Transactions of the Materials Research Society of Japan 29[6] 2881-2884 (2004)

Nano-Hydroxyapatite/Collagen/PLA bone scaffold reinforced by chitosan fibres

Q.L. Feng, X.M. Li

Biomaterials Laboratory, Department of Materials Science & Engineering, Tsinghua University, Beijing, 100084, China

Porous Nano-Hydroxyapatite /Collagen/PLA composite was prepared and reinforced by chitosan fibres as a scaffold for bone tissue engineering. The reinforced scaffold holds the characters of Nano-Hydroxyapatite / Collagen/ PLA composite, such as the porosity and the pore size. It was found that only if the length of the fibres was equal to or greater than a critical length, which was determined to be 1.2 mm by the experiments, could they reinforce the material. The compressive strength of the scaffold increased with the increase of the fibre's volume content when the fibres' length was 1.2 mm. We put forward an empirical formula to calculate the compressive strength of the intensified scaffold and figured out the modifying factor (F_σ) for the porous bone scaffold.

1. Introduction

Bone tissue engineering requires cellular components, scaffolds, and growth factors [1]. The scaffold lies at the heart of all the new tissue engineering approaches [2].

Regarding the scaffold, it is generally agreed that a highly porous microstructure with interconnected pores and a large surface area is conducive to tissue in growth. For bone regeneration, pore sizes between 100 and 350 um and porosities of more than 90% are preferred [3].

It is obvious that a bone scaffold should possess not only satisfactory porosity but also appropriate mechanical properties. It is very difficult to prepare a bone scaffold with satisfactory porosity and high mechanical property simultaneously. So it is an important subject to improve the mechanical property of the bone scaffold with satisfactory porosity and pore size.

Hydroxyapatite/collagen composite scaffold for bone tissue engineering has been very hot study field because hydroxyapatite and collagen are main components in natural bone. Recently, nano-hydroxyapatite/collagen (nHAC) was successfully developed in our lab [4]. It has been proved that the nHAC is biodegradable and biocompatible. But the mechanical property of the pure nHAC with satisfactory porosity is relatively low. So it is necessary for nHAC to compound with certain high molecular weight polymers to ensure the mechanical properties.

In this study, first of all, nHAC and polylactic acid (PLA) are compounded into a porous bone scaffold (nHAC/PLA) by the method developed in our lab [5].

Table 1 Length of the fibre, volume content of the fibre and porosity of the scaffold

Length of the fibres (mm)	Volume content of the fibres (%)	Porosity of the scaffold (%)
0.5±0.05	15	91.7
0.8±0.05		91.4
1.0±0.05		91.0
1.2±0.05		90.6
1.5±0.05		90.1
1.8±0.05		88.1
2.0±0.05		86.2
1.2±0.05	2	91.8
	5	91.7
	8	91.5
	12	91.0
	15	90.5
	18	89.2
	20	88.5
	22	82.5

Table 2 Volume content of the chitosan fibres and compressive strength of the reinforced scaffold

Length of the fibres (mm)	Volume content of the fibres (%)	Compressive strength of the scaffold(MPa)
1.2±0.05	2	2.11
	5	2.35
	8	2.55
	12	2.75
	15	3.21
	18	3.69
	20	4.04
	22	4.10

But the mechanical properties are much lower than natural bone.

Chitosan, an amino polysaccharide (poly-1, 4-d-glucoamine) derived from chitin by deacetylation, has been widely applied in biomedical applications, such as wound dressings and drug delivery systems [6]. In this paper, porous nano-hydroxyapatite/ collagen/PLA bone scaffold reinforced by the biodegradable and biocompatible chitosan fibres was prepared. The reinforced scaffold held the characters of nHAC, satisfactory porosity and good pore size. This work also showed the relation between the volume content of the fibres and compressive strength of the intensified scaffold.

2. Materials and methods

2.1 Materials

Chitosan fibres (80% deacetylated) were purchased from Donghua University, China. Polylactic acid (PLA) was purchased from Shandong medical appliance factory. Type I collagen, dioxane and other chemicals were purchased from Beijing Chemical Co.Lt d., China.

2.2 Synthesis of the nHAC powder

Firstly, type I collagen was dissolved in acetic acid and the collagen solution with a concentration of 0.02g/30mol was prepared. Afterwards, a solution containing PO_4^{3-} was added to the collagen solution until the content ratio of PO_4^{3-}/collagen reached 0.05mol/g. Secondly, a solution containing Ca^{2+} was added to the above solution until the mol ratio of Ca^{2+} /PO_4^{3-} arrived at 1.66. Thirdly, NaOH solution was added to the solution until pH (determined with pH meter) equaled to 6-8. Finally, after deposited for 2 days, the made-up solution was centrifuged and the separated deposition was lyophilized after rinsed by distilled water for 3 times. Subsequently, the lyophilized deposition was grinded into fine powder.

2.3 Preparation of the nHAC/PLA scaffold

Above all, PLA, with a molecular weight of one hundred thousand, was dissolved in dioxane at the concentration of 0.08g/ml. Then, nHAC powder was added to the solution until the mass of nHAC and PLA was the same. After that, the prepared liquid was dispersed ultrasonically for 30min. At last, the liquid was lyophilized for 12h.

2.4 Preparation of the scaffold reinforced by chitosan fibre

The chitosan fibres were added to nHAC/PLA solution gradually while the liquid was stirred by magnetic force. And then, the liquid was dispersed ultrasonically for 45min. Eventually, the liquid was lyophilized for 12h.

2.5 Measurement of the porosity

The porosity was measured by liquid substitution method. The liquid used in this study was isopropanol. The porosity was calculated by the form:

$$\varepsilon = (V_1 - V_3)/(V_2 - V_1) \quad (1)$$

Note: ε, the porosity of the scaffold; V_1, the volume of isopropanol before the scaffold was put in; V_2, the volume of the liquid after the scaffold was put in; V_3, the volume of isopropanol after the liquid was pressed into the pore of the sample and the sample was taken out of the liquid.

2.6 Measurement of the mechanical properties

The compression strength was measured by electronic universal material testing machine. The load was 10KN and the pressing velocity was 0.5mm/min. The size of the sample was Φ8.5×15mm. Modulus of compressibility was calculated according to the curve of stress-strain by Hooke's law.

2.7 Analysis of scanning electron micrograph (SEM)

The samples were observed by JSM-6460LV Scanning Electron Microscopy under the voltage of 20KV.

2.8 Analysis of x-ray diffraction (XRD)

Rigaku D/max x-ray diffractometer (Cu Kα, 40kV, 120mA, λ=1.5405Å, rate=4deg/min, pace=0.02deg) was applied.

3. Results and discussion

3.1 Analysis of XRD

The XRD patterns of hydroxyapatite (HA) crystal, nHAC, nHAC/PLA, natural bone and the reinforced scaffold were shown in figure 1. It can be observed from figure 1 that the main component of nHAC is HA. Because the HA in nHAC is nanophase, its diffraction peak broadens. From the two figures, we can see that the addition of chitosan fibres does not change nano-HA in the material, and that the reinforced scaffold holds the characters of nHAC. The XRD patterns of the reinforced scaffold and natural bone are very similar, which indicates that the omponents and crystal size of the reinforced scaffold and natural bone are very semblable and which will benefit the growth of cells and degradation of the

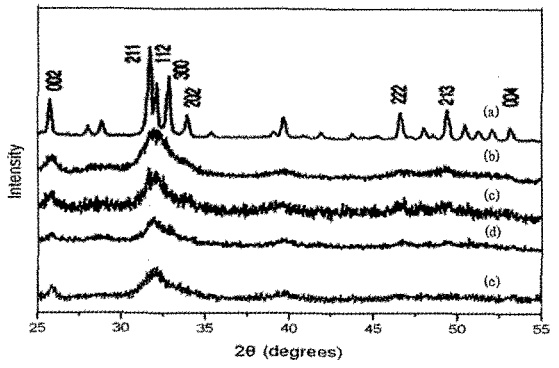

Fig 1 XRD patterns of materials: (a) HA crystal; (b) nHAC; (c) nHAC/PLA; (d) Natural bone; (e) the reinforced nHAC/PLA by chitosan fibres

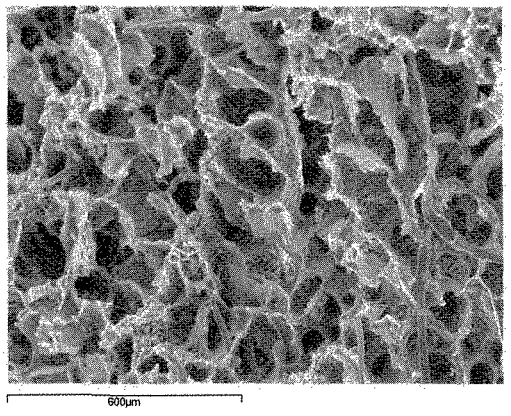

Fig 2 Scanning electron micrographs of the scaffold reinforced by chitosan fibres

scaffold.

3.2 Porosity of the reinforced scaffold

Fibres with certain volume content but different length, and certain length but different volume content are added to nHAC/PLA respectively. With the length and volume content of the fibres increasing, the porosity decreases, which is shown in table 1. It is observed that the porosity of the scaffold reinforced by chitosan fibres can reach 90%. The scanning electron micrographs of the scaffold, in which the volume content of the fibres is 15% and the length of the fibres is 1.2±0.05mm, are showed in figure 2. It is found that the pore size of the scaffold is 100~200um. Therefore, porosity and pore size of the reinforced scaffold is suitable for bone tissue engineering.

3.3 Compressive strength of the scaffold

Fibres with 15% volume content but different length were added and the compressive strength of the reinforced scaffold was measured. The results show that only if the length of the fibre is greater than or equal to a certain length, can it reinforce the material. According to the experimental results, the critical length of the chitosan fibre is 1.2±0.05mm.

From table 1, we can see that the porosity of the scaffold decreases, with the length of the fibre increasing. So the appropriate length of the fibres is decided to be 1.2 ± 0.05mm, guaranteeing the porosity of the fibres to be higher and the length of the fibres to be greater than or equal to the critical length.

Fibres with the same length of 1.2±0.05mm but different volume were added and the compressive strength of the reinforced scaffold was measured respectively, the result of which was shown in table 2. It is found that, along with increase of the fibre's volume content, the compressive strength of the scaffold increases.

There are some investigations on the relation between the fibres' volume content and the compressive strength of the intensified material, which is based on that the length of the fibres is greater than or equal to the critical length. Hancox N J has put up the formula (2a) under the assumptions that matrix material, which surrounds the fibres, is an ideal plastic body, and that matrix material and the fibres are both in linear elastic condition, and that orientations of the fibres are ideal and all the fibres are helpful for intensifying the material [7].

For the porous scaffold in this paper, the formula (2a) is apparently not exact. But the fibres are randomly distributed in the wall of the pores and the orientation of fibres with certain volume content is helpful for reinforcing the material. So it is possible to modify the formula (2a) to express the relation between the fibres' volume content and compressive strength of the porous scaffold. Using test data (E_f, 25GPa; E_m, 50MPa; σ_m,1.7MPa), many different experiment results, some of which is shown in table 1 and table 3, and the formula (2a), we put up another empirical formula shown as formula (2b) for the reinforced porous scaffold.

$$\sigma_c = 2V_f\sqrt{\frac{E_f E_m V_f}{3(1-V_f)}} + \sigma_m \qquad (2a)$$

$$\sigma_c = 2F_\sigma V_f\sqrt{\frac{E_f E_m V_f O}{3(1-V_f)}} + \sigma_m \qquad (2b)$$

Where σ_c is the compressive strength of the intensified material, V_f is the volume content of the fibre, E_f is the modulus of compressibility of the fibre, E_m is the modulus of compressibility of the matrix material, σ_m is the compressive strength of the matrix material, O is the porosity of the intensified material and F_σ is the modifying factor.

From the formula (2b), we can see that the compressive strength of the porous scaffold reinforced by the fibres is determined by the porosity of the scaffold and modifying factor besides the volume content of the fibre, the modulus of compressibility of the fibres and the modulus of compressibility of the matrix material.

The modifying factor (F_σ) is mainly influenced by the nature of the matrix material including continuity, pore size, uniformity and the form of the fibre. For the matrix material (nHAC/PLA) and the chitosan fibres applied in this study, the modifying factor F_σ is. 1/51.6

From table 1 and table 2, it can be seen that the appropriate volume content of the fibres is 15%, guaranteeing the porosity of the scaffold to be beyond 90% and the compressive strength of the scaffold to be maximum.

4. Conclusions

The reinforced scaffold holds the characters of nHAC. The components and crystal size of the reinforced scaffold and natural bone are very semblable, which will benefit the growth of cells and degradation of the scaffold.

The porosity of the reinforced scaffold is about 90% and the pore size is 100~200μm.

Only if the length of the fibres is greater than or equal to the critical length, can it reinforce the material. The appropriate length of the fibres is 1.2± 0.05mm for the porous matrix material (nHAC/PLA) in this paper. Along with increase of the fibres' volume content, the compressive strength of the scaffold increases. For the porous scaffold, the compressive strength can be calculated by the empirical formula:

$$\sigma_c = 2F_\sigma V_f \sqrt{\frac{E_f E_m V_f O}{3(1-V_f)}} + \sigma_m$$

For the scaffold and the chitosan fibres applied in the paper, the appropriate volume content of the fibres is 15%.

Acknowledgement

The authors are grateful to the financial support from the 863 project (2001AA326050) of China.

References

[1] Torun Kose G, Kenar H. Macroporous poly (3-hydroxybutyrate-co-3-hydroxyvalerate) matrices for bone tissue engineering. Biomaterials, 2003, 24:1949–1958

[2] Sikavitsas V, Bancroft G, Mikos A. Formation of three dimensional cell/polymer constructs for bone tissue engineering in a spinner .ask and a rotating wall vessel bioreactor. J Biomed Mater Res, 2002, 62:136–148.

[3] Yoshimoto H, Shin YM, Terai H, Vacanti JP.A biodegradable nanofibre scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials, 2003, 24: 2077–2082

[4] Wang RZ, Cui FZ, Lu HB, Ma CL, Li HD. Synthesis of Nanophase Hydroxyapatite collagen composite. Journal of Materials Science Letters, 1995, 14:490-492

[5] Liao Susan. The Study on Mineralized Collagen Based Materials for Bone Tissue Engineering: [dissertation]. Beijing: Tsinghua University, 2003

[6] Aiedeh K, Gianasai E, Orienti I, Zecchi V.Chitosan microcapsules as controlled release systems for insulin.J Microencapsul, 1997, 14:567–76.

[7] Hancox N.J. Fibre Composite Hybrid Materials. Applied Science Publishers LTD. 1981

(Received August 3, 2004; Accepted August 31, 2004)