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Research Article

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Study of human-like collagen adsorption on true bone ceramic

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ABSTRACT

An ideal bone graft material should have the characteristic of good biocompatibility. In this study, human-like collagen (HLC) was introduced into a scaffold of true bone ceramics (TBC) to structure a good bioactive scaffolds[1,2]. The cellular structure of scaffold and pore size was examined through scanning electron microscopy (SEM). The pores with diameter ranging from 300 to 500um distributed evenly in the scaffolds, and these pores were interconnected with each other. This study also was conducted to find the optimum condition of TBC absorbtion HLC. Experimental results showed that when the HLC solution pH was 4, concentration was 20 mg/ml and the temperature was 50 ℃, the HLC adsorption can reach the largest amount of 26.8 g/mg TBC. TBC/HLC scaffolds is expected to become a good bone defect repair material.

Keywords: True bone ceramic (TBC), Human-like collagen, protein adsorption

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INTRODUCTION

Caused by all kinds of trauma and bone disease brought great pain to the patient, bone defect of the traditional methods of autograft and allograft transplantation of there was a problem [3-5]. To some extent, restricted the clinical application. TBC is to use some fresh animal bone through prior removal of organic matter then high temperature calcination and made a good bone repair material. It comes from natural bone, the prior treatment and then calcination high temperature to removal the organic phase and retain the inorganic component of bone, so the TBC are similar to body tissue in structure. TBC has excellent histocompatibility. HLC obtained by high density fermentation technology is a new type of genetically engineered proteins, collagen is highly similar in structure to Human body, and has good biocompatibility, promote new cell formation and biodegradable properties such as excellent biological characteristics, and has no virus hidden danger and low and has been successfully applied to all aspects of the biomedical engineering [6,7].

In this experiment, we use TBC as the carrier, with the kind of person collagen protein on the biological modification, preparation of a new type of activity of the TBC, and the basic physical and chemical properties test and collagen composite condition of exploration with such people.

The experimental results show that calcination pretreatment can removal nearly 60% of organic matter. 700℃ calcination 3h can obtain ideal TBC. Type of person best conditions of collagen protein adsorption, pH of 4,

adsorption temperature is 50℃, protein solution concentration of 20mg/ml, in this condition, the protein adsorption capacity is 26.8μg.

EXPERIMENTAL SECTION

Materials

The HLC, produced in China xi 'an giant technology co., LTD. Animal bones were from commercially available cow vertebrae and coomassie brilliant blue G250 was purchased from Amresco. All other reagents and solvents are high purity grade or analytical grade.

Preparation of True bone ceramic (TBC)

Fresh bovine vertebra was cleaned and cut into strips. The material then was boiled and immersed in a 0.5mol/l sodium hydroxide for 12 h, 1:1 mixed solution of chloroform and methanol for 1 h, 30% hydrogen peroxide for 24 h, 2mol/l calcium chloride for 1 h and 8mol/l lithium chloride for 1 h to remove the organic components, followed by washing with deionized water to remove the remaining solution. Sintering was accomplished in two steps. Initially, the bone was placed in a furnace, and the temperature was increased to 700 °C at a heating rate of 5 °C/min and maintained for 3 h, and then cooling with the furnace. The bone was then immersed in 0.09 mol/l sodium pyrophosphate and 70 °C water baths for 72 h. The material then was sintered and temperature was increased to 1200 °C for an additional 1 h.

Basic performance test of TBC

Thermo gravimetric analysis：Taking same amount of fresh bone and bone after removing part of the organic matter in advance, drying, then do thermo gravimetric analysis.

Scanning electron microscope: apply a small amount of TBC, sprayed gold sample and scan electronic microscope.

Porosity and density determination: Using the liquid displacement method, the quality of W_1 TBC into the isopropyl alcohol volume for V_1, negative pressure pumping air into vacuum state, until no air bubbles, the record of isopropyl alcohol and the volume of a material for V_2, take out the samples, the rest of the volume of solution for V_3.

$$\rho = \frac{W_1}{V_2 - V_3} \quad ①$$

$$\varepsilon = \frac{V_1 - V_3}{V_2 - V_3} \times 100\% \quad ②$$

Optimal pH value of the TBC absorption HLC

Mixed concentration of 10 mg/ml of seven HLC solution of different pH gradient (4, 5, 6, 7, 8, 9, 10), 100mg of the TBC in 2ml of HLC solution in each pH gradient do three parallel samples, in the thermostatic oscillator frequency oscillation at 100r/min, at two different temperatures (20℃ and 40℃) under the static adsorption of HLC. Adsorption after 2h, then determination of protein concentrations before and after adsorption, calculation of protein adsorption equilibrium. Protein solution pH and adsorption capacity of pH–q relation curve, we can get a collagen adsorption curve in different pH conditions.

Isothermal adsorption curve of TBC absorption HLC

When the TBC and protein reaches an equilibrium in solution, the adsorption quantity should be linked to the protein concentration in the solution and temperature. Respectively in different reaction temperature (10℃, 20℃, 30℃, 40℃, 50℃) under the determination of the equilibrium adsorption from the HLC in TBC surface (2h). According to HLC concentration in the solution after adsorption and equilibrium adsorption quantity, make the TBC adsorption isotherm of HLC.

RESULTS AND DISCUSSION

Thermo gravimetric analysis (TGA)

Thermo gravimetric analysis was used to study the thermal changes of the fresh cow vertebrae ($1^{\#}$) and the cow vertebrae which partly removal of organic matter ($2^{\#}$). Fig.1 shows that the thermo gravimetric curves of $1^{\#}$ and $2^{\#}$ are similar. They both have two weightlessness platforms, and curves flatten after 700℃. The beginning and terminal temperature of the first weightlessness respectively are 70℃ and 170℃, the weightlessness rate is 5%, The beginning and terminal temperature of the second weightlessness respectively are 200℃and 600℃, the weightlessness rate respectively are 40% and 15%.

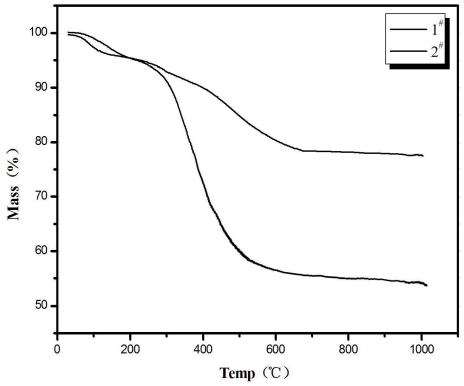

Fig.1: The TG curves of different samples. ($1^{\#}$. Raw bovine bone; $2^{\#}$. bovine bone that partly removal of organic matter)

Reasons of weightlessness for the first time may be the loss of free water and bound water. Reasons of the second weightlessness may be the loss of organic materials. Organic matter such as protein and lipid reaches its melting point began to evaporate with the increase of temperature and lose much weight. So removals of organic matter can removal most of the organic matter and improve burning efficiency, organic matter at 700℃ can be thought of have burn completely.

Scanning electron microscopy

The morphology of TBC was examined by scanning electron microscopy (SEM). The sample was sputter-coated with a thin gold layer and studied in Japan's Hitachi Company S-570 scanning electron microscope[8]. As Fig.2 shows, the TBC with natural trabecular bone, and bone trabecular gap inside the tube cavity system, keep the continuous porous structure of natural bone.

Fig.2 represents the SEM images of the TBC structure. They are shown that the pores with diameter ranging from 300~500μm distributed evenly in the TBC and these pores of TBC interpenetrated and interconnected with each other. Natural structure of the TBC for new blood vessels, blood vessels, the formation of new bone and surrounding tissue ingrowths to create a good environment.

Porosity and density of the TBC

Bone repair materials should have relatively high porosity, so that new cell ingrowths and nutrient transport, and application of liquid displacement method, we measured the TBC porosity is 80.65%, porosity is higher; TBC density for claiming measurements of bone mineral density of 0.48g/cm^3.

Optimal pH value of the TBC absorption HLC

HLC solution by soaking drawn into porous structure of the TBC, at the same time ionic strength and pH of the HLC solution make charging of HLC molecules and TBC inorganic calcium ion generate electrostatic force or make HLC and hydroxyapatite generate nonspecific adsorption. Figure. 3 shows that TBC adsorption ability of HLC solution under different pH conditions as follows: 4 > 9 > 5 > 8 > 7 > 10 > 6.

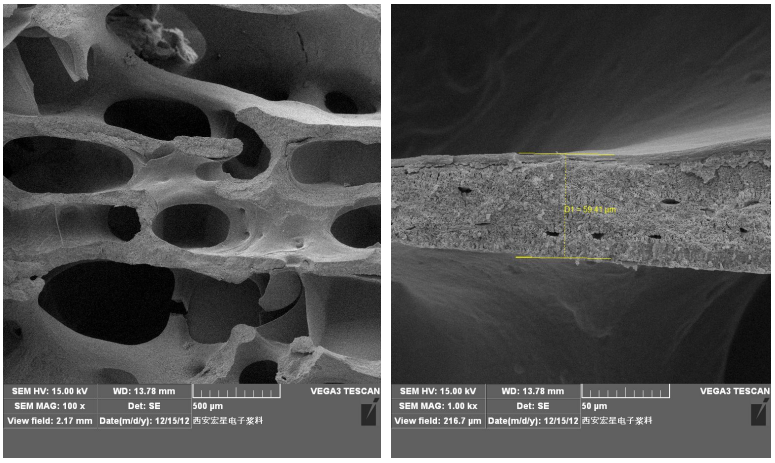

Fig.2: SEM micrographs of TBC

Main ingredients of TBC are high purity of hydroxyapatite (HA), two adsorption position on the surface of HA. TBC surface is positively charged in alkaline environment, which are negatively charged in acidic environments. Isoelectric point of HLC is 5.8, HLC molecules positively charged when pH is below the isoelectric point, which are negatively charged above isoelectric point. So when the solution pH is 4 and 5, HLC positively charged, when the solution pH value is 6, that close to protein isoelectric point, protein is electrically neutral, when the solution pH is 8, 9, 10, protein negatively charged. According to the charge on two kinds of material surface can judge the strength of their affinity adsorption force. The adsorption quantity are lowest when protein solution pH is 10, the reason might be that protein aggregation have taken place in alkali environment, so adsorption capacity will reduce. Fig.3 shows that protein optimal adsorption is pH 4.

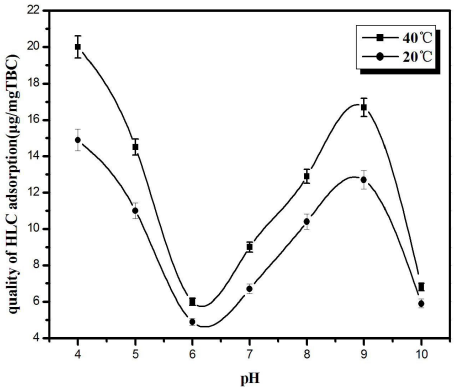

Fig.3: Effects of pH on the quality of HLC adsorption

Isothermal adsorption curve of TBC absorption HLC

HLC existence state in the solution is different under different temperature, molecular interactions will be different, too. As can be seen from the Figure. 4 also, with the increase of protein concentration, the adsorption quantity increases, but increases slowly after 15 mg/ml, so we can choose 20 mg/ml as optimal concentration of protein adsorption. Fig. 4 shows, TBC at different temperatures in bone absorption ability of HLC: 50℃ > 40℃> 20℃> 30℃> 10℃; As can be seen from the Fig.4 also, with the increase of protein concentration, the adsorption quantity increases, but increases slowly after 15 mg/ml, so the optional 20 mg/ml as optimal concentration of protein adsorption.

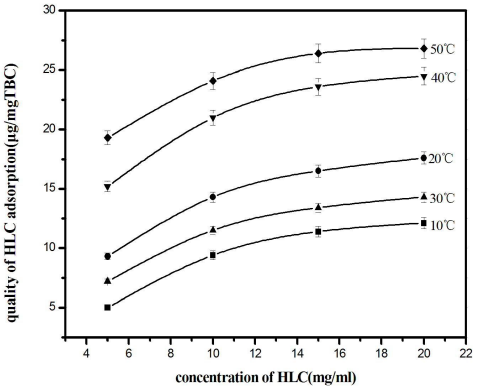

Fig.4: The adsorption of HLC on TBC under different temperature

Under different temperature, HLC existence state in the solution is different, and has different molecular interactions. When the temperature in the range of 3℃ to 22℃, the HLC molecular inter-atomic forces is weak, but the binding force of HLC molecules with the TBC is stronger, but when the temperature is very low, HLC since fibrosis formation of the fibers on the size is bigger, the adsorption quantity is less, so the protein adsorption quantity under the condition of 20℃ higher than 10℃. When the temperature is 22℃~38℃ temperature range, the interaction between HLC is greater than at low temperature, So HLC adsorption amount was lower than those of 20℃ under 30℃, When temperature more than 38℃, HLC loss between the molecular self accumulation ability; HLC structure more tend to form curls without rules, rather than a linear structure. At higher temperature, the hydrogen bonding interaction will be more damage, protein interaction force is abate, protein and bone force stronger, HLC ideal is 50℃; From isothermal adsorption curve can also see, with the increase of concentration of HLC, the adsorption quantity increases, but increases slowly after 15mg/ml, so the optional 20mg/ml as HLC adsorption optimal concentration.

CONCLUSION

By testing the basic properties of TBC, we can know that TBC is a kind of very potential material for replacing defects of bone, its main composition is high purity of hydroxyapatite, and it is similar to the natural bone in structure. Control the adsorption conditions (solution pH and temperature), is a convenient method to improve protein adsorption capacity. Under the Optimum condition (concentration of HLC solution is 20mg/ml, pH 4, temperature is 50℃)，the amount of absorption can reach to 26.8μg/mg TBC.

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