CONDUCTIVE HYDROGEL FOR PROMOTING BONE GROWTH, PREPARATION METHOD AND APPLICATION THEREOF

FIELD OF TECHNOLOGY

The present invention relates to the field of biomedical technology, specifically to a conductive hydrogel for promoting bone growth, preparation method, and application thereof.

BACKGROUND

Clinically, large-scale maxillofacial bone defects caused by trauma, tumors, inflammation, and other factors can severely affect patients' quality of life both physiologically and psychologically, making them extremely challenging problems. Bone regeneration for bone defects remains an urgent issue to be addressed. Bone substitute materials have been developed to promote the repair of bone defects and possess significant clinical potential, but certain problems still exist. Therefore, there is an urgent clinical need to find a novel bone defect repair material with biocompatibility and degradability. Electroactive hydrogels have shown great potential in the clinical application of bone defect repair. Electroactive hydrogels are intelligent materials with electrical stimulation response properties; they can mimic the mechanoelectric characteristics of bone, thereby generating corresponding electrical signals or deformation when stimulated by an electric field. This material combines the biocompatibility and injectability of hydrogels with the responsiveness of electroactive substances, providing new ideas for bone defect repair.
Electroactive hydrogels can simulate the mechanoelectric environment of bone and promote the proliferation and differentiation of bone cells through electrical stimulation, thereby accelerating the repair of bone defects. Studies have shown that hydrogels with piezoelectric properties can generate electrical signals under external force, which can stimulate bone cell activity and promote new bone formation.
The injectability of electroactive hydrogels allows them to be conveniently filled into bone defect sites, forming repair materials that closely integrate with surrounding bone tissue. Furthermore, by adjusting the degradation rate and mechanical properties of the hydrogel, it can be matched to the rate of bone regeneration, further improving repair efficiency.

Compared with traditional bone grafting methods, electroactive hydrogels have lower immunogenicity and better biocompatibility, which can reduce postoperative infections and complications such as rejection reactions.

Conductive hydrogels improve their electrical conductivity by introducing conductive substances (such as metal nanoparticles, carbon nanotubes, etc.). Such hydrogels can serve as electrode materials for electrical stimulation therapy, promoting bone regeneration by applying electrical stimulation to bone defect sites. Although electroactive hydrogels have shown great potential in bone defect repair, their clinical application still faces some challenges. For example, further improving the mechanical properties and stability of hydrogels and precisely controlling the conditions and parameters of electrical stimulation remain unresolved. In the future, with the continuous development of materials science and biomedical technology, electroactive hydrogels are expected to play a greater role in the field of bone defect repair.

SUMMARY

To solve the above technical problems, the first objective of the present invention is to provide a preparation method for a conductive hydrogel that promotes bone growth, the second objective is to provide a conductive hydrogel for promoting bone growth, and the third objective is to provide its application. The hydrogel exhibits excellent electrical conductivity, porous structure, biocompatibility, and mechanical properties, and possesses a favorable osteogenic effect. Its preparation process is simple and highly reproducible.

To achieve the above first objective, the present invention is implemented through the following technical solution: a preparation method for a conductive hydrogel that promotes bone growth, characterized by the following steps:

(1) Preparation of methacrylic anhydride-modified gelatin hydrogel prepolymer solution: dissolve the photoinitiator in deionized water to prepare a solution with a mass/volume concentration of 0.003–0.005 g/mL; dissolve methacrylic anhydride-modified gelatin (GelMA) in the solution to prepare the hydrogel prepolymer solution; this dispersion technique improves its dispersibility and stability.

(2) Preparation of CuNPs dispersion: add copper nanoparticles to deionized water and stir the mixture to obtain a copper nanoparticle dispersion.

(3) Preparation of photocrosslinked hydrogel: mix the methacrylic anhydride-modified gelatin prepolymer solution and CuNPs dispersion to obtain a methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel mixture; in the mixture, the mass/volume concentration of methacrylic anhydride-modified gelatin is maintained at 0.05 g/mL, and the copper nanoparticle concentration is 10 mmol/L–40 mmol/L; after removing bubbles, add the mixed solution into a mold and irradiate with ultraviolet light to prepare the photocrosslinked hydrogel.

The particle size of copper nanoparticles is 10–30 nm.

In the above solution: the degree of substitution of methacrylic anhydride-modified gelatin is 55–65%. This ensures its biocompatibility and molding properties.

In the above solution: the concentration of methacrylic anhydride-modified gelatin hydrogel prepolymer solution is 0.1 g/mL.

In the above solution: the concentration of CuNPs dispersion is 20 mmol/L–80 mmol/L, and the methacrylic anhydride-modified gelatin prepolymer solution and CuNPs dispersion are mixed at a volume ratio of 1:1.

In the above solution: ultraviolet irradiation time is 45–60 s. Wavelength is 405 nm; by adjusting the wavelength and irradiation time, hydrogel materials with ideal mechanical and electrical properties can be obtained.

In the above solution: the photoinitiator is LAP.

A conductive hydrogel for promoting bone growth prepared by the method described above.

The conductive hydrogel for promoting bone growth described above is used as a bone filler material for the treatment of bone defect repair.

In the present invention, methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel is used as a bone tissue engineering scaffold material, and the composition and ratio of the hydrogel scaffold material are controlled to ensure that it possesses excellent porous structure, biocompatibility, electrical conductivity, mechanical properties, and bioactivity, supporting cell growth and osteogenic differentiation within the hydrogel scaffold.

Mix GelMA with CuNPs dispersion, and under the action of photoinitiator LAP, use physical and chemical methods to uniformly composite all components and impart electrical conductivity to the hydrogel; then crosslink and cure. Under ultraviolet irradiation, GelMA crosslinks and cures, forming GelMA-CuNPs conductive hydrogel with a three-dimensional network structure and electrical conductivity.

This hydrogel exhibits excellent biocompatibility, antibacterial properties, and electrical conductivity, making it suitable for tissue engineering, cell culture, bioengineering, and other fields.

The hydrogel can serve as a cell scaffold material to promote cell growth and differentiation; additionally, its electrical conductivity can be used to monitor cell activity and physiological status, providing new methods for biomedical research.

The hydrogel can carry cells for cell culture and simultaneously load growth factors to promote osteogenic differentiation; it can serve as a bone filler material for the treatment and research of bone defect repair.

The photocrosslinked hydrogel prepared in this invention, in addition to possessing the morphology, water absorption, and plasticity of traditional hydrogels, also has the following improved effects:

1. The present invention processes and modifies methacrylic anhydride-modified gelatin and introduces copper nanoparticles to prepare a stable, mechanically strong, and biologically active photocrosslinked hydrogel.

2. The photocrosslinked hydrogel of the present invention, with the addition of appropriate CuNPs, possesses certain antibacterial and electrical conductivity properties.

3. The product of the present invention selects suitable irradiation time and wavelength to obtain hydrogel materials with ideal mechanical and electrical properties. By using different molds, rapid preparation of photocrosslinked hydrogels with various shapes and thicknesses can be achieved.

4. The photocrosslinked hydrogel prepared in this invention has a three-dimensional porous structure suitable for cell adhesion and growth; it possesses excellent biocompatibility, degradability, and low cytotoxicity, suitable for cell growth and proliferation; it has good mechanical properties, can withstand physiological pressure, and can promote osteogenic differentiation of cells.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the photocuring crosslinking process of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel.

FIG. 2 shows the scanning electron microscope images of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel (from left to right: GelMA, GelMA-CuNPs hydrogel), demonstrating that after the addition of copper nanoparticles, the hydrogel exhibits a loose and porous structure.

FIG. 3 shows the results of the conductivity experiment of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel.

FIG. 4 shows the results of live/dead cell staining and CCK8 experiments for methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel.

FIG. 5 shows the antibacterial experiment results of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel.

FIG. 6 shows the Micro-CT of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel implanted for 4 weeks in a mouse cranial infectious bone defect model.

FIG. 7 shows the H&E staining and Masson staining of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel implanted for 4 weeks in a mouse cranial infectious bone defect model.

DESCRIPTION OF THE EMBODIMENTS

The following describes the present invention in further detail with reference to the accompanying drawings and embodiments.

Embodiment 1: Preparation of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel

1. Preparation of methacrylic anhydride-modified gelatin hydrogel prepolymer solution: dissolve photoinitiator LAP in deionized water at 50°C to prepare a solution with a mass/volume concentration of 0.005 g/mL. Dissolve methacrylic anhydride-modified gelatin GelMA with a degree of substitution of 55–65% in the solution to prepare a prepolymer solution with a mass/volume concentration of 0.1 g/mL.

2. Preparation of CuNPs dispersion: copper nanoparticles (particle size, 10–30 nm) are separately prepared into deionized water (DI) solutions with concentrations of 20 mmol/L, 40 mmol/L, and 80 mmol/L, and the mixtures are stirred to obtain copper nanoparticle dispersions.

3. Preparation of photocrosslinked hydrogel: mix the methacrylic anhydride-modified gelatin prepolymer solution and CuNPs dispersion at a ratio of 1:1 to obtain a methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel mixture. In each group of conductive hydrogel mixtures, the mass/volume concentration of methacrylic anhydride-modified gelatin is maintained at 0.05 g/mL.

The concentrations of copper nanoparticles are 10 mmol/L, 20 mmol/L, and 40 mmol/L, respectively; after removing bubbles, add the mixed solution into a mold, irradiate with ultraviolet light for 45 s at a wavelength of 405 nm to obtain the photocrosslinked hydrogel.

Performance testing of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel

As shown in Figure 2, SEM images indicate that after the addition of copper nanoparticles, the hydrogel exhibits a loose and porous structure, providing a suitable growth space for bone cells, which can adhere and grow well, facilitating cell proliferation. Moreover, with the addition of copper nanoparticles, the pore size of the hydrogel gradually decreases, the uniformity of the pores increases, and the stability of the material continuously improves.

As shown in Figure 3, electrical performance testing demonstrates that with increasing copper nanoparticle content, the conductivity of the photocrosslinked hydrogel increases to a certain extent. The addition of 10 mmol/L copper nanoparticles can double the conductivity of the hydrogel. After the addition of copper nanoparticles, the electrical conductivity of the hydrogel is significantly improved.

Embodiment 2: Application of methacrylic anhydride-modified gelatin copper nanoparticle conductive hydrogel

1. Cell culture using methacrylic anhydride-modified gelatin copper nanoparticles: place the three synthesized hydrogels (with copper nanoparticle concentrations of 10 mmol/L, 20 mmol/L, and 40 mmol/L, respectively) into cell culture plates, digest macrophage RAW264.7 cells from the culture dish, add them to the cell culture plates containing hydrogel, and add DMEM basic medium containing 10% fetal bovine serum and 1% double antibiotics; cells grow, proliferate, and differentiate in this environment.

As shown in Figure 4, after cell culture on methacrylated gelatin copper nanoparticle hydrogel, live cell staining and CCK assay were performed. The results observed by laser confocal microscopy indicated that when 10 mmol/L copper nanoparticles were incorporated into the hydrogel, the cell morphology closely resembled that of cells grown in normal tissue. The prepared hydrogel exhibited good biocompatibility and low cytotoxicity, supporting cell adhesion and growth. When the concentration of copper nanoparticles exceeds 10 mmol/L and reaches 20 mmol/L or 40 mmol/L, certain cytotoxicity is observed.

Antibacterial experiment of methacrylated gelatin copper nanoparticle conductive hydrogel: The synthesized hydrogel (final copper nanoparticle content of 10 mmol/L) was placed in a cell well plate, and 1×10^{7} /mL suspensions of S. aureus and E. coli were inoculated into the wells, followed by culture in agar medium. After 2-4 hours, a certain amount of sample from the wells was taken, diluted to the same multiples, plated, and photographed. A certain amount of sample from the wells was taken for bacterial live/dead staining, and observed with confocal microscopy. As shown in Figure 5, the conductive hydrogel containing copper nanoparticles exhibits certain antibacterial properties.

To verify the biological effect of methacrylated gelatin copper nanoparticle conductive hydrogel, a defect was created on the right parietal bone of mice using a drill with a diameter of 2.5 mm. Then, 100 μL of suspension containing 1×10^8 CFU/mL Staphylococcus aureus was filled into the defect area, and different hydrogel materials were implanted. The experiment randomly divided the mice into the following groups: blank group, S. aureus (Staphylococcus aureus), S. aureus + CuNPS-GelMA, and S. aureus + CuNPS-GelMA (copper nanoparticle content of 10 mmol/L) + ES (ES refers to external electrical stimulation, using two needle-shaped metal electrodes connected via wires to a DS1102Z-E oscilloscope (RIGOL Technologies Co., Ltd.), providing external electric field stimulation (pulse electrical signal, 20 Hz, 100 mV mm^{-1}) at the mouse parietal bone defect site, with electrical stimulation applied for 1 hour every other day). After 4 weeks, the mice were euthanized, and cranial bone samples were collected and analyzed by Micro-CT scanning, HE staining, and Masson staining to evaluate bone regeneration efficiency. As shown in Figures 6 and 7: The results indicate that, compared with the blank group and S. aureus group, the bone repair in the defect area of the S. aureus + CuNPS-GelMA and S. aureus + CuNPS-GelMA + ES groups was significantly accelerated, demonstrating that methacrylated gelatin copper nanoparticle conductive hydrogel has a certain bone-promoting effect.

Example 3: Preparation of Methacrylated Gelatin Copper Nanoparticle Conductive Hydrogel

1. Preparation of methacrylated gelatin hydrogel prepolymer solution: Dissolve photoinitiator LAP in deionized water at 50°C to prepare a solution with a mass/volume concentration of 0.005 g/mL. Methacrylated gelatin GelMA with a degree of substitution of 55-65% is dissolved in the above solution to prepare a methacrylated gelatin hydrogel prepolymer solution with a mass/volume concentration of 0.1 g/mL.

2. Preparation of CuNPs dispersion: Copper nanoparticles (particle size, 10-30 nm) are formulated into deionized water (DI) solutions with a concentration of 20 mmol/L, and the mixture is stirred to obtain a copper nanoparticle dispersion.

3. Preparation of photocrosslinked hydrogel: The methacrylated gelatin prepolymer solution and CuNPs dispersion are mixed at a ratio of 1:1 to prepare a methacrylated gelatin copper nanoparticle conductive hydrogel mixture. In each group of conductive hydrogel mixtures, the mass/volume concentration of methacrylated gelatin is maintained at 0.05 g/mL. The concentration of copper nanoparticles is 10 mmol/L. After removing bubbles, the mixed solution is added to the mold and exposed to ultraviolet light for 60 s to obtain the photocrosslinked hydrogel.

Although the embodiments of the invention have been illustrated and described, it will be understood by those skilled in the art that various changes, modifications, substitutions, and variations can be made to these embodiments without departing from the principles and spirit of the invention, and the scope of the invention is defined by the appended claims and their equivalents.