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Application of Decellularized Bone Matrix and Chitosan Composite Scaffold in Bone Defect Repair

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Abstract: Repair materials for bone defects often have problems such as poor biocompatibility, immune rejection and unsatisfactory healing effects. How to develop a scaffold material that has good biocompatibility and can effectively promote bone regeneration has become one of the hot topics in current research. This paper tests a composite scaffold of decellularized extracellular matrix (dECM) and chitosan to explore its application in bone defect repair. First, composite scaffolds of dECM and chitosan with different ratios are prepared to optimize the structure and properties of the material. Then, the micromorphology, mechanical properties and biocompatibility of the composite scaffolds are systematically evaluated using scanning electron microscope (SEM), mechanical properties test and in vitro cell culture experiments. In the repair experiment of the mouse bone defect model, the composite scaffold with a 1:1 ratio shows the highest bone density (0.45 g/cm ^{3}) at 8 weeks. The mechanical test results show that the composite scaffold with a 1:1 ratio is superior to other groups in terms of compressive strength, tensile strength and elastic modulus, and is close to the mechanical properties of natural bone. Immunohistochemical analysis show that the expression of bone formation markers such as ALP (alkaline phosphatase) and HE (Hematoxylin and Eosin) is significantly increased in the 1:1 composite scaffold group. In the above data, the dECM and chitosan 1:1 composite scaffold shows good biological activity, mechanical properties and repair effect in bone defect repair, and has great clinical application potential.

1. Introduction

With the continuous development of modern medical technology, the treatment of bone defects has gradually shifted from traditional bone transplantation methods to the field of bone tissue engineering, especially in clinical repair, the requirements for biocompatibility, mechanical properties and degradability are constantly increasing. At present, common bone repair materials still face many challenges in terms of biocompatibility, immune rejection and healing effect. Therefore, how to develop scaffold materials that can effectively promote bone regeneration and have good biocompatibility has become one of the research hotspots. As a natural biomaterial,

dECM has high bioactivity and the characteristics of supporting bone cell growth. Chitosan has become a commonly used scaffold material due to its good biocompatibility, degradability and bioactivity. Combining dECM with chitosan can not only give play to the advantages of each of them, but also effectively improve the shortcomings of a single material, providing new ideas and solutions for bone defect repair.

This paper mainly studies the application of acellular bone matrix and chitosan composite scaffolds in bone defect repair, and systematically discusses the preparation method, structural characteristics and repair effect of composite materials in mouse bone defect models. By optimizing the ratio of dECM to chitosan, its advantages in biocompatibility and other aspects were explored. Studies have shown that dECM and chitosan composite scaffolds can effectively promote the repair of bone defects, especially in improving bone density and promoting new bone formation. In addition, this paper also conducts a detailed evaluation of the biodegradability and mechanical properties of the scaffold, providing theoretical basis and experimental support for its future clinical translation.

This paper first introduces the background of bone defect repair and the shortcomings of existing materials, and explains the research significance of dECM and chitosan composite scaffolds. Then, the experimental design and methods are described in detail, including the establishment of a mouse bone defect model, the preparation and characterization of composite scaffolds, and the setting of experimental evaluation indicators. Subsequently, the paper presents the experimental results and analyzes the effects of composite scaffolds with different ratios in bone repair. Finally, the paper summarizes the research findings, proposes directions for future research, and explores the potential of the composite scaffold in clinical applications.

2. Related Work

In recent years, research on bone tissue engineering has gradually focused on the development of biomaterials that can replace traditional bone transplants. Many previous attempts have used natural or synthetic biomaterials to make scaffolds to support bone. For example, Zhu et al. [1] elucidated the biomimetic design of scaffolds based on the transmission of bioactive factors in the bone physiological microenvironment and bone healing mechanism. In addition to thoroughly examining the benefits and drawbacks of bone tissue engineering scaffolds in terms of materials, fabrication techniques, and functions, Xue et al. [2] provided an in-depth introduction to the clinical state and difficulties of bone defect healing. Manzini et al. [3] aim to outline the roles of bone tissue and stem cells, and explore the bioactive molecules associated with these processes, which may contribute to the development of biomimetic bone substitutes and become practical alternatives in translational medicine. The purpose of bone tissue engineering is to repair and regenerate bone tissue and design bone grafts as bone substitutes. To this end, Guo et al. [4] used a variety of natural or synthetic polymers to develop polymer structures with biocompatibility and biodegradability. Lee et al. [5] summarized the performance indicators of ideal scaffolds, and divided the biomaterials of bone tissue engineering scaffolds into four categories, and elaborated on the mechanical and biological properties of each representative biomaterial. Dec et al. [6] aimed to comprehensively review the existing and new biomaterials for the treatment of bone tissue defects. The most recent developments in bone regeneration were thoroughly presented by Oliveira et al. [7], who concentrated on the use of polymer-based scaffolds for the spatiotemporal controlled administration of one or more growth factors in bone regeneration techniques. Aslam et al. [8] reviewed the latest progress of protein polymers used in cutting-edge bone tissue engineering in the past 3-5 years, focusing on the major challenges and future prospects. The above studies have solved the biocompatibility and biomechanical problems of bone repair materials to a certain extent, but there

are still problems such as poor material selectivity, insufficient functionality and imprecise control of biodegradability.

In order to solve the above problems, we are now beginning to focus on combining multiple materials to optimize their biological functions and mechanical properties. For example, Wang et al.[9]'s straightforward and inexpensive method could offer a viable approach to building an effective delivery system to fix bone abnormalities. Cui et al. [10] filled the peptide loaded hydrogel into the pores and constructed a functional scaffold with enhanced osteogenesis. Despite this, existing research still faces challenges such as insufficient optimization of material composite ratios and difficulties in clinical transformation. Therefore, this study will focus on optimizing the preparation process of ECM and shell composite scaffolds in order to overcome these shortcomings.

3. Methods

3.1 Preparation of dECM and Chitosan Composite Scaffolds

3.1.1 Preparation of acellular bone matrix

First, dECM is extracted from rat femurs. Fresh rat femurs are taken, muscles and soft tissues are removed, and the bone tissue is thoroughly washed with cold phosphate buffer to remove blood and fat. Then, a decellularization method is used to soak the bone tissue in 1% hexadecyltrimethylammonium bromide solution, and repeatedly washed to remove cell nuclei and cell residues, and finally acellular bone matrix is obtained. The decellularized bone matrix is freeze-dried to obtain bone matrix powder, which is stored in a -20 ℃ refrigerator for future use. This treatment can retain the three-dimensional structure of the bone matrix and some bioactive components, such as collagen, glycosaminoglycans, etc., providing a favorable cell scaffold environment for subsequent bone repair.

3.1.2 Preparation of chitosan solution

The chitosan solution is prepared by dissolving chitosan powder in 2% acetic acid solution. The solubility of chitosan is affected by its molecular weight and acetic acid concentration. Therefore, the viscosity and rheological properties of the solution are controlled by adjusting the concentration of acetic acid and the mass of chitosan. During the dissolution process, the solution needs to be heated to 50 ℃ under mild stirring conditions and maintained for a certain time to ensure that the chitosan is completely dissolved. The prepared chitosan solution is used for the subsequent preparation of the composite scaffold.

3.1.3 Preparation of dECM and chitosan composite scaffolds

The prepared dECM powder and chitosan solution are mixed in different mass ratios, and magnetic stirring is used to make the two fully and evenly dispersed. After being mixed evenly, the composite scaffold is prepared by electrospinning. In the specific operation, the mixed solution is loaded into a syringe, and a certain voltage difference is used to drive the solution to be sprayed onto a collection plate to form a fibrous scaffold structure. During the electrospinning process, the spray flow rate and voltage are adjusted to obtain a uniform scaffold structure with a high porosity. This scaffold has a good three-dimensional network structure and can provide a favorable microenvironment for the attachment, proliferation and differentiation of bone cells [11].

3.2 Structure and Performance Characterization

3.2.1 Microstructure characterization

Observation of the surface and pore structure of the composite scaffold through SEM not only clearly demonstrates the arrangement pattern of fibers on the surface of the scaffold, but also reveals the detailed distribution of pores. Experimental results show that different ratios of dECM and chitosan can successfully build unique three-dimensional network structures. As the proportion of dECM gradually increases, the porosity of the scaffold shows a significant increase trend, and the pore size distribution also shows a certain regularity, which provides cells with more abundant growth space and effective material exchange channels. Further analysis show that the fiber structure on the surface of the scaffold is not only dense but intricately intertwined, reflecting its superior mechanical stability. The optimized pore structure plays a crucial role in bone repair, determining the ability of cells to attach and migrate.

3.2.2 Mechanical properties characterization

The mechanical performance of bone repair scaffolds is one of the important indicators for evaluating their stability and load-bearing capacity in bone defect areas. In this study, we analyzed the mechanical properties of composite scaffolds by conducting compression and tensile tests. The results showed that with the increase of chitosan content, the elastic modulus and compressive strength of the composite scaffold were improved. However, there are significant differences in mechanical properties between dECM and chitosan composite scaffolds with different proportions [12]. Figure 1 shows the mechanical performance under various ratios.

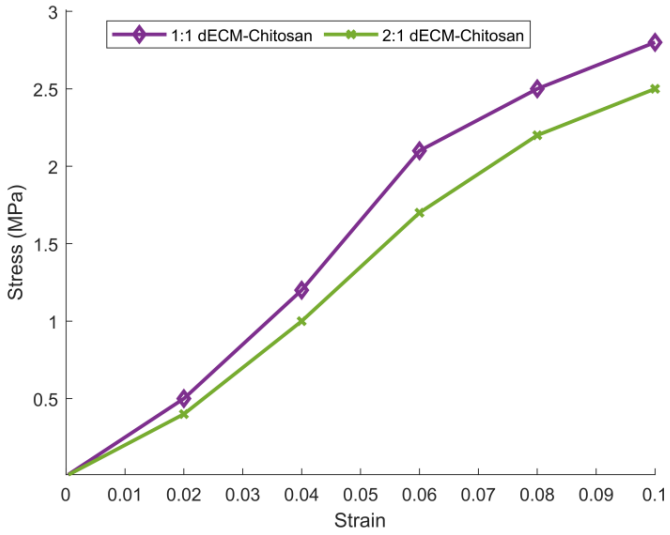

Figure 1: Tensile stress-strain curve

In Figure 1, the composite scaffold with a 1:1 ratio performs the best, with mechanical properties close to natural bone, and can effectively withstand loads. Studying scaffolds can better simulate the characteristics of natural bone tissue, with excellent load-bearing capacity and structural stability. These characteristics make it an ideal scaffold material for bone defect repair, which can effectively support the formation of new bone and the overall repair process.

3.3 Introduction to Cell Culture

3.3.1 Cell sources and culture conditions

The mechanical properties of bone repair scaffolds are one of the important indicators to evaluate their stability and load-bearing capacity in the bone defect area. In this study, we analyzed the mechanical properties of the composite scaffolds by performing compression and tension tests. The results showed that the elastic modulus and compressive strength of the composite scaffolds increased with the increase of chitosan content. However, there were significant differences in the mechanical properties of composite scaffolds with different ratios of dECM and chitosan [13]. The mechanical property curves in Figure 1 show the mechanical performance under various ratios.

3.3.2 Scaffold material preparation and cell seeding

First, the prepared dECM and chitosan composite scaffolds are sterilized. After the scaffolds are soaked in 70% ethanol for 30 minutes, they are placed under ultraviolet light for 2 hours for disinfection to ensure their sterility. Subsequently, the sterilized scaffolds are placed in a sterile culture dish and pre-moistened with DMEM culture medium for 24 hours to simulate the hydration in the actual in vivo environment. Next, MC3T3-E1 cells are inoculated on the surface of the scaffolds, with approximately 1×10^4 cells inoculated on each scaffold surface, and incubated in an incubator for 48 hours to observe the cell attachment.

3.3.3 Cell proliferation and activity detection

To evaluate cell proliferation and biocompatibility, this paper uses the MTT method (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) to detect cell activity. Samples are collected at a time point every 48 hours (day 1, day 3, day 5), and MTT solution is added to each well. After 4 hours of incubation, DMSO (Dimethyl Sulfoxide) is added to dissolve the reaction product, and finally its absorbance (Optical Density, OD value) is measured by a microplate reader. The rate and activity of cell proliferation can be judged by measuring the change in absorbance. A higher OD value indicates a stronger cell proliferation activity.

The results show that with the extension of culture time, the cells show good adhesion growth and expansion on the surface of the composite scaffold, especially on the composite scaffold with a 1:1 ratio, the number and morphology of cells change most significantly, indicating that the scaffold has a good supporting effect on cell growth.

4. Results and Discussion

4.1 Experimental Analysis

4.1.1 Experiment on evaluation of repair effect of mouse bone defect model

In the experiment to evaluate the repair effect of the mouse bone defect model, the effect of different ratios of dECM and chitosan composite scaffolds on the repair of mouse bone defects is evaluated, focusing on observing the bone healing in the bone defect area.

(1) Experimental grouping

The groups are blank control group (no scaffold implanted), dECM group, chitosan group, and dECM and chitosan composite scaffold group (different ratios, such as 1:1, 2:1). The number of samples in each group is 6 mice. The experimental time point is every two weeks after 0 weeks (immediately after surgery).

(2) Experimental steps

Establishment of mouse bone defect model: a certain area of bone tissue is removed from the femur of the mouse to form a bone defect. Scaffold implantation: dECM and chitosan composite scaffolds with different ratios are implanted in the defect area.

(3) Evaluation indicators

X-ray imaging involves regularly taking X-ray photos to quantitatively analyze the healing status of bone defect areas. Bone density analysis uses X-ray image analysis software to calculate the bone density of the bone defect area and evaluate the progress of bone repair. Histological analysis involves extracting bone tissue at different time points for HE staining and immunohistochemical analysis. The specific data is shown in Figure 2:

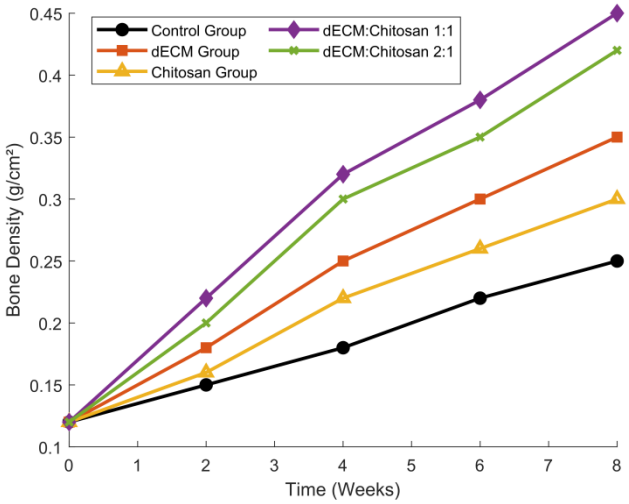

Figure 2: Evaluation of repair effect in mouse bone defect model

This study evaluates the effects of different ratios of dECM chitosan composite scaffolds in bone defect repair in mice. Experimental results show that all scaffold groups could promote the healing of bone defects, but the repair effect of the dECM and chitosan composite scaffold group is significantly better than that of the single material group. In particular, the 1:1 ratio composite scaffold show the highest bone density (0.45 g/cm ³) at 8 weeks, which is significantly higher than the blank control group (0.25 g/cm ³) and other individual material groups (such as the dECM group 0.35 g/cm ³, chitosan group 0.30 g/cm ³). These results show that the dECM and chitosan composite scaffold can effectively promote bone regeneration and has potential application value, especially in promoting bone defect repair.

4.1.2 Biomechanical testing experiments

The biomechanical testing experiment tested the mechanical properties of dECM and chitosan composite scaffolds with different ratios in bone defect repair, especially their compressive strength, tensile strength, and elastic modulus under mechanical loads, to verify the potential application of the scaffold in bone repair.

(1) Experimental grouping

The experimental grouping is the same as the above-mentioned experiment 1. The experimental time is to test the mechanical properties of each group of samples before and after the experiment to observe the mechanical changes of the materials.

(2) Experimental methods

Preparing each group of scaffolds and cutting them into standardized specimens (e.g., cubes or

cylinders) according to the size and shape of the scaffolds. Compression test and tensile test are performed, and each scaffold sample is tested using a universal material testing machine (such as Instron).

Compression test: evaluating the compressive strength of the scaffold under external force. Tensile test: evaluating the tensile strength and elongation of the scaffold. Elastic modulus: calculating the stiffness of the scaffold, that is, the ratio of stress to strain of the material during tension or compression. At least 6 repeated experiments are performed for each group to ensure the reliability of the data. Table 1 is the mechanical properties of each material measured at different time points:

Table 1: Biomechanical tests

Group	Compression Strength (MPa)	Tensile Strength (MPa)	Elastic Modulus (MPa)
Control Group	1.5	2.3	50
dECM Group	2	3	60
Chitosan Group	1.8	2.8	55
dECM & Chitosan 1:1 Group	2.5	4	70
dECM & Chitosan 2:1 Group	2.2	3.5	65

In Table 1, the dECM and chitosan 1:1 composite scaffold showed significant advantages in compressive strength and other aspects. Specifically, the compressive strength of the composite stent is 2.5 MPa, the tensile strength is 4.0 MPa, and the elastic modulus is 70 MPa, which are significantly higher than other groups. In the conclusion of the above data, when dECM and chitosan are 1:1, the composite scaffold has better mechanical properties, can better simulate the mechanical properties of natural bone tissue, and has the potential to be used in clinical applications for bone defect repair.

4.1.3 Histological analysis and immunohistochemical staining experiments

The effects of different ratios of dECM and chitosan composite scaffolds in the repair of bone defects in mice are evaluated by histological analysis and immunohistochemical staining, the bone tissue regeneration and the expression of related proteins are observed, and the effect of the scaffold on bone healing is analyzed.

(1) Experimental groups

The experimental grouping is the same as the above experiment.

(2) Organizational analysis

Using HE staining to observe the tissue repair in the bone defect area and evaluate the formation of new bone. Observing the bone tissue structure, cell proliferation, and differentiation in the repair area.

(3) Immunohistochemical staining

Analyzing the differences in bone formation among different groups by detecting the expression of bone formation markers through immunohistochemical staining. Selecting appropriate antibodies, stain osteogenic proteins, and evaluating their expression levels in different groups, as shown in Figure 3:

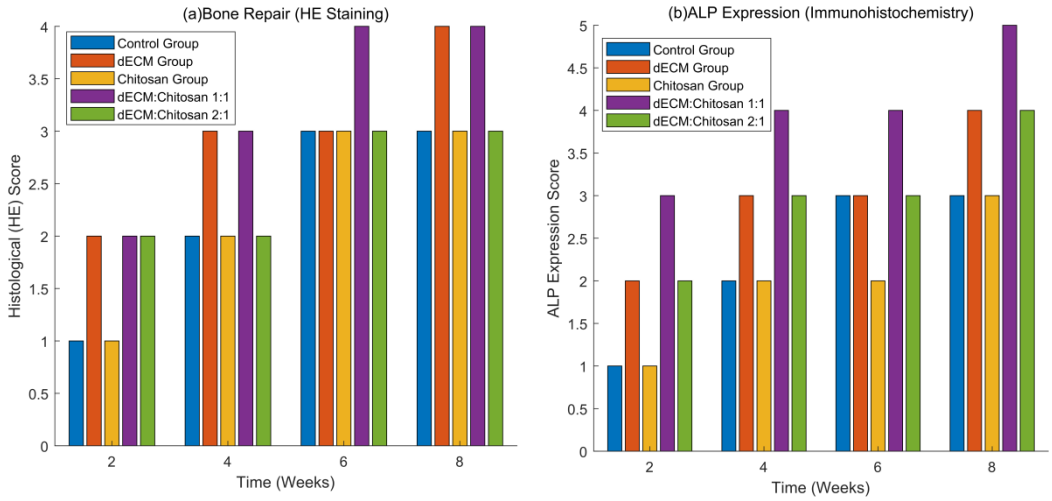

Figure 3: Histological analysis and immunohistochemical staining evaluation

Figure 3(a-b) HE staining score and ALP expression score respectively. According to the histological score (HE staining), at 8 weeks, the repair score of the 1:1 composite scaffold group reaches 4 points, which is higher than the blank control group and chitosan group which are 3 points. Immunohistochemistry results show that the 1:1 composite scaffold group also shows a significant increase in ALP expression, with a score of 5 at 8 weeks, which is significantly higher than other groups. In the above data conclusions, the dECM chitosan 1:1 composite scaffold has good potential in bone formation, exhibiting high biological activity and repair ability.

4.2 Experimental Discussion

This study verifies the effectiveness of dECM and chitosan composite scaffolds in bone defect repair through three experiments. The 1:1 ratio shows significant advantages, providing similar mechanical properties to natural bone, such as compressive strength and elastic modulus, providing strong support for repair. Histological and immunohistochemical analyzes further revealed increases in bone formation markers. Overall, the 1:1 dECM chitosan scaffold has strong biological and mechanical properties and shows great potential in clinical applications in bone defect repair.

5. Conclusion

This paper proposes a new type of bone repair scaffold by studying the application of dECM and chitosan composite materials in bone defect repair. In the above experimental conclusions, dECM and chitosan composite materials not only have good biocompatibility, but can also effectively promote the proliferation and differentiation of bone cells and improve the repair effect of bone defects. This composite material exhibits significant bone regeneration ability and possesses good mechanical properties and biodegradability, with broad clinical application potential. However, this paper also has certain shortcomings, mainly reflected in the lack of further research on the long-term in vivo degradation performance and immunogenicity of composite materials. In addition, the production process and stability of the stent need to be further optimized before large-scale clinical application. Future research can be carried out in the following directions: on the one hand, further exploring the immune response and long-term biodegradation process of the material to ensure its safety and effectiveness in the body; on the other hand, improving the design of the scaffold to enhance its repair ability in complex bone defect environments and promote its clinical transformation.

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References

[1] Zhu G, Zhang T, Chen M, et al. Bone physiological microenvironment and healing mechanism: Basis for future bone-tissue engineering scaffolds[J]. Bioactive materials, 2021, 6(11): 4110-4140.
[2] Xue N, Ding X, Huang R, et al. Bone tissue engineering in the treatment of bone defects[J]. Pharmaceuticals, 2022, 15(7): 879-884.
[3] Manzini B M, Machado L M R, Noritomi P Y, et al. Advances in Bone tissue engineering: A fundamental review[J]. Journal of biosciences, 2021, 46(4): 1-18.
[4] Guo L, Liang Z, Yang L, et al. The role of natural polymers in bone tissue engineering[J]. Journal of Controlled Release, 2021, 338(5): 571-582.
[5] Lee S S, Du X, Kim I, et al. Scaffolds for bone-tissue engineering[J]. Matter, 2022, 5(9): 2722-2759.
[6] Dec P, Modrzejewski A, Pawlik A. Existing and novel biomaterials for bone tissue engineering[J]. International Journal of Molecular Sciences, 2022, 24(1): 529-535.
[7] Oliveira É R, Nie L, Podstawczyk D, et al. Advances in growth factor delivery for bone tissue engineering[J]. International journal of molecular sciences, 2021, 22(2): 903-915.
[8] Aslam Khan M U, Aslam M A, Bin Abdullah M F, et al. Current perspectives of protein in bone tissue Engineering: bone structure, Ideal scaffolds, fabrication techniques, applications, Scopes, and future advances[J]. ACS Applied Bio Materials, 2024, 7(8): 5082-5106.
[9] Wang X, Yu T, Chen G, et al. Preparation and characterization of a chitosan/gelatin/extracellular matrix scaffold and its application in tissue engineering[J]. Tissue Engineering Part C: Methods, 2021, 23(3): 169-179.
[10] Cui Y, Wang J, Tian Y, et al. Functionalized decellularized bone matrix promotes bone regeneration by releasing osteogenic peptides[J]. ACS Biomaterials Science & Engineering, 2023, 9(8): 4953-4968.
[11] Zhou B, Jiang X, Zhou X, et al. GelMA-based bioactive hydrogel scaffolds with multiple bone defect repair functions: therapeutic strategies and recent advances[J]. Biomaterials Research, 2023, 27(1): 86-95.
[12] Hosseini S J, Parsaei H, Moosavifar M J, et al. Static magnetic field enhances the bone remodelling capacity of human demineralized bone matrix in a rat animal model of cranial bone defects[J]. Journal of Materials Chemistry B, 2024, 12(15): 3774-3785.
[13] Shen Y, Xu Y, Yi B, et al. Engineering a highly biomimetic chitosan-based cartilage scaffold by using short fibers and a cartilage-decellularized matrix[J]. Biomacromolecules, 2021, 22(5): 2284-2297.