Research Article

Biomimetic Design and Biocompatibility of Biomimetic Calcium Carbonate Nanocomposites for Skeletal Muscle Injury Repair

Yan Han

College of Physical Education, Xuchang University, Xuchang, 461000 Henan, China

Correspondence should be addressed to Yan Han; 22014015@xcu.edu.cn

Received 15 March 2022; Revised 19 April 2022; Accepted 28 April 2022; Published 20 May 2022

Academic Editor: Awais Ahmed

Copyright © 2022 Yan Han. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

As an important part of the human body, skeletal muscle is easily damaged in the process of exercise. The purpose of this study is to investigate the controllable construction and biocompatibility of biomimetic calcium carbonate nanocomposites in exercise skeletal muscle repair. In this study, adult healthy mice were selected as the research objects. First, the mice were anesthetized with 2.5% pentobarbital sodium. Then, the mice were fixed and hit with steel balls on the middle part of the gastrocnemius muscle abdomen to establish a skeletal muscle contusion model. The mice were killed by cervical dislocation under anesthesia. The injured gastrocnemius muscles were quickly removed to prepare the cell suspension. 0.8600 g chitosan was weighed and dissolved in 200 ml deionized water. Then, 0.0660 g of calcium chloride dihydrate was added and stirred to dissolve to prepare chitosan calcium carbonate composite. The calcium carbonate composite material with a length of 8.0 cm is cut into a square sheet of stainless steel. The stainless steel plate was sterilized and then placed in a 12 culture plate to dilute the full cells in the culture bottle. The cells were seeded in 12 well culture plates, and 100 μL cells and 600 μL medium were added to each well. The samples were cultured in the incubator (there were three control holes in each group for SEM test, three control holes for fluorescence measurement, and six control holes for MTT measurement). The plates were taken out and placed in a new 24 plate. 1000 μL medium was added. Then, 100 μL MTT solution was added into each well. The biocompatibility test was conducted for 4 h. The GSH PX level in the chitosan calcium carbonate composite group was lower than that in the natural recovery group (P < 0.01). The results showed that chitosan calcium carbonate composite could promote the growth of skeletal muscle cells.

1. Introduction

The research on biomimetic materials has long been a hot-spot in materials research. In recent years, biomimetic calcium carbonate nanocomposites have attracted more and more attention because of their special structure and good properties. On the basis of optimizing the material structure, the main purpose is to obtain a kind of composite bionic material with strong combination of organic and inorganic energy. On the other hand, through the biomimetic process, the ultimate goal is to make the mechanical properties of the composite materials obtained through formula adjustment controllable. The preparation methods include self-assembly, spin coating, chemical deposition, and compression molding. Bionic film and molding materials were obtained. In recent years, some people have obtained linear long materials with simulated structures using graphene as raw materials. Although the injured skeletal muscle has a strong regenerative capacity, severe trauma, such as loss of muscle tissue, exceeds the natural and complete recovery capacity of skeletal muscle, the clinical treatment has limited effect, and there is no satisfactory bone grafting program at present. Autologous bone is regarded as the “gold standard” of bone defect replacement material because of its excellent osteogenic properties, osteogenic induction, bone electrical conductivity, complete biocompatibility, nontoxicity, and no immune problems. Generally, with a history of trauma or overwork and fatigue, symptoms show localized muscle pain and tenderness.

Many reasons such as trauma and skull tumors can cause skull defects. Skull defects endanger the health of patients, so the repair of the defects is essential. At present,
repair materials include autologous bone, allogeneic bone, and artificial materials. Autogenous bone repair is the best, but its supply is insufficient and its application is limited. Allogeneic bone has severe rejection, which hinders its application. There are many kinds of artificial materials, such as hydroxyapatite and bioceramics, but they have serious side effects and are gradually eliminated. Currently, titanium alloy materials are the most widely used, with good biocompatibility, stable physical and chemical properties, and easy shaping. Carbon fiber has good mechanical properties and biocompatibility, which can improve the mechanical strength of other materials. Bioinert ceramics mainly refer to ceramic materials with stable chemical properties and good biocompatibility, such as alumina, zirconia, and medical carbon materials. The structure of these ceramic materials is relatively stable, the bonding force in the molecule is strong, and they all have high strength, wear resistance, and chemical stability.

Calcium carbonate (CaCO3) nanoparticles have barrier properties and biodegradability effects on polylactic acid (PLA). Afraimeh et al. prepare nanocomposite membranes with various CaCO3 nanoparticle contents (0, 3, 5, 10, and 15 wt%) by solution casting method. He uses constant volume and variable pressure equipment to evaluate the permeability of nitrogen (N2), oxygen (O2), and carbon dioxide (CO2) at different pressures and temperatures. In addition, he also observed that the gas permeability of the sample decreased by increasing the feed pressure and increased by increasing the temperature. There are too few experimental data in his research [1]. Acar et al. compared the effect of coffee dyeing on the color of 3 different CAD/CAM restoration materials and nanocomposite resins. He evaluated the color change (n = 5) of samples mixed with dental ceramics (VITA Enamic) and nanocomposite resins (Filtek Supreme Ultra Universal) due to thermal cycling. He thermally cycled samples with thicknesses of 0.5 to 0.7 mm and 1 to 1.2 mm for 5000 cycles. The color coordinates obtained from the spectroradiometer are used to calculate the CIEDE2000 color difference (AE00) due to thermal cycling in the coffee. Use ANCOVA to analyze the color difference between the thicknesses as covariates between materials. He used the Tukey-Kramer test to analyze the significant difference in average thickness. His research lacks comparative experiments [2]. Nguyen et al. completed the preparation and characterization of modified and unmodified polypropylene (PP-) wood powder (WP) composites under fixed processing conditions. He used different techniques to study the influence of the size and content of wettable powders and the content of compatibilizers on the properties of composite materials. Scanning electron microscope micrographs show that the distribution of WP in the PP matrix is relatively uniform. His research sample is too small [3]. De France et al. believe that although injectable hydrogels have multiple advantages in biomedical applications, their generally weak mechanical properties often limit their applications. He described that in situ gelling nanocomposite hydrogels based on poly(oligoethylene glycol methyl methacrylate) (POEGMA) and rigid rod-shaped cellulose nanocrystals (CNC) can overcome this challenge. By physically incorporating CNCs into the cross-linked POEGMA hydrogel, he can easily customize the macroscopic properties, including gelation rate, swelling kinetics, mechanical properties, and hydrogel stability. The strong adsorption of the aldehyde and hydrazide modified POEGMA precursor polymer on the surface of CNCs can promote the uniform dispersion of CNCs in the hydrogel and give physical cross-linking in the entire network. His research is not novel enough [4].

In this study, adult healthy mice were selected. First, the mice were anesthetized with 2.5% sodium pentobarbital, and the mice were fixed and then hit the middsections of the bilateral gastrocnemius muscles with steel balls to create skeletal muscle blunt contusion models. The mice were killed by cervical dislocation under anesthesia, and the injured gastrocnemius muscles of both sides were quickly taken to prepare the cell suspension. Weigh 0.8600 g of chitosan and dissolve it in 200 mL of deionized water. Then add 0.0660 g calcium chloride dihydrate, stir to dissolve, and prepare chitosan calcium carbonate composite material. The chitosan-calcium carbonate composite material prepared by using a stainless steel sheet as a base is cut into a square with a side length of 0.8 cm. Sterilize the stainless steel sheet and place it in a 12-well culture plate to dilute the overgrown cells in the culture flask. The cells were seeded in a 12-well culture plate, and 100 μL of cells and 600 μL of culture medium were added to each well. Incubate in an incubator (including three control holes for each group of samples when doing SEM test, three control holes for each group of samples when measuring fluorescence, and six control holes for each group of samples when measuring MTT). Ceramic artificial bone is the use of bio ceramics as a repair material for bone tissue defects or deletions and uses it to form a mesh scaffold structure to promote and guide bone tissue.

2. Skeletal Muscle Injury Repair Experiment

2.1. Bionic Materials. Inspired by biological materials, we use the principles of bionics to study the formation mechanism of biological materials and apply them to the synthesis of new materials in the future, to be able to design and synthesize organic materials, inorganic materials, organic-inorganic composite materials, functional, and even smart materials; it is a research hotspot that has developed rapidly in recent years and has developed into a popular research in multiple disciplines and fields such as chemistry, materials, and life sciences [5, 6]. Calcium carbonate material is loaded with rare earth metals or other functional components, which can realize the functions of comprehensive diagnosis, treatment or diagnosis and treatment, and is used for imaging diagnosis and treatment [7, 8]. The minimum energy parameter is expressed as follows:

$$E_{\text{snake}} = \int_0^1 EV(s)ds = \int_0^1 \left( E_{\text{int}}V(s) + E_{\text{imag}}V(s) + E_{\text{cim}}V(s) \right) ds.$$  \hspace{1cm} (1)

Among them, it can represent the internal energy of a
2.2. Nanomaterials. With the continuous progress and development of science and technology, nanotechnology is also constantly improving. Nowadays, nanotechnology can be seen everywhere in people’s daily life and is applied to all aspects of life. Many nanotherapeutic drugs in clinical and preclinical stages are polymer nanoparticles, which have been widely studied as therapeutic carriers. The design of polymer nanoparticles is based on their biocompatibility and biodegradability [17]. Most of these nanoparticles are formed by the self-assembly process of block copolymers, which are composed of two or more polymer chains with different hydrophilic properties [18, 19]. The resulting structure is very suitable for drug delivery. The hydrophobic core can carry a large number of therapeutic drugs, and the hydrophilic shell provides three-dimensional protection for the nanoparticles [20]. Used to express the compressive resistance of polymer nanomaterials, including

\[ \eta(X, \mu) = \frac{1}{2\pi^{1/2}|m|} e^{-1/2(X-\mu)\Sigma(X+\mu) + \mu K}. \]  

Among them, the larger the molecule, the stronger its cohesion [21, 22]. Other widely studied nanodrug delivery systems include metal nanoparticles. Metal-containing nanoshells are usually composed of inert metals (such as gold or titanium) and are used to control the release of chemotherapeutic drugs. These systems have unique characteristics [23]. Although these metal ions are inert and biocompatible, they will remain in the body in large quantities after administration, and the accumulation of metal particles formed by repeated administration may cause toxicity [24, 25]. For nanomedicine, its therapeutic effect is better, and the toxicity is lower [26]. In fact, the progress of nanomedicine is rapidly applied to clinical practice [27, 28].

2.3. Nanomaterial Preparation Experiment. PBT is milky white translucent to opaque, semicrystalline thermoplastic polyester, with high heat resistance, toughness, fatigue resistance, self-lubricating, low friction coefficient, not resistant to strong acid and alkali, resistant to organic solvents, flammable, and decomposed at high temperature [29]. Dilute the prepared nano-CaCO3 suspension with deionized water, and observe the particle dispersion in the solution with a transmission electron microscope. Take a part of the nano-CaCO3 suspension after multiple washing and drying, and then grind to obtain CaCO3 particles, which are combined with the initial nano-CaCO3 particles, and cellulose ether dispersant was analyzed by infrared spectroscopy together. The extruded strip was brittle under liquid nitrogen conditions, and the section was vacuum-plated. The morphology of the section was observed with a scanning electron microscope (SEM), and the accelerating voltage was 20 kV. For PC/Tinano2CaCO3 samples with the same thermal history. The pure PC sample is analyzed by gel permeation chromatography, the column PG1gel is 5 μm, tetrahydrofuran is selected as the solvent, the test temperature is \( T = 35^\circ\text{C} \), and PS is used as the standard sample. The sample is injected, the tensile performance is in accordance with the GB/T104021992 standard, and the bending performance is in accordance with the GB/T1934121998 standard. The impact performance is tested according to the GB/T183421996 standard.

After the resin matrix is filled with nano-CaCO3, the tensile strength and flexural strength have been improved to different degrees. The impact strength of PP composites has increased, and the impact strength of PBT and PC composites has decreased slightly. Combined with the GPC results, it can be considered soft, method does not cause mechanical and thermal degradation of the system matrix when preparing polymer \( \Pi \) nanoparticle composites, and is a new method for preparing nanocomposites of polar and nonpolar polymers. Especially for PP systems, the processing method can only rely on the addition of nanoparticles, which can simultaneously play a role in strengthening and toughening. Scanning electron microscopy photos of nanoporous silicon: a side view and by top view, as shown in Figure 1.


2.5. Selection of Experimental Subjects. The animals used in this experiment are adult healthy mice, a total of 96 mice, weighing 2.6-3.0 kg, and are half male and female. The food and activities of the experimental animals were good, the skin and mucous membranes were intact, and the epiphyses were closed. The experimental equipment is shown in Table 1.

2.6. Skeletal Muscle Blunt Injury Model. Skeletal muscle injuries are an important component of trauma in sports...
medicine, with injury rates ranging from 10% to 55%. Because treatment failure can delay an athlete’s return to play, which can be weeks or months, and lead to recurrence, the management of musculoskeletal injuries requires extreme measures. A simple and reproducible blunt skeletal muscle modelling method is adopted. After the mice were anesthetized by intraperitoneal injection of 2.5% sodium pentobarbital, they were fixed at the knee joint extension at 0° and the ankle joint extension at 90°. A solid stainless steel ball with a mass of 16.8 g and a diameter of 15.9 mm was placed on the top of a transparent tube. (Height 100 cm, inner diameter 16.0 mm) after free fall, it hits the striking device vertically. The bottom of the striking device hits the middle of the abdominal muscles of the mouse bilateral gastrocnemius muscles. The striking position is fixed (the striking area is 20 mm²), the skin is intact after injury, there is no tibiofibular fracture, and the anatomy confirmed that the damage rate was 100%. 

2.7. Animal Selection and Weighing. The genes of mice are very similar to those of humans. The mice are mammals, and their genomes are very similar to those of humans. The effect of using them in genetic experiments is very good, and the cause can be found through experiments on mice, to study the regularity of disease and treatment methods. Strictly according to the time point of the experimental grouping, the mice were killed by cervical dislocation under anesthesia. The injured gastrocnemius on both sides was quickly taken. The injured gastrocnemius muscle of the same part was taken in the control group, and the material was fixed. The excess connective tissue was carefully removed and rinsed with 4°C physiological saline. The filter paper was soaked and weighed, and the muscle weight/body weight was used to reflect the relative change of muscle wet weight. Among them, the right gastrocnemius of each group was made into paraffin specimens for histomorphological examination, part of the left gastrocnemius was tested immediately by flow cytometry, and the other was placed in liquid nitrogen for western blotting.

2.8. Flow Cytometric Detection of Skeletal Muscle Tissue. The fresh gastrocnemius tissue (n = 6/group) of 1 d and 3 d after blunt skeletal muscle injury was rinsed with PBS and cut into small pieces of about 1-2 mm³, and 30 times the amount of enzyme solution (0.1% trypsin mixed with 0.02% EDTA + 0.1 μg/mL collagenase), transfer the pipette to the erlenmeyer flask, place it in a 37°C incubator for 2 h, shake once every 5-10 min, and pass the digestion solution through 300 mesh filter through a strainer, centrifuge at 300 g for 5 minutes, and discard the supernatant. Wash with PBS solution containing 0.1% BSA by centrifugation for 1-2 times; adjust the cell concentration to 1 × 10⁶/mL with PBS solution containing 0.1% BSA. Add 3 μL CD11b antibody (PE labeled), 10 μL F4/80 antibody (FITC labeled), incubated for 30 minutes at room temperature in the dark, rinse with sterile PBS solution for 1-2 times, add 200 μL 0.5% sterile paraformaldehyde (PFA) solution for fixation, and flow cytometry detects the number of macrophages.

2.9. Preparation of Chitosan Calcium Carbonate Composite. Ethanol is an important organic solvent and is widely used
in medicine, paint, sanitary products, cosmetics, oils, and fats, accounting for about 50% of the total consumption of ethanol. Ethanol is an important basic chemical raw material. The cut stainless steel sheet was sonicated in absolute ethanol and ultrapure water for 5 minutes to remove oil stains and impurities on the surface and then dried naturally in a nitrogen atmosphere for later use. Weigh 0.8600 g of chitosan and dissolve it in 200 mL of deionized water, adjust the pH to 5.0 with a molar concentration of 2 M HCl solution, and then adjust the pH to 5.0 after stirring for 12 hours. Then take 50 mL of the above solution in a beaker, then add 0.0660 g calcium chloride dihydrate (that is, the final concentration is 9 mM), and stir to make it dissolved. Then add 0.0711 g of ammonium bicarbonate (that is, the final concentration is 18 mM), stir to dissolve. Then use the above solution as the mother solution for electrochemical deposition in a three-electrode system (reference electrode: Ag- AgCl electrode; counter electrode: Pt wire electrode; working electrode: stainless steel sheet). The stainless steel sheet obtained after the electrodeposition process is gently washed in distilled water and air-dried naturally in a clean and dry environment.

2.10. Cell Culture

(1) Cell culture refers to a method that simulates the in vivo environment (sterility, suitable temperature, pH, certain nutrient conditions, etc.) in vitro to make it survive, grow, reproduce, and maintain its main structure and function. Cut the bare stainless steel sheet and the chitosan-calcium carbonate composite material prepared with the stainless steel sheet as a base into a square with a side length of 0.8 cm

(2) Sterilize the front and back sides of the cut film with alcohol and UV, then place it in a 12-well culture plate, digest the overgrown cells in the culture flask, and then dilute. The cells were seeded in a 12-well culture plate, and 100 μL of cells and 600 μL of culture medium were added to each well. Incubate in an incubator with a content of 5% CO2 at 37°C (wherein, each group of samples is set with three control holes when doing SEM test, and each group of samples is set with three control holes when measuring fluorescence, and when measuring MTT, each group of samples is set 6 control wells)

2.11. Cytotoxicity Test. The CCK-8 method was used to study the toxicity of chitosan calcium carbonate composite materials on cells. The skeletal muscle cells in the logarithmic growth phase were seeded in a 96-well plate at a density of 8 × 103/well, grown adherently for 4h, then added a medium mixture containing different concentrations of chitosan calcium carbonate composite material (final concentration 5, 25, 50, 150, and 300 μg/mL), set a medium without nanoparticles as a control group, and continue to culture for 48h. After removing the nanoparticle medium and mixing the treatment solution, add 10 μL CCK-8 solution to each well. A microplate reader (Thermo, USA) was used to detect the absorbance at a wavelength of 450 nm, and the reference wavelength was 630 nm. Finally, the cell viability (%) was calculated according to the experimental data. Repeat the experiment three times.

2.12. Cell Fluorescence Microscopy Characterization. First, take out the composite material for cell culture from the culture plate on the first 1, 3, 5, 7, and 9 days, and wash it with 0.1 mol/L. PBS buffer three times, each washing for 2 s. Then fixed with 1% paraformaldehyde, and then add 1000 μL PBS and 50 μL, 0.1 mg/mL DAPI dye to each well for staining. Then continue to incubate in an incubator for 2 hours and rinse with PBS 3 times for a few seconds each time, and finally observe with a fluorescence microscope.

2.13. Biocompatibility Test of Composite Materials. First, take out the composite material for cell culture from the culture plate on days 1, 3, 5, 7, and 9. Then take out the slides, place them in a new 24-well culture plate, add 1000 μL of culture medium, then add 100 μL of MTT solution to each well, continue to incubate for 4h, wait until the culture solution is aspirated, and then add DMSO solution (1000 μL/well). Finally, 150 μL of solution is extracted from each well in the 96-well plate, the wavelength of 490 nm is selected, and the a value of each well is detected on the microplate reader.

2.14. Data Statistics. Statistical analysis method: in all experimental data, the measured data is represented by X ± S, and the comparison between and within groups is analyzed by single factor variance. Paired comparisons between groups were carried out by LSD test, and there were statistical differences when P ≤ 0.05.

3. Skeletal Muscle Injury Repair

3.1. Cell Detection and Analysis. Chemiluminescence method for cell viability test: ATP adenosine triphosphate combines adenine, ribose, and 3 phosphate groups. It releases more energy when hydrolyzed and is the most direct source of energy in the body. The ATP luminescence method analyzes cell activity and proliferation by detecting the content of ATP in the cell. The common ATP luminescence method uses exogenous firefly luciferin/luciferase to oxidize with intracellular ATP to generate photons and monitor the luminosity to detect ATP content to analyze cell activity and proliferation. The vitality value of the normal control group (A) was 55.5429, and it increased significantly on the 3rd day after injury (B); after the intervention of chitosan, calcium carbonate composite material, on the 5th day after injury, the chitosan calcium carbonate composite material group (C). The level of GSH-Px was significantly lower than that of the natural recovery group (D) (P < 0.01); on the 10th day after injury, the GSH-Px expression level of the chitosan calcium carbonate composite material group (E) was also significantly lower than that of the natural recovery group (E). Group (F) (P < 0.05), suggesting that the chitosan calcium carbonate composite material promotes the repair of injury. The cell detection results are shown in Table 2. It can be observed from Table 2 that the GSH-Px of normal rats is at a low level, and it rises rapidly after injury, significantly increases on the
3rd day after injury, and then begins to decline, and after the intervention of chitosan, calcium carbonate composite material, after injury on the 5th day, the GSH-Px level of the chitosan calcium carbonate composite group was lower than that of the natural recovery group (P < 0.01); during the decline process, the chitosan calcium carbonate composite group decreased faster than the natural recovery group. The difference was statistically significant (P < 0.01). The vitality value of the normal control group (A) was 195.1150, and it increased significantly on the 3rd day after injury (B); after the intervention of chitosan, calcium carbonate composite material, on the 5th day after injury, the chitosan calcium carbonate composite material group (C), the expression level of MPO was significantly lower than that of the natural recovery group (D) (P < 0.01); on the 10th day after injury, the MPO level of the chitosan calcium carbonate composite group (E) was significantly lower than that of the natural recovery group (F) (P < 0.01). The MPO of normal mice was at a low level, and it increased rapidly after injury. It increased significantly on the 3rd day after injury and then began to decrease. After the intervention of chitosan, calcium carbonate composite material, on the 5th day after injury, the chitosan calcium carbonate MPO level of the composite material group was lower than that of the natural recovery group (P < 0.01); during the decline process, the chitosan calcium carbonate composite material group decreased faster than the natural recovery group, and the difference was statistically significant (P < 0.01). Compared with the normal control group, the chitosan calcium carbonate composite material and the natural recovery group had significant expression of skeletal muscle cell mRNA (P < 0.05); after the intervention of the chitosan calcium carbonate composite material, the skeletal muscle cell mRNA expression level of the natural recovery group (D) was 1.45 times that of the chitosan calcium carbonate composite material group (C), and the difference was significant (P < 0.01); on the 10th day after injury, the mRNA expression level of skeletal muscle cells was natural. The recovery group (E) was 1.32 times that of the chitosan calcium carbonate composite material group (F), and the difference was significant (P < 0.05).

3.2. MTT Test Analysis of Skeletal Muscle Cells. The density of cells on the surface of the Chi-CaCO3NWs/SS composite material during different culture times is significantly smaller than that of the cells on the surface of the Chi-CaCO3NFs/SS composite material, indicating that ChiCaCO3NFs/SS can provide a good environment for cell growth and spreading, and is also conducive to further proliferation and differentiation of cells. The MTT test analysis result is shown in Figure 1. To further illustrate the good biocompatibility of the ChiCaCO3NFs/SS composite material, we conducted a comparative test of cell culture on a blank stainless steel plate. The density of the cells on the surface of the stainless steel plate is significantly higher than that of the Chi_CaCO3NWs/SS and Chi-CaCO3NFs/SS composite materials. It is much smaller, that is, the growth of cells on the surface of the chitosan-calcium carbonate composite material is clearly more vigorous than that on the blank stainless steel sheet, and this advantage becomes more obvious with the extension of the culture time. It shows that the composite material has a good promotion effect on cell growth. In addition, to further clarify the effect of the composite material on cell growth and understand the growth of MC3T3 cells on the surface of the composite material, we also performed the MTT test to quantitatively observe the difference between the two. The MTT test can indirectly but quantitatively reflect the number of cells.

It can be seen from Figure 2 that the activities of the three groups of cells are increasing with the extension of the culture time, but the Chi-CaCO3NWs/SS composite material and Chi-CaCO3NFs/SS cell activity increase of the chitosan-calcium carbonate nanocomposite is very obvious, indicating that the chitosan-calcium carbonate nanocomposite can promote the growth and proliferation of cells. Among them, the cell growth on the Chi-CaCO3NFs/SS (blue) composite material is the fastest, and it is on the ninth day. The cell activity is close to twice the cell activity on the blank stainless steel plate. It shows that the Chi-CaCO3NFs/SS composite material has a good promotion effect on cell growth.

3.3. Performance Analysis of Composite Materials. To determine the composition of the ChiCaCO3 composite material, we did an XRD test on it. As can be seen, there is a strong peak at 2θ = 43.8, which is a characteristic peak inherent in the stainless steel sheet substrate. The strong peaks at 2θ = 23.2, 29.6, 36.0, 39.6, 47.6, and 48.80 coincide with the standard peaks of calcium carbonate on the standard card, corresponding to the 12, 104, 110, 113, 018, and 116 crystal faces of calcite, respectively. The peaks obtained at 2θ = 24.0, 36.4 coincided with the peak positions of the standard peaks of chitosan and were identified as the characteristic peaks of chitosan. It shows that the Chi-CaCO3

<table>
<thead>
<tr>
<th>Substance time</th>
<th>GSH-Px</th>
<th>MPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group (A)</td>
<td>55.5429 ± 14.6182</td>
<td>195.1150 ± 34.4581</td>
</tr>
<tr>
<td>Day 3 after injury (B)</td>
<td>136.1833 ± 18.9154</td>
<td>360.2419 ± 26.0723</td>
</tr>
<tr>
<td>Day 5 after injury (C)</td>
<td>93.9429 ± 22.4785</td>
<td>265.4867 ± 15.4892</td>
</tr>
<tr>
<td>Day 5 after injury (D)</td>
<td>117.9429 ± 18.7348</td>
<td>3318584 ± 20.1084</td>
</tr>
<tr>
<td>Day 10 after injury (E)</td>
<td>68.5714 ± 20.2318</td>
<td>219.4896 ± 25.9215</td>
</tr>
<tr>
<td>Day 10 after injury (F)</td>
<td>94.4857 ± 15.7812</td>
<td>294.9852 ± 17.0487</td>
</tr>
</tbody>
</table>
composite material is composed of chitosan and calcite-type calcium carbonate. At the same time, it can be found that the peak of chitosan is relatively weak, mainly because chitosan is organic, and it is difficult to form stable crystals under this condition. To prove that the CaCO3-ChiNFs/SS composite material has certain mechanical properties, we compared the tensile strength of the smooth stainless steel sheet and the stainless steel sheet as the substrate for electrochemical power deposition to obtain the CaCO3-ChiNFs/SS composite material. From the experimental results, it shows that the tensile strength of the smooth stainless steel sheet cut to a certain shape is $727.1 \pm 14.66$ MPa, the tensile strength of the CaCO3-ChiNFs/SS composite material under the corresponding conditions has been significantly improved, and its strength reached $1317.2 \pm 3.65$ MPa. First of all, it shows that the uniform and compact structure of CaCO3-ChiNFs itself has strong integrity, which is the result of the interweaving and close integration of the fiber network that combines the advantages of organic and inorganic materials. This is also similar to the structure of "cement mortar" composed of alternate organic-inorganic shells. At the same time, it also shows that the use of stainless steel sheet as a base not only facilitates the on-demand tailoring of materials but also increases the mechanical properties of the body as a bone tissue material. For biological materials, the properties of the surface of the material can determine part of its biological activity. When the material has proper wettability, it can promote the spread and growth of cells, because the water-soluble surface can provide a humid environment for healthy cell growth. The wettability of the smooth stainless steel sheet, CaCO3-chiNWs/SS, and CaCO3-chiNFs/SS surfaces was tested. The contact angle of the smooth stainless steel sheet is $93 \pm 7.0^\circ$, the contact angle of CaCO3-chiNWs/contact angle of SS is $64.3 \pm 7.0^\circ$, and the minimum contact angle of CaCO3-chiNFs/SS is $32.4 \pm 4.6^\circ$.

This result shows that CaCO3-chiNFs/SS has good water wettability, and its surface state can be cell adhesion and spreading. Provide a good environment, which is more hopeful as a skeletal muscle repair and replacement material to provide cells with a more suitable platform for their growth, proliferation, and differentiation, as shown in Figure 3.

3.4. Biocompatibility Analysis of Composite Materials. The skeletal muscle cells were cultured on the surface of ChiCaCO3NFs/SS that had been sterilized beforehand. After the cells had grown for 3 days, they were pretreated, and then the morphology of the cells was characterized by a scanning electron microscope. Skeletal muscle cells are laid flat on the surface of the three-dimensional network fibers. They are larger in size, and the cell bodies and network fibers are firmly combined. And it can be seen that the cells protrude many pseudopods, which have penetrated into the network fibers. It shows that Chi-CaCO3NFs/SS can provide a good environment for cell growth and spread, and it is also conducive to the further proliferation and differentiation of cells. As we all know, the surface properties and nanostructures of
biological materials can significantly affect cell growth and viability. Therefore, we did a comparative experiment, cultured skeletal muscle cells on the surface of Chi-CaCO3NWs/SS composite material and under the same experimental conditions, and then performed SEM characterization. The cells appear round and become thicker, that is, the cells are not spread well, but exist in the form of clumps. The results show that the surface of the Chi-CaCO3NWs/SS composite material cannot provide for the spread, growth, and differentiation of the cells. Surface environment very well. The above results show that the three-dimensional network fiber of Chi-CaCO3NFs/SS has good biocompatibility, making it a potential material for repairing damaged bone in tissue engineering.

Hemolysis test was used to evaluate whether Chi-CaCO3NFs/SS has a damaging effect on red blood cells. The red blood cells were exposed to 5 different gradient concentrations of Chi-CaCO3NWs/SS solution, and normal saline and double distilled water were set as negative and positive controls, respectively. After incubating for 1 h, 2 h, and 3 h, they were observed under a microscope. It can be seen that compared with the negative control group, even with the highest concentration (300μg/mL) of nanoparticles incubated for 3 h, the number and morphology of red blood cells did not change significantly. On the contrary, due to the lysis of red blood cells, the double-distilled water treatment group has almost no intact red blood cells remaining under the 1 h microscope. After the red blood cells are lysed, hemoglobin will be released, and the supernatant after centrifugation will show red. Clearly, this phenomenon is more obvious in the double-distilled water treatment group. At the same time, the hemoglobin content in the supernatant is directly proportional to its OD value. The t-test analysis of the OD value of the supernatant in each EP tube found that, compared with the negative control group, the red blood cells incubated with the gradient concentration of Chi-CaCO3NWs/SS solution; there was no statistical difference in the OD value of the serum ($P > 0.05$); on the contrary, there was a significant statistical difference compared with the positive control group ($P < 0.01$). The biocompatibility analysis result of the composite material is shown in Figure 4.

3.5. Cytotoxicity Analysis. To confirm the biocompatibility of the Chi-CaCO3NWs/SS composite material, the CCK-8 method was used to detect its effect on cell viability. After the mouse skeletal muscle cells were treated with Chi-CaCO3NWs/SS composite for 48 hours, the cell activity showed a downward trend with the increase of nanoparticle concentration. When the concentration is lower than 50ug/mL, compared with the control group, the activity of the two cells is only slightly reduced, but there is no statistical difference ($P > 0.05$). When the concentration was increased to 50μg/mL, the difference in toxic effects was statistically significant ($P < 0.05$). The activities of the two cell types were 83.6% and 87.5%, which were still above 80%. The results of the cytotoxicity analysis are shown in Figure 5. Based on the concentration required for cytotoxicity detection and cell imaging of Chi-CaCO3NWs/SS, in other experimental projects, we set the maximum concentration to 50 μg/mL. The blood test results showed that the skeletal muscle toxicity sensitive indicators ALP and ALT had no abnormal changes compared with normal rats, and the HE staining results did not find obvious pathological changes; the nephrotoxicity sensitive indicators urea nitrogen and creatinine levels were also within the normal range. The sections show that the glomerulus and renal tubules are structurally intact; cardiotoxicity is often used as an important indicator of drug toxic side effects. The STAINED picture shows that myocardial cells are tightly arranged and orderly, and the structure of each part is complete; there are no other organ tissue sections. Appears noticeable pathological changes. It can be seen from the cell proliferation experiment that skeletal muscle cells can grow, divide, and proliferate well after inoculation. The cell activity of the experimental group and the negative control group increased significantly with time, and there was no significant

![Figure 4: Biocompatibility analysis results of composite materials.](image-url)
difference between the two groups in the statistical analysis. And the RGR of the experimental group and the negative control group was ≥100%, and the cytotoxicity was 0; while the positive control group had RGR < 30%, the cytotoxicity was 4, and the cell growth status was poor. After 24 hours of culture, the growth rate of cell viability of L929 in the experimental group was significantly higher than that in the normal culture group. It can be seen that C2C12 has higher cell activity and proliferation rate than Lg29.

4. Conclusion

The power of exercise comes from skeletal muscle contraction, which is a high energy consumption process. As a result, the body’s normal energy balance relationship develops in the direction of imbalance. To prevent the influence of energy imbalance on the body, the mechanisms that reverse this change trend are activated. At present, the research on skeletal muscle injury and repair is no longer limited to muscle satellite cells themselves. The role of immune cells in the repair of skeletal muscle damage is gradually being recognized, and its role and mechanisms are in the preliminary stage of exploration and become a new research focus. Skeletal muscle during strenuous exercise or acute injury can produce a large number of free radicals and neutrophil infiltration in the damaged muscles, followed by a series of inflammatory reactions caused by macrophage invasion. Biomimetic nanomaterials can solve this problem well.

In this study, adult healthy mice were selected. First, the mice were anesthetized with sodium pentobarbital, and the mice were fixed with steel balls to strike the midsections of the bilateral gastrocnemius muscles to create skeletal muscle blunt injury models. The mice were killed by cervical dislocation under anesthesia, and the injured gastrocnemius muscles of both sides were quickly taken to prepare the cell suspension. Weigh and dissolve chitosan in deionized water. Then add calcium chloride dihydrate, stir to dissolve, and prepare chitosan calcium carbonate composite material. The chitosan–calcium carbonate composite material prepared by using a stainless steel sheet as a base is cut into a square with a side length. Sterilize the stainless steel sheet and place it in a culture plate to dilute the overgrown cells in the culture flask and cultivate them in an incubator.

The skeletal muscle cells were cultured on the surface of ChiCaCO3NFs/SS which had been sterilized beforehand, and then, the cell morphology was characterized by scanning electron microscope. Skeletal muscle cells are laid flat on the surface of the three-dimensional network fibers. They are larger in size, and the cell bodies and network fibers are firmly combined. And it can be seen that the cells protrude many pseudopods, which have penetrated into the network fibers. It shows that Chi-CaCO3SS can provide a good environment for cell growth and spread, and it is also conducive to the further proliferation and differentiation of cells. In summary, there are many types of calcium carbonate medical materials, which combine the inherent good biocompatibility, osteoconductivity, and good hardness of calcium carbonate materials, as well as the advantages of polymers, collagen, biomolecules, metals, and other materials to make up for the shortcomings of calcium carbonate material are high brittleness. The successful application of calcium carbonate medical composite materials expands the application and development space of calcium carbonate materials in the field of biomedicine. The above results indicate that the material has no inhibitory effect on cell growth and is a qualified nontoxic material. It can be seen that the mice can tolerate the experimental concentration of Chi-CaCO3NWs/SS. This new type of nanomedicine carrier will not negatively affect its growth and development, nor will it cause histopathological changes in major organs. Normal skeletal muscle injury often occurs in high-intensity exercise training, more than habitual activities or physical labor. The repair of skeletal muscle injury has always been a basic issue of long-term attention and research in the sports medicine community. Many researchers have carried out research from different angles. Many aspects of research have been carried out, but many mechanistic problems have not been completely solved.
Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References


