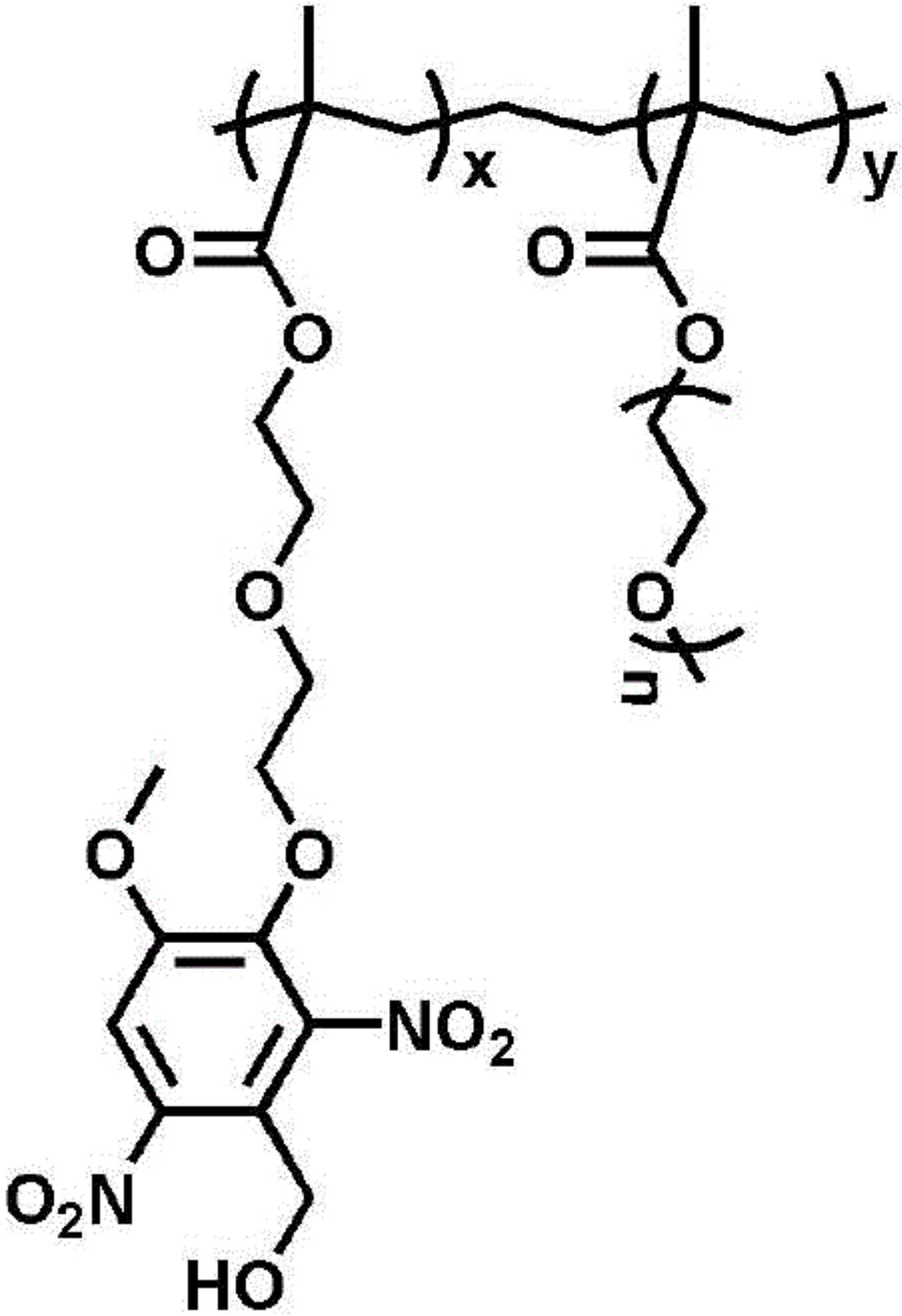












wherein, P_1 Is a hydrophilic or water-soluble natural or synthetic polymer, or P_1 Independently selected from a plurality of hydrophilic or water-soluble natural or synthetic polymers;

the hydrophilic or water-soluble natural high polymer comprises natural polysaccharide substances and modifications or degradation products thereof, and proteins and modifications, modifications and degradation products thereof;

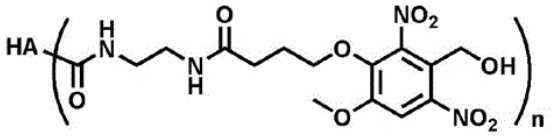
the natural polysaccharide substance comprises hyaluronic acid, carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, alginic acid, dextran, agarose, heparin, chondroitin sulfate, ethylene glycol chitosan, propylene glycol chitosan, chitosan lactate, carboxymethyl chitosan or chitosan quaternary ammonium salt;

the protein comprises various hydrophilic or water-soluble animal and plant proteins, collagen, serum protein, silk fibroin and elastin, and the protein degradation product comprises gelatin or polypeptide;

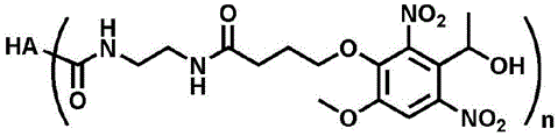
the hydrophilic or water-soluble synthetic polymer comprises two-arm or multi-arm polyethylene glycol, polyethyleneimine, synthetic polypeptide, polylysine, polyglutamic acid, polyacrylic acid, polymethacrylic acid, polyacrylamide, polymethacrylamide, polyvinyl alcohol and polyvinylpyrrolidone;

n≥2。

2. the o-nitrobenzyl-based photo trigger modified polymer derivative of claim 1, having a structure selected from the group consisting of:



组分 A-1



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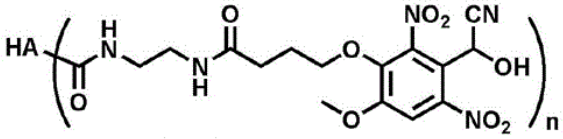

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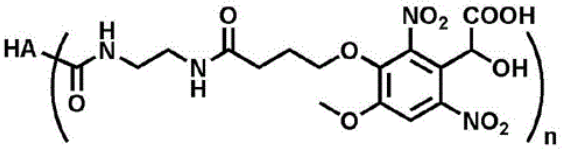

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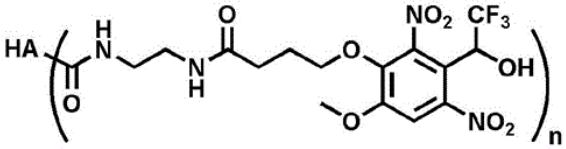

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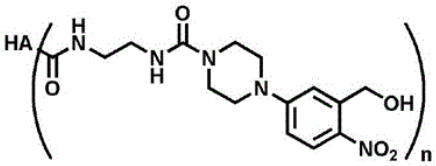

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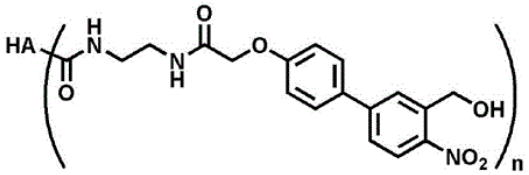

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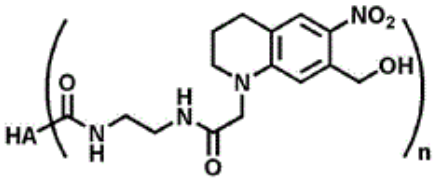

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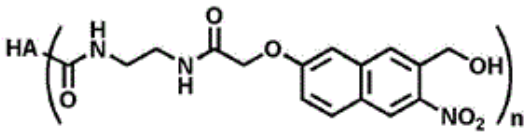

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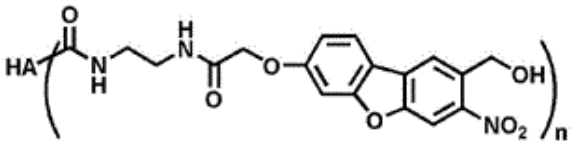

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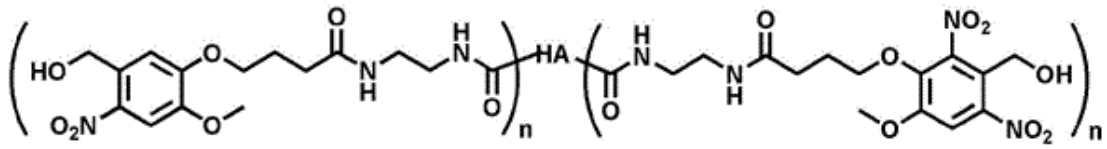

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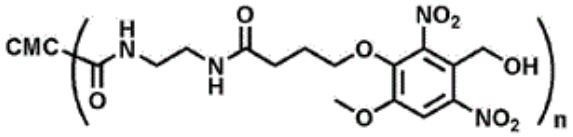
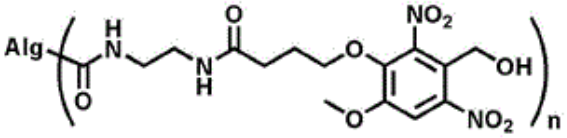
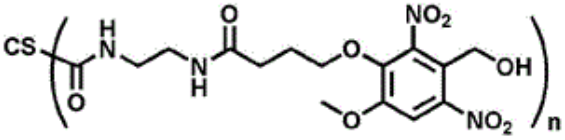


组分 A-36



组分 A-37



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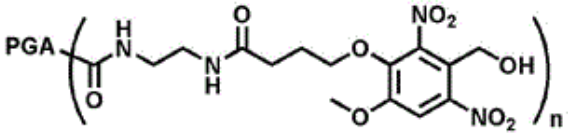

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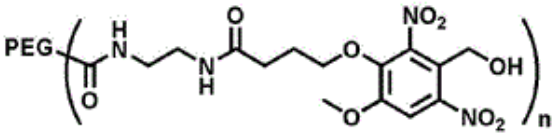

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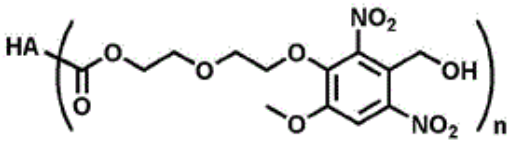

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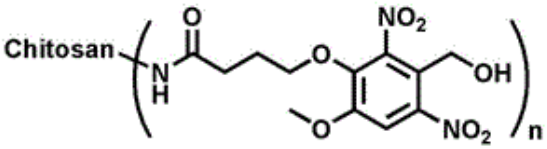

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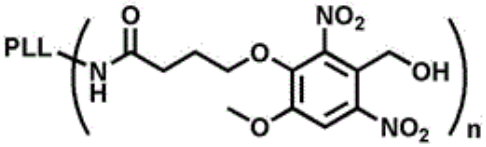

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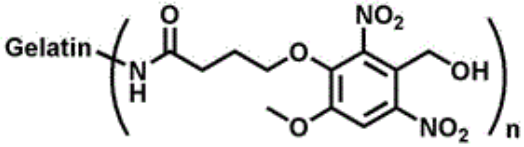

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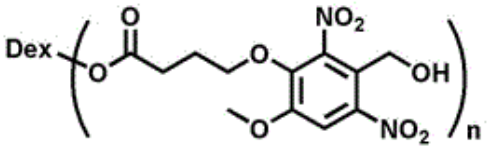

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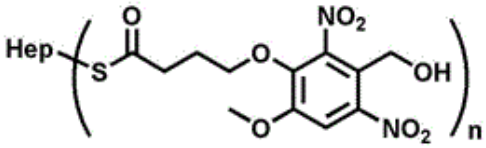

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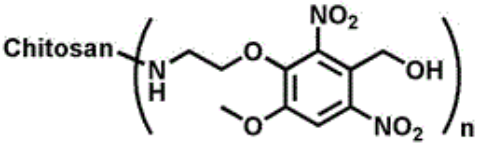

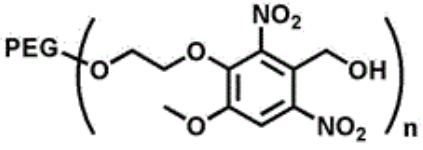

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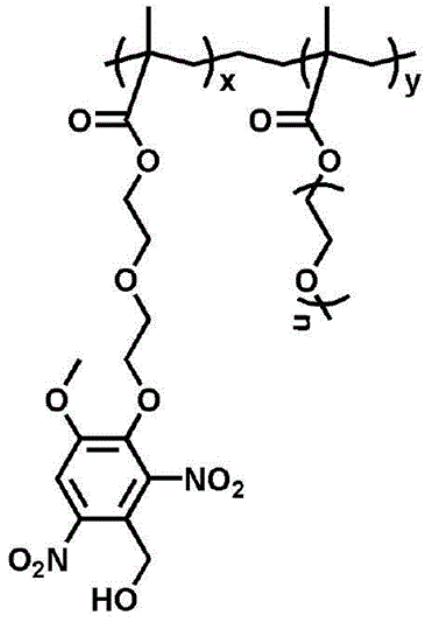


组分 A-48

组分 A-49

in the component A, n is more than or equal to 2, and HA is hyaluronic acid; CMC is carboxymethyl cellulose; alg is alginic acid; CS is chondroitin sulfate; PGA is polyglutamic acid; PEG is polyethylene glycol; chitosan is Chitosan; gelatin is Gelatin; PLL is polylysine; dex is dextran; hep is heparin.

3. The method for preparing the o-nitrobenzyl light trigger modified macromolecule derivatives of claim 1, wherein the method is chemical labeling method or artificial polymerization method,

the chemical labeling method is a method for connecting macromolecules and chemical groups contained in the o-nitrobenzyl light trigger by utilizing a chemical reaction, and comprises the following labeling methods:

the macromolecule containing carboxyl and the o-nitrobenzyl micromolecule containing hydroxyl, sulfydryl or amido are marked,

the macromolecule containing hydroxyl and the o-nitrobenzyl micromolecule containing carboxyl or bromine are marked,

marking a macromolecule containing amido and an o-nitrobenzyl micromolecule containing carboxyl or bromine;

the artificial polymerization method is to use o-nitrobenzyl derivative functional monomer to copolymerize with other comonomer, and the polymerization method comprises a random free radical polymerization method and a controlled free radical polymerization method.

4. The method for preparing the o-nitrobenzyl light trigger modified polymer derivative of claim 3, wherein any one of the following methods is selected:

A. dissolving a water-soluble macromolecule containing carboxyl in distilled water, adding an o-nitrobenzyl micromolecule containing active functional group hydroxyl or sulfydryl or amido, adding a condensing agent 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride and an activating agent hydroxybenzotriazole, stirring for reaction, adding reaction liquid into a dialysis bag after the reaction is finished, dialyzing with a dilute hydrochloric acid solution, and then freeze-drying to obtain an o-nitrobenzyl modified macromolecule derivative;

B. dissolving a water-soluble macromolecule containing carboxyl in 2- (N-morpholine) ethanesulfonic acid MES buffer solution, stirring until the macromolecule is completely dissolved, dissolving an o-nitrobenzyl micromolecule containing hydroxyl, sulfydryl or amino in dimethyl sulfoxide, adding the reaction solution, dissolving 4- (4, 6-dimethoxytriazine-2-yl) -4-methylmorpholine hydrochloride in MES buffer solution, adding the MES buffer solution into the reaction solution for reaction, pouring the reaction solution into a dialysis bag, dialyzing with deionized water, and freeze-drying to obtain an o-nitrobenzyl modified macromolecule derivative;

C. dissolving a water-soluble polymer containing hydroxyl or amino in distilled water, adding an o-nitrobenzyl micromolecule containing active functional group carboxyl, adding a condensing agent 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride and a catalyst pyridinium p-toluenesulfonate, stirring at room temperature for reaction, pouring a reaction solution into an insoluble solvent for re-precipitation after the reaction is finished, dissolving in water, dialyzing by using a dialysis bag, and freeze-drying to obtain an o-nitrobenzyl modified polymer derivative;

D. dissolving a water-soluble polymer containing hydroxyl or amino in distilled water, adding an o-nitrobenzyl micromolecule containing active functional group bromine, adding potassium carbonate as alkali, reacting, pouring a reaction solution into an insoluble solvent for re-precipitation after the reaction is finished, dissolving the reaction solution in water, dialyzing by using a dialysis bag, and freeze-drying to obtain an o-nitrobenzyl modified polymer derivative;

E. polymerizing the o-nitrobenzyl polymerizable monomer derivative and one or more polymerizable comonomers to obtain an o-nitrobenzyl modified synthetic copolymer;

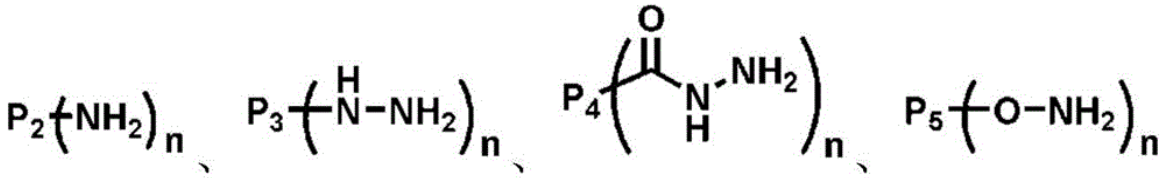
the o-nitrobenzyl polymerizable monomer derivative is an acrylate compound, a methacrylate compound, an acrylamide compound or a methacrylamide compound,

at least one of the polymerizable comonomers must be a water-soluble comonomer, and the polymerizable comonomer is polyethylene glycol methacrylate, polyethylene glycol acrylate, methacrylic acid, acrylic acid, hydroxyethyl acrylate, acrylamide.

5. A method for preparing a photo-coupled cross-linked hydrogel material comprises the following steps: dissolving the component A-the o-nitrobenzyl type optical trigger modified macromolecule derivative of claim 1 in a biocompatible medium to obtain a solution A; dissolving the component B-containing primary amine, diamine, hydrazide or hydroxylamine polymer derivative in a biocompatible medium to obtain a solution B; uniformly mixing the solution A and the solution B to obtain a hydrogel precursor solution; under the irradiation of a light source, aldehyde groups or ketone groups generated by the o-nitrobenzyl in the component A under the excitation of light and primary amine, diamine, hydrazide or hydroxylamine groups in the component B are crosslinked in a Schiff base mode to form hydrogel.

6. The method for producing a photo-coupled crosslinked hydrogel material according to claim 5,

the component B-containing primary amine, diamine, hydrazide or hydroxylamine polymer derivatives have structural formulas B-I, B-II, B-III and B-IV respectively:



式 B-Ⅰ　　式 B-Ⅱ　　式 B-Ⅲ　　式 B-Ⅳ

wherein n is more than or equal to 2, P_2、P_3、P_4、P_5Is hydrophilic or water-soluble natural high polymer or synthetic polymer;

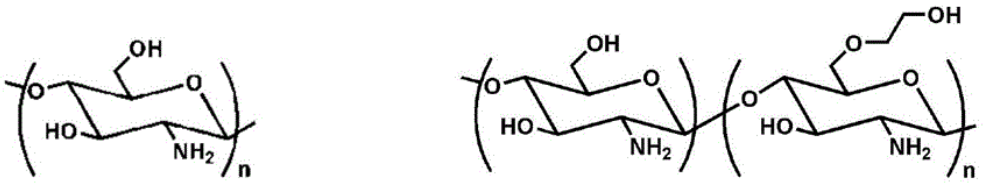
the hydrophilic or water-soluble natural high polymer comprises natural polysaccharide substances and modifications or degradation products thereof, and proteins and modifications, modifications and degradation products thereof;

the natural polysaccharide substance comprises hyaluronic acid, carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, alginic acid, dextran, agarose, heparin, chondroitin sulfate, ethylene glycol chitosan, propylene glycol chitosan, chitosan lactate, carboxymethyl chitosan or chitosan quaternary ammonium salt;

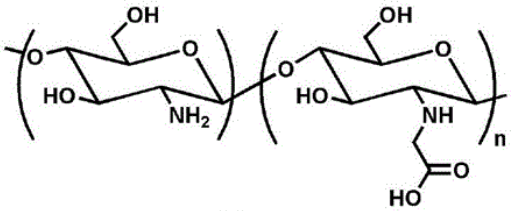
the protein comprises various hydrophilic or water-soluble animal and plant proteins, collagen, serum protein, silk fibroin and elastin, and the protein degradation product comprises gelatin or polypeptide;

the hydrophilic or water-soluble synthetic polymer comprises two-arm or multi-arm polyethylene glycol, polyethyleneimine, synthetic polypeptide, polylysine, polyglutamic acid, polyacrylic acid, polymethacrylic acid, polyacrylamide, polymethacrylamide, polyvinyl alcohol and polyvinylpyrrolidone.

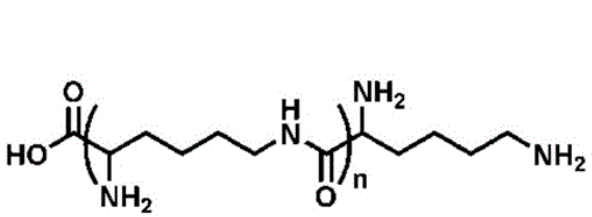
7. The method for preparing a photo-coupled crosslinked hydrogel material according to claim 5, wherein said component B is selected from the following structures:

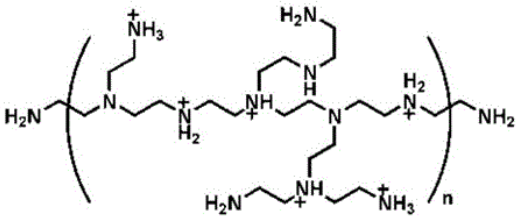


组分 B-1　　组分 B-2

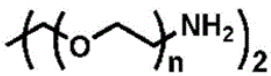


组分 B-3

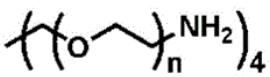




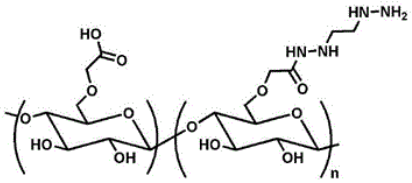
组分 B-5



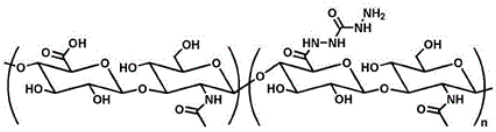
组分 B-6



组分 B-7

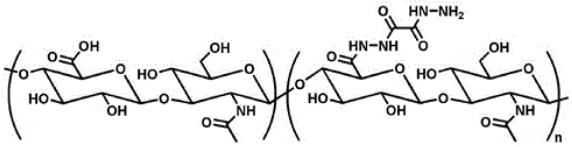


组分 B-8

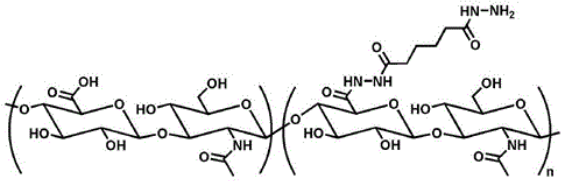


组分 B-10

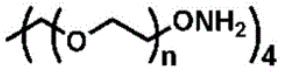
组分 B-11



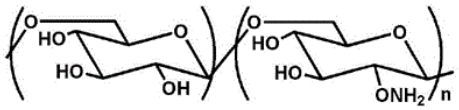
组分 B-12



组分 B-13



组分 B-14



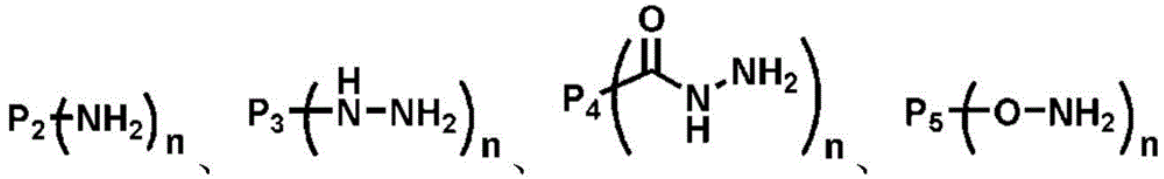
组分 B-15

in the component B-1 to the component B-15, n is more than or equal to 2.

8. A photo-coupled crosslinked hydrogel material prepared by the method of claim 5 or 6 or 7.

9. A kit for use in the preparation of the photo-coupled crosslinked hydrogel material of claim 8, comprising: component A-a polymeric derivative modified with an o-nitrobenzyl-based photo-trigger according to claim 1 or 2; component B-contains primary amine, diamine, hydrazide or hydroxylamine polymer derivatives; and instructions for hydrogel preparation and use;

the component B-containing primary amine, diamine, hydrazide or hydroxylamine polymer derivatives have structural formulas B-I, B-II, B-III and B-IV respectively:



式 B-Ⅰ 式 B-Ⅱ 式 B-Ⅲ 式 B-Ⅳ

wherein n is more than or equal to 2, P_2、P_3、P_4、P_5Is hydrophilic or water-soluble natural high polymer or synthetic polymer;

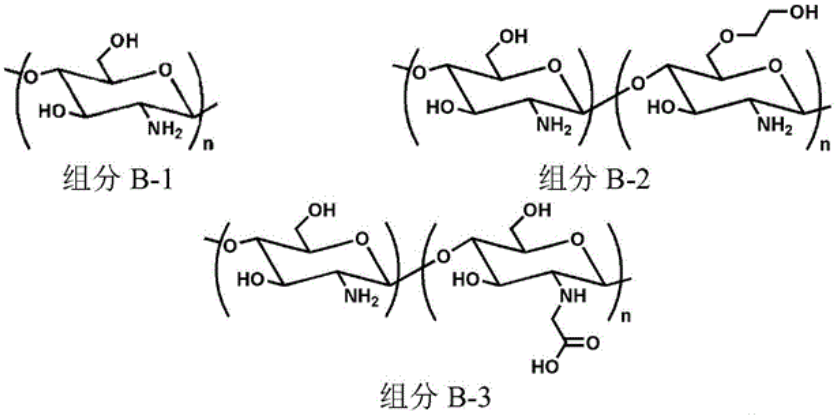
the hydrophilic or water-soluble natural high polymer comprises natural polysaccharide substances and modifications or degradation products thereof, and proteins and modifications, modifications and degradation products thereof;

the natural polysaccharide substance comprises hyaluronic acid, carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, alginic acid, dextran, agarose, heparin, chondroitin sulfate, ethylene glycol chitosan, propylene glycol chitosan, chitosan lactate, carboxymethyl chitosan or chitosan quaternary ammonium salt;

the protein comprises various hydrophilic or water-soluble animal and plant proteins, collagen, serum protein, silk fibroin and elastin, and the protein degradation product comprises gelatin or polypeptide;

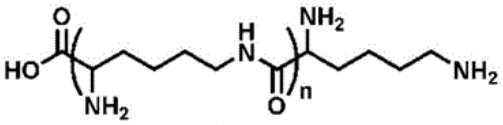
the hydrophilic or water-soluble synthetic polymer comprises two-arm or multi-arm polyethylene glycol, polyethyleneimine, synthetic polypeptide, polylysine, polyglutamic acid, polyacrylic acid, polymethacrylic acid, polyacrylamide, polymethacrylamide, polyvinyl alcohol and polyvinylpyrrolidone.

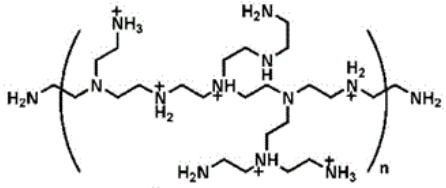
10. A kit for use in the preparation of the photo-coupled crosslinked hydrogel material of claim 8, comprising: component A-a polymeric derivative modified with an o-nitrobenzyl-based photo-trigger according to claim 1 or 2; component B-contains primary amine, diamine, hydrazide or hydroxylamine polymer derivatives; and instructions for hydrogel preparation and use; the component B is selected from the following structures:

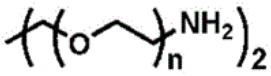

组分 B-1

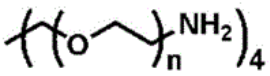
组分 B-2

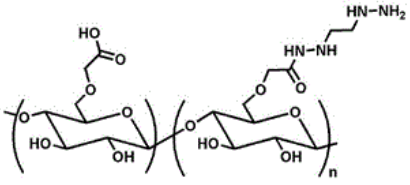
组分 B-3

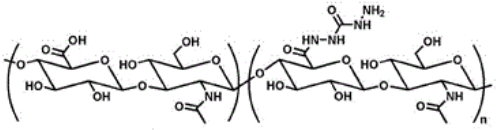

组分 B-5

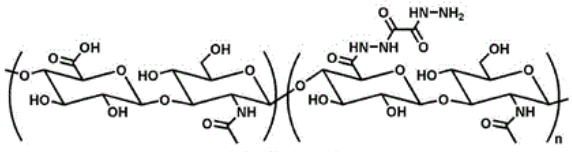

组分 B-6

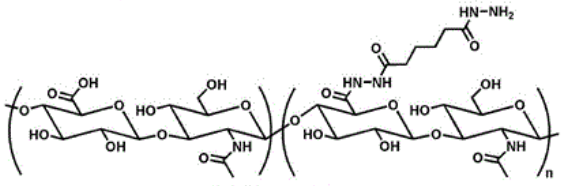

组分 B-7

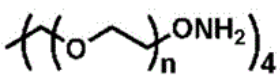

组分 B-8

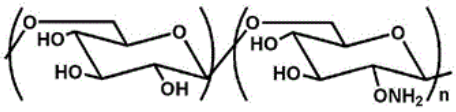

组分 B-10


组分 B-11


组分 B-12


组分 B-13


组分 B-14


组分 B-15

in the component B-1 to the component B-15, n is more than or equal to 2.

11. Use of the photo-coupled crosslinked hydrogel material according to claim 8, comprising the following applications:

the photo-coupling cross-linked hydrogel is used for preparing postoperative wound surface sealing products,

the photo-coupled cross-linked hydrogel is used for preparing tissue fluid leakage plugging articles,

the application of the photo-coupled cross-linked hydrogel in preparing hemostatic materials,

the photo-coupled cross-linked hydrogel is used for preparing tissue engineering scaffold materials-bone repair materials,

the application of the photo-coupled cross-linked hydrogel as a 3D printing material-biological ink,

the light-coupled cross-linked hydrogel is applied to preparation of cell, protein and drug carriers.

12. Use of the photo-coupled cross-linked hydrogel material according to claim 8 for preparing a scaffold material for tissue engineering-cartilage repair material.

Description

Preparation method, raw materials, product and application of photo-coupled cross-linked hydrogel material

Technical Field

The invention belongs to the field of biological materials, and particularly relates to a preparation method, raw materials, a product and application of a photo-coupled cross-linked hydrogel material.

Background

The hydrogel is a polymer material with a three-dimensional network cross-linked structure and high water content, and can be widely applied to the fields of tissue engineering and regenerative medicine because the hydrogel has excellent biocompatibility and certain mechanical strength and can be highly fitted with the microenvironment of biological tissues. The in-situ cured hydrogel has excellent tissue forming property in clinical application. Currently, the hydrogel curable in situ mainly includes temperature sensitive type (e.g. LeGoo, hydroxybutyl chitosan, etc.), two-component injection type (e.g. fiber Glue, adheus AutoSpray, etc.), photosensitive type (e.g. FocalSeal, ChonDux, etc.) and so on according to different gelling mechanisms.

The photosensitive hydrogel material has the advantages of non-physical contact of light, accurate and controllable space and time and the like in the gelling process, and has more practical clinical operability. Free radical initiated photopolymerization crosslinking (Hubbell et al. U.S. Pat. No.6060582A, issued May 9,2000) and its further developed thiol-ene reaction (Christopher Bowman et al. U.S. Pat. NO. US 72888B 2, issued October 30,2007) are two typical ways of preparing hydrogels by current photo-initiated free radical polymerization crosslinking. However, in a system for initiating polymerization crosslinking by free radicals, a small-molecule photoinitiator is required to participate, and free radicals generated by illumination inevitably cause damage to cells or biological tissues; in addition, free radicals are extremely sensitive to oxygen, so that the thin-layer in-situ hydrogel is difficult to construct; more importantly, the hydrogel constructed in situ by the method has almost no tissue adhesion capability, so that the clinical fixation of the hydrogel becomes a trouble and is a barrier for the clinical transformation of the technology.

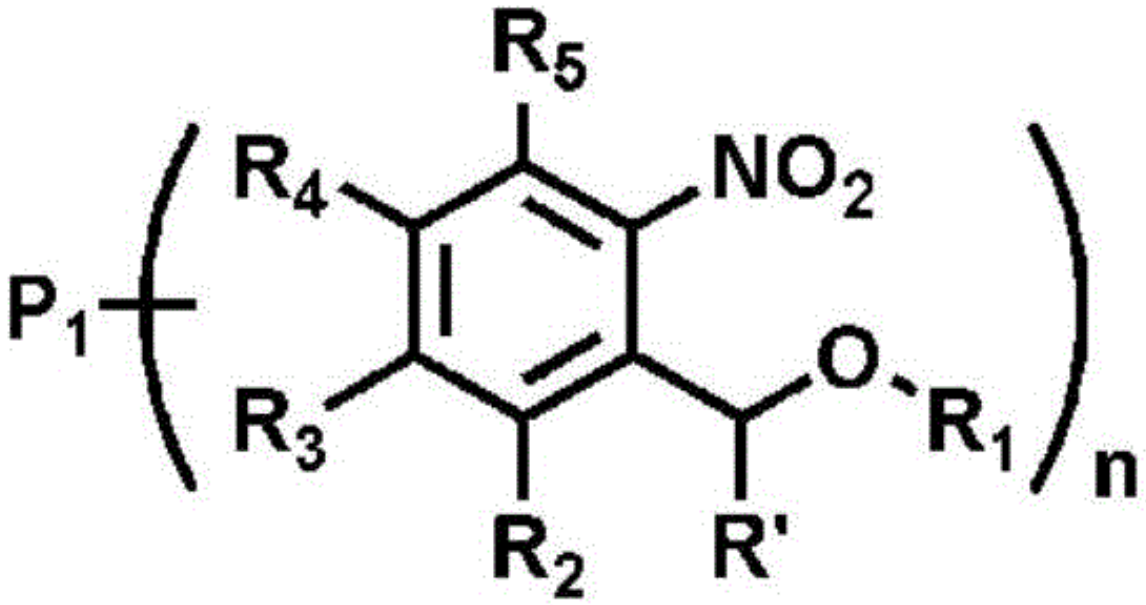
In order to avoid the defects of a free radical initiated polymerization crosslinking mode, the Zhujoing research group proposed in 2014 a non-free radical photo-coupling crosslinking technology (Yunlong Yang; Jieyuan Zhang; Zhenzhen Zhu Liu, Qining Lin; Xialin Liu, Chunyan Bao, Yang Wang; Linying Zhu, Adv. Mater.2016,28,2724; Linying Zhu et al. PCT.No. 82725A 1, issued Jun 2,2016) for further crosslinking a polyamine-based polymer derivative to prepare hydrogel based on o-nitrobenzyl alcohol irradiated by ultraviolet light (365nm) to generate aldehyde groups, wherein the generation of free radicals is completely avoided in the process of clinically carrying out gel forming by using the technology, so that the toxicity and oxygen inhibition of the free radicals can be effectively solved, the biocompatibility is excellent, and the gel layer is thin and adjustable; meanwhile, aldehyde groups generated by irradiation of o-nitrobenzyl alcohol can be crosslinked with protein amino groups rich on the surface of the tissue, so that chemical bond bonding and fixation of the glue layer and the tissue are realized, and the problems of tissue adhesion, integration and the like of the traditional photosensitive hydrogel are solved. However, the system has some disadvantages, such as a shorter light curing wavelength (365nm), a slower light crosslinking speed (about 30s for initial gel forming time and about 2min for complete gel forming time), a more complicated synthesis, and the like, thereby limiting the clinical transformation of the non-radical light coupling crosslinking technology.

Disclosure of Invention

In order to further exert the advantages of good biocompatibility and strong tissue adhesion of the non-free radical photo-coupling crosslinked hydrogel material and overcome the defects of the non-free radical photo-coupling crosslinked hydrogel material, the invention further improves the molecular structure of the original ortho-nitrobenzyl photo-trigger, and synthesizes a series of ortho-nitrobenzyl photo-triggers with novel structures for constructing the photo-coupling crosslinked hydrogel.

The first purpose of the invention is to provide an o-nitrobenzyl type photo-trigger modified macromolecule derivative.

The o-nitrobenzyl type photo trigger modified macromolecule derivative has a structure shown in a formula I:



式 I

wherein R' is selected from hydrogen, halogen atom, hydroxyl, sulfhydryl, amino, nitryl, cyano, aldehyde group, ketone group, ester group, amide group, phosphonic acid group, phosphonate group, sulfonic acid group, sulfonate group, sulfone group, sulfoxide group, aryl group, heteroaryl group, alkyl group, alkylidene group, modified alkyl group or modified alkylidene group, etc.,

R_1selected from hydrogen, ether bond substituent, ester bond substituent, carbonate bond substituent, carbamate bond substituent, mercapto formate bond substituent or phosphate bond substituent, etc.,

R_2, R_3, R_4, $R_5$is freely selected from hydrogen, halogen atom, hydroxyl, sulfhydryl, amine group, nitryl, cyano, aldehyde group, ketone group, ester group, amide group, phosphonic acid group, phosphonate group, sulfonic group, sulfonate group, sulfone group, sulfoxide group, aryl group, heteroaryl group, alkyl group, alkylidene group, modified alkyl group or modified alkylidene group, etc.,

$P_1$and R_2, R_3, R_4, R_5In which any one or more radicals are linked, in which formula I, P_1Is a hydrophilic or water-soluble natural or synthetic polymer, or P_1Independently selected from a plurality of hydrophilic or water-soluble natural or synthetic polymers;

in formula I, n is more than or equal to 2, namely a single P_1The average number of o-nitrobenzyl optical triggers (namely structures in brackets in the formula I) on the macromolecular chain is more than or equal to 2;

when R', R_2Selected from hydrogen, P_1Is not connected to R_4。

Further, the alkyl is a saturated or unsaturated aliphatic straight chain or branched chain alkyl with 1-30 carbon atoms;

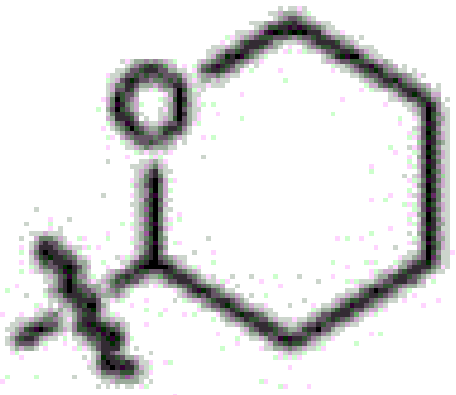
the alkylene is a saturated or unsaturated aliphatic straight chain or branched chain alkylene with 1-30 carbon atoms;

any carbon atom of the modified alkyl is selected from halogen atom, -OH, -SH, -NO_2-CN, -CHO, -COOH, ester group, amide group, aryl group, arylene group, -CO-, -O-, -S-, -SO-, -$_2$A group obtained by replacing at least one group of primary amino, secondary amino, tertiary amino, quaternary ammonium base, saturated or unsaturated monocyclic or bicyclic cycloalkylene and bridged lipid heterocycle, wherein the modified alkyl has 1-30 atoms, and the carbon-carbon single bond of the modified alkyl can be optionally replaced by a carbon-carbon double bond or a carbon-carbon triple bond;

any carbon atom of the modified alkylene group being an alkylene group substituted by a halogen atom, -OH, -SH, -NO_2-CN, -CHO, -COOH, ester group, amide group, aryl group, arylene group, -CO-, -O-, -S-, -SO-, -$_2$A group obtained by replacing at least one group of primary amino, secondary amino, tertiary amino, quaternary ammonium base, saturated or unsaturated monocyclic or bicyclic cycloalkylene and bridged lipid heterocycle, wherein the modified alkylene has 1 to 30 atoms, and a carbon-carbon single bond of the modified alkylene can be optionally replaced by a carbon-carbon double bond or a carbon-carbon triple bond;

the ether bond substituent is selected from the following structures:

-$(CH_2)_xCH_3$、-$(CH_2CH_2O)_xCH_3$、-$(CH_2)_x(CH_2CH_2O)_yCH_3$or is



And the like, wherein x and y are integers of 0 or more;

the ester bond substituent is selected from the following structures:

-$CO(CH_2)_xCH_3$、-$CO(CH_2CH_2O)_xCH_3$、-$CO(CH_2)_x(CH_2CH_2O)_yCH_3$and the like, wherein x and y are integers of 0 or more;

the carbonate bond substituent is selected from the following structures:

-$COO(CH_2)_xCH_3$、-$COO(CH_2CH_2O)_xCH_3$、-$COO(CH_2)_x(CH_2CH_2O)_yCH_3$and the like, wherein x and y are integers of 0 or more;

the carbamate bond substituent is selected from the following structures:

-$CONH(CH_2)_xCH_3$、-$CONH(CH_2CH_2O)_xCH_3$、-$CONH(CH_2)_x(CH_2CH_2O)_yCH_3$and the like,wherein x and y are integers not less than 0;

the mercapto formic ester bond substituent is selected from the following structures:

-$COS(CH_2)_xCH_3$、-$COS(CH_2CH_2O)_xCH_3$、-$COS(CH_2)_x(CH_2CH_2O)_yCH_3$and the like, wherein x and y are integers of 0 or more;

the phosphate ester bond substituent is selected from the following structures:

-$POOO(CH_2)_xCH_3$、-$POOO(CH_2CH_2O)_xCH_3$、-$POOO(CH_2)_x(CH_2CH_2O)_yCH_3$and the like, wherein x and y are integers of 0 or more;

the aryl is a 5-10-membered aromatic monocyclic ring or aromatic condensed bicyclic ring structure;

the heteroaryl is a 5-10-membered aromatic monocyclic ring or aromatic condensed bicyclic ring structure containing at least one heteroatom selected from O, S, N or Si on the ring;

the halogen atoms are each independently selected from F, Cl, Br, I.

For the structures of formula I, there are also some preferred structures, namely R_2, R_3, R_4, R_5At least two of which are linked to each other, together with the carbon atoms, to form a saturated or unsaturated aliphatic or aliphatic heterocyclic ring, or to form an aromatic or aromatic heterocyclic ring.

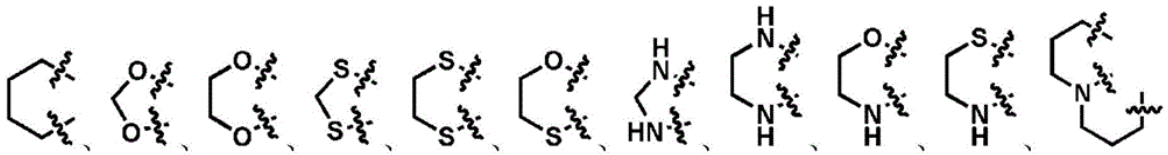
The alicyclic ring is a saturated or unsaturated 3-to 10-membered monocyclic or polycyclic alicyclic ring;

the aliphatic heterocyclic ring is a saturated or unsaturated 3-to 10-membered monocyclic or polycyclic aliphatic heterocyclic ring containing at least one hetero atom selected from O, S, N or Si in the ring, and when the aliphatic heterocyclic ring contains an S atom, it is optionally-S-, -SO-or-SO_2-; h on the alicyclic or alicyclic ring can be optionally substituted by a halogen atom, nitro, aryl, alkyl or modified alkyl;

the aromatic ring is a 5-10-membered aromatic monocyclic ring or an aromatic condensed bicyclic ring;

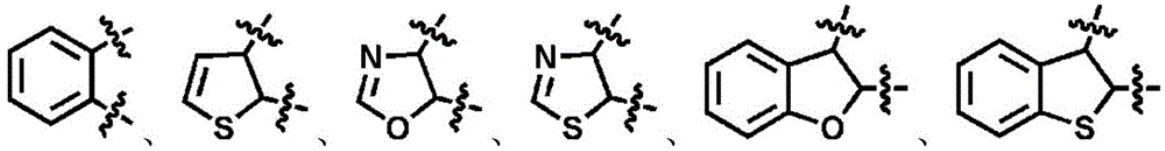
the aromatic heterocyclic ring is a 5-to 10-membered aromatic monocyclic ring or an aromatic condensed bicyclic ring which contains at least one heteroatom selected from O, S, N or Si on the ring; the H on the aromatic ring or the aromatic heterocyclic ring can be optionally substituted by a halogen atom, a nitro group, an aryl group, an alkyl group or a modified alkyl group.

Further, preferred structures of alicyclic or alicyclic rings include:



etc.;

further, preferred structures of the aromatic ring or the aromatic heterocyclic ring include:



etc.;

when R in the structure of formula I_2, R_3, R_4, R_5Wherein at least two are linked to each other to form, together with the carbon atom, a saturated or unsaturated aliphatic or aliphatic heterocycle, or an aromatic or aromatic heterocycle, P_1May also be linked to R_2, R_3, R_4, R_5Saturated or unsaturated alicyclic or alicyclic heterocyclic ring formed between them, or aromatic ring or aromatic heterocyclic ring formed between them.

For P_1And R_2, R_3, R_4, R_5Is linked to any one or more radicals of R, or to R_2, R_3, R_4, R_5A saturated or unsaturated alicyclic or alicyclic ring or an alicyclic or aromatic ring formed therebetween, or an aromatic ring or an aromatic heterocyclic ring formed therebetween,

the bond being selected from the group consisting of bonds P obtained from hydroxy groups$_1$-O-; or a linkage P obtained from the mercapto group$_1$-S-; or a linkage P obtained from the class of amine groups$_1$-NH-; or a linkage P obtained from alkanes$_1$-; or a connecting bond P obtained from the ester bond group$_1$-COO-; or a linkage P obtained from amide bonds$_1$-CONH-, one end of the bond with P_1Connected and the other end is connected with the benzene ring of the molecule shown in the formula I.

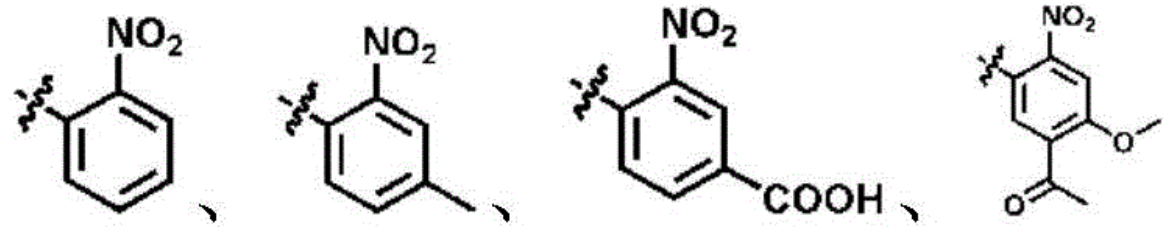
Ortho-nitrobenzyl-based optical trigger modifiedPolymer P in the Polymer derivative$_1$The natural polymer which can be hydrophilic or water-soluble comprises natural polysaccharide substances and a modifier or a degradant thereof, protein and a modifier or a degradant thereof and the like, the natural polysaccharide substances comprise hyaluronic acid, carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, alginic acid, dextran, agarose, heparin, chondroitin sulfate, glycol chitosan, propylene glycol chitosan, chitosan lactate, carboxymethyl chitosan or chitosan quaternary ammonium salt and the like, the protein comprises various hydrophilic or water-soluble animal and plant proteins, collagen, serum protein, fibroin and elastin, the protein degradant comprises gelatin or polypeptide and the like, and the hydrophilic or water-soluble synthetic polymer comprises two-arm or multi-arm polyethylene glycol, polyethyleneimine, synthetic polypeptide, polylysine, polyglutamic acid, polyacrylic acid, polymethacrylic acid and the like, Polyacrylates, polymethacrylates, polyacrylamides, polymethacrylamides, polyvinyl alcohols, polyvinyl pyrrolidones, and the like.

In the grafted or polymerized water-soluble or hydrophilic polymer derivative, the average number of the o-nitrobenzyl type photosites on a single polymer chain is more than or equal to 2 (namely n is more than or equal to 2).

The polymer derivative modified by the o-nitrobenzyl type optical trigger can be a hydrophilic or water-soluble polymer simultaneously containing one or more than one different groups, or a mixture of hydrophilic or water-soluble polymers containing one or more than one different groups. The hydrophilic or water-soluble polymer refers to a hydrophilic or water-soluble natural polymer or a hydrophilic or water-soluble synthetic polymer.

Some preferred structures of R' include:

-H、$-CH_3$、$-CH_2CH_3$、-CH = CH-CH = $CH-CH_3$、-F、-Cl、-Br、-I、$-CF_3$、$-CCl_3$、$-CBr_3$、$-Cl_3$、-CN、-COOH、-Ph、



and the like.

R_2, R_3, R_4, R_5Some preferred structures of (a) include:

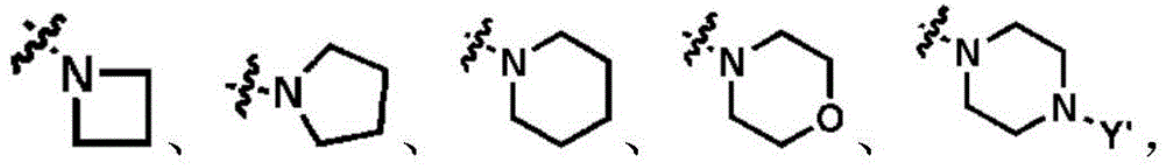
-H、-OH、-SH、$-NH_2$、-F、-Cl、-Br、-I、$-CF_3$、$-CCl_3$、$-CBr_3$、$-Cl_3$、$-NO_2$、-CN、-CHO、-COOH、$-COONH_2$、$-SO_3$h, etc.;

alkyl substituents preferably have the structure, e.g. straight-chain alkyl- $(CH)_2)_xCH_3$Branched alkyl radical- $(CH)_2)_x(CY'Y'')_yCH_3$(Y ', Y' is hydrogen, alkyl or modified alkyl), etc., wherein x and Y are not less than 0 and are integers;

preferred ether substituents are of the structure, e.g. -O $(CH)_2)_xCH_3$、$-O(CH_2CH_2O)_xCH_3$、$-O(CH_2)_x(CH_2CH_2O)_yCH_3$And the like, wherein x and y are integers of 0 or more;

preferred thioether substituents are of the structure, e.g. -S $(CH)_2)_xCH_3$、$-S(CH_2CH_2O)_xCH_3$、$-S(CH_2)_x(CH_2CH_2O)_yCH_3$And the like, wherein x and y are integers of 0 or more;

preferred structures for the aminyl-like substituents are, for example, -NH $(CH)_2)_xCH_3$、$-NH(CH_2)_x(CY'Y'')_yCH_3$、$-N(CY'Y'')_x(CY'Y'')_y$、

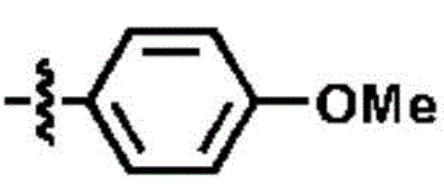


(Y, Y' are hydrogen, alkyl or modified alkyl), etc., wherein x and Y are not less than 0 and are integers;

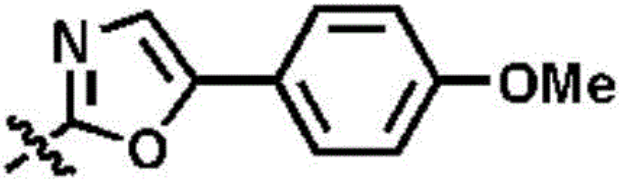
the ester substituent is preferably of the structure, e.g. -COO $(CH)_2)_xCH_3$、$-COO(CH_2CH_2O)_xCH_3$、$-COO(CH_2)_x(CH_2CH_2O)_yCH_3$And the like, wherein x and y are integers of 0 or more;

preferred structures for the amide substituent are, for example, -CONH $(CH)_2)_xCH_3$、$-CONH(CH_2CH_2O)_xCH_3$、$-CONH(CH_2)_x(CH_2CH_2O)_yCH_3$And the like, wherein x and y are integers of 0 or more;

preferred aromatic substituents are of the structure-Ph,

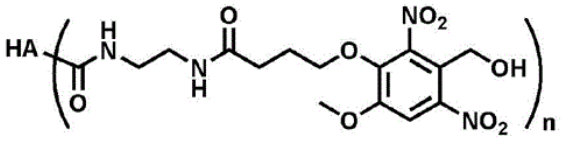


、

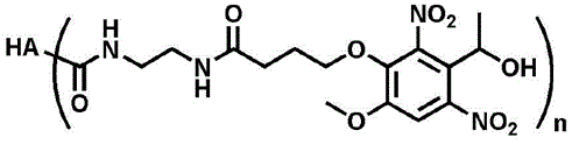


And the like.

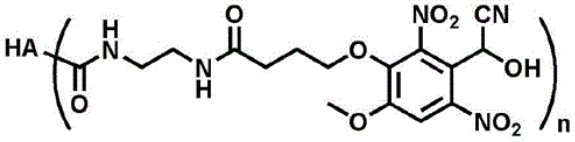
Further preferably, the formula I can be selected from the following structures in component A-1 to component A-49:



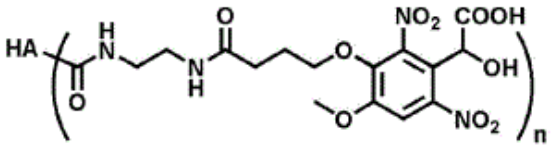
组分 A-1



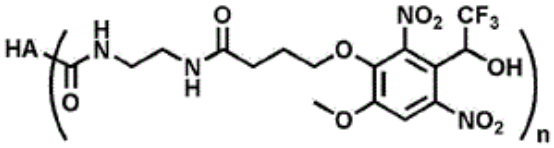
组分 A-2



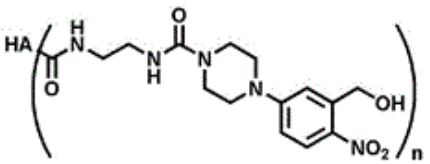
组分 A-4



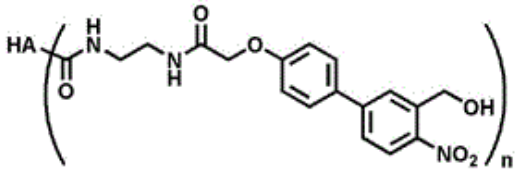
组分 A-5



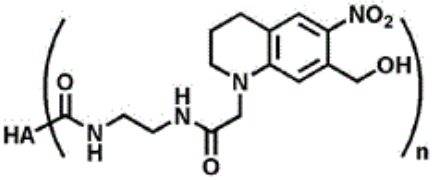
组分 A-6



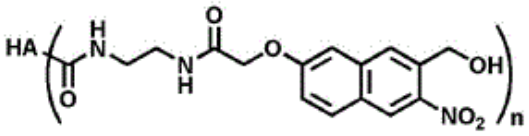
组分 A-21



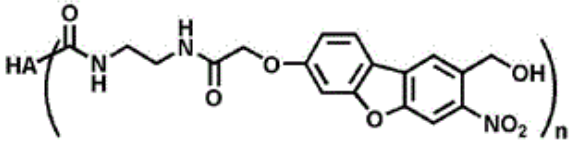
组分 A-22



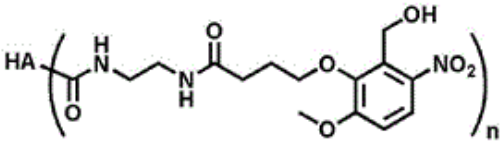
组分 A-25



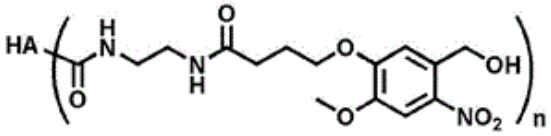
组分 A-26



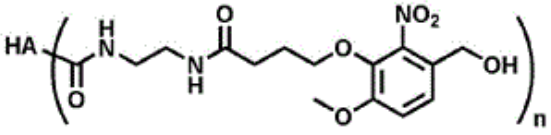
组分 A-27



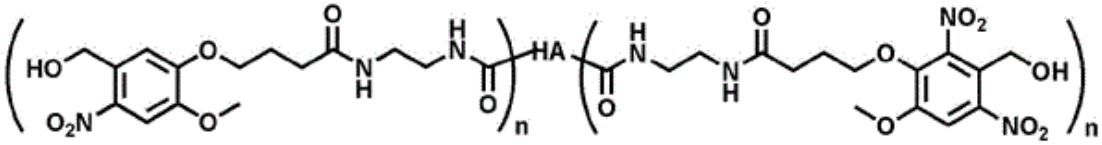
组分 A-28



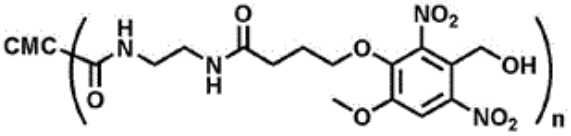
组分 A-29



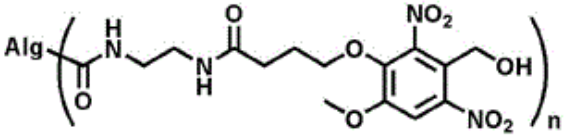
组分 A-30



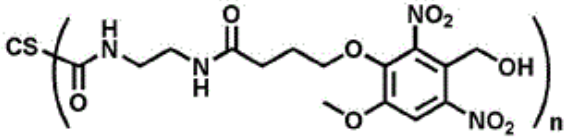
组分 A-32



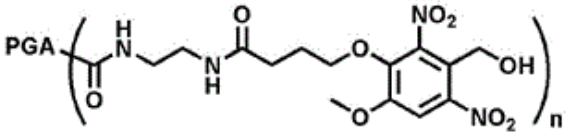
组分 A-36

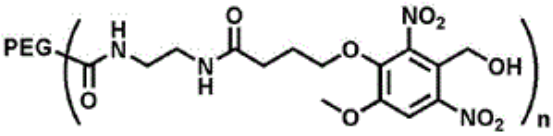


组分 A-37

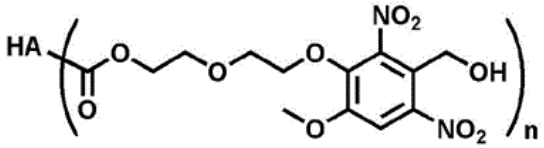


组分 A-38

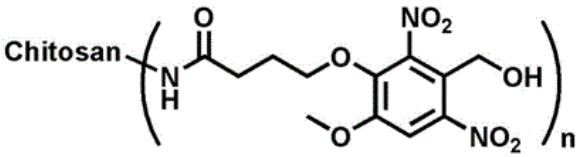




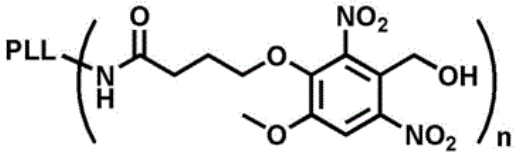
组分 A-39



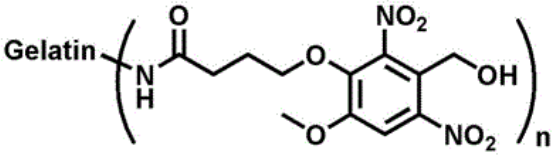
组分 A-40



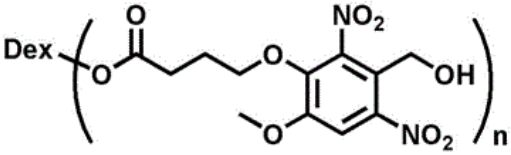
组分 A-41



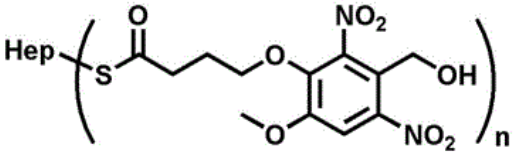
组分 A-42



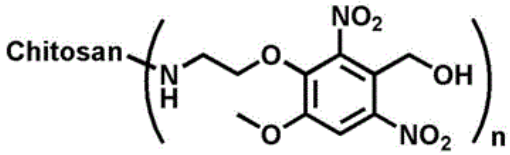
组分 A-43



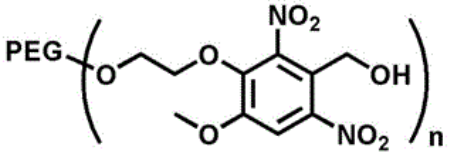
组分 A-44



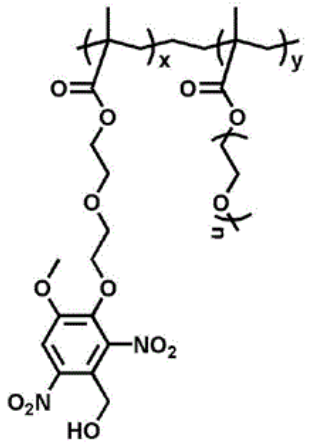
组分 A-45



组分 A-46



组分 A-47



组分 A-48

组分 A-49

in the component A-1-component A-49, n is more than or equal to 2, and HA is hyaluronic acid; CMC is carboxymethyl cellulose; alg is alginic acid; CS is chondroitin sulfate; PGA is polyglutamic acid; PEG is polyethylene glycol; chitosan is Chitosan; gelatin is Gelatin; PLL is polylysine; dex is dextran; hep is heparin.

The invention aims at providing a component A-o-nitrobenzyl type photo-trigger modified polymer derivative which is mainly characterized in that:

1) the introduction of substituent groups or conjugated systems on benzene rings enables the absorption wavelength of the light trigger to be red-shifted, the molar absorption coefficient is increased, a 365nm purple light band light source used in photocuring is changed into a 405nm blue light band light source, the light intensity required by photocuring is weakened, and the heat sensation and cytotoxicity generated by a strong ultraviolet light source are greatly reduced;

2) the connection position of the o-nitrobenzyl optical trigger and the polymer is more flexible, the connection position of the polymer can be selected according to different substituents introduced on a benzene ring, compared with the prior molecular structure, the synthesis is simpler and more flexible, and the application cost of the hydrogel can be greatly reduced. Therefore, the novel o-nitrobenzyl molecules further improve the photolysis wavelength and the photolysis efficiency on the basis of the original molecular structure, and are simpler and more convenient to synthesize the macromolecular derivatives.

The second purpose of the invention is to provide a preparation method of the o-nitrobenzyl type photo-trigger modified macromolecule derivative.

In the invention, the preparation method of the o-nitrobenzyl light trigger modified macromolecule derivative is a chemical labeling method or an artificial polymerization method.

Wherein, the chemical labeling method is to connect the macromolecule and the chemical groups contained in the o-nitrobenzyl light trigger by using the chemical reaction, and can be the labeling of the macromolecule containing carboxyl and the o-nitrobenzyl micromolecule containing hydroxyl/sulfhydryl/amido (the references are O.P.Oommen, S.Wang, M.Kisiel, M.Sloff, J.Hilborn, O.P.Varghese, adv.Funct.Mater.2013,23,1273.); or hydroxyl-containing macromolecules and carboxyl-containing or bromine-containing o-nitrobenzyl small molecule markers (references k.peng, i.tomatsu, a.v.korobko, a.kros, Soft Matter 2010,6, 85; l.li, n.wang, x.jin, r.ding, s.nie, l.sun,

q.wu, y.wei, c.gong, Biomaterials 2014,35, 3903.); the labeling method may be a labeling method such as labeling of an amine group-containing polymer and a carboxyl group-containing or bromine-containing o-nitrobenzyl small molecule (refer to L.Li, N.Wang, X.jin, R.Deng, S.Nie, L.Sun, Q.Wu, Y.Wei, C.Gong, Biomaterials 2014,35, 3903).

The artificial polymerization method is to use o-nitrobenzyl derivative functional monomer to copolymerize with other comonomer, and can be a random radical polymerization method, and also can be a controlled radical polymerization method (such as ATRP polymerization and RAFT polymerization method).

Some practical preparation methods of the o-nitrobenzyl type photo-trigger modified macromolecule derivatives are as follows:

the first practical preparation method is as follows: dissolving a water-soluble polymer containing carboxyl in distilled water, adding an o-nitrobenzyl micromolecule containing active functional group hydroxyl or sulfydryl or amido, adding a condensing agent 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and an activating agent hydroxybenzotriazole (HOBt), and stirring at room temperature for 24-48 h. After the reaction is finished, adding the reaction solution into a dialysis bag, dialyzing for 2-3d by using a dilute hydrochloric acid solution, and then freeze-drying to obtain the o-nitrobenzyl modified macromolecule derivative.

A second practical preparation method is as follows: the water-soluble polymer containing carboxyl is stirred to be completely dissolved in 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH is 5.2), an o-nitrobenzyl micromolecule is dissolved in dimethyl sulfoxide and then added into the reaction solution, 4- (4, 6-dimethoxytriazine-2-yl) -4-methylmorpholine hydrochloride (DMTMM) is dissolved in the MES buffer solution and added into the reaction solution in three times (every 1h) for reaction at 35 ℃ for 24 h. And then pouring the reaction solution into a dialysis bag, dialyzing with deionized water for 2-3d, and freeze-drying to obtain the o-nitrobenzyl modified polymer derivative.

In the first and second embodiments, the water-soluble polymer having a carboxyl group may be polyethylene glycol, polysaccharide having a carboxyl group (e.g., hyaluronic acid, carboxymethyl cellulose, alginic acid, etc.), protein or polypeptide having a carboxyl group (e.g., gelatin, etc.), and preferably multi-arm carboxyl polyethylene glycol, hyaluronic acid, carboxymethyl cellulose, gelatin. Further preferred is hyaluronic acid.

A third practical preparation method is as follows: dissolving a water-soluble polymer containing hydroxyl or amino in distilled water, adding an o-nitrobenzyl micromolecule containing active functional group carboxyl, adding a condensing agent 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and a catalyst pyridinium p-toluenesulfonate (DPTS), and stirring at room temperature for 24-48 h. After the reaction is finished, pouring the reaction solution into an insoluble solvent for re-precipitation (for example, the modified polyethylene glycol derivative can be poured into ether for re-precipitation, and the polysaccharide macromolecular derivative can be poured into ethanol for re-precipitation), then dissolving into water, dialyzing for 2-3d by using a dialysis bag, and freeze-drying to obtain the o-nitrobenzyl modified macromolecular derivative.

A fourth practical preparation method is: dissolving a water-soluble polymer containing hydroxyl or amino in distilled water, adding an o-nitrobenzyl micromolecule containing active functional group bromine, adding potassium carbonate as an alkali, and reacting at room temperature for 24-48 h. After the reaction is finished, pouring the reaction solution into an insoluble solvent (for example, the modified polyethylene glycol derivative can be poured into ether, and the modified polysaccharide macromolecular derivative can be poured into ethanol) for re-precipitation, then dissolving into water, dialyzing for 2-3d by using a dialysis bag, and freeze-drying to obtain the o-nitrobenzyl modified macromolecular derivative.

In the third and fourth embodiments, the water-soluble polymer containing a hydroxyl group or an amine group may be a polyethylene glycol containing a hydroxyl group or an amine group, or a natural polysaccharide or protein/polypeptide, preferably a multi-arm hydroxypolyethylene glycol, a multi-arm aminopolyethylene glycol, an ethylene glycol chitosan, a propylene glycol chitosan, a carboxymethyl chitosan, a chitosan lactate, or a natural polysaccharide, or polylysine, gelatin, or the like, and more preferably an ethylene glycol chitosan, a multi-arm hydroxypolyethylene glycol.

In the reaction, the mol ratio of the carboxyl, hydroxyl or amino in the water-soluble polymer to the micromolecule o-nitrobenzyl derivatives is preferably 1: 0.1-2; the mol ratio of the amino-modified o-nitrobenzyl micromolecules to 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and the activating agent hydroxybenzotriazole (HOBt) is preferably 1:2: 1.5; the mol ratio of the amino-modified o-nitrobenzyl small molecule to the 4- (4, 6-dimethoxytriazine-2-yl) -4-methylmorpholine hydrochloride (DMTMM) is preferably 1: 7.5; the mol ratio of the carboxyl modified o-nitrobenzyl micromolecule to 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and the catalyst DPTS is preferably 1:2: 1.5; the molar ratio of the brominated o-nitrobenzyl small molecules to potassium carbonate is preferably 1: 2.

A fifth practical preparation method is: the o-nitrobenzyl polymerizable monomer derivative and one or more polymerizable comonomers are polymerized to obtain the o-nitrobenzyl modified synthetic copolymer. Purifying the product by multiple dissolving-reprecipitation.

The o-nitrobenzyl polymerizable monomer derivative may be an acrylate compound, a methacrylate compound, an acrylamide compound, or a methacrylamide compound, preferably a methacrylate compound and an acrylamide compound, and more preferably a methacrylate compound.

At least one of the polymerizable comonomers is required to be a water-soluble comonomer, and any water-soluble polymerizable monomer such as polyethylene glycol methacrylate (PEG-MA), polyethylene glycol acrylate, methacrylic acid (MAA), Acrylic Acid (AA), hydroxyethyl acrylate, Acrylamide (AM) and the like can be used, and polyethylene glycol methacrylate (PEG-MA) is preferable. Other comonomers are selected for different applications.

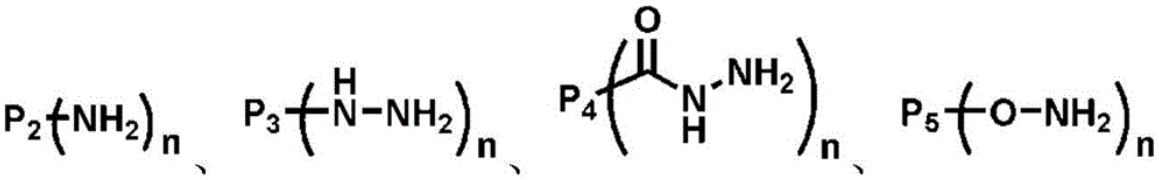
The polymerization molar ratio of the o-nitrobenzyl polymerizable monomer derivative to the water-soluble comonomer may be 1:20 to 1:2, preferably 1:9 to 1:3, and more preferably 1: 4.

The polymerization method may be random radical polymerization or controlled radical polymerization (for example, RAFT polymerization, ATRP polymerization, etc.). Random radical polymerization is preferred. The o-nitrobenzyl polymerizable monomer derivative and a comonomer are co-dissolved in a certain solvent, a free radical initiator is added to be fully dissolved, and after three times of freezing-vacuumizing circulation operation, the o-nitrobenzyl polymerizable monomer derivative and the comonomer react overnight under the heating condition. After the reaction is finished, pouring the reaction liquid into anhydrous ether for precipitation, and obtaining the o-nitrobenzyl-containing copolymer after multiple dissolving-reprecipitation purification processes and vacuum drying. (reference G.Delaittre, T.Pauloehrl, M.Bastmeyer, C.Barner-Kowoollik, Macromolecules 2012,45, 1792-.

A third object of the present invention is to provide a method for preparing the photo-coupled crosslinked hydrogel material. The photo-coupled cross-linked hydrogel material is prepared by taking the o-nitrobenzyl photo-trigger modified polymer derivative as a raw material.

The preparation method of the photo-coupled cross-linked hydrogel material comprises the following steps: dissolving the component A-o-nitrobenzyl type photo-trigger modified macromolecule derivative in a biocompatible medium to obtain a solution A; dissolving the component B-containing primary amine, diamine, hydrazide or hydroxylamine polymer derivative in a biocompatible medium to obtain a solution B; uniformly mixing the solution A and the solution B to obtain a hydrogel precursor solution; under the irradiation of a light source, aldehyde groups or ketone groups generated by the o-nitrobenzyl in the component A under the excitation of light and primary amine, diamine, hydrazide or hydroxylamine groups in the component B are crosslinked in a Schiff base mode to form hydrogel.

The component B-containing primary amine, diamine, hydrazide or hydroxylamine polymer derivatives have structural formulas B-I, B-II, B-III and B-IV respectively:



式 B-Ⅰ　　式 B-Ⅱ　　式 B-Ⅲ　　式 B-Ⅳ

wherein n is more than or equal to 2, P_2、P_3、P_4、P_5Is hydrophilic or water-soluble natural polymer or synthetic polymer, etc.

Wherein, the primary amine containing polymer derivative has a structure shown in a formula B-I, and represents a water-soluble or hydrophilic polymer containing n amino groups. The diamine-containing high polymer derivative has a structure shown in a formula B-II, and represents a water-soluble or hydrophilic high polymer containing n diamine groups. The hydrazide-containing polymer derivative has a structure shown in formulas B-III, and represents a water-soluble or hydrophilic polymer containing n hydrazide groups. The hydroxylamine-containing polymer derivative has a structure shown in a formula B-IV and represents a water-soluble or hydrophilic polymer containing n hydroxylamine groups.

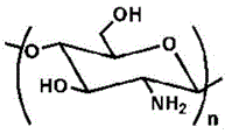
The water-soluble or hydrophilic polymer refers to a hydrophilic or water-soluble natural polymer and a modified substance thereof, or a hydrophilic or water-soluble synthetic polymer and a modified substance thereof.

The hydrophilic or water-soluble natural high polymer comprises natural polysaccharide substances and a modifier or a degradation product thereof, protein and a modifier or a degradation product thereof and the like, wherein the natural polysaccharide substances comprise hyaluronic acid, carboxymethyl cellulose, methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, alginic acid, dextran, agarose, heparin, chondroitin sulfate, ethylene glycol chitosan, propylene glycol chitosan, chitosan lactate, carboxymethyl chitosan or chitosan quaternary ammonium salt and the like, the protein comprises various hydrophilic or water-soluble animal and plant proteins, collagen, serum protein, silk fibroin and elastin, and the protein degradation product comprises gelatin or polypeptide and the like.

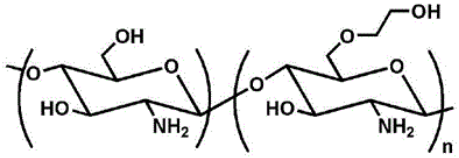
The hydrophilic or water-soluble synthetic polymer includes two-arm or multi-arm polyethylene glycol, polyethyleneimine, synthetic polypeptide, polylysine, polyglutamic acid, polyacrylic acid, polymethacrylic acid, polyacrylate, polymethacrylate, polyacrylamide, polymethacrylamide, polyvinyl alcohol, polyvinylpyrrolidone, etc.

The primary amine, diamine, hydrazide or hydroxylamine-containing polymer derivative may be a hydrophilic or water-soluble polymer containing one or more different groups at the same time, or a mixture of hydrophilic or water-soluble polymers containing one or more different groups.

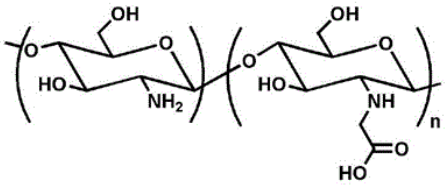
Preferably, the formula B-I can be selected from the following structures in component B-1 to component B-9; the formula B-II can be selected from the structures in the following component B-10; the formula B-III can be selected from the structures in the following components B-11 to B-13; the formula B-IV can be selected from the following structures in the component B-14 to the component B-15:



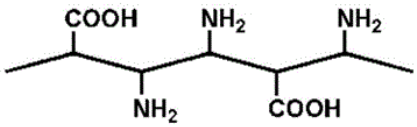
组分 B-1



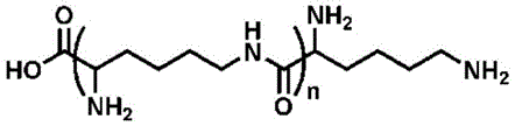
组分 B-2



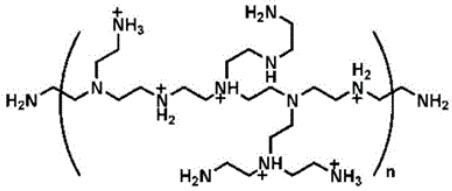
组分 B-3



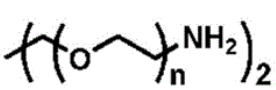
组分 B-4



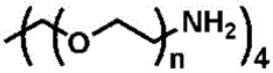
组分 B-5



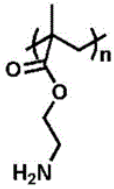
组分 B-6



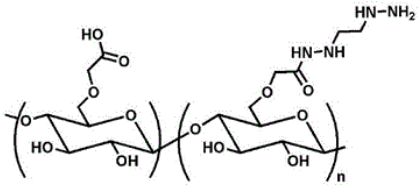
组分 B-7



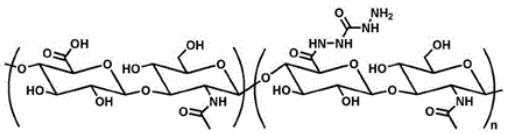
组分 B-8



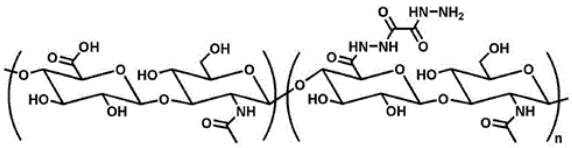
组分 B-9



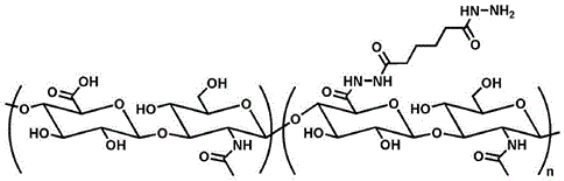
组分 B-10



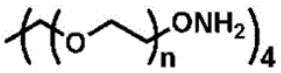
组分 B-11



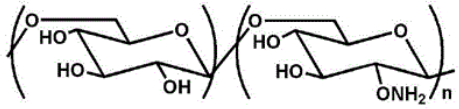
组分 B-12



组分 B-13



组分 B-14



组分 B-15

in the component B-1 to the component B-15, n is more than or equal to 2, and the component B-1 is chitosan; the component B-2 is glycol chitosan; the component B-3 is carboxymethyl chitosan; the component B-4 is gelatin; the component B-5 is polylysine; the component B-6 is polyethyleneimine; the component B-7 is two-arm amido polyethylene glycol; the component B-8 is four-arm amido polyethylene glycol; the component B-9 is an amino polymer; the component B-10 is diamine modified carboxymethyl cellulose; the component B-11 to the component B-13 are hydrazide modified hyaluronic acid; the component B-14 is four-arm hydroxylamine polyethylene glycol; component B-15 is hydroxylamine modified dextran.

Some practical preparation methods of the primary amine, diamine, hydrazide or hydroxylamine-containing polymer derivatives in the present invention are as follows:

the amino-modified water-soluble polymer can be artificially synthesized polyamine polymer and its modifier (such as polyethyleneimine PEI, dendrimer PAMAM, two-arm or multi-arm amino polyethylene glycol), or natural amino-containing polysaccharide hydrophilic or water-soluble polymer and its modifier or degradant (such as ethylene glycol chitosan, propylene glycol chitosan, chitosan lactate, carboxymethyl chitosan, chitosan oligosaccharide, etc.); or biological or protein extracted after microbial expression and its modified or degraded product (such as collagen, serum protein and gelatin); or hydrophilic or water-soluble polypeptide (such as polylysine) containing two or more amino groups, or acrylate, methacrylate, acrylamide or methacrylamide polymer and modified substances thereof, which are artificially synthesized or expressed and extracted by microorganisms. Preferably gelatin and glycol chitosan.

The preparation method of the diamine modified macromolecule derivative comprises the following steps: dissolving the water-soluble polymer containing carboxyl and diamine in distilled water, adding condensing agent 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and activating agent hydroxybenzotriazole (HOBt), and stirring at room temperature for 24-48 h. And after the reaction is finished, pouring the reaction solution into a dialysis bag, dialyzing for 2-3d by using a dilute hydrochloric acid solution, and then freeze-drying to obtain the diamine modified high-molecular derivative.

The water-soluble polymer containing carboxyl groups may be carboxyl polyethylene glycol or carboxyl group-containing polysaccharide (such as chitosan lactate, carboxymethyl chitosan, hyaluronic acid, alginic acid, carboxymethyl cellulose, etc.), preferably multi-arm carboxyl polyethylene glycol or hyaluronic acid, and more preferably hyaluronic acid.

In the above reaction, the molar ratio of carboxyl groups in the water-soluble polymer to small molecule diamine is preferably 1: 0.1-2; the molar ratio of the diamine small molecules to 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and the activating agent hydroxybenzotriazole (HOBt) is preferably 1:2: 1.5.

The preparation method of the hydrazide modified macromolecule derivative comprises the following steps: dissolving the water-soluble polymer containing carboxyl and dihydrazide in distilled water, adding condensing agent 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and activating agent hydroxybenzotriazole (HOBt), and stirring at room temperature for 24-48 h. After the reaction is finished, pouring the reaction solution into a dialysis bag, dialyzing for 2-3d by using a dilute hydrochloric acid solution, and then freeze-drying to obtain the hydrazide modified high-molecular derivative.

The water-soluble polymer containing carboxyl groups may be carboxyl polyethylene glycol or carboxyl group-containing polysaccharide (such as chitosan lactate, carboxymethyl chitosan, hyaluronic acid, alginic acid, carboxymethyl cellulose, etc.), preferably multi-arm carboxyl polyethylene glycol or hyaluronic acid, and more preferably hyaluronic acid.

In the above reaction, the small molecule dihydrazide may be any dihydrazide such as carbodihydrazide, oxalic dihydrazide, malonic dihydrazide, succinic dihydrazide, glutaric dihydrazide, adipic dihydrazide, pimelic dihydrazide, etc., preferably carbodihydrazide, oxalic dihydrazide, adipic dihydrazide, etc., more preferably carbodihydrazide. The mol ratio of the carboxyl in the water-soluble polymer to the small molecule dihydrazide is preferably 1: 0.1-2; the molar ratio of the dihydrazide small molecules to 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) and the activating agent hydroxybenzotriazole (HOBt) is preferably 1:2: 1.5.

The preparation method of the hydroxylamine modified macromolecule derivative comprises the following steps: dissolving a polymer containing hydroxyl and N-hydroxyphthalimide in a dichloromethane solution, adding triphenylphosphine, slowly dropwise adding diisopropyl azodicarboxylate, reacting for 16-24h, precipitating the polymer in diethyl ether, dissolving the polymer in the dichloromethane solution again, adding hydrazine hydrate, and reacting for 1-3h to obtain the hydroxylamine modified macromolecular derivative.

The above-mentioned hydroxyl group-containing polymer may be polyethylene glycol, and polysaccharides (such as dextran and chitosan), preferably multi-arm hydroxyl polyethylene glycol.

In the above reaction, the molar ratio of the hydroxyl group in the polymer to N-hydroxyphthalimide, triphenylphosphine, diisopropylazodicarboxylate, and hydrazine hydrate is preferably 1:10:10:10: 10.

In the method for preparing the hydrogel, the biocompatible medium is selected from distilled water, physiological saline, buffer solution or cell culture medium solution, and different media can be selected according to different applications.

In the method for preparing the hydrogel, which is object of the present invention, in the hydrogel precursor solution formed by uniformly mixing the solution a and the solution B, the molar ratio of the o-nitrobenzyl group to the primary amine, diamine, hydrazide or hydroxylamine group in the component B may be 1:0.02 to 50, preferably 1:0.1 to 10, and the total concentration of the polymer may be 0.1 wt% to 60 wt%, preferably 1 wt% to 10 wt%.

In the preparation method of the hydrogel, the wavelength of the light source is determined according to the absorption wavelength of the o-nitrobenzyl-based optical trigger, and may be 250-500nm, preferably 300-450nm, and more preferably 395 or 405 nm.

The preparation method adopts the technical principle that: the o-nitrobenzyl type photo-trigger generates aldehyde group or ketone group under the light excitation, and is crosslinked with a macromolecule derivative containing primary amine, diamine, hydrazide and hydroxylamine group in a Schiff base mode to form hydrogel, and the crosslinking mode is that active group is generated under the light excitation to carry out coupling reaction with other corresponding groups, and the crosslinking mode can be called as light coupling crosslinking. The method for preparing the hydrogel through the photo-coupling crosslinking overcomes the bottlenecks of biotoxicity, thin-layer gelling, tissue adhesion and integration and the like of free radical crosslinking, solves the problems of short photocuring wavelength, low photo-crosslinking rate, complex synthesis and the like in the existing non-free radical photo-coupling crosslinking, and is expected to substantially promote the clinical application of the photo-in-situ gel technology.

A fourth object of the present invention is to provide a product obtained by the method for preparing a photo-coupled crosslinked hydrogel material.

The present invention provides a hydrogel prepared by the method described in the third objective, which may be referred to as a photo-coupled crosslinked hydrogel.

It is a fifth object of the present invention to provide a kit for the preparation of a photo-coupled crosslinked hydrogel material, comprising: the component A is a macromolecular derivative modified by an o-nitrobenzyl optical trigger shown as a formula I; the component B-primary amine, diamine, hydrazide and hydroxylamine high molecular derivatives shown in the formulas B-I, B-II, B-III and B-IV; and instructions for hydrogel preparation and use.

Further, the kit of the present invention may further comprise a biocompatible medium such as distilled water, physiological saline, a buffer solution and a cell culture medium.

Furthermore, the specification in the kit of the present invention describes the application of hydrogel, including the application thereof in postoperative wound closure, tissue fluid leakage closure, hemostatic materials, tissue engineering scaffold materials, 3D printed bio-ink and as a cell, protein or drug carrier.

A sixth object of the present invention is to provide the use of the product obtained by the method for preparing the photo-coupled crosslinked hydrogel material.

The invention provides application of the photo-coupling cross-linked hydrogel in preparing postoperative wound surface sealing products.

The invention also provides application of the photo-coupled cross-linked hydrogel in preparation of tissue fluid leakage plugging products.

The invention also provides application of the photo-coupled cross-linked hydrogel in preparation of a hemostatic material.

The invention also provides application of the photo-coupled cross-linked hydrogel in preparation of a tissue engineering scaffold material-cartilage repair material.

The invention also provides application of the photo-coupled cross-linked hydrogel in preparing a tissue engineering scaffold material-bone repair material.

The invention also provides application of the photo-coupled cross-linked hydrogel as a 3D printing material-biological ink.

The invention also provides application of the photo-coupled cross-linked hydrogel in preparation of cells, proteins and drug carriers.

Compared with the prior art, the invention has the following innovation points:

(1) the introduction of substituent groups or conjugated systems on benzene rings enables the absorption wavelength of the light trigger to be red-shifted, the molar absorption coefficient is increased, a 365nm purple light band light source used in photocuring is changed into a 405nm blue light band light source, the light intensity required by photocuring is weakened, and the heat sensation and cytotoxicity generated by a strong ultraviolet light source are greatly reduced;

(2) the connection position of the o-nitrobenzyl optical trigger and the polymer is more flexible, the connection position of the polymer can be selected according to different substituents introduced on a benzene ring, compared with the prior molecular structure, the synthesis is simpler and more flexible, and the application cost of the hydrogel can be greatly reduced;

(3) the gel has adjustable chemical structure, composition and degradability, strength and thickness, can flexibly adjust the composition and property of a gel material according to different applications, can particularly form thin gel in situ on a wound surface, is particularly suitable for sealing and repairing the wound surface after operation, is also suitable for tissue fluid leakage plugging, can be used as a hemostatic material, a tissue engineering scaffold material, a biological ink for 3D printing, and can provide an in-situ carrier for cells, proteins or medicaments, and is effectively applied to regenerative medicine.

Drawings

Note: NB_0For the o-nitrobenzyl-based photoinitiators for the construction of hydrogels reported in the literature (Yunlong Yang; Jieyuan Zhang; Zhenzhen Liu; Qianing Lin; Xiaolin Liu; Chunyan Bao; Yang Wang; Linying Zhu. adv. Mater.2016,28,2724.). NB is an o-nitrobenzyl type photosetting initiator in the component A-1 of the present invention. Wherein, HA-NB_0Is NB_0The marked hyaluronic acid macromolecule derivative HA-NB is the component A-1.

FIG. 1 shows ortho-nitrobenzyl-based photosriggers $(NB)_0$And NB) in comparison to the uv absorption wavelength and molar absorptivity.

FIG. 2 is a hydrogel precursor solution (2% HA-$NB)_0$2% CMCh and 2% HA-NB/2% CMCh) light-illuminated gel real-time rheology profile.

FIG. 3 is a hydrogel (2% HA-$NB)_0$2% CMCh and 2% HA-NB/2% CMCh).

FIG. 4 is a graph showing the biocompatibility test of the hydrogel (component A-1/component B-3).

FIG. 5 is a visual diagram of the wound surface sealing effect of the hydrogel (component A-1/component B-3).

FIG. 6 is a visual representation of the effect of hydrogel (component A-1/component B-3) as a hemostatic material.

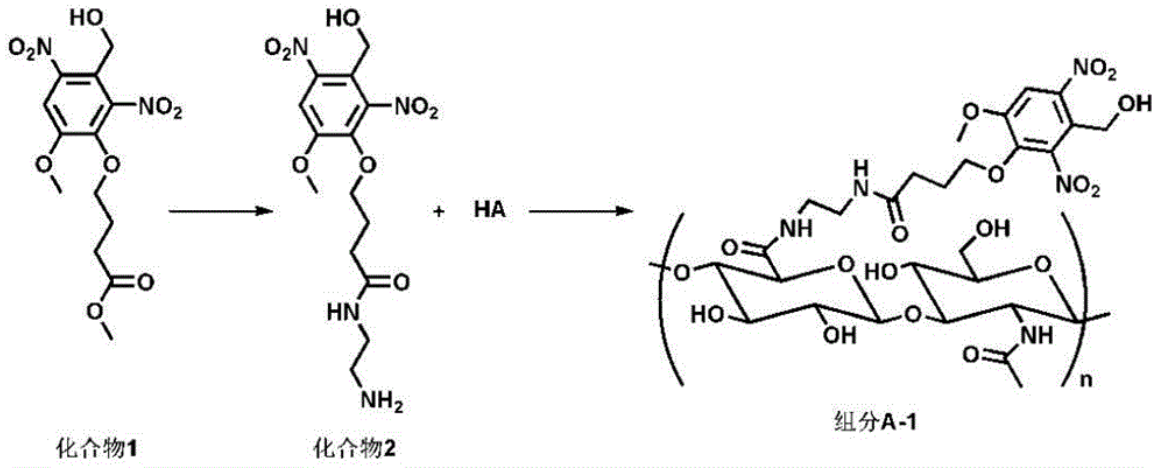
FIG. 7 is a visual chart of the effect of hydrogel (component A-1/component B-3) as cartilage tissue engineering scaffold material.

FIG. 8 is a visual representation of the effect of hydrogel (component A-1/component B-3) as a bio-ink.

Detailed Description

The present invention is described in more detail below with reference to examples. The present invention will be further described with reference to the drawings and examples, which are only for describing the best mode of carrying out the invention and are not intended to limit the scope of the invention. Any other changes and modifications that may occur to those skilled in the art without departing from the spirit and scope of the present invention are also encompassed by the present invention.

The first embodiment is as follows: synthesis of component A-1

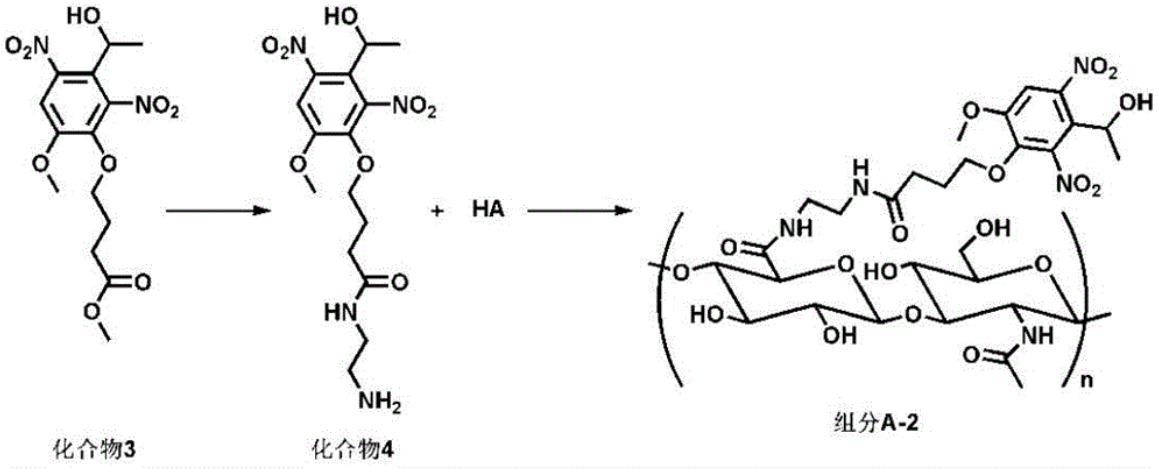


(1) Synthesis of Compound 1: as per reference Sarit s.agasti.; apiwat chompoosor; synthesized by the method disclosed in Vincent m.rotello.j.am.chem.soc.2009,131,5728.

(2) Synthesis of Compound 2: after dissolving compound 1(1g, 2.9mmol) and ethylenediamine (1.1mL) in methanol (50mL) and refluxing overnight, the reaction was carried out under reduced pressure and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 2(0.92g, 85% yield) was obtained.^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]373.1373.

(3) Synthesis of component A-1: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer (pH 5.2), stirred until completely dissolved, compound 2(75mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer and added to the reaction mixture three times (every 1h) and reacted at 35 ℃ for 24 h. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-1(1.82g), wherein the labeling rate of compound 2 can be calculated to be about 3.06% according to nuclear magnetic hydrogen spectrum.

Example two: synthesis of component A-2

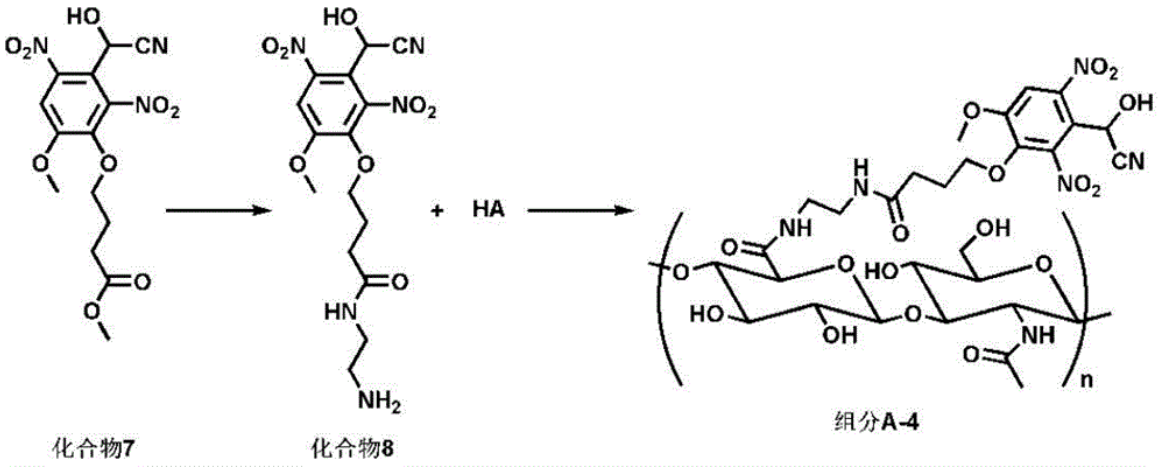


(1) Synthesis of Compound 3: as per reference James f.cameron; jean M.J.Frechet.J.Am.chem.Soc.1991,113,4303.

(2) Synthesis of Compound 4: compound 3(1g, 2.8mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 4(0.86g, 80% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(m,1H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H),1.33(d,J = 6.9Hz,3H).MS(ESI):[M+H]387.1553.

(3) Synthesis of component A-2: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 4(77mg, 0.2mmol) was weighed and dissolved in 10mL of dimethylsulfoxide DMSO before being added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was weighed and dissolved in 3mL of MES buffer solution, and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-2(1.65g), wherein the labeling rate of compound 4 is calculated to be about 3.26% according to nuclear magnetic hydrogen spectrum.

Example four: synthesis of component A-4

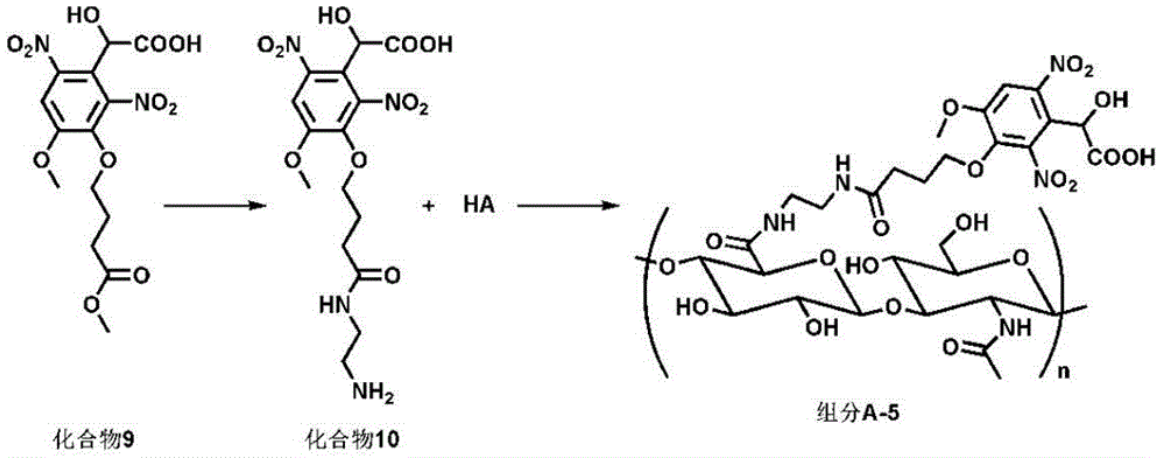


(1) Synthesis of compound 7: according to the reference Isabelle Aujard; chouaha benbrahim.; ludovic julilen. chem. eur.j.2006,12,6865. the disclosed methods were used for the synthesis.

(2) Synthesis of compound 8: after dissolving compound 7(1g, 2.7mmol) and ethylenediamine (1.1mL) in methanol (50mL) and refluxing overnight, the reaction was rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 8(0.95g, 88% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,1H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]398.1326.

(3) Synthesis of component A-4: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 8(80mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer solution and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-4(1.92g), wherein the labeling rate of compound 8 is calculated to be about 3.14% according to nuclear magnetic hydrogen spectrum.

Example five: synthesis of component A-5

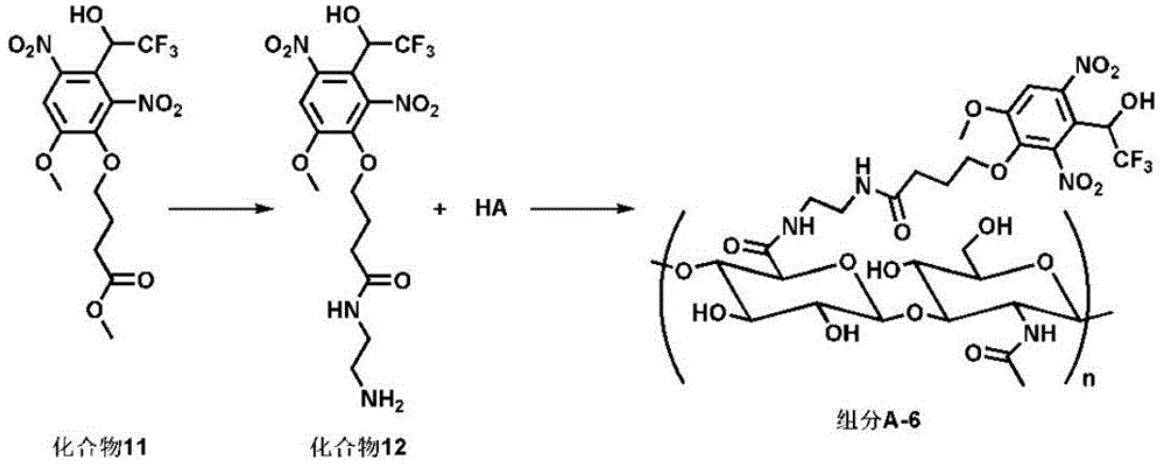


(1) Synthesis of compound 9: as per reference Alexander g.russell; dario m.bassani.; john s.snaith.j.org.chem.2010,75,4648.

(2) Synthesis of compound 10: after compound 9(1g, 2.6mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL) and reacted overnight under reflux, the crude product was dissolved in methanol and reprecipitated in ethyl acetate by rotary evaporation under reduced pressure. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 10(0.79g, 74% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,1H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]417.1244.

(3) Synthesis of component A-5: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 10(83mg, 0.2mmol) was weighed and dissolved in 10mL of dimethylsulfoxide DMSO before being added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was weighed and dissolved in 3mL of MES buffer solution, and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-5(1.73g), wherein the labeling rate of compound 10 can be calculated to be about 3.03% according to nuclear magnetic hydrogen spectrum.

Example six: synthesis of component A-6

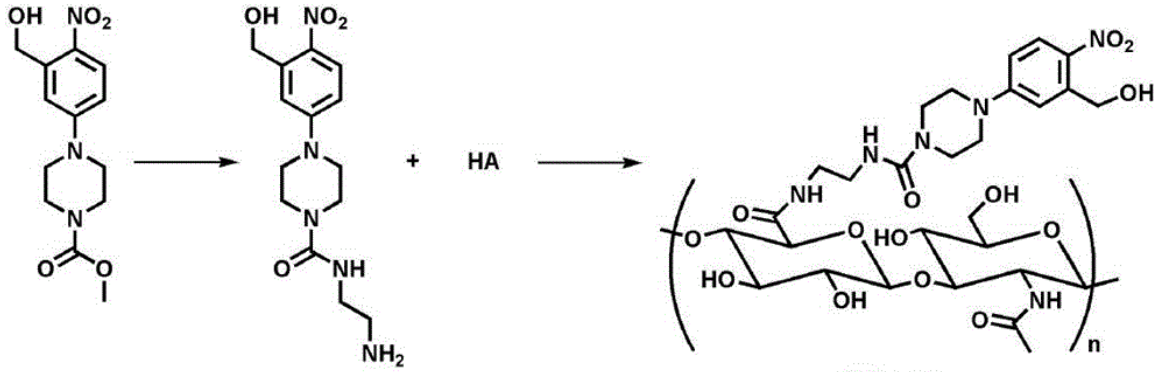


(1) Synthesis of compound 11: as in the reference Alexandre spec; the synthesis was carried out according to the method disclosed in Maurice Goeldner.Angew.chem.Int.Ed.2004,43,2008.

(2) Synthesis of compound 12: compound 11(1g, 2.4mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), refluxed overnight for reaction, then rotary evaporated under reduced pressure, the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 12(0.66g, 62% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,1H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]441.1274.

(3) Synthesis of component A-6: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer (pH 5.2), stirred until completely dissolved, compound 12(88mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer and added to the reaction mixture three times (every 1h) and reacted at 35 ℃ for 24 h. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-6(1.63g), wherein the labeling rate of compound 12 is calculated to be about 2.15% according to nuclear magnetic hydrogen spectrum.

Example twenty one: synthesis of component A-21

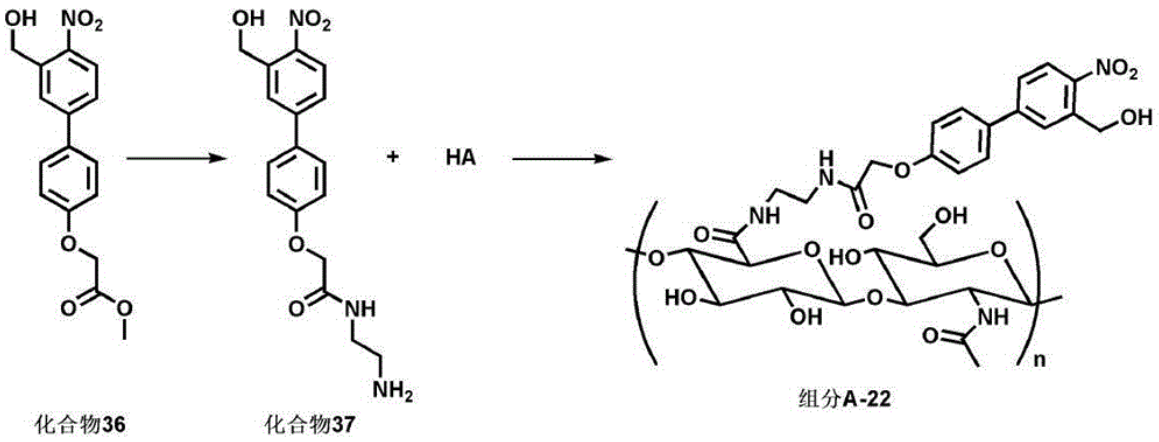


(1) Synthesis of compound 34: as per reference Emmanuel riguet; the synthesis was carried out according to the method disclosed in Christian g.bochet.org.lett.2007,26,5453.

(2) Synthesis of compound 35: compound 34(1g, 3.4mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 35(0.83g, 76% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 8.05(d,J = 9.54Hz,1H),7.24(d,J = 2.72Hz,1H),6.92(dd,J = 9.54,2.72Hz,1H),4.85(s,2H),3.56-3.68(m,4H),3.49-3.56(m,2H),3.42-3.49(m,2H),3.32(t,J = 5.9Hz,2H),2.82(t,J = 5.9Hz,2H).MS(ESI):[M+H]324.1632.

(3) Synthesis of component A-21: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 35(65mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer solution and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-21(1.87g), wherein the labeling rate of compound 35 is about 3.42% according to nuclear magnetic hydrogen spectrum.

Example twenty two: synthesis of component A-22

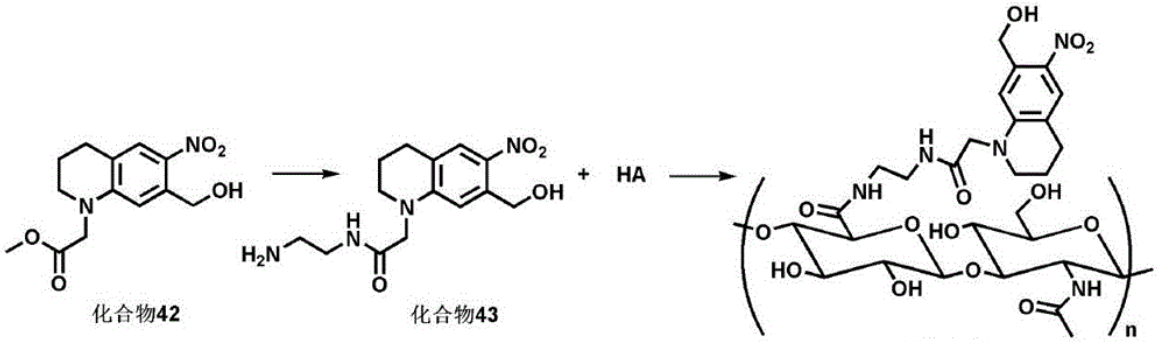


(1) Synthesis of compound 36: according to the reference Isabelle Aujard; chouaha benbrahim.; ludovic julilen. chem. eur.j.2006,12,6865. the disclosed methods were used for the synthesis.

(2) Synthesis of compound 37: compound 36(1g, 3.2mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 37(0.78g, 72% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 8.05(d,J = 9.54Hz,1H),7.28(d,J = 8.00Hz,2H),7.24(d,J = 2.72Hz,1H),6.92(dd,J = 9.54,2.72Hz,1H),6.78(d,8.00Hz,2H),4.96(s,2H),4.83(s,2H),3.32(t,J = 5.9Hz,2H),2.82(t,J = 5.9Hz,2H).MS(ESI):[M+H]346.1454.

(3) Synthesis of component A-22: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 37(69mg, 0.2mmol) was weighed and dissolved in 10mL of dimethylsulfoxide DMSO, and then added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was weighed and dissolved in 3mL of MES buffer solution, and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-22(1.73g), wherein the labeling rate of compound 37 is calculated to be about 3.16% according to nuclear magnetic hydrogen spectrum.

Example twenty-five: synthesis of component A-25

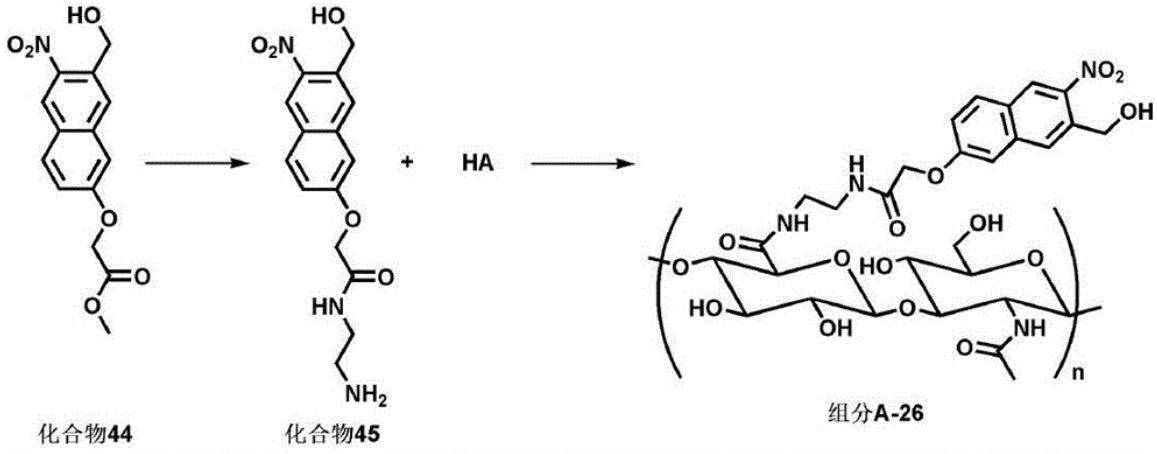


(1) Synthesis of compound 42: as per reference Emmanuel riguet; the synthesis was carried out according to the method disclosed in Christian g.bochet.org.lett.2007,26,5453.

(2) Synthesis of compound 43: compound 42(1g, 3.6mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 43(0.93g, 85% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 7.71(s,1H),7.22(s,1H),4.96(s,2H),4.24(s,2H),3.32(t,J = 5.9Hz,2H),3.27-3.21(m,2H),2.82(t,J = 5.9Hz,2H),2.75(t,J = 6.3Hz,2H),2.00-1.91(m,2H).MS(ESI):[M+H]309.1522.

(3) Synthesis of component A-25: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 43(62mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer solution and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-25(1.84g), wherein the labeling rate of compound 43 is calculated to be about 3.23% according to nuclear magnetic hydrogen spectrum.

Example twenty-six: synthesis of component A-26

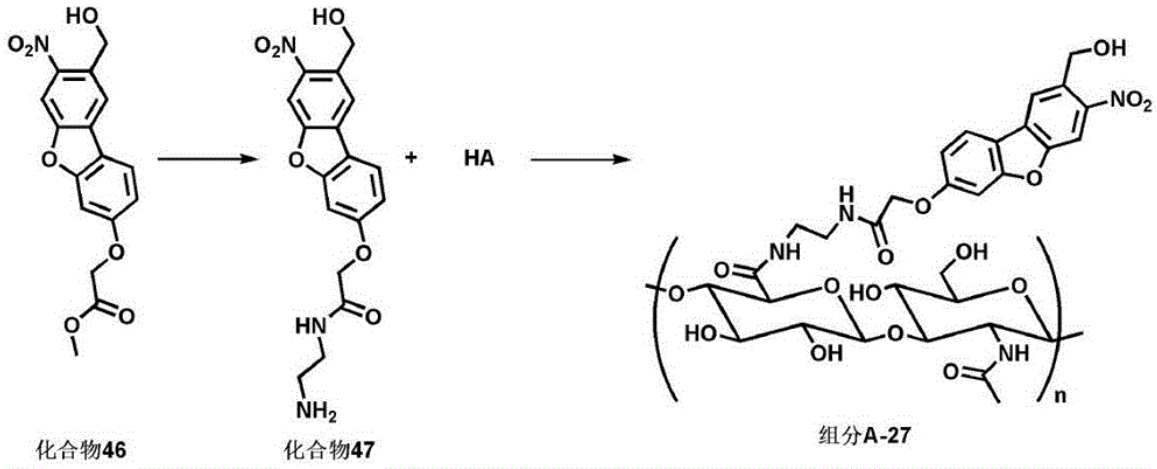


(1) Synthesis of compound 44: according to Singh, a.k., reference; khade, p.k.tetrahedron.2005,61,10007.

(2) Synthesis of compound 45: compound 44(1g, 3.4mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 45(0.68g, 62% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 8.31-7.12(m,5H),4.96(s,2H),4.83(s,2H),3.32(t,J = 5.9Hz,2H),2.82(t,J = 5.9Hz,2H).MS(ESI):[M+H]320.1254.

(3) Synthesis of Components A-26: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer (pH 5.2), stirred until completely dissolved, compound 45(64mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer and added to the reaction mixture three times (every 1h) and reacted at 35 ℃ for 24 h. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-26(1.73g), wherein the labeling rate of compound 45 can be calculated to be about 3.12% according to nuclear magnetic hydrogen spectrum.

Example twenty-seven: synthesis of component A-27



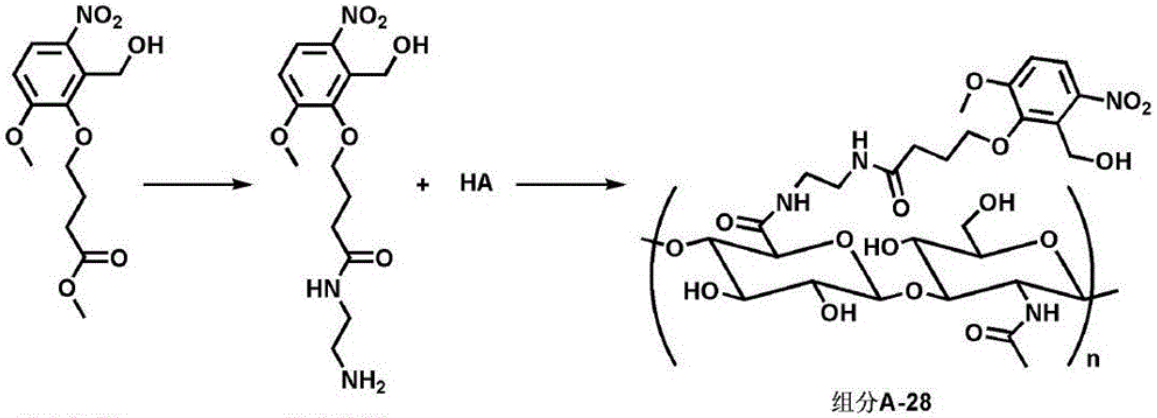
化合物**46** 化合物**47** 组分**A-27**

(1) Synthesis of compound 46: as per reference Felix friedrich; mike heilemann; synthesis was performed according to the method disclosed in Alexander heckel. chem. commu.2015, 51,15382.

(2) Synthesis of compound 47: mixing Compound 46(1 g)3.0mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), refluxed overnight for reaction, then rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 47(0.68g, 63% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 8.31-7.12(m,5H),4.96(s,2H),4.83(s,2H),3.32(t,J = 5.9Hz,2H),2.82(t,J = 5.9Hz,2H).MS(ESI):[M+H]360.1254.

(3) Synthesis of component A-27: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer (pH 5.2), stirred until completely dissolved, compound 47(72mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer and added to the reaction mixture three times (every 1h) and reacted at 35 ℃ for 24 h. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-27(1.71g), wherein the labeling rate of compound 47 is calculated to be about 2.93% according to nuclear magnetic hydrogen spectrum.

Example twenty-eight: synthesis of component A-28



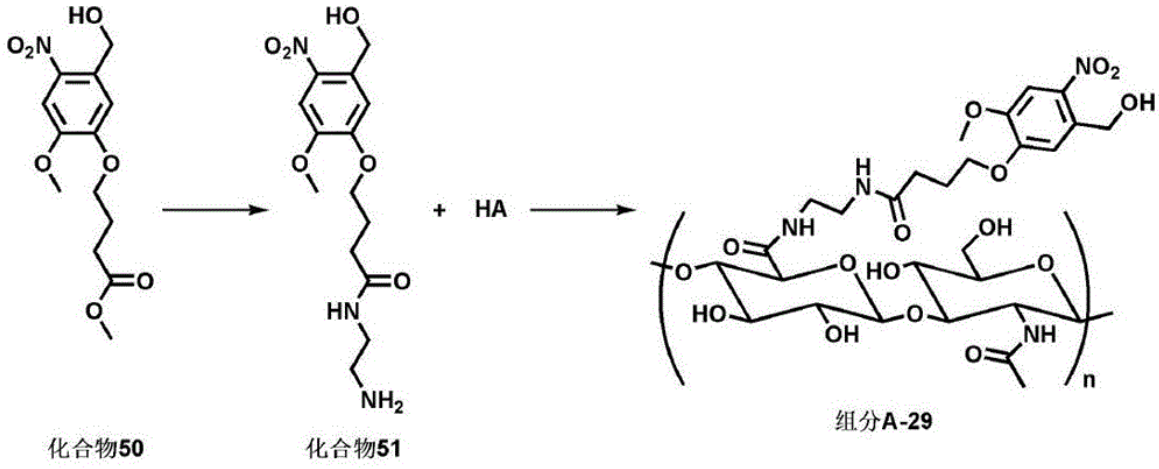
化合物**48** 化合物**49** 组分**A-28**

(1) Synthesis of compound 48: according to reference Grazyna Groszek; agnieszka Nowak-krol.; the synthesis was carried out according to the method disclosed in barbarara filipek. eur.j.med.chem.2009,44,5103.

(2) Synthesis of compound 49: compound 48(1g, 3.3mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 49(0.94g, 86% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 8.04(s,1H),7.42(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]328.1507.

(3) Synthesis of component A-28: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer (pH 5.2), stirred until completely dissolved, compound 49(65mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer and added to the reaction mixture three times (every 1h) and reacted at 35 ℃ for 24 h. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-28(1.92g), wherein the labeling rate of compound 49 can be calculated to be about 3.54% according to nuclear magnetic hydrogen spectrum.

Example twenty-nine: synthesis of component A-29



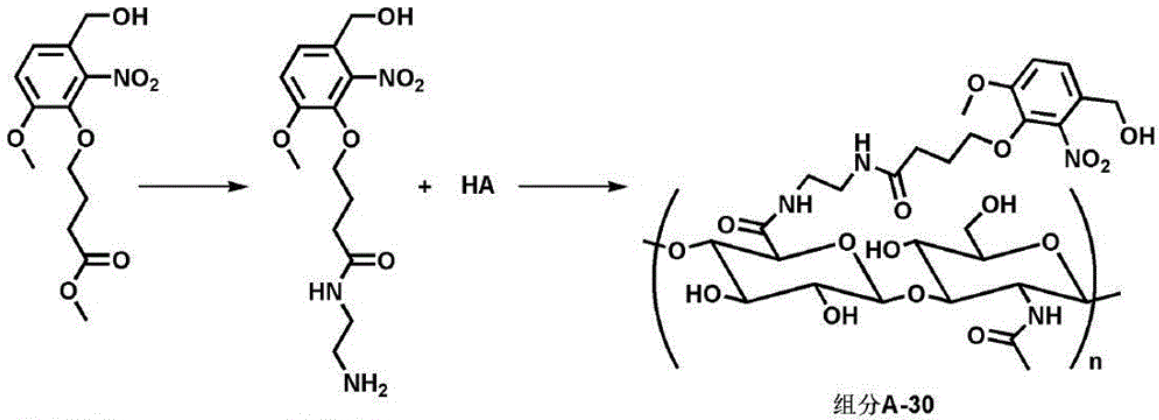
化合物**50** 化合物**51** 组分**A-29**

(1) Synthesis of compound 50: as per reference Thomas f.greene; shu wang.; synthesis was performed by the method disclosed in Mary j. meagan.j. med.chem.2016,59,90.

(2) Synthesis of compound 51: compound 50(1g, 3.3mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, then rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 51(0.97g, 89% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 7.95(s,1H),7.12(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]328.1507.

(3) Synthesis of component A-29: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 51(65mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer solution and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-29(1.86g), wherein the labeling rate of compound 51 is about 3.38% according to nuclear magnetic hydrogen spectrum.

Example thirty: synthesis of component A-30



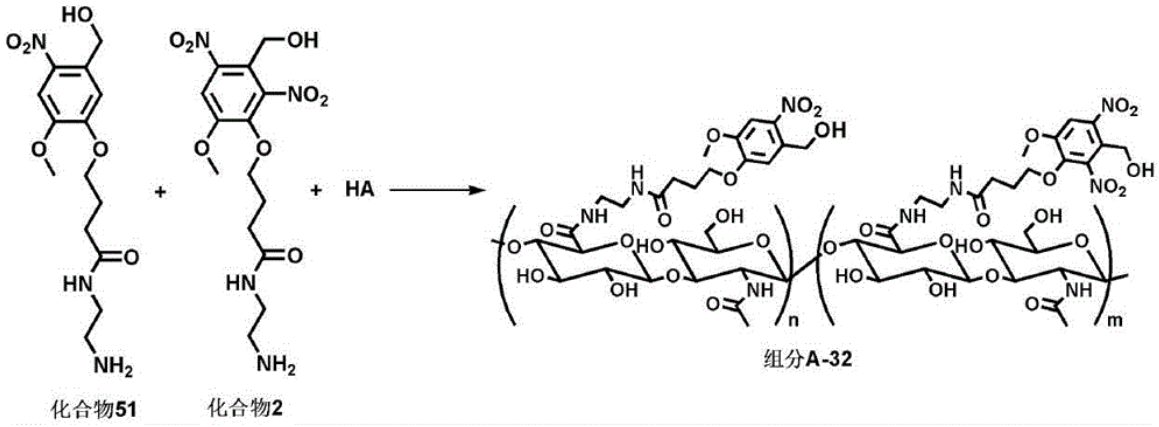
化合物**52** 化合物**53** 组分**A-30**

(1) Synthesis of compound 52: as per reference Yu-shan.; mohane Selvaraj coumar; Hsing-Pang Hsieh.J.Med.chem.2009,52,4941.

(2) Synthesis of compound 53: compound 52(1g, 3.3mmol) and ethylenediamine (1.1mL) were dissolved in methanol (50mL), reacted overnight under reflux, rotary evaporated under reduced pressure, and the crude product was dissolved in methanol and reprecipitated in ethyl acetate. After multiple dissolution-reprecipitation, filtration and vacuum drying, compound 53(0.91g, 83% yield) was obtained. ^{1}H NMR(400MHz,$CDCl_3$):δ = 7.64(s,1H),7.02(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.32(dd,J = 11.6,5.7Hz,2H),2.82(t,J = 5.9Hz,2H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]328.1507.

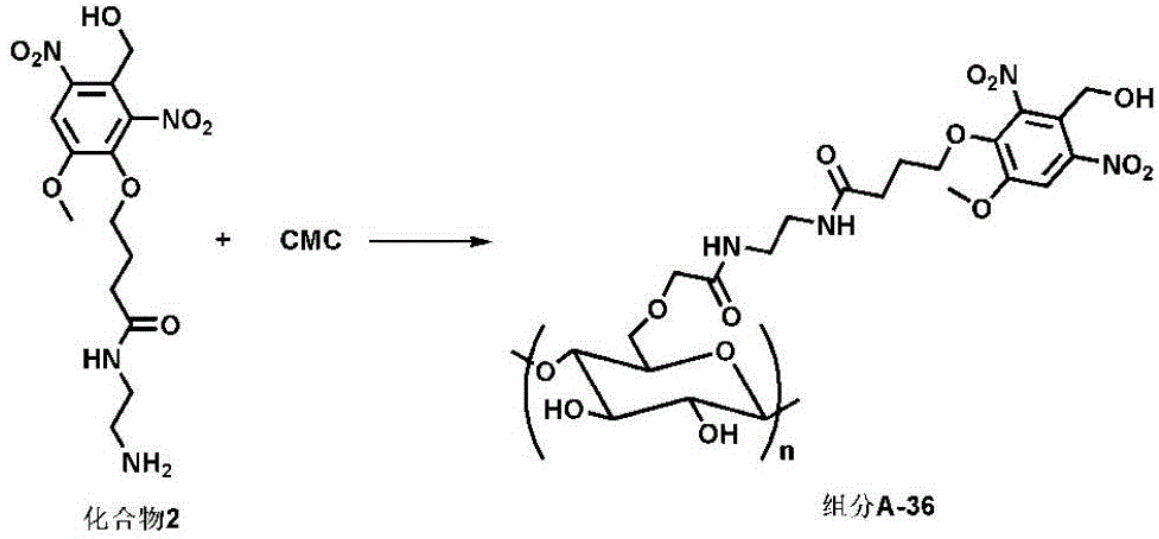
(3) Synthesis of component A-30: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 53(65mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction solution, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer solution and added to the reaction solution three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive hyaluronic acid derivative A-30(1.85g), wherein the labeling rate of compound 53 can be calculated to be about 3.41% according to nuclear magnetic hydrogen spectrum.

Example thirty-two: synthesis of component A-32



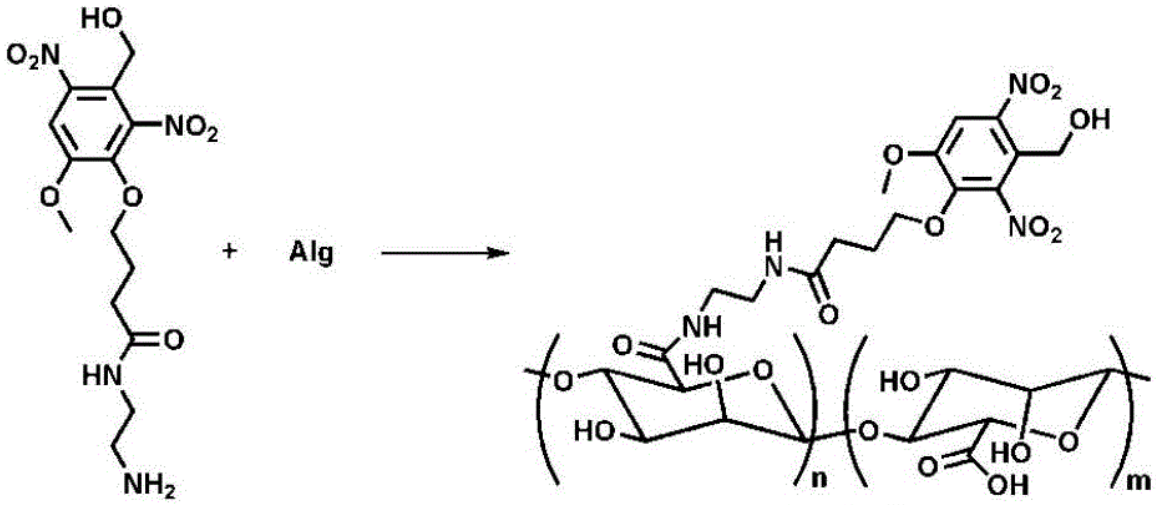
Synthesis of component A-32: hyaluronic acid (2g, 340kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer (pH 5.2), stirred until completely dissolved, NB mixture (compound 2/compound 51, 60mg, 1:1) was weighed and dissolved in 10mL of dimethylsulfoxide DMSO before adding to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was weighed and dissolved in 3mL of MES buffer, and added to the reaction mixture three times (every 1h) for 24h at 35 ℃. Then the reaction solution was poured into a dialysis bag (MWCO 7000), dialyzed with deionized water for 2-3d, and freeze-dried to obtain the photosensitive hyaluronic acid derivative A-32(1.85g), and the labeling rate of the NB mixture (Compound 2/Compound 51) was calculated to be about 3.45% from the nuclear magnetic hydrogen spectrum.

Example thirty-six: synthesis of component A-36



Synthesis of component A-36: carboxymethyl cellulose (2g, 90kDa) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), and stirred until completely dissolved, Compound 2(75mg, 0.2mmol) was dissolved in 10mL of DMSO and added to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer solution and added to the reaction mixture three times (every 1h) for 24h at 35 ℃. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive carboxymethyl cellulose derivative A-36(1.92g), wherein the labeling rate of compound 2 is calculated to be about 2.82% according to nuclear magnetic hydrogen spectrum.

Example thirty-seven: synthesis of component A-37

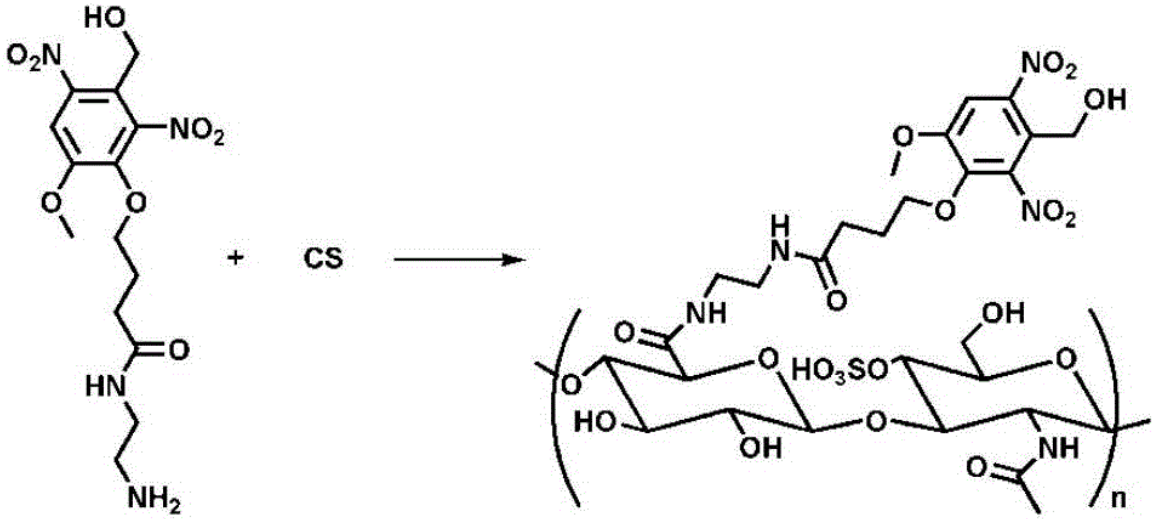


化合物2

组分A-37

Synthesis of component A-37: alginic acid (2g) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 2(75mg, 0.2mmol) was weighed and dissolved in 10mL of dimethyl sulfoxide DMSO, then added to the reaction mixture, 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was weighed and dissolved in 3mL of MES buffer solution, added to the reaction mixture three times (every 1h), and reacted at 35 ℃ for 24 h. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive alginic acid derivative A-37(1.89g), wherein the labeling rate of compound 2 is about 3.13% according to nuclear magnetic hydrogen spectrum.

Example thirty-eight: synthesis of component A-38

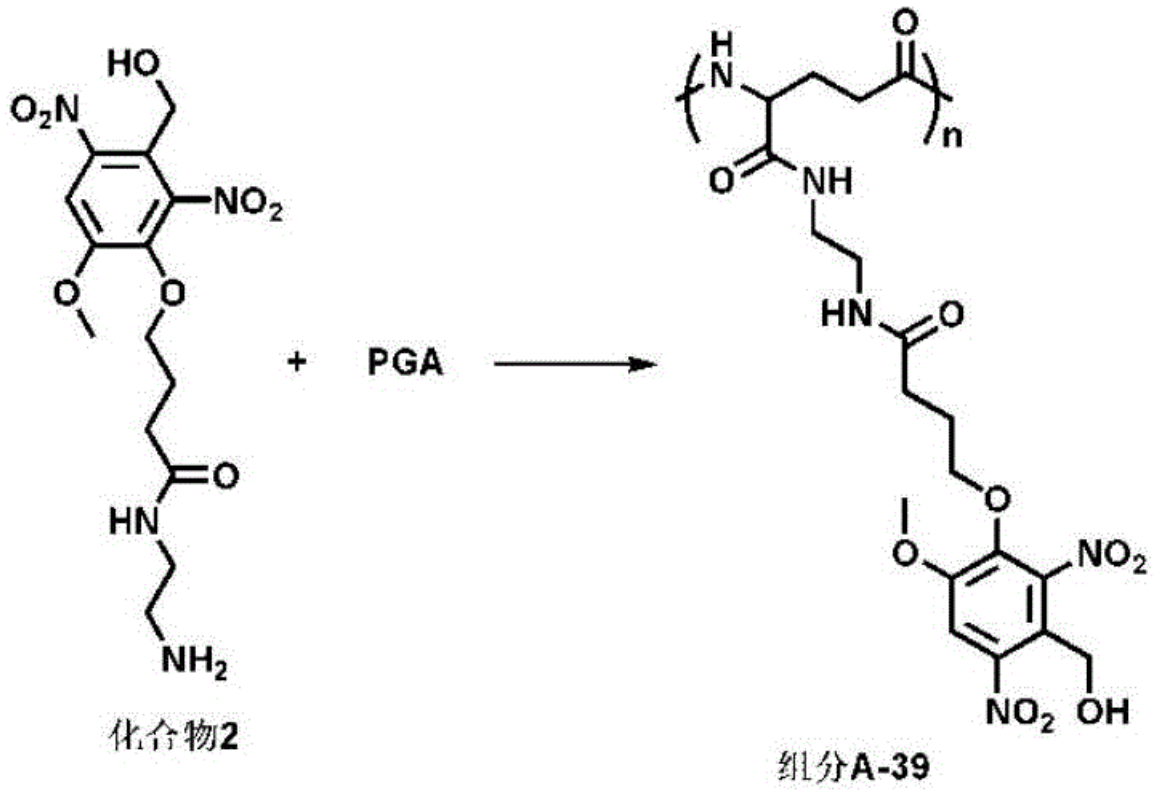


化合物2

组分A-38

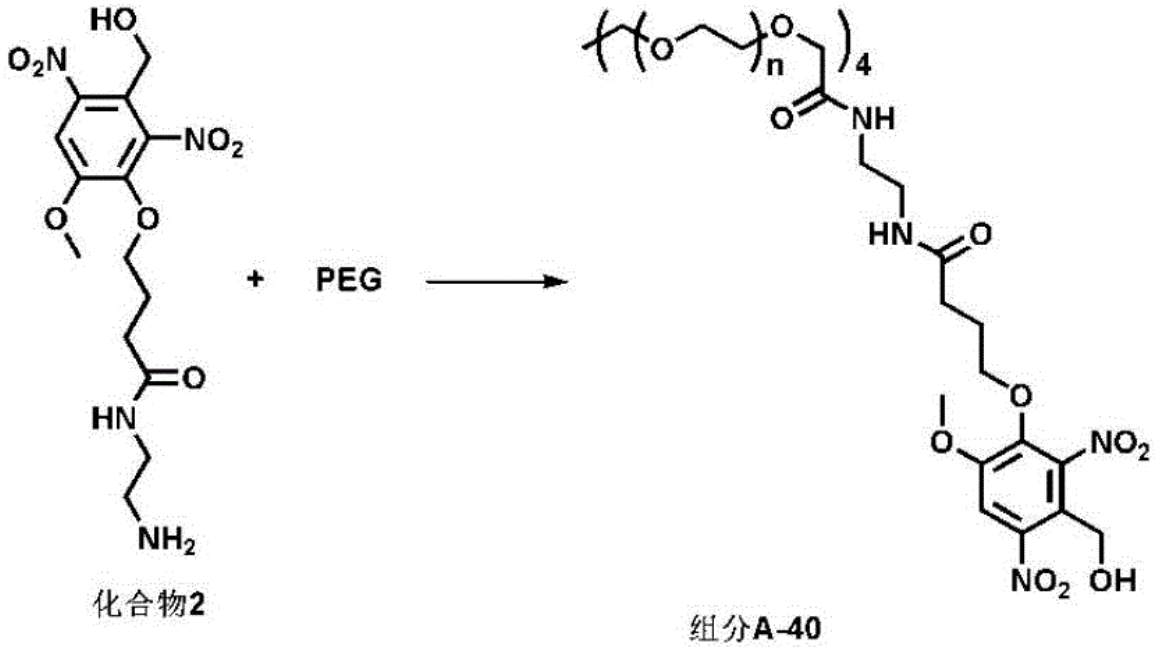
Synthesis of component A-38: chondroitin sulfate (2g) was dissolved in 100mL of 0.01mol/L2- (N-morpholine) ethanesulfonic acid MES buffer solution (pH 5.2), stirred until completely dissolved, compound 2(75mg, 0.2mmol) was dissolved in 10mL of dimethylsulfoxide DMSO and added to the reaction mixture, and 4- (4, 6-dimethoxytriazin-2-yl) -4-methylmorpholine hydrochloride DMTMM (0.4g, 1.5mmol) was dissolved in 3mL of MES buffer solution and added to the reaction mixture three times (every 1 hour) and reacted at 35 ℃ for 24 hours. Then pouring the reaction solution into a dialysis bag (MWCO 7000), dialyzing with deionized water for 2-3d, and freeze-drying to obtain photosensitive chondroitin sulfate derivative A-38(1.74g), wherein the labeling rate of compound 2 can be calculated to be about 2.73% according to nuclear magnetic hydrogen spectrum.

Example thirty-nine: synthesis of component A-39



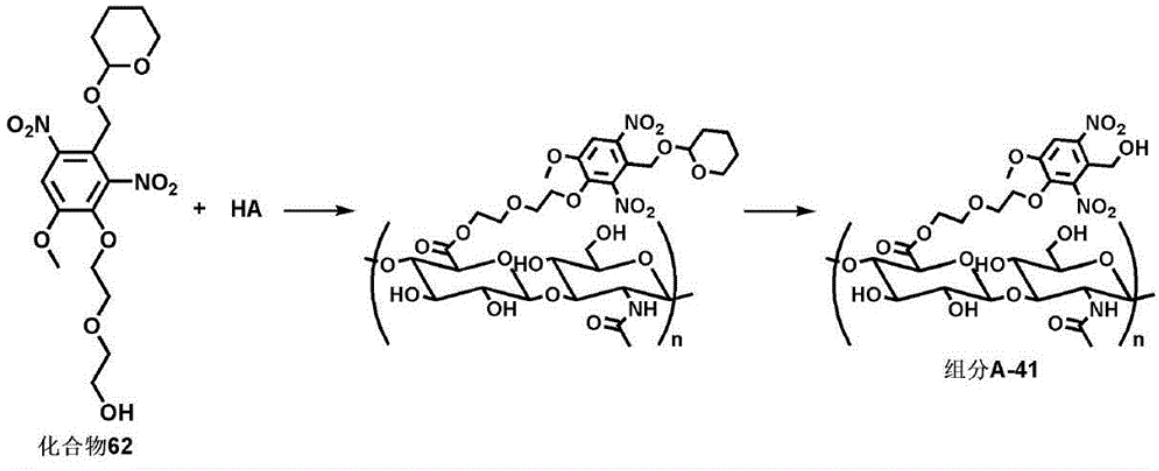
Synthesis of component A-39: polyglutamic acid PGA (1g) was dissolved in 50mL of distilled water until completely dissolved, hydroxybenzotriazole (HOBt, 0.3g, 2.3mmol) was added, then compound 2(0.6g,1.6mmol) and 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, 0.5g, 2.6mmol) dissolved in methanol were added to the above solution for reaction at room temperature for 48 hours, and after dialysis for 1 day with a dilute hydrochloric acid solution containing sodium chloride (pH 3.5), followed by dialysis for 1 day with pure water, followed by lyophilization, photosensitive polyglutamic acid derivative a-39(0.92g) was obtained, and from its nuclear magnetic hydrogen spectrum, the modification degree of compound 2 was calculated to be about 18%.

Example forty: synthesis of component A-40



Synthesis of component A-40: dissolving a four-arm polyethylene glycol carboxylic acid derivative 4-PEG-COOH (0.5g, 10kDa) in 20mL of anhydrous dimethyl sulfoxide DMSO until the four-arm polyethylene glycol carboxylic acid derivative is completely dissolved, weighing a compound 2(130mg, 0.4mmol) to be dissolved in 5mL of anhydrous dimethyl sulfoxide DMSO, adding the reaction solution, adding 0.2mL of triethylamine TEA, adding benzotriazole-1-yl-oxypyrrolidinyl phosphorus PyBop (210mg, 0.4mmol) hexafluorophosphate, reacting at room temperature for 24h, then re-precipitating in diethyl ether, dissolving the crude product in water, pouring into a dialysis bag (MWCO 3500), dialyzing with deionized water for 2-3d, and freeze-drying to obtain a photosensitive polyethylene glycol derivative A-40(0.45g), wherein the labeling rate of the compound 2 can be calculated to be about 98% according to a nuclear magnetic hydrogen spectrogram.

Example forty one: synthesis of component A-41



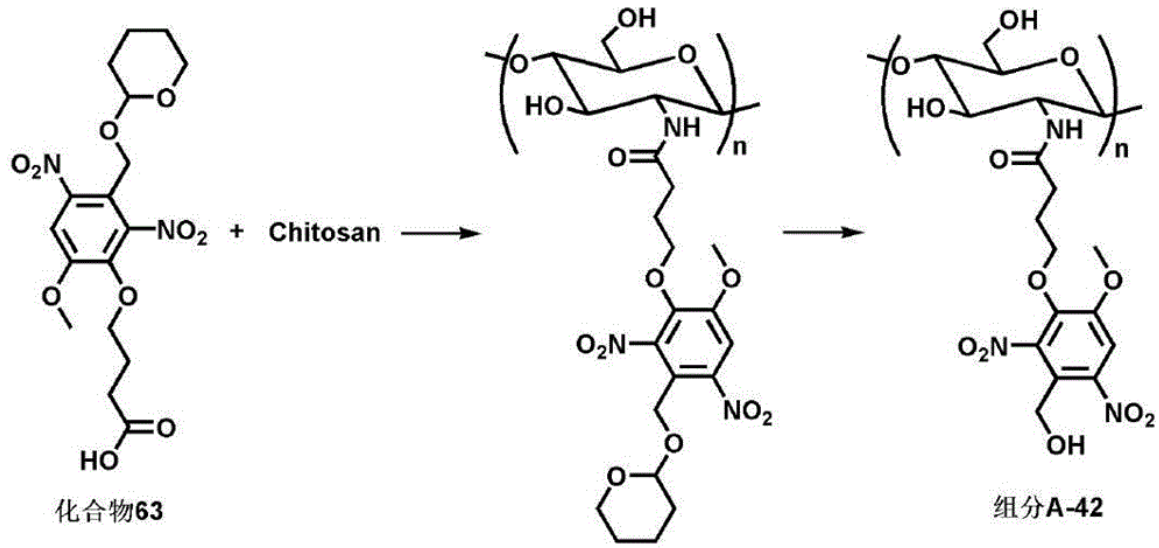
(1) Synthesis of compound 62: as per reference Pauloehrl, t.; delaittre, g.; bruns, m.; mei β ler, m.;

Börner,

H.G.; baselayer, m.; synthesis was performed according to the method disclosed in Barner-kowoollik, c.angelw.chem.int.ed.2012, 51,9181.^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.90-3.80(m,1H),3.79(t,J = 6.1Hz,2H),3.70(t,J = 7.2Hz,2H),3.63-3.52(m,1H),3.56(t,J = 7.2Hz,2H),2.00-1.34(m,6H).MS(ESI):[M+H]417.1527.

(2) Synthesis of component A-41: hyaluronic acid Hyaluronic acid (1g, 340kDa) was dissolved in 50mL of water, and compound 62(0.2g, 0.54mmol), EDC-HCl (0.76g, 3.96mmol) and DPTS (0.12g, 0.48mmol) were added to the above solution in this order, and the reaction was stirred at room temperature for 48 h. After the reaction is finished, pouring the reaction solution into cold ethanol for multiple times of reprecipitation and purification, drying the collected precipitate, dissolving the dried precipitate in anhydrous DMSO, and adding p-toluenesulfonic acid to remove the dihydropyran protecting group to obtain the photosensitive hyaluronic acid derivative A-41(0.82 g). From its nuclear magnetic hydrogen spectrum, the modification degree of compound 62 was calculated to be about 9.5%.

Example forty two: synthesis of component A-42



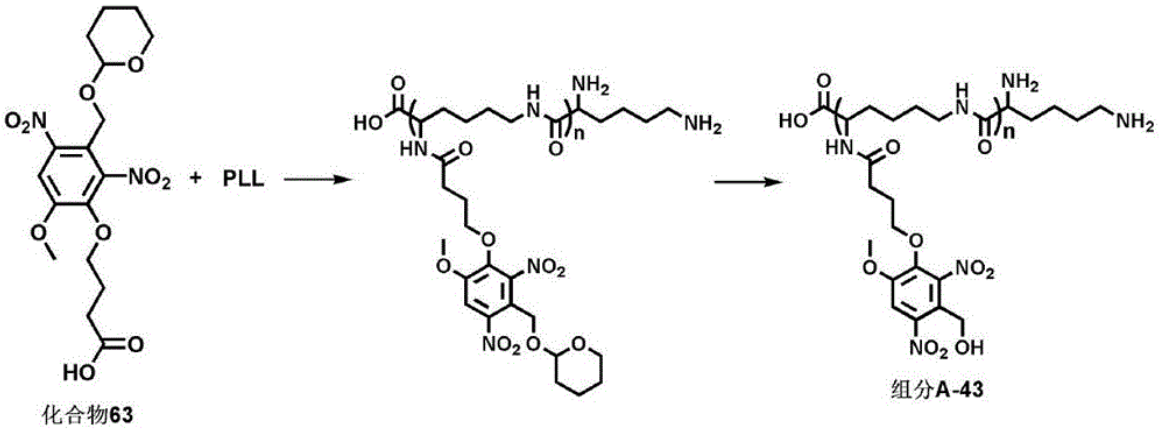
(1) Synthesis of compound 63: as per reference Pauloehrl, t.; delaittre, g.; bruns, m.; mei β ler, m.;

Börner,

H.G.; baselayer, m.; synthesis was performed according to the method disclosed in Barner-kowoollik, c.angelw.chem.int.ed.2012, 51,9181.^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.90-3.80(m,1H),3.63-3.52(m,1H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H),2.00-1.34(m,6H).MS(ESI):[M+H]415.1312.

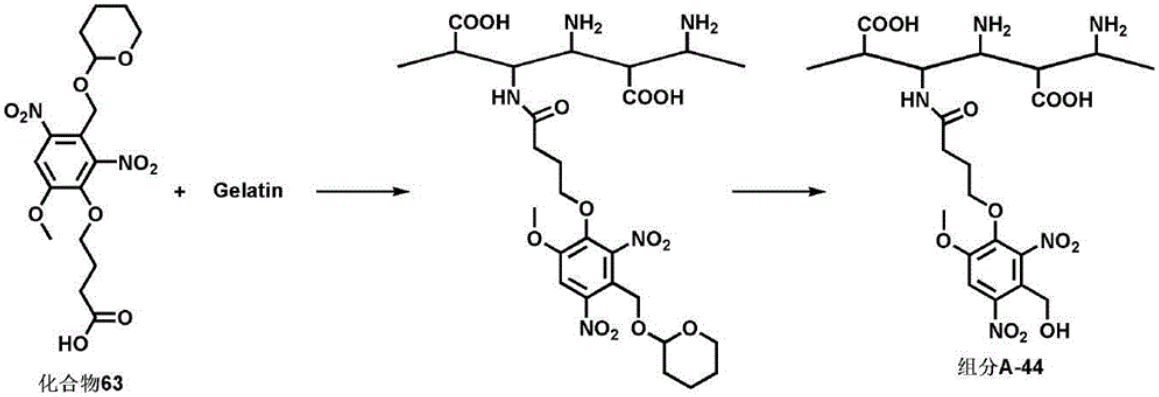
(2) Synthesis of component A-42: 1g of chitosan was added to 75mL of isopropanol to form a suspension of chitosan, and then Compound 63(0.2g, 0.48mmol), EDC-HCl (0.76g, 3.96mmol) and NHS (0.46g, 4.0mmol) were added to the above solution in that order, and the reaction was stirred at room temperature for 48 h. After the reaction was completed, the mixture solution was filtered, and the filtrate was dialyzed with a methanol/water mixed solvent three times, twice with methanol, and then lyophilized to obtain compound 63-labeled chitosan (0.92 g). Dissolving the chitosan marked by the compound 63 in DMSO, adding p-toluenesulfonic acid to remove dihydropyran protection to obtain the photosensitive chitosan derivative A-42, and calculating the modification degree of the compound 63 to be about 11.5% according to a nuclear magnetic hydrogen spectrum diagram.

Example forty-three: synthesis of component A-43



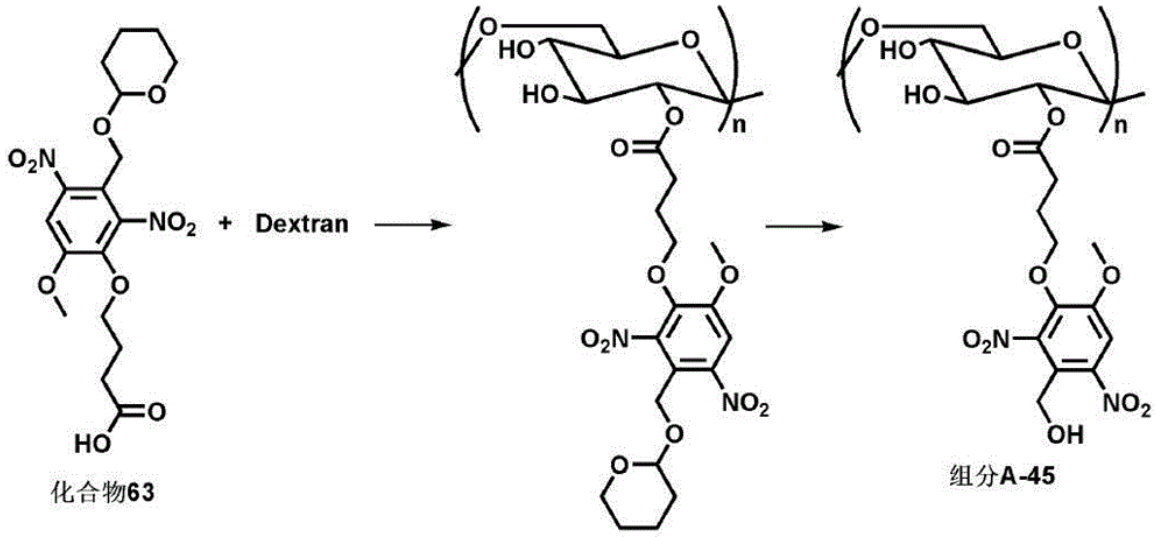
Synthesis of component A-43: polylysine PLL (1g) was dissolved in 50mL of water, and Compound 63(0.2g, 0.48mmol), EDC-HCl (0.76g, 3.96mmol) and NHS (0.46g, 4.0mmol) were added to the above solution in that order, and the reaction was stirred at room temperature for 48 h. After the reaction is finished, pouring the reaction solution into cold ethanol for multiple times of reprecipitation and purification, drying the collected precipitate, dissolving the dried precipitate in anhydrous DMSO, and adding p-toluenesulfonic acid to remove the dihydropyran protecting group to obtain the photosensitive polylysine derivative A-43(0.85 g). From its nuclear magnetic hydrogen spectrum, the modification degree of compound 63 was calculated to be about 12.6%.

Example forty-four: synthesis of component A-44



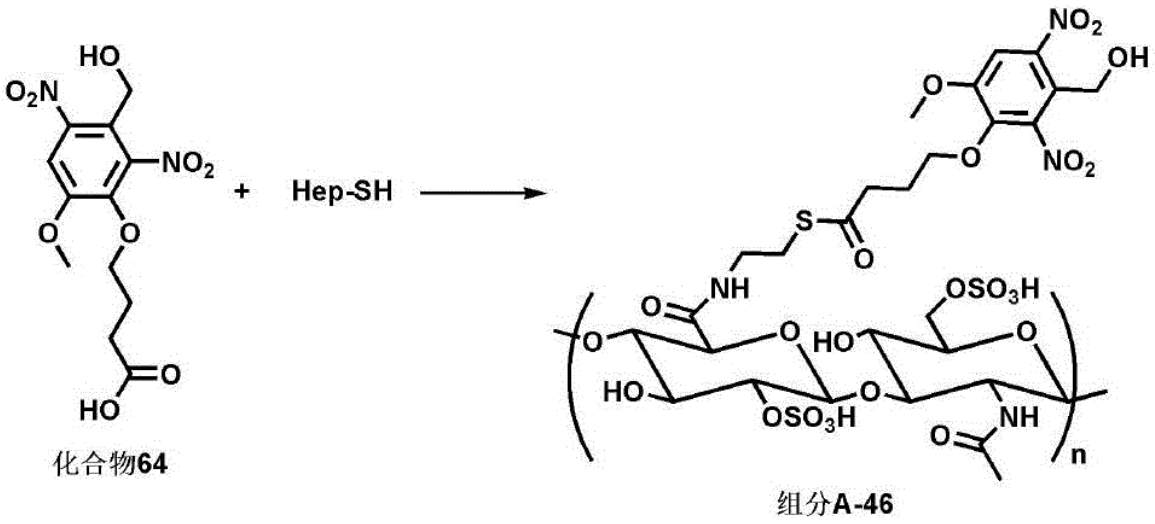
Synthesis of Components A-44: gelatin (1g) was dissolved in 50mL of distilled water until completely dissolved, and Compound 63(0.2g, 0.48mmol), EDC-HCl (0.76g, 3.96mmol) and NHS (0.46g, 4.0mmol) were added to the above solution in this order, and the reaction was stirred at room temperature for 48 hours. After the reaction is finished, pouring the reaction solution into cold ethanol for multiple times of reprecipitation and purification, drying the collected precipitate, dissolving the dried precipitate in anhydrous DMSO, adding p-toluenesulfonic acid to remove a dihydropyran protecting group to obtain a photosensitive gelatin derivative A-44(0.91g), and calculating the modification degree of the compound 63 to be about 15.3% according to a nuclear magnetic hydrogen spectrum diagram.

Example forty-five: synthesis of component A-45



Synthesis of Components A-45: dextran (1g) was dissolved in 50mL water, and compound 63(0.2g, 0.48mmol), EDC-HCl (0.76g, 3.96mmol) and DPTS (0.12g, 0.48mmol) were added to the above solution in that order and the reaction was stirred at room temperature for 48 h. After the reaction is finished, pouring the reaction solution into cold ethanol for multiple times of reprecipitation and purification, drying the collected precipitate, dissolving the dried precipitate in anhydrous DMSO, and adding p-toluenesulfonic acid to remove the dihydropyran protecting group to obtain the photosensitive glucan derivative A-45(0.89 g). From its nuclear magnetic hydrogen spectrum, the modification degree of compound 63 was calculated to be about 18.2%.

Example forty-six: synthesis of component A-46

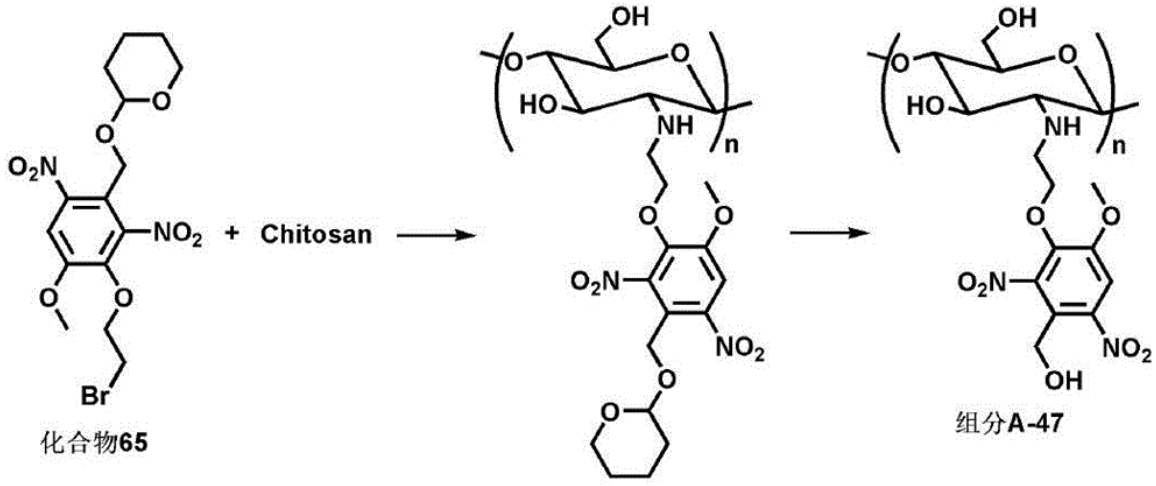


(1) Synthesis of compound 64: as per reference Pauloehrl, t.; delaittre, g.; bruns, m.; mei β ler, m.;

H.G.; baselayer, m.; synthesis was performed according to the method disclosed in Barner-kowoollik, c.angelw.chem.int.ed.2012, 51,9181.^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),2.44(t,J = 7.2Hz,2H),2.26-2.17(m,2H).MS(ESI):[M+H]331.0743.

(2) Synthesis of component A-46: sulfhydryl modified heparin Hep-SH (1g) is dissolved in 50mL distilled water until completely dissolved, hydroxybenzotriazole (HOBt, 0.3g, 2.3mmol) is added, then compound 64(0.5g,1.6mmol) and 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, 0.5g, 2.6mmol) dissolved in methanol are added into the solution for reaction at room temperature for 48h, then dilute hydrochloric acid solution (pH 3.5) containing sodium chloride is dialyzed for 1d, and then pure water is dialyzed for 1d, and freeze drying is carried out to obtain photosensitive heparin derivative A-46(0.85g), and according to the nuclear magnetic spectrum diagram, the modification degree of compound 64 can be calculated to be about 14.2%.

Example forty-seven: synthesis of component A-47

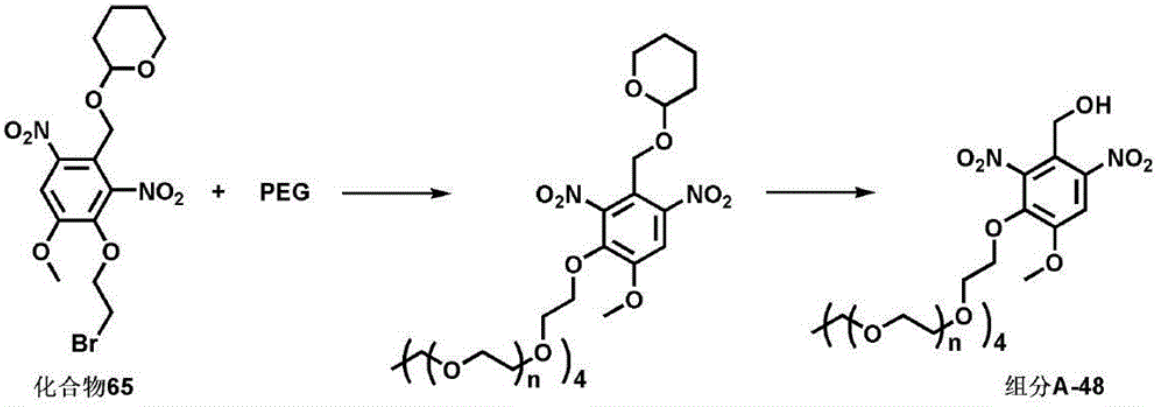


(1) Synthesis of compound 65: as per reference Pauloehrl, t.; delaittre, g.; bruns, m.; mei β ler, m.;

H.G.; baselayer, m.; synthesis was performed according to the method disclosed in Barner-kowoollik, c.angelw.chem.int.ed.2012, 51,9181.^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.90-3.80(m,1H),3.63-3.52(m,1H),3.04(t,J = 7.2Hz,2H),2.00-1.34(m,6H).MS(ESI):[M+H]436.0318.

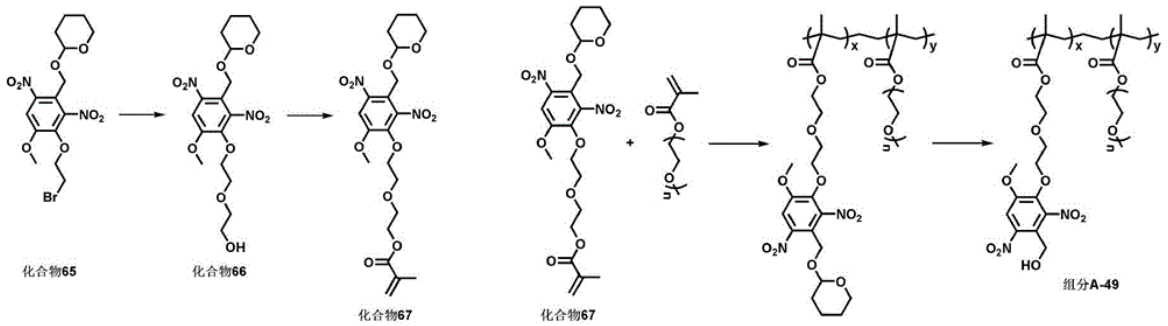
(2) Synthesis of Components A-47: 1g of chitosan was added to 75mL of isopropanol to form a suspension of chitosan, and 25mL of NaOH solution (10mol/L) was slowly added to the suspension of chitosan five times and stirring was continued for about half an hour. Compound 65(0.2g) was then added to the above solution and reacted at 60 ℃ for 3 h. After the reaction was completed, the mixture solution was filtered, and the filtrate was dialyzed with a methanol/water mixed solvent three times, twice with methanol, and then lyophilized to obtain compound 65-labeled chitosan (0.97 g). Dissolving chitosan marked by a compound 65 in DMSO, adding p-toluenesulfonic acid to remove dihydropyran protection to obtain a photosensitive chitosan derivative A-47(0.86g), and calculating the modification degree of the compound 65 to be about 19.2% according to a nuclear magnetic hydrogen spectrum diagram.

Example forty-eight: synthesis of component A-48



Synthesis of Components A-48: mixing PEG-4 OH: (1g, 0.05mmol) in anhydrous acetonitrile and addition of K_2CO_3(55.3mg, 0.4mmol) was stirred for 30min, then compound 65(0.17g, 0.4mmol) was added and the reaction was continued at room temperature for 24 h. After the reaction is finished, most of the solvent is removed, the solvent is re-precipitated in ether and washed for a plurality of times, then the polyethylene glycol marked by the compound 65 is dissolved in DMSO, p-toluenesulfonic acid is added to remove the protection of dihydropyrane, and the photosensitive polyethylene glycol derivative A-48(0.82g) can be obtained, and according to a nuclear magnetic hydrogen spectrum diagram, the modification degree of the compound 65 can be calculated to be about 95%.

Example forty-nine: synthesis of component A-49

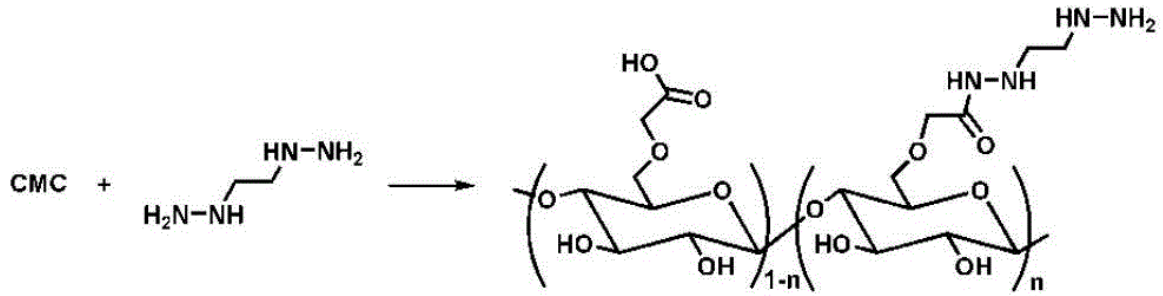


(1) Synthesis of compound 66: compound 65(0.5g, 1.29mmol) and ethylene glycol (0.24g, 3.87mmol) were dissolved in anhydrous acetonitrile and K was added$_2CO_3$(0.5g, 3.87mmol) as base was refluxed overnight. After the reaction was completed, the solvent was evaporated under reduced pressure and purified by column chromatography to obtain compound 66(0.34g, 72%).^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.90-3.80(m,1H),3.79(t,J = 6.1Hz,2H),3.70(t,J = 7.2Hz,2H),3.63-3.52(m,1H),3.56(t,J = 7.2Hz,2H),2.00-1.34(m,6H).MS(ESI):[M+H]417.1527.

(2) Synthesis of compound 67: compound 66(0.64g,1.72mmol) and triethylamine (0.34g, 3.44mmol) were dissolved in dry dichloromethane, methacryloyl chloride (0.27g, 2.58mmol) was slowly added dropwise to the solution under ice-bath conditions, and after completion of the addition, the reaction was allowed to proceed overnight at room temperature. After completion of the reaction, the solvent was evaporated under reduced pressure and purified by column chromatography to obtain compound 67(0.49g, 65%).^{1}H NMR(400MHz,$CDCl_3$):δ = 7.91(s,1H),6.25(s,1H),5.68(s,1H),4.96(s,2H),4.13(t,J = 6.1Hz,2H),3.99(s,3H),3.90-3.80(m,1H),3.79(t,J = 6.1Hz,2H),3.70(t,J = 7.2Hz,2H),3.63-3.52(m,1H),3.56(t,J = 7.2Hz,2H),2.00-1.34(m,6H),1.87(s,3H).MS(ESI):[M+H]485.1742.

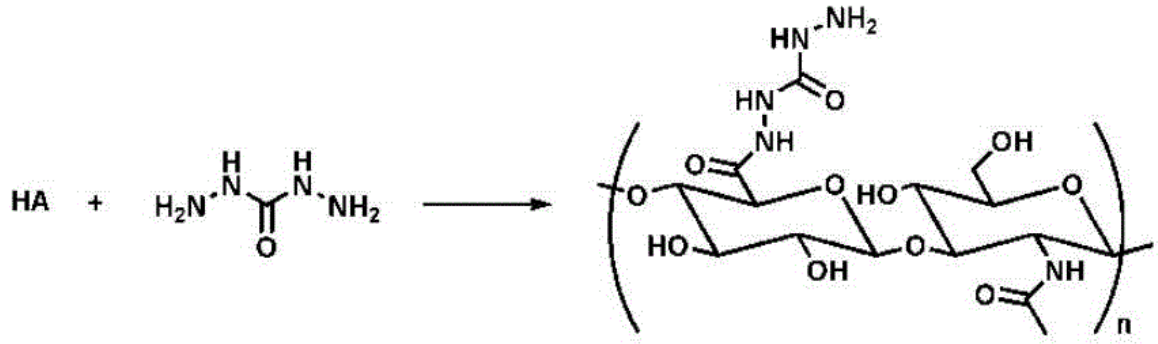
(3) Synthesis of Components A-49: weighing the compound 67(0.28g, 0.63mmol), the comonomer PEG-MA (0.882g, 2.52mmol) and the initiator azobisisobutyronitrile (11mg), adding into a Schlenk tube, adding anhydrous THF for dissolving, and reacting the reaction system for 24 hours at 75 ℃ after multiple freezing-vacuumizing cycles. After the reaction is finished, pouring the reaction solution into cold ether for repeated precipitation and purification, drying the collected precipitate, dissolving the dried precipitate in anhydrous DMSO, and adding p-toluenesulfonic acid to remove the dihydropyran protecting group to obtain the photosensitive copolymer A-49(0.81 g). From the nuclear magnetic hydrogen spectrum, the content of compound 67 in the copolymer was calculated to be about 15.2%. The molecular weight of the synthetic polymer is about 25kDa according to GPC, and n is 12, x is 10 and y is 40 according to the charge ratio.

Example fifty: synthesis of component B-10



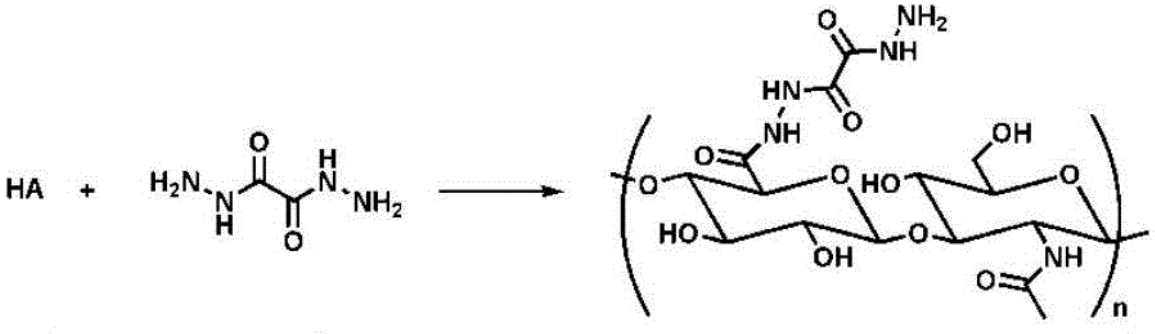
Synthesis of component B-10: carboxymethyl cellulose CMC (400mg) is dissolved in 50mL of distilled water till complete dissolution, hydroxybenzotriazole (HOBt, 153mg), diamine (90mg) and 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, 90mg) are added into the solution to react for 48 hours at room temperature, dilute hydrochloric acid solution (pH 3.5) containing sodium chloride is firstly used for dialysis for 1d, then pure water is used for dialysis for 1d, and freeze drying is carried out to obtain the diamine modified carboxymethyl cellulose (410 mg). The grafting rate of diamine tested by the TBNS method was about 12%.

Example fifty one: synthesis of component B-11



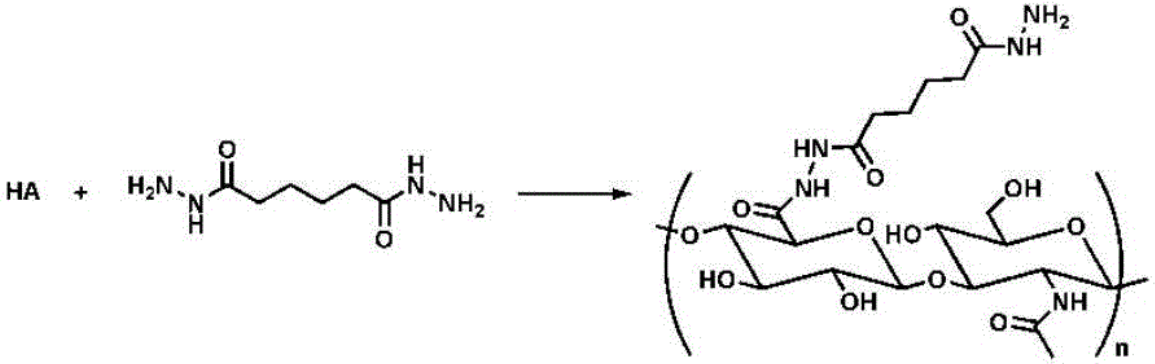
Synthesis of component B-11: hyaluronic acid HA (400mg) was dissolved in 50mL of distilled water until completely dissolved, hydroxybenzotriazole (HOBt, 153mg), carbodihydrazide (CDH, 90mg) and 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, 90mg) were added to the above solution to react at room temperature for 48 hours, and then dialyzed with a dilute hydrochloric acid solution (pH 3.5) containing sodium chloride for 1 day, then dialyzed with pure water for 1 day, and then freeze-dried to obtain HA-CDH (410 mg). The TBNS method measures hydrazide grafting of about 15%.

Example fifty two: synthesis of component B-12



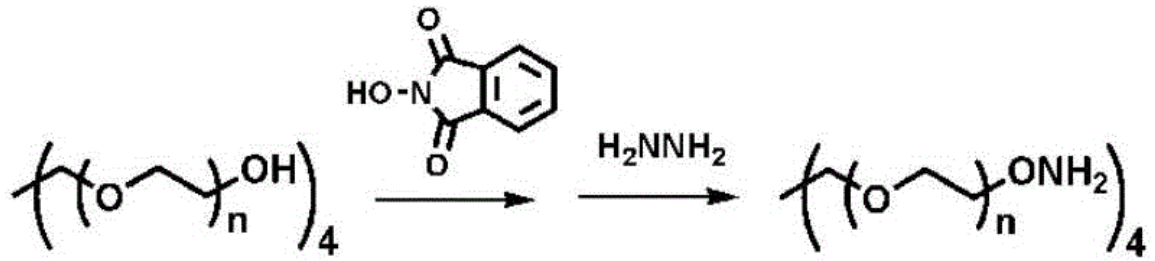
Synthesis of component B-12: hyaluronic acid HA (400mg) was dissolved in 50mL of distilled water until completely dissolved, hydroxybenzotriazole (HOBt, 153mg), oxalic acid dihydrazide (ODH, 90mg) and 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, 90mg) were added to the above solution to react at room temperature for 48 hours, and then dialyzed with a dilute hydrochloric acid solution (pH 3.5) containing sodium chloride for 1 day, then dialyzed with pure water for 1 day, and freeze-dried to obtain HA-ODH (410 mg). The TBNS method measures hydrazide grafting of about 11%.

Example fifty three: synthesis of component B-13



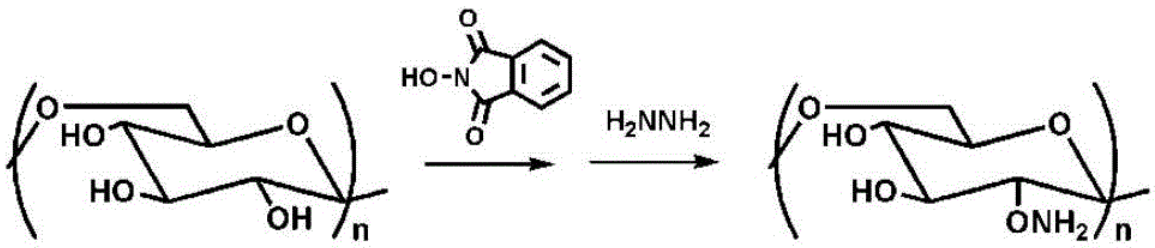
Synthesis of component B-13: hyaluronic acid HA (400mg) was dissolved in 50mL of distilled water until completely dissolved, hydroxybenzotriazole (HOBt, 153mg), adipic acid dihydrazide (ADH, 90mg) and 1-ethyl- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, 90mg) were added to the above solution to react at room temperature for 48h, and then dialyzed with a dilute hydrochloric acid solution (pH 3.5) containing sodium chloride for 1d, then dialyzed with purified water for 1d, and freeze-dried to obtain HA-ADH (410 mg). The TBNS method measures hydrazide grafting of about 14%.

Example fifty-four: synthesis of component B-14



Synthesis of component B-14: four-armed hydroxypolyethylene glycol (PEG-4OH, 2g, 97.3. mu. mol) and N-hydroxyphthalimide (634.6mg,3.89mmol) was dissolved in dry dichloromethane, then triphenylphosphine (1.02g, 3.89mmol) was slowly added under ice bath conditions and reacted for about 30 min. Diisopropyl azodicarboxylate (765.9. mu.L, 3.89mmol) was dissolved in dry dichloromethane and slowly added dropwise to the above solution and reacted at room temperature for 1 d. After the reaction is finished, the N-hydroxyphthalimide modified four-arm polyethylene glycol is re-precipitated by ether. The material (0.25g, 11.8. mu. mol) was then redissolved in acetonitrile and hydrazine monohydrate (22.9. mu.L, 473. mu. mol) was added and stirring continued for 2 h. Dichloromethane was then added to the mixture solution and filtered with suction. Removing solvent from the filtrate by rotary evaporation under reduced pressure to obtain hydroxylamine-modified four-arm polyethylene glycol (PEG-4 $ONH)_2$)。

Example fifty-five: synthesis of component B-15



Synthesis of component B-15: dextran (Dextran, 2g, 97.3. mu. mol) and N-hydroxyphthalimide (634.6mg, 3.89mmol) were weighed out and dissolved in dry dichloromethane, followed by slow addition of triphenylphosphine (1.02g, 3.89mmol) under ice-bath conditions and reaction for about 30 min. Diisopropyl azodicarboxylate (765.9. mu.L, 3.89mmol) was dissolved in dry dichloromethane and slowly added dropwise to the above solution and reacted at room temperature for 1

d. After the reaction was completed, the N-hydroxyphthalimide-modified dextran was reprecipitated with diethyl ether. The material (0.25g, 11.8. mu. mol) was then redissolved in acetonitrile and hydrazine monohydrate (22.9. mu.L, 473. mu. mol) was added and stirring continued for 2 h. Dichloromethane was then added to the mixture solution and filtered with suction. Removing solvent from the filtrate by rotary evaporation under reduced pressure to obtain hydroxylamine modified dextran $(Dex\text{-}ONH)_2$)。

Example fifty-six: ultraviolet absorption test of o-nitrobenzyl optical trigger

In this example, o-nitrobenzyl-based phototrigger NB_0And NB in methanol (80. mu. mol L)$^{-1}$) In the method, 3.0mL of the sample is transferred to an ultraviolet colorimetric cell, and the ultraviolet-visible absorption spectrum of the sample is measured in an ultraviolet-visible spectrophotometerAnd respectively calculating the molar absorption coefficients of the molecules according to Lambert-beer law. As shown in FIG. 1, the absorption wavelength of the o-nitrobenzyl-based photo-trigger NB (370nm) in the component A-1 is significantly longer than that of the o-nitrobenzyl-based photo-trigger NB reported in the literature$_0$(360nm) and can reach more than 405nm, and the molar absorption coefficient is also improved to a certain extent. Therefore, the molecules can be irradiated into glue by a light source of 405nm, and the defect of 365nm light source irradiation of the original molecules is effectively overcome. In addition, the maximum absorption wavelengths of other differently structured light triggers are shown in table 1.

TABLE 1

Components	Maximum absorption wavelength λ max (nm)	NB_0	360
A-1	370	A-2	372
A-4	375	A-5	375
A-6	378	A-21	386
A-22	410	A-25	385
A-26	450	A-27	480
A-28	367	A-29	367
A-30	367	A-49	370

Note: NB_0For the o-nitrobenzyl-based photoinitiators for the construction of hydrogels reported in the literature (Yunlong Yang; Jieyuan Zhang; Zhenzhen Liu; Qianing Lin; Xiaolin Liu; Chunyan Bao; Yang Wang; Linying Zhu. adv. Mater.2016,28,2724.). NB is an o-nitrobenzyl type photosetting initiator in the component A-1 of the present invention. Wherein, HA-NB_0Is NB_0The marked hyaluronic acid macromolecule derivative HA-NB is the component A-1.

Example fifty-seven: preparation of hydrogel by photo-coupling crosslinking method

Different hydrogel precursor solutions were prepared according to the method of the invention, operating at 37 ℃ as shown in Table 2.

TABLE 2

浓度 B / A	组分 B-1	组分 B-2	组分 B-3	组分 B-4	组分 B…	组分 B-15
组分 A-1	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%
组分 A-2	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%
组分 A-4	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%
组分 A…	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%
组分 A-49	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%	1-20wt%

The different gel solutions were separately treated at 405nm (20 mW/cm)^2) Irradiating for a certain time under the condition to obtain the hydrogel with different chemical compositions. Different gel materials have different biological effects, and the composition of the gel material can be specifically selected according to different applications.

Note: the component A … is a component A-5-A-48; the component B … is a component B-5 to B-14.

The preferred mass concentration range for the hydrogel precursor solution is 1-20 wt% in Table 1.

Example fifty-eight: rheology test of photo-coupled crosslinked hydrogels

In this example, the rheometry was carried out using an HAAKE MARS rheometer, test platform at 37 ℃

Rheological measurements were performed. This example investigated the effect of uv exposure time, exposure intensity and mass concentration of the polymeric derivative on gel formation time and storage modulus of the hydrogel. FIG. 2 shows the gel formation curves of hydrogel precursor solutions prepared from component A-1 (i.e., HA-NB) and component B-3 (i.e., carboxymethyl chitosan CMCh) in the mass ratio of 2% wt: 2% wt under light irradiation, and 2% HA-NB_0And 2% CMCh (storage modulus, loss modulus, and gel point when G' exceeds G "in the rheology test). As seen from FIG. 2, the hydrogel precursor solution constructed by the component A-1(HA-NB) prepared in the first example starts to gel in about 15s until the gel is completely formed in about 60s, and the modulus in the complete gel forming process

can reach about 1000Pa, so that the hydrogel (HA-NB) constructed by the original o-nitrobenzyl photoinitiator is obviously superior to the hydrogel (HA-NB) constructed by the original o-nitrobenzyl photoinitiator in gel strength and gel forming speed$_0$) The performance of (c). In addition, the strength of the gel is proportional to the mass concentration of the gel solution, and the greater the mass concentration of the gel, the greater the strength of the resulting gel. Other different materialsThe gel point and gel strength of the constituent hydrogel systems also varied, and the specific data are shown in table 3.

TABLE 3

Hydrogel Material composition (A/B)	Glue point(s)	Gel Strength (Pa)
Component A-1/component B-3 (2% wt: 2% wt)	15	650
Component A-1/component B-4 (2% wt: 2% wt)	25	430
Component A-1/component B-11 (2% wt: 2% wt)	14	1230
Component A-2/component B-3 (2% wt: 2% wt)	13	730
Component A-32/component B-3 (2% wt: 2% wt)	11	1650
Component A-37/component B-3 (2% wt: 2% wt)	19	540
Component A-39/component B-3 (2% wt: 2% wt)	24	380
Component A-42/component B-3 (2% wt: 2% wt)	15	670
Component A-44/component B-3 (2% wt: 2% wt)	26	320
Component A-49/component B-3 (2% wt: 2% wt)	18	660
HA-NB$_0$/CMCh(2%wt：2%wt)	30	200
Component A-1/component B-3 (4% wt: 4% wt)	12	2200
Component A-1/component B-10 (2% wt: 2% wt)	16	580
Component A-1/component B-14 (2% wt: 2% wt)	15	950
Component A-28/component B-3 (2% wt: 2% wt)	15	680
Component A-36/component B-3 (2% wt: 2% wt)	17	450
Component A-38/component B-3 (2% wt: 2% wt)	26	430
Component A-40/component B-3 (2% wt: 2% wt)	22	780
Component A-43/component B-3 (2% wt: 2% wt)	25	450
Component A-45/component B-3 (2% wt: 2% wt)	24	580

Example fifty-nine: adhesion testing of photo-coupled crosslinked hydrogels

In this example, fresh pig intestine was sampled in a few portions and cut into pieces of intestine of 3.5cm × 2.5 cm. Then the glass is fixed on a toughened glass sheet with the size of 6.5cm multiplied by 2.5cm by 502 glue. Taking the above toughened glass sheets, and smearing 150 μ L of hydrogel precursor solution with certain components on one of the casing-bonded surfaces. Then, another glass sheet is placed on top of the glass sheet, and the upper and lower sheets are adhered to the sausage casingAre completely opposite. At this time, excess extruded hydrogel precursor solution was wiped off. Then using a 405nm LED light source (20 mW/cm^2) Irradiating the sausage casing part for 5min to make the hydrogel precursor solution form gel in situ between two sausage casings. After the glue is completely formed, one end of the glass sheet is vertically fixed, and the other end of the glass sheet is connected with a container capable of containing water through a string. A constant amount of water was then added to the container until the two pieces of glass were separated. Thereafter, the mass of the water and container at that time was recorded, converted to gravity, i.e., the pulling force F at which the glass sheet broke, and the tissue adhesion of the hydrogel was calculated using the following formula:

hydrogel tissue adhesion force F/A

Where A is the bonding area of the casing and the schematic of the test setup is shown in FIG. 3. The tissue adhesion of hydrogel systems composed of other different materials also varied, and the specific data are shown in Table 4.

TABLE 4

水凝胶材料组成（A/B）	组织粘附力（kPa）
HA-NB$_0$/CMCh（2%wt：2%wt）	24
组分 A-1/组分 B-3（2%wt：2%wt）	46
组分 A-1/组分 B-3（4%wt：4%wt）	82
组分 A-1/组分 B-4（2%wt：2%wt）	28

组分 A-1/组分 B-10（2%wt：2%wt）	27
组分 A-1/组分 B-11（2%wt：2%wt）	34
组分 A-1/组分 B-14（2%wt：2%wt）	27
组分 A-2/组分 B-3（2%wt：2%wt）	41
组分 A-28/组分 B-3（2%wt：2%wt）	39
组分 A-32/组分 B-3（2%wt：2%wt）	47
组分 A-36/组分 B-3（2%wt：2%wt）	37
组分 A-37/组分 B-3（2%wt：2%wt）	32
组分 A-38/组分 B-3（2%wt：2%wt）	29
组分 A-39/组分 B-3（2%wt：2%wt）	28
组分 A-40/组分 B-3（2%wt：2%wt）	27
组分 A-42/组分 B-3（2%wt：2%wt）	34
组分 A-43/组分 B-3（2%wt：2%wt）	33
组分 A-44/组分 B-3（2%wt：2%wt）	27
组分 A-45/组分 B-3（2%wt：2%wt）	28
组分 A-49/组分 B-3（2%wt：2%wt）	29

Example sixty: mechanical property test of photo-coupled crosslinked hydrogel

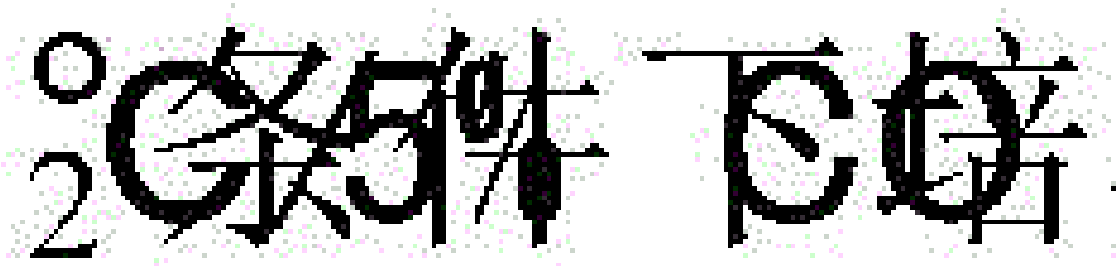
In this example, a GT-TCS-2000 tensile machine is used for mechanical property tests (including tensile tests and compression tests), a tensile test sample is a dumbbell-shaped test sample with the length of 20mm, the width of 3mm and the thickness of 2mm, the test speed is 5mm/min, a compression test sample is a cylindrical test sample with the diameter of 10mm and the height of 3mm, the test speed is 1mm/min, and the hydrogel precursor solution prepared by the component A-1 (namely HA-NB) and the component B-3 (namely carboxymethyl chitosan CMCh) prepared in the first example in the mass ratio of 2 wt% to 2 wt% is used for preparing the hydrogel under the light irradiation, so as to test the tensile property and the compression property of the hydrogel. The hydrogel can be stretched to about 150 percent, and the tensile strength is about 40 kPa; the compression is about 75 percent, and the compression strength is about 500 kPa. The mechanical properties of hydrogel systems composed of other different materials are different, and specific data are shown in table 5.

TABLE 5

水凝胶材料组成（A/B）	压缩变形率（%）	压缩强度（kPa）
HA-NB_0/CMCh（2%wt：2%wt）	45	200
组分 A-1/组分 B-3（2%wt：2%wt）	75	500
组分 A-1/组分 B-3（4%wt：4%wt）	73	460
组分 A-1/组分 B-4（2%wt：2%wt）	71	420
组分 A-1/组分 B-10（2%wt：2%wt）	64	380
组分 A-1/组分 B-11（2%wt：2%wt）	65	370
组分 A-1/组分 B-14（2%wt：2%wt）	61	320
组分 A-2/组分 B-3（2%wt：2%wt）	72	460
组分 A-28/组分 B-3（2%wt：2%wt）	68	410
组分 A-32/组分 B-3（2%wt：2%wt）	67	400
组分 A-36/组分 B-3（2%wt：2%wt）	65	380
组分 A-37/组分 B-3（2%wt：2%wt）	62	370
组分 A-38/组分 B-3（2%wt：2%wt）	64	340
组分 A-39/组分 B-3（2%wt：2%wt）	62	330
组分 A-40/组分 B-3（2%wt：2%wt）	60	310
组分 A-42/组分 B-3（2%wt：2%wt）	58	300
组分 A-43/组分 B-3（2%wt：2%wt）	52	280
组分 A-44/组分 B-3（2%wt：2%wt）	53	270
组分 A-45/组分 B-3（2%wt：2%wt）	56	290
组分 A-49/组分 B-3（2%wt：2%wt）	54	240

Example sixty one: photo-coupled crosslinked hydrogel biocompatibility test

In this experiment, the component A-1 (namely, HA-NB) and the component B-3 (namely, carboxymethyl chitosan CMCh) prepared in example one were evaluated by a CCK-8 kit. First, fibroblast HDFs were seeded in a 96-well plate at a cell density of 5X 10^3Cells/well, then medium is added at 37



And (5) cultivating for 24 hours. Each set of test samples was dissolved in cell culture fluid, added to a well plate in which cells were cultured, and cultured for another 24 hours, then the cell fluid in the well was aspirated, 100. mu.L of medium and 10. mu.L of CCK-8 solution were added to each well, and the cells were incubated for another 2 hours. Finally, absorbance at 450nm was measured in each well using a microplate reader. Cell viability was calculated as follows:

cell visual (%). x 100% (mean of absorbance in experimental group/mean of absorbance in control group)

As shown in FIG. 4, the photo-coupled crosslinked hydrogel has better biocompatibility.

In addition, in the in vivo immune inflammatory reaction test, taking the component A-1 (namely HA-NB) and the component B-3 (namely carboxymethyl chitosan CMCh) prepared in the first embodiment as examples, the hydrogel is implanted into the subcutaneous tissues of rabbits, and the inflammatory reaction of the hydrogel on organisms is analyzed by tissue section staining at different time points.

The biocompatibility of hydrogel systems composed of other different materials also varied, and the specific data are shown in table 6.

TABLE 6

Hydrogel Material composition (A/B)	Survival rate (%)	Hydrogel Material composition (A/B)	Survival rate (%)	Component A-1/component B-3	98	Component A-37/component B-3	94
Component A-1/component B-3	93	Component A-38/component B-3	93				

Component A-1/component B-10	94	Component A-40/component B-3	92	Component A-1/component B-4	91
Component A-1/component B-14	91	Component A-43/component B-3	93	Component A-1/component B-11	95
Component A-28/component B-3	98	Component A-45/component B-3	97	Component A-2/component B-3	92
Component A-36/component B-3	96			Component A-32/component B-3	97
Component A-39/component B-3	97				
Component A-42/component B-3	94				
Component A-44/component B-3	92				
Component A-49/component B-3	93				

The relationship between the component A and the component B in the hydrogel material with different components is 2 wt percent: 2% wt.

Example sixty two: application of photo-coupled cross-linked hydrogel in postoperative wound surface sealing

In this example, a skin complete defect wound of 1.8cm in diameter was constructed on the back skin of SD rats. 400 μ L of hydrogel precursor solution (2% component A-1/2% component B-3) was then filled into the wound site. Due to the good fluidity of the solution, the wound can be sufficiently filled and penetrated by the hydrogel precursor solution. Then, hydrogel is prepared in situ at the skin defect under the irradiation of 405nm LED light source, and the wound surface is sealed (as shown in figure 5). Next, the repairing effect of the skin wound on the back of SD rat cleaned with physiological saline only within 7 days was compared with the in situ formed hydrogel, the pre-formed hydrogel and the SD rat. The wound repair rate of the hydrogel formed in situ is obviously higher than that of the other two groups, and the wound shrinkage area is the largest at 7d, so that a good repair effect is achieved. It is difficult for the pre-formed hydrogel material to adequately fill the wound site; in addition, the tissue has no seamless interface with covalent connection, and the tissue has poor integration. The new cells and tissues are difficult to rapidly enter the hydrogel material, so that the new cells and tissues can fully play the role of the scaffold material. Thus, the repair rate and effectiveness of the pre-formed hydrogel is inferior to that of the in situ formed hydrogel. The wound repair rate without hydrogel filling is the slowest, which indicates that the photocrosslinking hydrogel has promotion effect on wound repair as a cell scaffold material.

Other hydrogel systems (component A: component A-1-component A-49; component B: component B-1-component B-15) made of different materials belong to the photo-coupled cross-linked hydrogel, and can be applied to postoperative wound surface sealing.

Example sixty-three: application of photo-coupled cross-linked hydrogel in intestinal leakage plugging

New Zealand male white rabbits were divided into two groups for cecal leakage plugging experiments: a: hydrogel treated (2% component A-1/2% component B-3) group; b: untreated control group. In the experiment, a leakage model is manufactured at the cecum of a rabbit, then the hydrogel precursor solution is smeared at the wound, after full permeation, the hydrogel is formed in situ by illumination, and the hydrogel can be firmly adhered to the defect without additional fixation after being formed. After 4 weeks of surgery, rabbits were sacrificed in the experiment by intravenous air and cecum was extracted to evaluate the effect of the experimental repair. The results show that no leakage occurred in caecum blocked with hydrogel, whereas severe leakage occurred in caecum not treated with hydrogel. After several weeks of repair, the original part with caecum defect is obviously repaired after being treated by the hydrogel, so the hydrogel not only can effectively block the leakage, but also is beneficial to repairing the postoperative damaged tissue.

Other hydrogel systems (component A: component A-1-component A-49; component B: component B-1-component B-15) made of different materials belong to the photo-coupled cross-linked hydrogel, and can be also applied to intestinal leakage plugging.

Example sixty-four: application of photo-coupled cross-linked hydrogel in hemostatic material

Adopt SD rat, evaluate the hemostatic effect of aquogel, divide into three groups and carry out the liver hemostasis experiment: a: a gelatin sponge group; b: hydrogel (2% component A-1/2% component B-3); c positive control group. Experimental rats were anesthetized by intraperitoneal injection of chloral hydrate (4% aqueous solution) in a dose of 0.9ml/100g, and after deep anesthesia, the rat chest area was shaved with a shaver and sterilized with iodine. An approximately 4cm long incision was then made along the midline of the chest, and the chest was opened to expose a liver site. An approximately 2cm incision was made in the left lobe of the liver. Group a is stopped bleeding by gelatin sponge; b, adding a hydrogel precursor solution at the cut to cover the cut, and illuminating with 405nm LED for 2min to form gel for hemostasis; group c was left untreated, the liver cut was allowed to ooze blood to clot naturally, the oozed blood was aspirated with gauze, and the amount of bleeding, and the bleeding time, were recorded by weight loss (as shown in fig. 6). After the experiment, the gelatin sponge adhered to the section of the group a was left together in the rat body and sutured. Group b hydrogels were cross-linked in situ at the incision and the cut wound was isolated, the liver was placed back into the chest and sutured. Group c was stitched directly without treatment. After 14d, SD rats were observed for liver recovery, and the rats were sacrificed by intraperitoneal injection of excess anesthetic chloral hydrate (4% aqueous solution, 2.7ml/100g), the chest was opened along the midline of the chest, and the recovery of the liver was observed in three groups of rats and recorded by photography. Meanwhile, the tissues of the injured part of the liver are sampled, the sample is fixed for 2 days by 4% formalin solution, and after dehydration treatment, the sample is embedded by paraffin, and then the tissue section operation is carried out by a microtome, wherein the thickness of the sample is 5 mu m. And finally, H & E staining is carried out on the specimen, and the record is observed by taking a picture through an optical microscope. The experimental result shows that the liver of the group b recovers well, the hydrogel degrades completely, no adhesion occurs, and the liver incision grows new liver tissues. The gelatin sponges remained undegraded in the rats in group a, and the rats generally had severe organ-to-omentum adhesion. The c group is commonly in the condition of liver and omentum adhesion. H & E staining shows that the surfaces of the livers of the experimental groups are smooth and mellow, abundant in vascularity and clear in liver interfaces. The liver with adhesion is stained by H & E to find that the liver interface is uneven, the liver and the omentum tissue are adhered together, and inflammatory cells are deposited on the interface.

Other hydrogel systems (component A: component A-1-component A-49; component B: component B-1-component B-15) composed of different materials belong to the photo-coupled cross-linked hydrogel, and can also be applied to hemostatic materials.

Example sixty-five: application of photo-coupled cross-linked hydrogel in cartilage tissue engineering

New Zealand male white rabbits were used and divided into three groups for articular cartilage repair experiments: a: a hydrogel (2% component A-1/2% component B-3) group coated with chondrocytes, i.e., Gel + chondrocyte group; b: the simple hydrogel group, i.e., Gel group; c: the untreated Control group, i.e., Control group. In the experiment, the hydrogel precursor solution can fully permeate and fill the defect of the rabbit articular cartilage, and is firmly adhered to the defect after being irradiated by light without additional fixation. After 12 weeks of operation, rabbits in the experiment were sacrificed by intravenous air, and the injured joint was extracted to evaluate the effect of the experimental repair. The result of a general observation picture of the rabbit articular cartilage injury part shows that after 12 weeks, a Gel + chondrocyte group grows smooth new cartilage tissues at the joint defect part and is well integrated with the old cartilage tissues; cartilage was also repaired to some extent in the Gel group, but the contour of cartilage trauma at the time of surgery was also seen; in the Control group, cartilage tissue was not substantially repaired, and the lesion was still clearly hollow (as shown in FIG. 7). Next, the repair of each group of cartilage was further evaluated by H & E staining. H & E staining results show that the Gel + chondrocyte group and the Gel group both have new tissue generation and are well integrated with old cartilage tissues; however, the thickness of the new tissue of the Gel + chondrocyte group is better than that of the Gel group, and the surface is smooth; whereas in the Control group it is difficult to find evidence of significant neogenesis. In addition, the composition of the new cartilage was analyzed by safranin-O and immunohistochemical staining. In both Gel + chondrocyte and Gel groups, the new cartilage tissue showed safranin-O staining activity, demonstrating that the new cartilage tissue contains the glycoprotein component of normal cartilage. Meanwhile, the new cartilage tissues of the Gel + chondrocyte group and the Gel group both showed the staining activity of type II collagen, which proves that the cartilage tissues contain a large amount of type II collagen. The results of the above safranin-O and immunohistochemical staining demonstrate that the new cartilage tissue is hyaline cartilage when cartilage repair is performed using the novel photo-crosslinked hydrogel material.

Other hydrogel systems (component A: component A-1-component A-49; component B: component B-1-component B-15) made of different materials belong to the photo-coupled cross-linked hydrogel, and can be also applied to cartilage tissue engineering.

Example sixty-six: application of photo-coupled cross-linked hydrogel in bone defect repair

SD rats were used for cranial repair experiments and were randomly divided into 3 groups: a: hydrogel + hydroxyapatite test panel; b: hydrogel (2% component A-1/2% component B-3); c: control group without material treatment. In the experiment, 4% chloral hydrate solution (0.9mL per gram of body weight) was used for abdominal anesthesia and iodine disinfection. Then, the scalp at the skull of the rat was opened using a surgical blade. A complete skull defect model with the diameter of 5mm is symmetrically manufactured at the left and the right of the mouse skull by using dental trephine. In an experimental group, 200 mu L of hydrogel precursor solution is filled in the SD rat skull defect to fully permeate to the wound edge; using a 405nm LED light source (20 $mW/cm)^2$) Lighting for 30s to completely gelatinize; finally, the scalp of the rat was sutured with sutures. In the control group, after the SD rat skull defect model is manufactured, the scalp is directly sutured withoutAny other processing. The SD rats were kept in a sterile, 37 ℃ environment for a period of 8 weeks. Then, the repair of the skull of the SD rat in each group was evaluated by micro-CT scanning imaging. The results showed that in the control group without any treatment, the SD rats had essentially no repair of the skull defect, while the hydrogel-filled skull defect had new bone formation at the edge, but the amount of new bone tissue was small, and most of the defect was not well repaired, while the hydrogel + hydroxyapatite-filled skull defect had essentially repaired, and a large amount of new bone tissue was formed at the defect. Histological staining analysis was then performed on tissue sections of the skull using Van Gieson staining. The results showed that all the cranial defects of the SD rats treated with hydrogel and hydroxyapatite showed complete new bone tissue growth, while the cranial defect treated with hydrogel alone showed only a small amount of new bone tissue growth, most of the bone tissue in the defect remained defective, and almost no new bone tissue growth in the control group. The tissue staining result further proves that the hydrogel wrapped with hydroxyapatite has good repairing effect on bone defects.

Other hydrogel systems (component A: component A-1-component A-49; component B: component B-1-component B-15) made of different materials belong to the photo-coupled cross-linked hydrogel and can be also applied to bone defect repair.

Example sixty-seven: biological ink for applying photo-coupled cross-linked hydrogel to 3D printing

The 3D printing technology is a three-dimensional molding technology rapidly developed in recent years, and has been widely used, and currently, the 3D printing technology includes fused deposition type (FDM), light solidification molding (SLA), laser sintering type (SLS), continuous liquid level manufacturing type (CLIP), and the like. However, the mode suitable for cell-carrying printing is mainly FDM mode at present, and the material for cell-carrying printing is mainly hydrogel material, so that the development of 3D printed bio-ink-printable hydrogel material and the improvement of the resolution of hydrogel material printing are basic problems of research in the field. Taking the component a-1 and the component B-3 prepared in the first embodiment as an example, uniformly mixing hydrogel precursor solution with a certain mass concentration with cells, placing the mixture into a low-temperature printing barrel, controlling the printing temperature to be about 25 ℃, adjusting the viscosity of bio-ink by the temperature to obtain an optimal printing state, determining appropriate printing pressure and printing speed, performing bio-printing with different structures, crosslinking the hydrogel by light (or printing and lighting at the same time) after printing is completed, thereby obtaining hydrogel with cells and structures, and performing 3D cell culture (as shown in fig. 8).

Other hydrogel systems (component A: component A-1-component A-49; component B: component B-1-component B-15) made of different materials belong to the photo-coupled cross-linked hydrogel, and can also be applied to 3D printing biological ink.

Example sixty-eight: application of photo-coupled cross-linked hydrogel in wrapping and releasing drugs

The hydrogel is a cross-linked polymer network which can swell but does not dissolve in water, and is very good in biocompatibility due to the fact that the hydrogel is mainly composed of water, and is particularly suitable for carriers of medicines and bioactive macromolecules. The drug or the bioactive macromolecules wrapped in the hydrogel material realize the effect of sustained release of the drug through the diffusion effect of molecules and the degradation effect of the material. The specific description of drug encapsulation and release is as follows: dissolving the component A-1 and the component B-3 prepared in the first embodiment in physiological saline to prepare a hydrogel precursor solution with a certain mass concentration, adding a certain amount of drug molecules, placing 200 mu L of the solution in a circular mold to form hydrogel by light irradiation, then placing the hydrogel precursor solution in a 24-hole cell culture plate, adding a certain amount of physiological saline to perform a drug release experiment, and analyzing the release amount of the drug in the solution by ultraviolet test to evaluate the release effect of the material on the drug.

Other hydrogel systems (component A: component A-1-component A-49; component B: component B-1-component B-15) made of different materials belong to the photo-coupled cross-linked hydrogel, and can be applied to wrapping and releasing of drugs.

The embodiments described above are described to facilitate an understanding and use of the invention by those skilled in the art. It will be readily apparent to those skilled in the art that various modifications to these embodiments may be made, and the generic principles described herein may be applied to other embodiments without the use of the inventive faculty. Therefore, the present invention is not limited to the above embodiments, and those skilled in the art should make improvements and modifications within the scope of the present invention based on the disclosure of the present invention.

Patent Citations (3)

Publication number	Priority date	Publication date	Assignee	Title
CN105131315A *	2014-11-27	2015-12-09	华东理工大学	Non-radical photochemical crosslinked hydrogel material preparation method, product and application
CN105153362A *	2015-08-07	2015-12-16	天津大学	Photosensitive hydrogel as well as preparation method and application thereof
CN106349465A *	2016-08-31	2017-01-25	电子科技大学	Light and temperature double-respond copolymer and synthesizing method and hydrogel system thereof
Family To Family Citations				

* Cited by examiner, † Cited by third party

Non-Patent Citations (6)

Title
" Tissue-Integratable and Biocompatible Photogelation by the Imine Crosslinking Reaction";Yunlong Yang等;《Advanced Materials》;20160203;第28卷;第2724-2730页 *
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"Photo-responsive polyethyleneimine microcapsules cross-linked by ortho-nitrobenzyl derivatives";Huiying Li等;《Journal of Colloid and Interface Science》;20151022;第463卷;第22-28页 *
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* Cited by examiner, † Cited by third party

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Publication number	Priority date	Publication date	Assignee	Title
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Family To Family Citations				
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CN110003408A *	2019-03-13	2019-07-12	上海大学	Biocompatibility porous frozen gel micro-ball and preparation method thereof
CN110183595A *	2019-03-13	2019-08-30	上海大学	Alkyloxy-ethers tree shaped polymer/gelatin-compounded freezing gel, preparation method and application
CN109824565B *	2019-03-25	2022-10-25	华东理工大学	Light-responsive multifunctional chemical cross-linking agent and preparation method and application thereof
CN110183549B *	2019-05-05	2021-01-26	湖北三江航天江河化工科技有限公司	light/pH double-responsiveness sodium alginate derivative and preparation method and application thereof
CN112126069B *	2019-06-24	2021-12-21	中国科学院苏州纳米技术与纳米仿生研究所	Rapidly curable hydrogel based on collagen and hyaluronic acid, preparation method and application thereof
CN110540661B *	2019-09-30	2022-03-29	湖北工程学院	Composite hydrogel of silk fibroin and polyvinyl alcohol, and preparation method and application thereof
CN110680959B *	2019-10-31	2021-12-03	江苏地韵医疗科技有限公司	Hydrogel for repairing multiple cross-linked meniscus and preparation method thereof
CN113144275B *	2020-01-22	2023-02-28	鸡西代悦科技有限公司	Hydrogel adhesive and preparation method and application thereof
CN113398321B *	2020-03-17	2022-04-12	天津大学	Porous hemostatic sponge with high liquid absorption rate and high resilience, and preparation method and application thereof
CN111748088B *	2020-05-31	2021-07-27	中山光禾医疗科技有限公司	High-strength and high-toughness photo-crosslinking hydrogel material and preparation method and application thereof
CN112898599A *	2021-02-02	2021-06-04	深圳市第二人民医院(深圳市转化医学研究院)	Three-dimensional network bionic hydrogel and preparation method and application thereof
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CN107964056B	2021-03-19	Preparation method, raw materials, product and application of photo-coupled cross-linked hydrogel material
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Priority And Related Applications

Priority Applications (1)

Application	Priority date	Filing date	Title
CN201711132951.4A	2017-11-15	2017-11-15	Preparation method, raw materials, product and application of photo-coupled cross-linked hydrogel material

Applications Claiming Priority (1)

Application	Filing date	Title
CN201711132951.4A	2017-11-15	Preparation method, raw materials, product and application of photo-coupled cross-linked hydrogel material

Legal Events

Date	Code	Title	Description
2018-04-27	PB01	Publication	
2018-04-27	PB01	Publication	
2018-05-22	SE01	Entry into force of request for substantive examination	
2018-05-22	SE01	Entry into force of request for substantive examination	
2018-08-31	TA01	Transfer of patent application right	
2018-08-31	TA01	Transfer of patent application right	**Effective date of registration**: 20180814 **Address after**: 100085, 4 floor 22, 1 Street, ten Street, Haidian District, Beijing. **Applicant after**: Zhong Rong Yun Da (Beijing) Technology Co., Ltd. **Address before**: 200237 No. 130, Meilong Road, Shanghai, Xuhui District **Applicant before**: East China University of Science and Technology
2019-03-01	TA01	Transfer of patent application right	
2019-03-01	TA01	Transfer of patent application right	**Effective date of registration**: 20190212 **Address after**: 528403 Linhai Industrial Park, Cuiheng New District, Zhongshan City, Guangdong Province **Applicant after**: Zhongshan Guanghe Medical Technology Co., Ltd. **Address before**: 100085, 4 floor 22, 1 Street, ten Street, Haidian District, Beijing. **Applicant before**: Zhong Rong Yun Da (Beijing) Technology Co., Ltd.
2021-03-19	GR01	Patent grant	
2021-03-19	GR01	Patent grant	