Research Article

Compatibility of Carbon Composite Biomaterials for Repairing Bone Tissue Injury in Wushu Training

Jingfeng Ren

Department of Sports, Henan University of Technology, Zhengzhou 450000, Henan, China

Correspondence should be addressed to Jingfeng Ren; renjinfeng1986@haut.edu.cn

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With the improvement of economic level, more and more people begin to pursue a healthy lifestyle, among which sports have become an important way of modern people’s sports, but in the process of sports, there will inevitably be some injuries, especially in martial arts training, and bone is the most vulnerable part. Because of the special physiological characteristics of cartilage tissue, it is difficult to recover after injury. This problem has become the main health problem in real life, which greatly affects people’s health and quality of life. At present, the technology of carbon composite biological nanomaterials is more and more mature, and the bioactive composite materials are composed of polymers and bioactive components, which have very good biocompatibility. In recent years, the bioactive composite materials have been applied to clinical practice and achieved very good results. Based on this, this paper considers linking the bioactive carbon composite biomaterials with the recovery of bone tissue damage, and according to the biocompatibility of bioactive composite materials, it is applied to the repair of bone tissue injury in clinical practice. In this paper, two kinds of carbon composite biomaterials, calcium carbonate composite and graphene composite, were synthesized by electrochemical method and photocatalytic reduction method. The crystal of calcium carbonate complex and graphene composite was extracted by changing the experimental time and the parameters of the solution in the experiment, and then the two carbon composite materials were analyzed as biomineralization complex biocompatibility, cell growth, and cell activity of the complex as a drug carrier for bone tissue injury. The experimental results show that the two kinds of carbon composite biomaterials can provide a very good interface for cell adhesion and spreading. This experiment proves that the artificial bone made of carbon composite biological nanomaterials has good biocompatibility, and the biocompatibility of carbon composite biological nanomaterials can be directly applied to clinical bone tissue repair surgery.

1. Introduction

Nanotechnology refers to the study of the characteristics of the laws of motion of electrons, atoms, and molecules and processes materials and materials in the range of 0.1 to 100 nanometers [1]. The size of nanomaterials is closely related to organisms. For example, the linearity of the RNA protein complex, which is one of the life elements, is between 15 and 20 nm, and the size of various viruses in organisms is also in the nanorange. Nanobiomedical materials are the intersection of nanomaterials and biomedical materials. Nanoparticles are combined with other materials to make various composite materials. With the deepening of research and the development of technology, nanomaterials have begun to penetrate into many disciplines, showing great potential application value, and have obtained preliminary application in some fields [2]. In recent years, the theoretical and experimental research of biological nanomaterials has become the focus of attention, especially the biochemistry, biophysics, and biomechanics of nucleic acids and proteins, and their intelligent composites have become the frontier of interdisciplinary subjects of life science and materials science. Carbon nanomaterials are carbon materials with at least one dimension less than 100 nm on the dispersed phase scale. There are three types of carbon nanomaterials: carbon nanotubes, carbon nanofibers, and carbon nanospheres. Carbon element is one of the most closely related elements in nature, and in recent years, the research of carbon nanotechnology is more and more, a variety of carbon nanotransistors and
sensors emerge endlessly, and carbon nanotechnology is widely used in many fields [3, 4].

Bone defect mainly includes bone defect and defect repair. The bone defect is a common clinical disease and one of the prominent problems in orthopedic treatment. Bone tissue injury belongs to cartilaginous injury. Soft tissue contusion is the injury of muscle, ligament, and other tissues as well as peripheral nerves and blood vessels. These tissues are affected by different internal and external damage factors, leading to tissue damage and tissue physiological dysfunction [5]. Soft tissue injury is usually affected by the pressure of external mechanism. When it reaches a certain strength, it will cause injury and produce symptoms. Generally, it can be divided into acute injury and chronic cumulative injury. When soft tissue becomes blunt or severely injured by violence, it may lead to local soft tissue contusion and laceration. Soft tissue injury is a common disease in human motion system. Due to acute injury and chronic cumulative injury, soft tissue injury can lead to different conditions and degrees of symptoms of neck, shoulder, back, and waist, legs, and limbs. In recent decades, it has been found that a large part of diseases originates from soft tissue muscles and ligaments, nerves, blood vessels, etc., which directly cause great damage [6]. Tissue repair refers to the repair and replacement of local tissues and cells caused by pathogenic factors. These pathogenic factors lead to injury and death through the regeneration of adjacent healthy cells and restore tissue integrity. Soft tissue injury repair technology begins with human anatomy and physiopathology. The physiological and pathological changes of soft tissue in the lesion site are observed and analyzed visually, the target is accurately located, and the lesion tissue is completely repaired by manual operation. The damaged tissue can return to its normal state and relieve the pain completely. There are three stages in the repair and repair process of soft tissue injury repair technology: local inflammatory reaction stage, cell proliferation and differentiation and granulation tissue formation stage, and tissue repair and shaping stage. The repair process after injury mainly depends on the fibroblasts in the loose connective tissue inside the tendon. These cells will proliferate and synthesize collagen. Due to excessive secretion, it also fills the tissue gap of tendon (internal ligament) and finally forms collagen fiber, which makes tendon and ligament degenerate and harden.

Bionics is science applied and realized in engineering. Bionics is a scientific method to construct technical systems by imitating the functions and behaviors of all systems in the biological world. It breaks the boundary between biology and machines and conveys various systems [7–9]. Our ancestors imitated the birds flying in the sky, invented the plane, imitated the animals in nature, and invented the car, radar, and so on. The development of modern civilization is inseparable from the science of bionics. In 2019, a Swiss researcher developed a kind of bionic artificial leg, which allows patients to contact naturally, and does not need the brain to control walking all the time. The purpose of bionics is to analyze biological processes and structures and their analysis for future design purposes. The idea of bionics is based on natural evolution and coevolution. Human technology is optimized and coordinated with each other. It is undoubtedly a good opportunity to simulate the adaptive function of organisms to the environment. The research scope of bionics includes mechanical bionics, molecular bionics, energy bionics, and information technology control bionics. The bionics studied in this paper is carbon composite biological nanomaterials. It imitates the human learning process and creates a machine called "perceptron." The machine can learn and change the weight of the connection between components through training, so as to realize pattern recognition. In addition, it also studies and simulates the control mechanisms in biological systems, such as dynamic balance, motion control, animal orientation and navigation, and bionics of man-machine systems [10].

Bio compatibility refers to the reaction characteristics of organisms to nonactive substances and usually refers to the compatibility between the substances and the main body. After implanting biomaterials into the human body, they will have an impact and effect on the specific biological tissue environment, the biological tissue will also have an impact and effect on the biomaterials, and the circulation between the two will continue until the balance is reached or the implants are removed [11]. Bio compatibility has always been a theme in the research of biomaterials. Biocompatibility mainly depends on the nature and use of the material. The properties of materials and products, including shape, size and surface roughness, residual toxic low molecular substances during polymerization or material preparation, pollution of material processing technology, degradation products of materials in vivo, etc., are all related to the properties of materials and substances. Short-term contact with the human body will produce toxicity, irritation, teratogenicity, and local inflammation to cells and the whole body. Long-term exposure may have mutagenic, teratogenic, and carcinogenic effects, and abnormal coagulation and hemolysis will be caused by blood contact. Therefore, biocompatibility is an important index to be considered and evaluated when considering the use of materials in the biomedical field. In this paper, it was confirmed that the activation of peripheral blood mononuclear cells by carbon composite biomaterials was small. Carbon composite biomaterial technology is helping to improve the immune compatibility, mechanical properties, and biocompatibility of cartilage. This work can solve the problem of biocompatibility between soft tissue repair and carbon composite biomaterials. It is suitable for clinical practice and has important research value and application value.

2. Electrochemical Synthesis of Calcium Carbide Complex

Calcium carbonate is an inorganic mineral widely existing in nature and an important component of bone tissue. Due to the diversity of calcium carbonate forms, crystal morphology is easy to control in the process of biomineralization [12]. Generally, the technology of calcium carbonate is to synthesize the chitosan calcium carbonate denuded shell structure by layered deposition method and immerse the
prepared chitosan membrane in calcium chloride solution to form chitosan and calcium carbonate film in the CO₂ atmosphere. However, the mechanical properties of the composites prepared by the above methods are not high and the preparation process is complex, which limits its application as scaffold materials. Therefore, in this part of the experiment, we use the electrochemical method, which is not only simple but also highly efficient. We control the number of reactants and electrochemical parameters to realize the carbon composite biological nanomaterials composite. Because of its good biocompatibility, nanotechnology provides a very good theoretical framework for cell culture and bone tissue repair.

2.1. Main Reagents of Experimental. The main reagents used in experiments and tests are listed in Table 1.

2.2. Main Instruments of Experimental. The main instruments of experimental and equipment used in experiments and tests are listed in Table 2.

2.3. Preparation of Calcium Carbide Complex. Hydroxyapatite is the main inorganic component of alumina in bone tissue. It has good biocompatibility and high bioactivity and can form a chemical bond with bone tissue. However, the brittleness and processing difficulty of hydroxyapatite restrict its industrial application. Poly-caprolactone (PCL) is a biodegradable polyester material with good biocompatibility and physical and mechanical properties but lacks biological activity [13]. Natural bone is mainly composed of nano HA and collagen, which can be considered as a dual-phase composite material containing nanocrystals. Therefore, from the perspective of bionics, the combination of matrix and hydroxyapatite with organic polymer composites, especially the preparation of degradable polymers, can enhance the secondary performance, develop the advantages and avoid the disadvantages, and learn from each other to obtain the ideal bone repair materials.

In this experiment, nano calcium carbonate was modified by the wet method. The slurry with a solid content of 20% was prepared by adding 5 g nano calcium carbonate and anhydrous ethanol into a three-port flask. After ultrasonic vibration for 30 minutes, 10% silane coupling agent KH-570 (i.e., calcium carbonate mass) was added at 80°C, then mechanically stirred for 1.5 hours, and then filtered and washed. Finally, the modified nano calcium carbonate was prepared by vacuum drying, grinding, and sieving. Then, 0.100 g chitosan was added to the solution of 45 ml microcomputer controlled electronic universal testing machine, the pH value was adjusted to 3 as mixed acid, and then ultrasonic dispersion ultrasonic instrument was used for 2 h. Finally, a certain amount of licio 4 was added for ultrasonic dispersion for 2 h. An appropriate size of stainless steel iron was cut, washed in anhydrous ethanol or distilled water, and finally blow-dried with nitrogen for standby. A three-electrode, namely, Ag-AgCl electrode, Pt electrode, and stainless steel iron sheet was prepared. The dry stainless steel shoe was used as the working electrode. The electrochemical material solution parameters, electrochemical voltage parameters, and time by cyclic voltammetry were adjusted, and finally, calcium carbonate complex was got.

Using the same preparation, separation, and purification methods mentioned above, the control experiment of extracting calcium carbonate complex crystal from pure water without adding licio 4 was carried out. Sodium carbonate solution and calcium chloride solution were mixed in equal volume to form calcium carbonate crystal through a chemical reaction. During the experiment, the reaction time of calcium carbonate crystal was changed. The reaction time was 2 hours, 6 hours, 12 hours, 1 day, 2 days, 4 days, and 7 days.

2.4. Performance Representation. Dissolution test: a certain amount of pure PMMA samples and nanocomposite materials with different calcium carbonate contents were put into a single port flask containing chloroform for 3D-4D to observe the dissolution rate, and scanning electron microscope observation and analysis: the cross-section of the composite was sprayed with gold, and the cross-section morphology was observed by scanning electron microscopy (SEM).

FT-IR analysis: after the sample and KBr powder were pressed, the composition changes were analyzed and studied.

Mechanical property test of composite materials: 100D electronic universal testing machine controlled by computer was used to test bending strength and tensile strength according to GB 1039–1992, and the impact strength test was conducted by JB 6 impact testing machine according to GB 1039–1992.

3. Study on Graphene Calcium Carbide Complex

Graphene is a kind of two-dimensional carbon nanomaterials. It is a hexagonal honeycomb lattice composed of carbon atoms of SP hybrid orbitals. Graphene has good solubility in nonpolar solvents, and it can also adsorb and desorb various atoms and molecules [14–16]. The PZ orbitals...
perpendicular to the plane of each carbon atom form a large polyatomic bond throughout the carbon layer, which provides excellent electrical and optical properties. Graphene has good toughness and can be bent, but the graphite paper composed of graphene has a lot of holes, so the graphite paper is very fragile. However, after oxidation to get functional graphene, and then from the functionalized graphene to make graphite paper, it will be very solid and reliable. Compared with carbon nanotubes, graphene is more suitable for the study of biomaterials. The edge of graphene is longer, which is easier to be doped and chemically modified than carbon nanotubes. It is easier to accept functional groups and has better biocompatibility. In addition, the structure of graphene is very stable, and the carbon bond is only 1.42, so the force between atoms is very strong. At room temperature, even if the surrounding carbon atoms collide, the interference of electrons inside graphene is very small [17, 18]. The research and application of graphene at high temperatures makes graphene applied in many fields and has made a series of progress in chemistry, materials, biology, and semiconductor. So, it is necessary to study graphene. In terms of biology, graphene has been used to accelerate the osteogenic differentiation of human bone marrow mesenchymal stem cells, and the epitaxial graphene on silicon carbide is used to make biosensors. Graphene can also be used as a nerve interface electrode without changing or destroying the signal strength or scar tissue formation. Due to the flexibility [19], biocompatibility, and conductivity of the graphene electrode, the stability is much higher than that of the tungsten electrode or silicon electrode. Graphene oxide can effectively inhibit the growth of E. coli without harming human cells. However, the industrialization of graphene is still in the early stage, and some applications are not enough to reflect the “ideal” properties of graphene. However, many researchers around the world are exploring the application of “killer mace.” In the future, graphene will face many challenges in the detection and certification work, so we still need to innovate in means and methods.

3.1. Preparation of Graphene Composites. The experimental instruments and reagents in this part of the experiment are shown in Tables 1 and 2.

Graphene nanocomposites were prepared by the photocatalytic reduction method. Graphene and tetra butyric acid were used as raw materials, and sodium dodecylbenzene sulfonate and polyethylene glycol were used as surfactants. Ethanol, glacial acetic acid, deionized water, and ammonium nitrate were selected as solvents, and the volume ratio was 20:1:1:0.2. Graphite oxide was added to TiO₂ colloid obtained by hydrolysis of isopropyl titanium, and nanographite oxide dispersion was obtained by ultrasonic treatment. Finally, graphene nanocomposites were obtained by reducing graphite oxide under UV irradiation. Due to the strong conjugation between trimethylene and graphene, the derivatives of trimethylene can be easily grafted onto graphene. Without destroying the internal structure of graphene, the dispersion of graphene in organic solvents can be effectively improved. It provides the conditions for further functionalization and application of graphene. Firstly, the surface of polystyrene was treated with a cationic surfactant solution, filtered and dried to obtain the surface treated polystyrene powder. The surface-treated polystyrene powder was dispersed and stirred to obtain the mixture. The mixture was sprayed and dried at high temperatures to obtain polystyrene/graphene mixture powder. Finally, the mixed powder was added to hydrazine hydrate solution, reduced, washed, and dried to obtain the polystyrene/graphene composites.

3.2. High-Temperature Modification of Graphene Composites. Modification of graphene oxide dispersion in water: graphite oxide prepared by the Hummers method was dispersed in water for ultrasonic treatment, and hydrazine hydrate was added at 100°C. The partially reduced graphite oxide was prepared, and then the partially reduced graphite oxide was treated with epoxy resin/hardener (4:1) acetone solution and stirred for several hours. After the reaction, the graphite oxide was dried to a suitable shape at 60°C and annealed in nitrogen at 250°C for 2 h to completely reduce the unreduced graphite oxide, so as to improve its conductivity. Finally, the electromagnetic shielding effect of graphene/epoxy resin composite is less than or equal to 21 dB, which basically meets the commercial application requirements of 20 dB [17]. Modification of graphene oxide by manganese dioxide: in this method, graphene was prepared by an improved Hummer method, and the mixture of graphene and manganese dioxide was prepared by Yanjing microwave method. The mixture of graphene and manganese dioxide was prepared by a redox reaction. Firstly, 100 ml and 1.65 mg/ml
graphene solution was prepared by ultrasonic wave for 1 h. 95 g potassium permanganate was added to the ultrasonic graphene solution and stirred for 10 min. Then, the stirred solution was heated in a microwave oven for 5 minutes, cooled to room temperature, washed with deionized water and absolute ethanol several times, and finally dried in a drying oven at 100 °C for 12 hours. This method is a low cost, suitable for mass production, and has practical significance. The electrochemical test shows that the impedance curve of the mixture of graphene and manganese dioxide is a typical electrochemical characteristic curve. With the decrease of frequency, the impedance curve gradually becomes diffusion control. In addition, the capacitor formed by the electrode also has good electrochemical capacitance characteristics. The first charge-discharge ratio is 237 F/g, which is higher than that of graphene or nano manganese dioxide prepared by traditional methods.

Sodium borohydride was used to modify graphene: firstly, carboxyl and carbonyl groups around graphene were used together with sodium borohydride and then reacted with p-aminobenzene sulfonic acid in an ice bath for 2 h, and then the sulfonic group was grafted onto graphene oxide and then reduced with hydrazine at 100 °C for 24 h. The solubility of layered graphene in water was 2 mg/ml.

4. Results Analysis
4.1. Biocompatibility of CaCO3 Composites. In this experiment, in order to further study the biocompatibility of CaCO3 composites, we tested MC3T3 cells in different time periods; the time selected is 2 hours, 6 hours, 12 hours, 1 day, 2 days, 4 days, and 7 days. The growth of cells was observed at different time periods. The control experiment used in this paper was the cell active cell and growth experiment on the blank stainless steel sheet. The experimental group was to observe the growth of cells on cnt-CaCO3 composite material. The experimental results are shown in Figure 1.

As we all know, the MTT method can reflect the number of cells indirectly. With the prolongation of culture time, the cell activity of both groups was improved, but the cell activity of CaCO3 composite material was significantly enhanced, which indicated that calcium carbonate nanocomposite could promote cell growth and proliferation. It can be seen from Figure 1 that the cell activity on stainless steel sheet and CaCO3 composite material has little difference, less than 0.05, at two hours after the experiment. However, with the growth of time, after 12 hours, the growth of cells on the stainless steel sheet began to be significantly lower than that on the CaCO3 composite. On the fourth day, the cell activity on the CaCO3 composite was more than twice that of the blank stainless steel sheet. On the seventh day, the difference was more. The cell activity on the stainless steel sheet was only 0.3, but the activity on CaCO3 composite was 0.8. The results show that carbon nanotubes prepared by this method are simple and effective nanocomposites, which can promote the growth and proliferation of cells.

Next, we analyze the growth trend of cell activity on CaCO3 composite and stainless steel sheet. The MTT growth trend of cells on stainless steel sheet and CaCO3 composite materials during the experiment can be seen in Figure 2. It can be seen from Figure 2 that in the experiment on CaCO3 composite, the cell activity has been increasing, the maximum value of cell activity is 1, the minimum value is 0, the cell growth rate is very fast, and the slope of the straight line is also increasing between the two adjacent times. On the seventh day of the experiment, the cell activity had reached 0.8, but our experiment only lasted for a week. It is believed that in the next two days, the cell activity can reach nearly 100%. On the contrary, the cell activity on the stainless steel sheet is also increasing, but the increasing rate is slower than that on the CaCO3 composite.

4.2. Biocompatibility of Graphene Composites. The biocompatibility of graphene composites is determined by its molecular structure. This is because the edge of the graphene molecular structure is longer, it is easier to doping and chemical modification than carbon nanotubes, and it is easier to accept functional groups, so it has better biocompatibility. We analyzed the biocompatibility of graphene composites and observed cell growth and cell activity on graphene composites.

0.1 mol/l H3PO4 buffer (PBS, pH = 7.4) was used as the supporting electrolyte. CV was used in all electrochemical experiments. Ag-AgCl electrode was used as a reference electrode, platinum electrode as a counter electrode, and God/AuNPs/chit/GNS/GC as a working electrode. Before the experiment, 8 ml PBS buffer was put into a small beaker and reduced with high purity nitrogen for 30 min. During
the experiment, the N₂ saturated atmosphere was maintained in the small beaker.

According to the change of cell activity on graphene shown in Figure 3, with the increase of time, the cell activity increases in Figure 3 are a network graph, and the scale of each net represents 0.2. From the graph, we can see an obvious change between the experiment for two hours and seven days. The cell activity changes from 0.2 to 0.8 directly, indicating that graphene has good biocompatibility.

The advantage of this experiment is that there is no special requirement for the template, catalyst, surfactant, and other special materials. Graphene has good biocompatibility, bioactivity, and diversity. We hope to apply it to cell adhesion, in vitro cell culture, tissue compatibility scaffold, nanobiosensor, and other medical fields.

It can be seen from Figure 4 that after the experiment, the proportion of total cell activity in carbon composite materials and stainless steel iron sheet is 4:1, which indicates that the growth rate of cells in carbon composite materials is faster, which is the same as the experimental hypothesis. Under the action of strong acid and oxidant, carboxyl groups and hydroxyl groups are introduced on the surface and both ends of carbon nanotubes. In the process of preparing carbon nanotubes calcium carbonate composites, the carboxyl groups on the wall of carbon nanotubes combine with calcium ions in CaCl₂. Due to the presence of CO₃²⁻, calcium ions are easy to form calcium carbonate, which is mainly controlled by diffusion. With the development of electrochemical reaction, the acidity of the electrolyte increases with the release of H₂. The surface of the working electrode is covered with more calcium ion carbon nanotubes, which weakens the repulsion force of the electric field to carbonate, indirectly promotes the diffusion of CO₃₂⁻ calcium ion, and accelerates the formation of calcium carbonate. This indicates that CO₃²⁻ provides a good environment for cell growth and diffusion and promotes the further proliferation and differentiation of fine cells. The growth of cells on the surface of carbon nanotubes/calcium carbonate composite is obviously better than that of the blank stainless steel sheet, and this advantage is more obvious with the extension of culture time. This experiment shows that the composite has a good promotion effect on cell growth.

5. Conclusions

In this paper, two carbon composite models, calcium carbonate composite and graphene composite, were established by using carbon composite as a template. The biocompatibility, cell growth, and cell activity of two kinds of carbon composites as a biomineralization complex as a drug carrier for bone tissue injury were studied. In this experiment, carbon composite materials were extracted by a one-step electrochemical method. The method is simple and fast and only needs to change the time and solution parameters. Under the effect of the electric field, positively charged chitosan moves to the cathode and forms calcium carbonate complex crystal on the stainless steel plate. The extraction of
graphene carbon composites by photocatalytic reduction method can be understood as the solubility of carbon composites in aqueous solution depends on the pH value of the solution. Nanocarbon composite scaffolds can promote the adhesion and proliferation of bone marrow mesenchymal stem cells, promote the secretion of osteoblasts in the extracellular matrix, and enhance the calcification of tissue-engineered bone. In microenvironment, it is more conducive to the economic growth and differentiation of bone marrow mesenchymal stem cells and promotes and accelerates the regeneration and repair of bone tissue injury. Bone tissue engineering is developing rapidly in the field of sports medicine supporting the quality of treatment. With the continuous development of composite material research and the continuous improvement of artificial bone material production technology, the structure, performance, and various biological activities of biomedical materials similar to human tissue can be obtained in future research centers, which brings new hope for the development of artificial bone repair materials for bone defect repair. The experiment in this paper also proved that the carbon composite biomaterial has very good biocompatibility in the repair of bone tissue injury, which can be applied to clinical practice.

Data Availability
No data were used to support this study.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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