Research Note

Preparation and Characterization of Integrated Condylar Biomimetic Scaffolds: A Pilot Study

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Abstract: We constructed the bionic mandibular condyle scaffold composite with Chitosan, polycaprolactone and hydroxyapatite CS-PCL-HA and investigated the feasibility in condylar tissue engineering applications. The condyle mold was fabricated by resin materials with rapid prototyping methods and the bionic mandibular condyle scaffold was constructed by solution casting-ice drain. The microstructure, porosity, infrared and X-ray diffraction of the condyle scaffold were studied. The exterior of scaffold was white and hard as condyle, consisting of cartilage scaffold and subchondral bone scaffold. The scanning electron microscopy showed that the scaffold composite were highly bionic in three-dimensional structures with 70-85 % porosity and 40-200 μm aperture, which can be controlled by the size and the number of ice particles. Infrared spectrum showed that with the reduction of HA content, the peak strength also decreased, while X-ray diffraction results demonstrated that with the increase of HA content, the diffraction intensity reduced relatively. The compressive strain and bending strain were increased with the growing of the HA content. In conclusion, the special design of scaffolds has good performance of synthetic materials, which is expected to be applied in autologous tissue engineering as condyle scaffold material.

Key words: Mandibular condyle, Tissue engineering, Bionic scaffold composite

Introduction

Temporomandibular joint is the only joint in the maxillofacial region, which is flexible, stable and complex, producing various important activities related to mastication, swallowing, speaking and facial expressing with the function of maxillofacial muscle. Epidemiological investigation showed that 6-12 % individuals had temporomandibular joint disorders¹⁾, which could cause the destruction of bone and condyle. In addition, the condyle defect and damage caused by condyle tumor and trauma have to be treated surgically with autogenous bone transplantation or artificial material substitution. However, autogenous bone transplantation could hardly match with the shape of condyle and could damage the bone donor site. The artificial material substitution, on the other hand, has problems of mechanical toughness requirements, degradation period and postoperative infection to achieve the ideal scheme^{2,3)}. The structure of an osteochondral biphasic scaffold is required to mimic native tissue, which owns a calcified layer associated with mechanical and separation function.⁴⁾ With the development of tissue engineering, the research on two phase composite materials has provide us new ideas to find new artificial

condyle materials. The ideal tissue engineering scaffolds should have good biocompatibility, surface activity, plasticity and suitable biodegradability and have mechanical strength as well, which is necessary in providing mechanical support at the early stage of new tissue growth. Biphasic scaffold consists of cartilage and bone. The upper phase cartilage bracket mainly works as temporary extracellular matrix to provide attachment, proliferation, differentiation and the site of metabolism for cartilage cell, while the lower bone material is similar to bone tissue in the structure and biomechanical properties, and its function is good for proliferation and differentiation of osteoblasts. In this research, we constructed a bipolar integrated bionic scaffold with upper and lower layers of different aperture and porosity by solution casting-ice drain methods. This special design has make it happen that the materials and porosity between the cartilage and the transitional areas of two under layer bones have a gradient change to avoid fracture and separation due to sudden changes in material, realizing the close integration between the two. We have tested its performance to explore the feasibility of its application in autologous subcondylar bone and cartilage tissue engineering.

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Materials and Methods

Reagents and instruments

Figure 1. SEM micrographs showed subchondral part of the scaffold (left) and cartilage part of the scaffold (right).

CS was from Sinopharm Chemical Reagent Co. (Beijing, China). PCL was purchased from Aladdin Reagent Co. (Shanghai, China). HA was from Shanghai Jimian Co. (Shanghai, China). Tetrahydrofuran was purchased from West Reagent Co. (Chengdu, China). Thermostatic magnetic mixer was provided from Changzhou Guohua Electric Appliance Co. (Changzhou, China). Condylar die was from Shanghai Jiao Tong University (Shanghai, China). Scanning electron microscope was Philips (Japan). Universal testing machine was Toshiba (Japan). Infrared spectrometer was provided from Bruker Co. (Germany). Automatic X-ray diffractometer was provided from Peking University Instrument Factory (Beijing, China).

Preparation of the porogenic agent

Ice particles were adopted as porogen in the study. The distilled water was rapidly sprayed into the container with liquid nitrogen through capillary, which were solidified into ice particles in no time. Because of the role of surface tension, the ice particles were prepared as a ball type. In subzero environment, ice particle were selected into 2 group of 50-100 μm diameter and 100-200 μm diameter and were saved in vacuum under the -80 °C for the backup.

Preparation for porous scaffold

At room temperature, the PCL and CS were dissolved in 20ml tetrahydrofuran (THF) solution with the proportion of 4:1 and the mixture were divided into a, b, c, d four groups, in which adding 40 %, 50 %, 60 %, 70 % HA respectively. The mixed solution was stirred under magnetic stirring apparatus for 30 min and was pre-froze in refrigerator (-20 °C) for a period of time. The prepared Ice particles then were added to the mixture respectively and mixed evenly. The size and the amount of ice

particles of the mixture were adjusted , which were then frozen to a thick mixture in -20 °C. The mixture was added gradiently into condylar mold from small size and amount of porogens to the large ones and were again froze and dried under -20 °C. After removing the solvent THF, the mixture was finally dried at room temperature to remove the porogenic agent to get the HA-PCL-CS composite scaffolds. The reserved of ice particles were layered mixed in the bracket respectively in preparation of porous scaffolds, to make the aperture in the range of 50-200μm after drying.

Infrared spectrum detection

The scaffold was dried and cut into 0.5 g, grinding into powder in agate mortar for 10min and sieving (2 μm). The quality of powder after sieving should be 0.05~0.08 g, which was tableted in compression mold and then was put into the sample pool. EQUINOX55 type Fu Liye transform infrared spectrometer was used to analysed the scaffold.

X-ray Diffraction

The sample should be ground into smooth and deoxidize surface and cleaned with alcohol. The scaffold was placed on the test rig to determine its XRD curve with the use of MASL2XD2 type automatic X- ray diffraction, to further study on the crystal properties.

Mechanical property experiment

Three point flexural and compressive strength of the scaffold were tested by universal material tester (Toshiba Cor., Japan). Bending test conditions were loading speed (V= 10 mm/min), span length (L=30 mm), pressure head diameter (d = 4 mm) and the calculation formula was $R = (3F \cdot L) / (2b \cdot h^3)$. Compressive

Figure 2. The results of infrared spectrum detection of four groups (a, b, c, d). The characteristic absorption peaks of P-O bond of HA were appeared at about 1038 cm^{-1} wavelength, while at the 1375 cm^{-1} wavelength and 1820 cm^{-1} wavelength.

strength test condition was the loading rate ($v = 5 \text{ mm/min}$). If the scaffold material fractured before its deformation was less than 25 %, then the fracture strength became compressive strength. Otherwise, the compressive strength was 25 % of its deformation intensity.

Result

SEM characteristics of scaffolds

The appearance of the scaffold was like condylar in shape and texture, which was hard and honeycomb in surface. The electron microscopic observations (Fig. 1) showed that the scaffold was in the form of multi mesh structure, which the pore size of the cartilage in the upper part of scaffold was about 100-200 μm and the porosity is about 85 %, while the pore size of the lower bone part was about 50-100 μm and the porosity is about 70 % (The size of the aperture was determined by the porogen. The size of lower porogen was 50-100 μm , while the upper porogen size was 100-200 μm . The SEM observation results only showed that the support structure was a porous network structure, but because of the experiment error such as porogenic agent melting, the multiple aperture was mixed, including 10-40 μm). Hydroxyapatite was uniformly distributed in the PCL-CS matrix in good dispersion and PCL was stacked in a semi crystalline mode, in which there was a small part with smaller pore about 10-40 μm . Observed on the whole, the scaffold was formed to be a three-dimensional structure in which aperture and porosity became larger gradually from lower to the upper part.

Infrared spectrum detection

As Fig. 2 showed that the characteristic absorption peak of P-O bond of HA were appeared at about 1038 cm^{-1} wavelength, while at the 1375 cm^{-1} wavelength and 1820 cm^{-1} wavelength, the characteristic absorption peak of methyl and C=O double bond of PCL were appeared separately. In the interval, C-O bond, N-H bond and the amide bond of CS and PCL were overlapped, showing an irregular wave that the hydroxyl of CS showed a

Figure 3. The X ray diffraction diagrams of the scaffolds with different content of HA.

Figure 4. The influence of HA content on the compressive strain and bending strain of the scaffold.

characteristic absorption peak at the 3450 cm^{-1} wavelength. With the reduction of HA content, the peak strength also decreased.

X-ray Diffraction

Fig. 3 showed the X ray diffraction diagrams of the scaffolds with different content of HA. Since PCL was a semi crystalline polymer, it showed strong diffraction peak in the 22.4 degree and 24.9 degree. With the increase of HA content, the strength of diffraction peak reduced relatively, illustrating the degree of crystallinity decreased relatively.

Mechanical property test

Fig. 4 showed the influence of HA content on the compressive strain and bending strain of the scaffold. In the experiment the porosity and pore size were largely decided by the porogen, therefore we considered that the mechanical properties of the four groups (a, b, c, d) were only affected by the content of HA. According to the experimental results, the compressive strain and bending strain were increased with the growing of the HA content and the fracture strain were decreased with the increase of the HA content.

Discussion

Temporomandibular joint is the most complex joints of the human body, which could bring difficulty in repairing the defect of condyle lesions.⁶⁾ With the development of rapid prototyping technology, the joints with complex shapes could be fabricated under the support of the new technical. Nowadays there have been a lot of researches about bone scaffold⁷⁾, but the relatively ideal material has yet to be found somewhere, due to the toughness and strong wear resistance of condylar, which would put forward higher requirements on materials for scaffold³⁾.

Some scholars have made personalized mandibular condyle biphasic scaffold with calcium phosphate cement - poly lactic acid -glycolic acid copolymer. Bone cement with high brittleness and low mechanical strength, has the problem of less-reliable connection between cartilage and bone⁵⁾. In this study, we used HA-CS-PCL as scaffold material to construct the integration of condylar structure. Chitosan and polycaprolactone are biodegradable materials with good biocompatibility and its degradation products are carbon dioxide and water, which could be absorbed by the body and excreted⁸⁾. Polycaprolactone was stacked and folded to a slender crystalline region, while the molecular chains crossed each other in the amorphous region, being linear semi crystalline polymers. Polycaprolactone with good flexibility and mechanical relaxation has a good compressive property and creep property to be an ideal material of condylar defect implantation, which has a degradation half-life more than a year⁹⁾. The hydroxyl of chitosan reasoned in its strong hydrophilicity, but relatively poor mechanical properties. Hydroxyapatite has the chemical composition that is similar to natural bone inorganic mineral composition, which has good mechanical strength and high biological activity, but poor fatigue resistance and easy to migrate. The three together, compensating each other, could achieve a relatively ideal scaffold.

The study fabricated integrated bionic condyle scaffold model with the methods of solution casting - ice drain, with no porogen residuing. The electron microscope showed that the porosity of the scaffold was 60-80 %, of which the pore sizes were increased gradually from 50 μm of the basal layer to 150-200 μm of the upper part. This kind of design could solve the problem of unreliable connection between cartilage and bone. According to the cell homing theory, the scaffold has the potential to induce cells, such as mesenchymal stem cells, to differentiate into bone progenitor cell¹⁰⁾, which could reconstruct the organization structure from the constructed structure. Chang H Lee etc.¹¹⁾ have demonstrated in their animal experiment that the scaffold containing cytokines could be successfully implanted *in vivo* under the seed cell free culture. The pore size and the connectivity were keys to the design of the scaffold.¹²⁾ Research showed that fibrous tissue could be induced to grow in pores of 15 to 50 μm diameter, while pores of 50 ~ 100 μm diameter could stimulate the formation

of osteoid tissue and pores of 100 ~ 400 μm could directly induce the mineralization of bone. Therefore, the integrated bionic condyle scaffold structure was expected to form a biphasic scaffold by increasing the inducing factors, which required to be further studied in animal experiments in the future.

Studies have shown that when PCL and CS were at the proportion of 4:1, the composite structure material remained the crystalline structure of PCL with toughness and hydrophilicity. According the studied results, we adjusted the proportion of HA in HA-PCL-CS. As shown in Figure 4, the compressive strain and bending strain were increased with the growing of the HA content. And the tensile strength of the composite scaffold was about 8.12 MPa, which was thought to meet the requirement of mechanical needs, since the tensile strength was studied to be $2.50 + 0.72$ MPa that condylar cartilage could withstand¹³⁾. In addition, with contents of CS and PCL, the scaffold was of benefit to inducing osteogenesis and being biocompatibility in function. But along with the degradation of PCL-CS and reconstruction of the articular cartilage, whether there would be changes in mechanical properties requiring further animal and clinical experiment. The porosity of the scaffold was in inverse proportion to the content of HA, between which a new balance should be found. Plus, whether the scaffold can adapt to the mechanical properties of condyle and functional requirements of chewing and whether the cell clusters of nutrition and waste support exchanging could be ideally achieved in the scaffold were to be explored in further animal experiments. Overall, the composite scaffold made by the method was expected to become the condylar tissue engineering scaffold with good comprehensive properties.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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