doi: 10.13241/j.cnki.pmb.2014.10.008

Experimental Research of the Novel Macroporous Chitin / Alginate-nanohydroxyapatite Composite Scaffolds*

XIE Jing-sen[1], SUN Jian[2△], LI Ya-li[2], CHEN Li-qiang[2], WEI Hua-sha[3], LIU Feng[1]

(1 The Medical College of Qingdao University, Qingdao, Shandong, 266021, China;

2 The Affiliated Hospital of Medical College Qingdao University, Qingdao, Shandong, 266021, China;

3 Qingdao Eighth People's Hospital of Stomatology, Qingdao, Shandong, 266000, China)

ABSTRACT Objective: It is the emphasis and difficulty to manufacture excellent scaffold for bone tissue engineering research. The purpose of the study was to analyze the effect of chitin to the porosity, water retention, degradation rate and biomechanical characteristics of composite scaffolds. **Methods:** Chitin solution was mixed with sodium alginate solution, and then the mixture was added a certain quality of hydroxyapatite. We divided the mixture into two groups according to the different chitin solution mass fraction: sca1 (0 % chitin), sca2 (50 % chitin). The surface structure and the pore size was observed under the Scanning electron microscopic. Then we calculated the porosity, degradation rate, water content and biomechanical properties. **Results:** Two groups of scaffold materials showed a multi-pore structure. The average pore size were 121.2 ± 12.6 μm and 213.3 ± 27.3 μm. The porosity were (90.53 ± 1.62) % and (87.73 ± 1.22) %. Statistical analysis showed that two groups of material porosity difference were statistically significant ($P<0.05$). The degradation rates of two groups of scaffold materials at six weeks were (59.12 ± 1.93) % and (22.91 ± 0.953) %. Statistical analysis showed that the degradation rate of the material differences between the two groups were statistically significant ($P<0.05$). Water content of two groups of scaffold materials were (95.52 ± 1.17) %and (90.42 ± 0.85) %. Statistical analysis showed that the water content of the material differences between the two groups were statistically significant ($P<0.05$). Biomechanical properties of the second group increased significantly. **Conclusion:** It could be seen from the experimental data that chitin could increase the pore size, improved stability to degradation and the biomechanical strength of materials. Therefore, chitin may has important research value in bone tissue engineering field.

Key words: Chitin; Sodium alginate; Nano-hydroxyapatite; Composite scaffold materials; Bone tissue engineering

Chinese Library Classification(CLC): R318.08 Document code: A

Article ID: 1673-6273(2014)10-1834-05

Introduction

With the rapid development of science and tissue engineering, bone tissue repair research has made great progress in recent years. Alginate is a natural anionic polymer. It is widely used in bone tissue engineering because of its good hydrophilicity and biodegradability under normal physiological conditions. Alginate could increase the mechanical strength of the stent and could be formed in a short time frame. It is easier to adjust the porosity of the bracket [1,2]. Hydroxyapatite is the main inorganic component of bone and teeth. The formula is $[Ca_{10}(PO_{46})(OH)_2]$. Due to its good biocompatibility, bioactivity, bone conduction and human bone mineral components similarity, hydroxyapatite is widely used in the field of biomedical materials. Experiments showed that, ALP could reach the highest activity and composite material could have good effect to promote cell proliferation when the content of hydroxyapatite in the composite material is between 30 % to 40 %. However, the ability of cells were reduced when the content of hydroxyapatite reaches to a certain extent[3-5]. Chitin is a non-toxic, biodegradable and biocompatible natural cationic polymer. Chitin has a crucial role in hierarchical controlling in the mineralization process[6]. PT Sudheesh Kumar, who has made the application of chitin nanocomposite scaffolds, showing good cell adhesion and protein adsorption [7]. Therefore, in the medical field, chitin could play an important role in bone repair and tissue engineering. For example, chitin and hydroxyapatite scaffolds prepared by mixing can increase the mechanical properties of the stent [8], and it could be used as a bone repair and reconstruction of bone. In this study, we analyzed the effect of chitin to the porosity, water retention, degradation rate and biomechanical characteristics of composite scaffolds, providing experimental evidence to the use of chitin in bone tissue repair applications.

1 Materials and methods

1.1 Materials and equipment

Chitin (Shanghai PuZhen Biological Technology Co., Ltd. C-

*Foundation items: Natural Science Foundation of Shandong Province (ZR2012HM069)

Author introduction: XIE Jing-sen, (1986-), male, master, Mainly engaged in oral and maxillofacial surgery research,

Tel: 18753251727, E-mail:xiejingsen0532@163.com

△ Corresponding author: SUN Jian, E-mail: sunjianqy@126.com

(Received: 2013-08-15 Accepted: 2013-09-10)

hina), Epichlorohydrin (Shanghai PuZhen Biological Technology Co., Ltd. China), Sodium alginate (Qingdao Xiangyu Seaweed Co., Ltd. China), nano-hydroxyapatite (Nanjing Emperor Nano Materials Co.,Ltd. China), Germany CHRIST ALPHA1-4-type freeze-drying machine, low temperature thermostat bath (Shanghai Bilang instrument Co., Ltd. China), Japan Shimadzu AGS-J universal material testing machine, CJJ-931 magnetic stirrer (Jiangsu Jintan Universal scientific Instrument Factory. China), JMS-840 scanning electron microscope (JEOL CO Japan).

1.2 Preparation of Chitin/Alginate −nanohydroxyapatite composite scaffolds

8 g of chitin was dispersed into 192 g of 6 wt % NaOH/4 wt % urea/90 wt % water mixture stirring for 10 min. The mixed solution was agitated at -20 ℃ for 48 h, then it could obtaine a transparent chitin solution with a concentration of 4 wt %. The prepared chitin solution was stored at -20 ℃ environment. 4 g SA was dissolved in the same solvent and stirred for 3 h at room temperature to obtain a 4 wt % polymer concentration. The chitin and SA solutions were mixed rapidly by changing the weight ratio of chitin to SA by w/w % of 0:10 and 5:5, which was coded as sca1 and sca2. ECH (1 ml) as a cross-linker and nanohydroxyapatite (400 mg) were added to the chitin/SA mixture (10 g). The resultant mixtures were stirred at 25 ℃ for 4 h to yield a homogeneous solution, and then reacted at 60 ℃ for 2 h. Preparation of solidifying liquid: The 5 wt % $CaCl_2$ and 5 wt % HCl solution were formulated into a uniform mixture. Adding a certain amount of solidifying liquid in the molds, and then the two groups of mixture were put in a mold to solidify at the same time. Cured for 30 minutes, the two groups of gel-like solid were placed in dialysis bag washed to neutral. Put the neutral gel-like solid into -80 ℃ refrigerator for 12 h. The two groups of gel-like solid were placed in a freeze drier (-52 ℃) lyophilized for 48 h. Stripping at room temperature to obtain chitin/alginate-nanohydroxyapatite composite scaffolds.

1.3 SEM images of composite scaffolds

The fracture surface (cross-section) of the composite scaffolds were sputtered with gold, and then were observed and photographed under scanning electron microscopic.

1.4 Determination of porosity

Good porosity of the scaffold is conducive to the transport of the blood and cell growth. Nanocomposite scaffolds' porosity were determined by the liquid displacement method. Took a certain volume (V_1) and weight (W_1) nanocomposite scaffolds of each group, immersed in ethanol (ρ) to saturation, removed and wiped the surface of the liquid with filter paper, measured the weight (W_2), and then the porosity was calculated according to the following formula:

$$\text{Porosity} = (W_2 - W_1)/\rho\ V_1 \times 100\ \%$$

1.5 Determination of water retention

A certain mass of each scaffold was immersed in PBS solution for 24 h. Took the scaffolds out of the PBS solution and weighted its weight (W_1), and the scaffolds were freeze-dried, then weighed its weight (W_2), so the water retention was calculated according to the following formula:

$$\text{Water retention} = (W_1 - W_2)/W1 \times 100\ \%$$

1.6 Determination of degradation rate

Vitro scaffold degradation rate is a very important studying parameter, ideal scaffold material can degrade slowly and eventually completely decomposed absorbed or eliminated by the body, gradually replaced by the newly generated tissue. Weighed two groups of certain quality scaffolds (W_1), placed 2 mg/ml lysozyme in PBS solution (pH 7.4, temperature 35 ℃), respectively took the scaffolds out of the solution in one week, two weeks, three weeks, four weeks, five weeks, six weeks. Then washed them with distilled water, freeze-dried and re-weighed (W_2) repeatedly. Therefore, the calculation formula of degradation rate is:

$$\text{Degradation rate} = (W_1 - W_2)/W_1 \times 100\ \%$$

1.7 Biomechanical testing

Scaffold's mechanical characteristic is an important parameter. The scaffold's maximum load were measured by the universal testing machine. Each scaffold material were cut into 10 mm × 5 mm × 5 mm size. Tensile test at room temperature, specimen length is 5 mm, and a tensile speed of 2 mm/min. We calculated modulus of elasticity after measuring the maximum load values of the scaffold material ($\overline{X} \pm S$, n=5).

1.8 Statistical analysis

Used SPSS13.0 software package for data analysis. T test was used for statistical analysis. Differences were considered statistically significant when the p-value was less than 0.05. The results were represented with $\overline{X} \pm S$.

2 Results

2.1 Appearance of the material

2.2 Scanning electron micrographs

2.3 Pore size is shown in table one

Table 1 Dimensions of the pores of the two groups observed on the surfaces of sections

Specimen	Pore size (μm)	
	Rang (minimum-maxmum)	Average (Mean± Standard deviation)
Sca 1 (0 %chitin)	86.2-159.2	121.2± 12.6
Sca 2 (50 %chitin)	152.4-269.5	213.3± 27.3

Fig.1 The appearance of the two groups, sca1 represented the 0 %chitin group, sac2 represented the 50 %chitin group

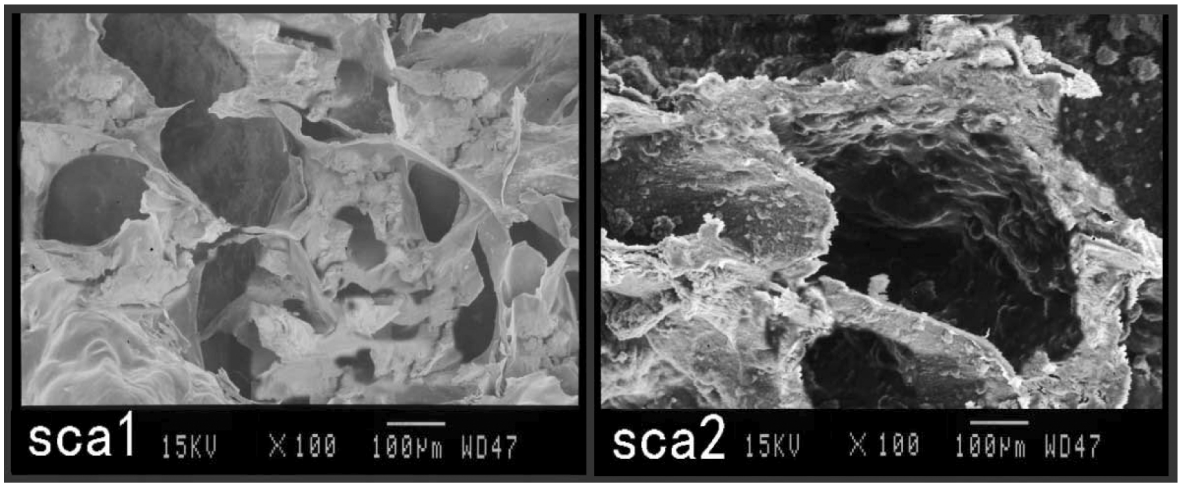

Fig.2 The SEM images of two groups(100×)

2.4 The test results of two groups

2.4.1 Porosity Porosity can be seen from Table 2, the porosity of sca1 and sca2 were (90.53 ± 1.62) % and (87.73 ± 1.22) % respectively. The higher the porosity, proved more conducive to cell growth[9]. After statistical analysis, the difference of porosity between the two groups were statistically significant($P < 0.05$).

2.4.2 Water retention Water retention can be seen from Table2, the water retention of sca1 and sca2 were (95.52 ± 1.17) % and (90.42± 0.85) % respectively. By statistical analysis, the difference of water retention between the two groups were statistically significant($P < 0.05$).

Table 2 The porosity and water retention of two groups(%)

	Porosity	Water retention
Sca 1 (0 %chitin)	90.53± 1.62	95.52± 1.17
Sca 2 (50 %chitin)	87.73± 1.22	90.42± 0.85

2.4.3 Degradation rate of two material in vitro Two material were put into PBS solution which Containing 2 mg/mL lysozyme, measuring degradation rate in vitro after 1, 2, 3, 4, 5, 6 weeks respectively. The results were shown in Table 3. After statistical analysis, the difference of degradation rates between the two groups were statistically significant ($P < 0.05$).

2.4.4 Biomechanical testing Elastic modulus is equal to the stress/strain. The differences between the two groups were statistically significant. The tensile propertiesthe of the group which containing 50 % Chitin have better performance than that without chitin.

Fig.1 Comparison of degradation rate of two groups for 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks

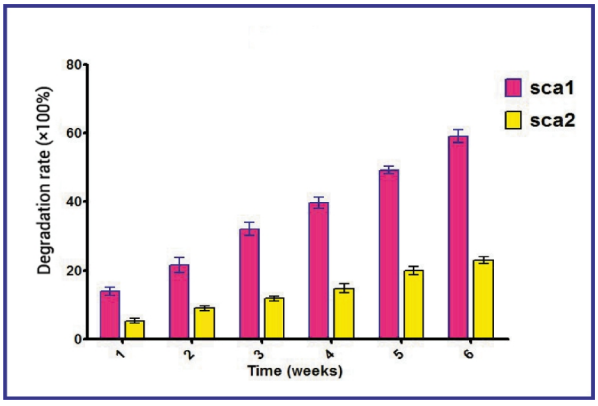

Table 3 Mechanical properties of the two groups

	Maximum lord (N)	Young modulus (MPa)
Sca 1 (0 %chitin)	12.178	6.41± 0.31
Sca 2 (50 %chitin)	64.231	34.56± 0.48

3 Discussion

It has been the thorny issue of clinical to repair bone defects caused by tumors, trauma or deformity. Severe bone defects, the bone tissue can not be self-repair. Meanwhile, the rapid development of modern tissue engineering has opened up new ways to treat bone defects. So it is a trend to find a bone substitute material autologous to repair bone defect.

Ideal tissue engineering scaffold material should have the following conditions[10, 12]: ① has good biocompatibility. ② osteoinductive and osteoconductive. ③ biodegradability and degradation adjustability. ④ suitable mechanical strength. ⑤ open porosity. ⑥ plasticity and workability. In this study, two groups of scaffolds were prepared, which sca1 group does not contain chitin, while sca2 group containing 50 % chitin. Two groups contain the same amount of hydroxyapatite to improve stent strength. Groups contain alginate, sodium alginate non-toxic, non-immunogenic, preferably the viscosity, hydrophilic, are able to provide a good three-dimensional cells and to maintain a good environment for the growth shape, thus completing the demineralized bone matrix modeling[13-15].

Studies have shown that the need for porous scaffolds [16], the porosity of 85 % or more[9], is conducive to cell adhesion. Meanwhile, scaffold material should have good biodegradability. The experimental results showed that: composite scaffold by freeze-drying showed spongy, with a high moisture content, and hardness, not easy to be broken. Judging from the appearance of the two groups, they have good plasticity, can be prepared to more regular shape. Scanning electron microscopy showed sca2 group aperture greater than sca1 group. The average pore size of Sca2 group was about 210 μm, more conducive to the growth of cell adhesion. Except by the pore size of freeze-dried outer[17], may also be set with sca2 alkaline ions in the dialysis processes. Porosity and moisture content in the two groups were 85 %, while the porosity sca2 group and water content below sca1 group, but still able to meet the needs of cell growth. Sca2 group was significantly lower than the degradation rate sca1 group, indicating that degradation of chitin material can improve the stability of the bone defect. It is more conducive to play a supporting role substitute materials, while the degradation of the material is stable in favor of bone formation. It is an important indicator to evaluate whether the scaffolds practical. The experimental results showed that: chitin scaffolds significantly improved biomechanical properties. Sca2 group is higher than sca1 group in maximum stress, strain and elastic modulus.

In summary, Chitin/Alginate-hydroxyapatite composite scaffolds have shown its superiority in tissue engineering materials. But its actual effect of bone defect repair remains to be further through animal bone defect repair in vivo experiments confirmed. Chitin is a resource-rich, biological material with excellent performance. Because of its intrinsic antibacterial activity and other fine features, it shows a great prospect in repairing bone defect filled with bone tissue engineering scaffolds.

References

[1] J. F. A. Valentea, T. A. M. Valentea, P. Alvesb, et al. Alginate based scaffolds for bone tissue engineering[J]. Materials Science and Engineering: C, 2012, 32(8): 2596-2603

[2] J.A. Sowjanya, J. Singh, T. Mohita, et al. Biocomposite scaffolds containing chitosan/alginate/nano-silica for bone tissue engineering[J]. Colloids and Surfaces B: Biointerfaces, 2013, 109: 294-300

[3] ManjubalaI, S.Scheler, Bossert, et al. Mineralisation of chitosan scaffolds with nano-apatite formation by double diffusion technique [J]. Acta Biomater, 2006, 2(2): 75-84

[4] Teng S H, Lee E J, Yoon B H, et al. Chitosan/nanohydroxyapatite composite membranes via dynamic filtration for guided bone regeneration [J]. Biomed Mater ResA, 2009, 88(3): 569-580

[5] Venugopal J R, Giri Dev V R, Senthilram T, et al. Osteoblast mineralization with composite nanofibrous substrate for bone tissue regeneration [J]. Cell Biol Int, 2011, 35(1): 73-80

[6] Katrin Spinde, Martin Kammer, Katja Freyer, et al. Biomimetic Silicification of Fibrous Chitin from Diatoms[J]. Chem. Mater, 2011, 23(11): 2973-2978

[7] P. T. Sudheesh Kumar, C. Ramya, R. Jayakumar, et al. Drug delivery and tissue engineering applications of biocompatible pectin-chitin/nano CaCO3 composite scaffolds[J]. Colloids and Surfaces B: Biointerfaces, 2013, 106: 109-116

[8] Huang, Z. H, Dong, et al. Electrochemistry assisted reacting deposition of hydroxyapatite in porous chitosan scaffold [J]. Material Letters, 2008, 62: 3376-3378

[9] Cao Yi-lin. Tissue Engineering [M]. Beijing: Science Press, 2007: 625-636

[10] Gross Karlis A, Rodrí guez-Lorenzo L. M. Biodegradable composite scaffolds with an interconnected spherical network for bone tissue engineering[J]. Biomaterial, 2004, 25(20): 4956

[11] Akeda K, An H. S. Platelet-rich plasma stimulates porcine articular chondrocyte p roliferation and matrix biosynthesis [J]. Osteoarthr Carti, 2006, 14(12): 1272

[12] Datta N, Pham Q. P, Sharma U, et al. In vitro generated extracellular matrix and fluid shear stress synergistically enhance 3D osteoblastic differentiation[J]. Proc Natl Acad Sci, 2006, 103(8): 2488

[13] Park H, Kang SW, Kim BS, et al. Shear- reversibly crosslinked alginate hydrogels for tissue engineering [J]. Macromol Biosci, 2009, 9(9): 895-901

[14] Park H, Lee K Y. Alginate/h, Yaluronate hydrogels for cartilage regeneration [J]. Control Release, 2011, 152 (Suppl 1): e233-234

[15] Place ES, Rojo L, Gentleman E, et al. Strontium - and zinc -alginate hydrogels for bone tissue engineering[J]. Tissue Eng Part A, 2011, 17 (21- 22): 2713-2722

[16] Uebersax L, Hagenmuller H, Hofmann S, et al. Effect of scaffold design on bone morphology in vitro[J]. Tissue Eng, 2006, 12 (12): 3417-3429

[17] Zhao Jie, Lian Xiao-Feng, Hou Tie-Sheng, et al. Anterior debridement and bone grafting of spinal tuberculosis with one-stage instrumentation anteriorly or posteriorly [J]. International Orthopaedics, 2007, 31(6): 859-863

甲壳素/海藻酸钠-纳米羟基磷灰石多孔复合支架材料的实验研究*

解京森[1] 孙 健[2△] 李亚莉[2] 陈立强[2] 魏华莎[3] 刘 峰[1]

(1 青岛大学医学院 山东 青岛 266021;2 青岛大学医学院附属医院 山东 青岛 266021;
3 青岛市第八人民医院 山东 青岛 266000)

摘要 目的:制备性能优良的复合支架一直是骨组织工程学研究的重点和难点。比较分析甲壳素对复合支架材料的孔隙率、含水量、降解率及生物力学特性的影响。**方法:**将甲壳素溶液与海藻酸钠溶液充分混合,然后将一定质量的羟基磷灰石加入混合液。根据甲壳素溶液在混合液中的质量分数不同分为两组:sca1(0 %chitin)、sca2(50 % chitin)。扫描电镜下观察材料的表面结构以及检测材料的孔径。测量并计算出复合支架材料的孔隙率、降解率、含水量以及生物力学性能。**结果:**两组支架材料均表现为多孔隙结构,平均孔径大小分别为:121.2± 12.6 μm、213.3± 27.3 μm。孔隙率分别为:(90.53± 1.62)%、(87.73± 1.22)%,统计学分析显示,两组材料孔隙率的差异比较有统计学意义(P< 0.05)。两组支架材料第 6 周的降解率分别:(59.12± 1.93)%、(22.91± 0.953)%,统计学分析显示,两组材料降解率的差异比较有统计学意义(P< 0.05)。两组含水量分别为:(95.52± 1.17)%、(90.42± 0.85)%,统计学分析显示,两组材料含水量的差异比较有统计学意义(P< 0.05)。第二组生物力学特性显著提高。**结论:**从本实验的实验数据可以看出,甲壳素可以增大材料的孔径,提高材料的降解稳定性,提高材料的生物力学强度。因此,甲壳素在骨组织工程领域具有重要的研究价值,同时为今后的进一步实验提供一定的实验依据。

关键词:甲壳素;海藻酸钠;纳米羟基磷灰石;复合支架材料;组织工程

中图分类号:R318.08 **文献标识码:**A **文章编号:**1673-6273(2014)10-1834-05

* 基金项目:山东省自然科学基金 (ZR2012HM069)

作者简介:解京森(1986-),男,硕士,主要从事口腔颌面外科学研究,
电话:18753251727,E-mail:xiejingsen0532@163.com
△通讯作者:孙健,E-mail: sunjianqy@126.com

(收稿日期:2013-08-15 接受日期:2013-09-10)