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# Preparation of porous biodegradable poly(lactide-co-glycolide)/ hyaluronic acid blend scaffolds: Characterization, *in vitro* cells culture and degradation behaviors

**Chih-Ta Lee · Yu-Der Lee**

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**Abstract** A series of poly(lactide-co-glycolide) (PLGA)/ hyaluronic acid (HA) blend with different HA composition were used to fabricate scaffolds successfully. The pores of the three dimensional scaffold were prepared by particle leaching and freeze drying. The pore size was about 50–200 $\mu$m and the porosity was about 85%. The characterizations of the scaffold, such as mechanical properties, hydrophilicity and surface morphologies were determined. Mouse 3T3 fibroblast was directly seeded on the scaffolds. The cell adhesion efficiency, cell morphology observed by scanning electron microscopy (SEM) and the degradation behavior of the blend scaffold were evaluated. In summary, the results show that the adhesion efficiency of cells on the PLGA/HA blend scaffold is higher than that on the PLGA scaffold. Moreover, the incorporation of HA in PLGA not only helps to increase the cell affinity but also tends to lead the water and nutrient into the scaffold easily.

## 1 Introduction

Biodegradable materials have been of great interest in the field of medical science such as drug carriers [1–4], synthetic prosthesis [5–8] and medical devices [9–11] etc. Recently, the use of new materials in tissue engineering has been studied intensively. In view of their widespread uses reported in the literature [12], the development of tissue engineering is especially notable during the last decade. In the past, the route of therapy is to perform organ transplantation when the organs of human body suffer from damages or failure of their functions. The shortcomings of therapy are twofold: the number of donors could be insufficient and the problems that are associated with immunity. Moreover, other approaches, i.e., artificial prosthesis and medical devices, are not capable of replacing all the functions of damaged organ or tissue such as caused by skin [13], cartilage [14], bone [14] and heart valve [15]. Therefore, many efforts have focused on the studies of tissue engineering.

The requirements of scaffold to be used in tissue engineering are biodegradable, biocompatible in addition to desirable mechanical properties [16]. Moreover, this scaffold should be non-toxic with respect to cells and it can also be metabolized in terms of time. Presently, biodegradable materials can be classified into three categories [17]: polyester to be produced by microorganisms [18], synthesized polymer [19–21] such as aliphatic polyesters, and natural polysaccharides [22]. For the former two types, polyesters are materials with poor hydrophilicity and they don't possess natural cell binding sites. In order to ameliorate the cells' affinity of scaffold, polysaccharides were introduced to improve both the hydrophilicity of scaffold [23–24] and the cells adhesion to scaffolds. As mentioned above, scaffolds can be fabricated from these materials to be adsorbed onto the surface via ECM molecules.

In like fashion, poly(lactide-co-glycolide) (PLGA) exhibits good biodegradation properties, biocompatibility, high mechanical strength and excellent shaping and molding properties. Further, there are many synthetic methods, the feasibilities of which have been investigated, for example, polycondensation of diols and carboxylic acid [25], self-condensation of hydroxyl acids [26] and ring opening polymerization [27–28]. However, these materials have poor hydrophilicity. As such, many researches have focused their studies on synthesizing block copolymer, such as poly(lactic acid-co-ethylene glycol) (PLA-co-PEG)[29], and they are indeed successful in ameliorating the hydrophilicity of the polyester.

C.-T. Lee · Y.-D. Lee (✉)
Department of Chemical Engineering National Tsing Hua University 101, Section 2 Kuang Fu Road, Hsin Chu, Taiwan 300, ROC
email: ydlee@che.nthu.edu.tw

On the other hand, Hyaluronic acid (HA) is a naturally occurring anionic polysaccharide consisting of alternation 1, 4-linked units of 1, 3-linked glucoronic acid and N-acetylglucosamine [30] and it is the only non-sulfated glycosaminoglycan (GAG) in the extra-cellular matrix (ECM) [31]. It is capable of influencing cell adhesion [32].

In this paper, PLGA/HA blending materials were fabricated. The addition of polysaccharide (hyaluronic acid) is to improve the ability of cell attachment and provide the scaffold with a surrounding similar to its original environment. The salt leaching and frozen dried methods were chosen to form porous structure. In addition, the properties, including mechanical, hydrophilicity, in-vitro biocompatibility and in-vitro degradation behavior will be investigated. In the mean time, the properties of the scaffold were characterized with respect to the composition of HA.

## 2 Materials and methods

### 2.1 Materials

PLGA (lactide/glycolide copolymer, 85:15 mole ratio) was purchased from Purac Inc. with an average molecular weight of 580000 g/mole. Hyaluronic acid was purchased from Sigma. Sodium chloride was obtained from Aldrich Chemical Co. Dimethyl Sulfoxide(DMSO) was obtained from Aldrich Chemical Co. Deionized distilled water was acquired by using MillPore and at 18.2 m$\Omega$ resistance.

### 2.2 Preparation of PLGA/hyaluronic acid (HA) scaffolds and films

Scaffolds of PLGA/HA blends were prepared by solution mixing of HA (0–10%) in PLGA. HA was first dissolved in water to form a 1wt% solution, then DMSO was added to replace water and then stirred vigorously. After that, water was removed by freezing/drying step. Suitable amount of PLGA was dissolved in DMSO at about 50°C to form another DMSO solution. The two DMSO solutions were evenly mixed and poured into a Teflon mold with NaCl salt used as a porogen material. The mixed solutions were kept in the room temperature for 48 hrs and were subsequently frozen/dried for 48 h to remove the remaining solvent. The resulted product was then immersed in distilled water in an ultrasonic bath for 12 hrs to leach out the salt and then the porous scaffolds with thickness of 3 mm were obtained. The combination of different pore-forming methods might form internal- connected pores and can avoid the closed pores. The pore size was about 50–200 $\mu$m and the porosity was about 85%.

To prepare the polymeric films, the mixed polymer DMSO solution was cast on a Teflon plate. Then solvent was evaporated at room temperature for 48 h. After most of the solvent had been air-dried at room temperature, the residual solvent was removed in vacuum at 50°C for another 24 hrs until a constant weight was obtained. In contrast, film of PLGA was also prepared by using chloroform as a casting solvent. The thickness of the films was about 0.47 mm.

### 2.3 Measurements of mechanical properties

The mechanical properties of blended films were measured by Shimadzu AGS 2000G equipment. The sample (length, 20 mm; width, 10 mm) end was clipped vertically and elongated at a rate of 1mm/min, and the load-displacement and modulus curve were recorded.

### 2.4 Analysis of hydrophilicity

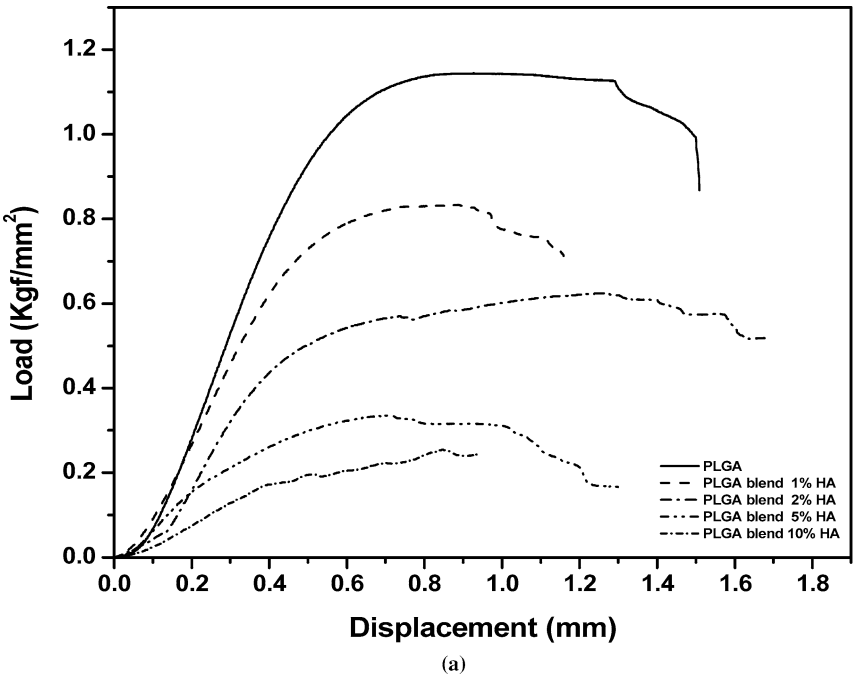
Contact angles (FACE CA-D Contact Angle Meter, Kyowa Kaimenkagaku Co.) of polymer films to water were measured on the surface of the sample. Deionized water was used for the measurement. The blended materials were coated on a glass plate. Water was dropped on the glass and a picture was taken by a digital camera. Five independent measurements at different sites were averaged. Then, the contact angle was analyzed by a computer.

### 2.5 Cell culture

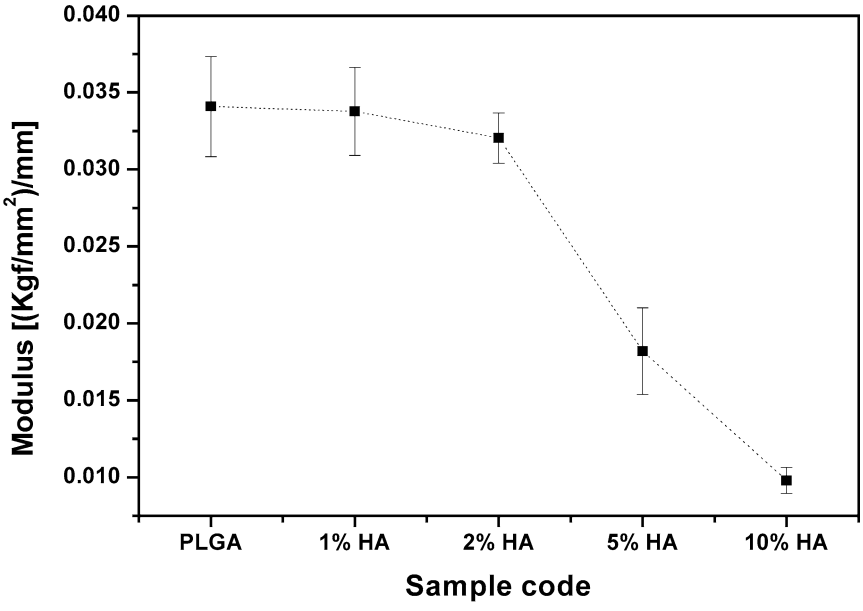
#### *2.5.1* In-vitro *cells culture*

The *in vitro* cytotoxicity of matrices was evaluated on the basis of cell morphology and cell viability. Polymer films were cut into circular shape of 10 mm in diameter and placed in a 24-well culture dish, and then sterilized in 70% ethanol for 4 hrs. After sterilized, the matrices were washed with phosphate-buffered saline (PBS, pH = 7.4) for several times.

Cells (mouse 3T3 fibroblasts) were cultured in 24 wells tissue culture flasks. Cells were seeded on the matrices films with 1–2 $\times$ $10^5$ cells per well and cultured for different time interval in growth medium containing Dulbecco's Modified Eagles Medium (DMEM), 10% (v/v) fetal calf serum, 1% penicillin/streptomycin/ amphotericin-B, and 10% PBS. The cells of seeded disc were maintained in a humidified incubator at an atmosphere with 5% $CO_2$ at 37°C for different periods of time. Then the culture medium was removed. Subsequently, the residual culture medium and unattached cells were removed by washing with PBS buffer solution. After the attached cells on the disks were digested by trypsin, the cell attachment efficiency was determined by counting the number of cells remained in the matrices. A cell viability count was performed using the trypan blue exclusion stain.




(a)




(b)

**Fig. 1** (a) Load-displacement curves of PLGA/HA blending scaffolds. (b)modulus for scaffolds containing different HA

*2.5.2 Cells culture on PLGA/HA blend scaffolds*

Scaffolds with different composition of HA were cut into small discs. The diameter of the disc was about 10 mm. The scaffolds were sterilized with 70% ethanol for 60 min and disinfected via ultraviolet light for 1hr. Further, the scaffolds were washed with PBS several times and kept in a 24 well cell culture plate. Finally, 1–2 $\times$ $10^6$ cells (mouse 3T3 fibroblast) were evenly seeded on the scaffolds. The culture plate was maintained at 37°C under 5% $CO_2$ condition. After three days, the cells were digested by trypsin and number of cells remained on the scaffolds was counted. The morphology of cells was observed by SEM. To be meaningful, the culture medium was refreshed every day.

*2.5.3 Morphology observation by SEM*

The surface and internal structure of the porous scaffold was observed via a scanning electron microscopy (JSM-5600, JEOL).

Mouse 3T3 fibroblasts were cultured on scaffolds with different PLGA/HA composition. They were cultured for

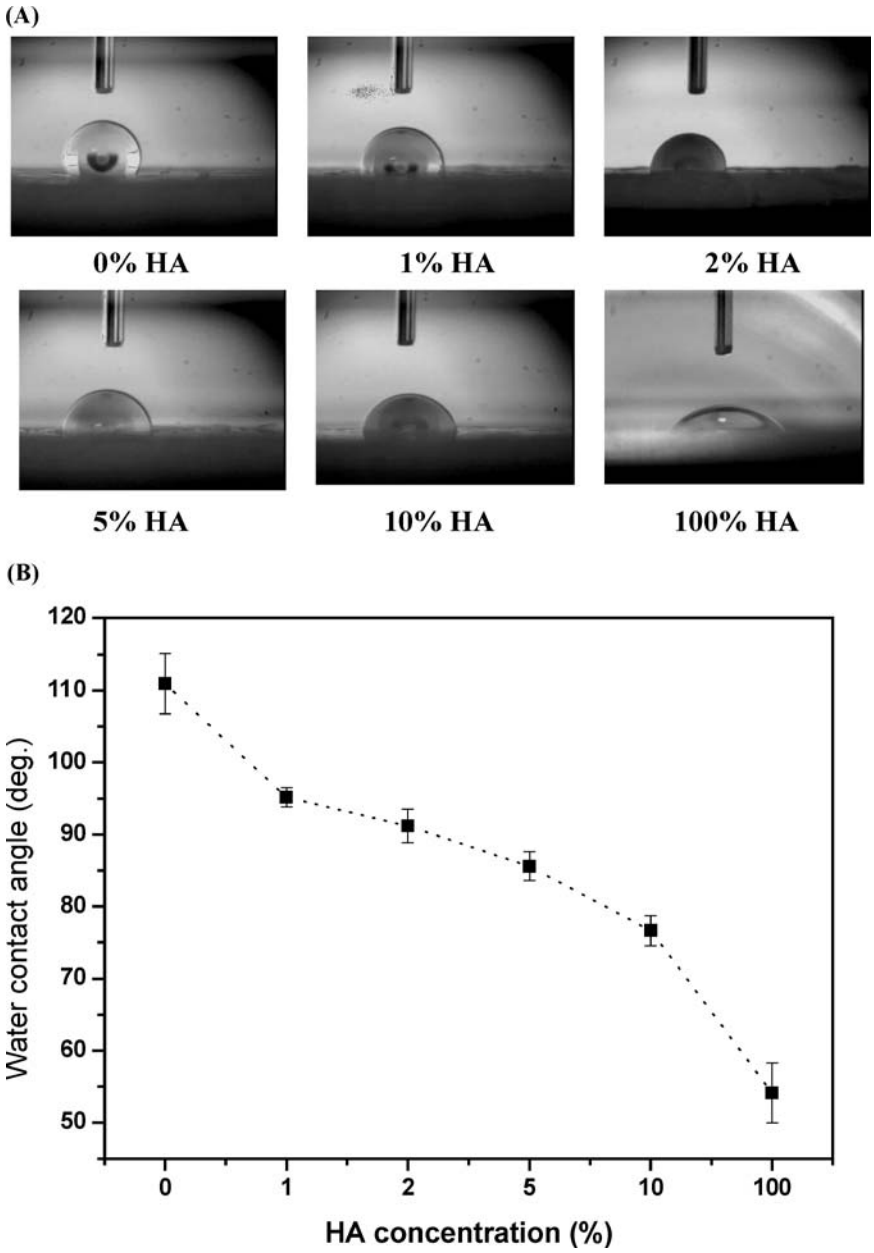



**Fig. 2** (A) photographs of water contact angles for scaffolds with different HA concentration (wt%) and (B) the variation of the contact angles of the blend scaffolds with different HA concentration

periods of time and then fixed with 2% glutaraldehyde in PBS. First, the scaffold was immersed in 2% glutaraldehyde for 3 hrs at 4°C and then rinsed with 0.1 M cacodylate buffer solution (pH 7.4) twice. After removing the fixative, the cells were post-fixed in 1% osmium tetroxide (1% w/v osmium tetroxide in 0.1 M PBS) for 40 min at room temperature. After that, these materials were dehydrated by treating with a series of graded ethanol solutions (50% for 1 h, then 75%, 85%, and 95%, each for 20 min), and then the fixed samples were dried by critical point dryer (Hitachi, HCP-2). Before morphologic observations, the scaffold samples were coated with gold using a sputter coater (SPI-MODULE sputter coater) in vacuum. And the cells distributed on the scaffold were observed under Olympus Invert-microscopy (Nikon, TS1000). The samples were fixed by 10% formalin at 4°C, and they were subsequently dehydrated in the graded ethanol (50%,75%, 85%, and 95%, respectively) and embedded in paraffin. They were then cut into slices and washed with xylene to removed paraffin. After H&E stained, the slices were observed under invert microscopy.

### 2.6 *In-vitro* degradation

The degradation behavior was assessed after preset time periods. Polymer matrix degradation was determined by measuring changes in water up-take and its mass immediately after

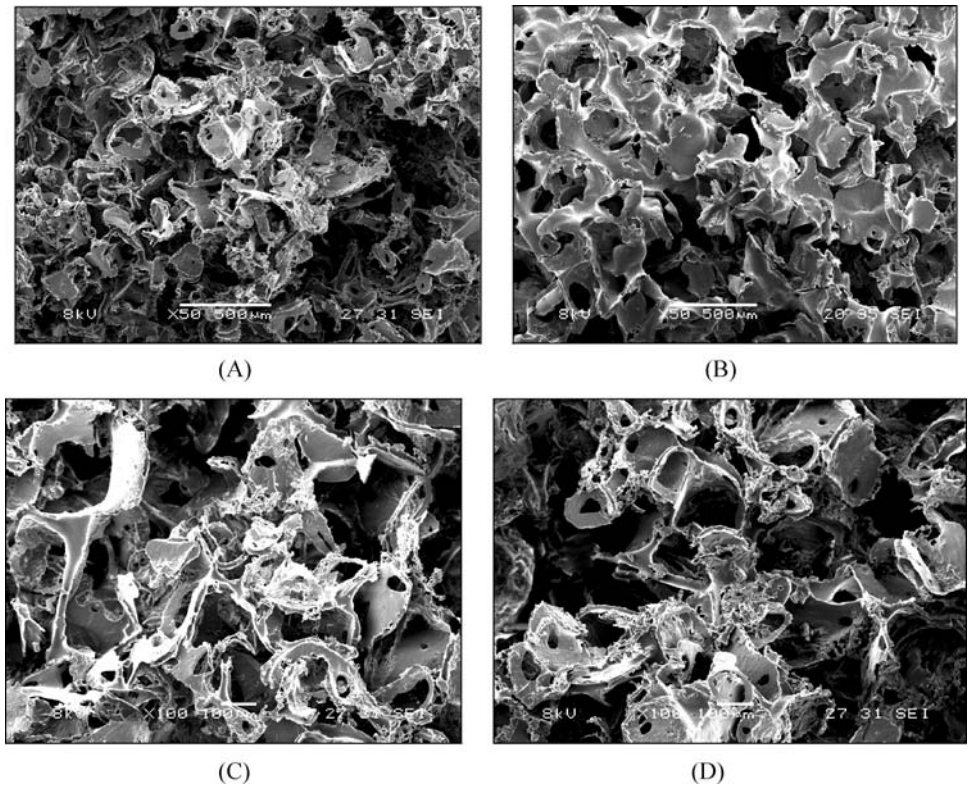



**Fig. 3** SEM photographs of PLGA and PLGA/HA blend scaffolds(thickness about 3 mm). (A) surface of PLGA; (B) surface of PLGA/HA blend; (C) cross section of PLGA scaffold; (D) cross section of PLGA/HA blend scaffold

incubated at 37°C for periods of time. Scaffolds were immersed in 30 ml phosphate buffer saline (pH 7.4) and stirred in a thermostat (SB-302) at 15 rpm and 37°C. After preset time intervals, the samples were recovered to determine the water uptake and then dried in vacuum until a constant weight was obtained in order to determine the weight loss. Scaffold morphologies were also observed by scanning electron microscopy after degradation.

### *2.6.1 Water-uptake*

The dry films were immersed in PBS at 37°C and recovered at different periods of time up to 46 days. The water on the surface was removed by filter paper, and the water-uptake was calculated from the weight increase of the film.

$$\text{Water} - \text{uptake} = \frac{W_w - W_t}{W_t} \times 100\%$$

$Ww$: the weight of the wet film. $Wt$: the weight of dry film(after remove the water)

### *2.6.2 Weight loss*

After the films were recovered at each time interval, three samples were weighted after water was removed from the films and they were dried in vacuum until a constant weight was obtained. Then the weight of the dry film was measured ($W_t$) and compared with its original weight ($W_0$). The weight lost by degradation could be denoted as follows.

$$\text{Weight loss} = \frac{W_0 - W_t}{W_0} \times 100\%$$

Where $W_0$ is the original weight of the film.

## 3 Result and discussions: Characterizations of PLGA/HA blend scaffolds

### 3.1 Mechanical properties

The porous scaffolds must have enough mechanical strength to support cells during the cell proliferating interval. The mechanical properties of the blend polymers were investigated, including load-displacement and modulus of each sample (as shown in Fig. 1). From Fig.1 (b), the modulus of PLGA/HA blend scaffolds decreases gradually with increasing content of HA. However, it does not change significantly as the composition of HA is less then 2%. This might be due to the hydrophilic nature of hyaluronic acid. As it blends with PLGA, it will affect the modulus of PLGA and it is found that the addition of 2 wt% HA seems to be the effective concentration. Thus, PLGA/HA blend scaffold with 2% HA was chosen for in-vitro degradation and in-vitro cells culture studies.

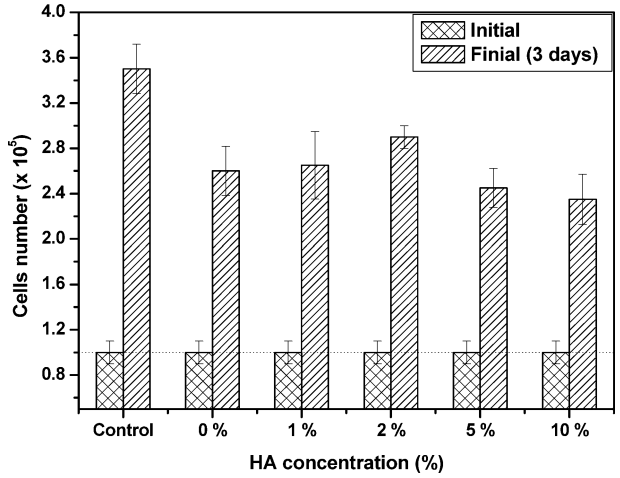



**Fig. 4** The cells survival of the PLGA/HA blend with different hyaluronic acid concentrations. The each column on the left with value of cell number $1\times10^5$/ml corresponds to the initial state at each concentration. All the data on the right above the dotted line are for final states corresponding to the initial states on the left

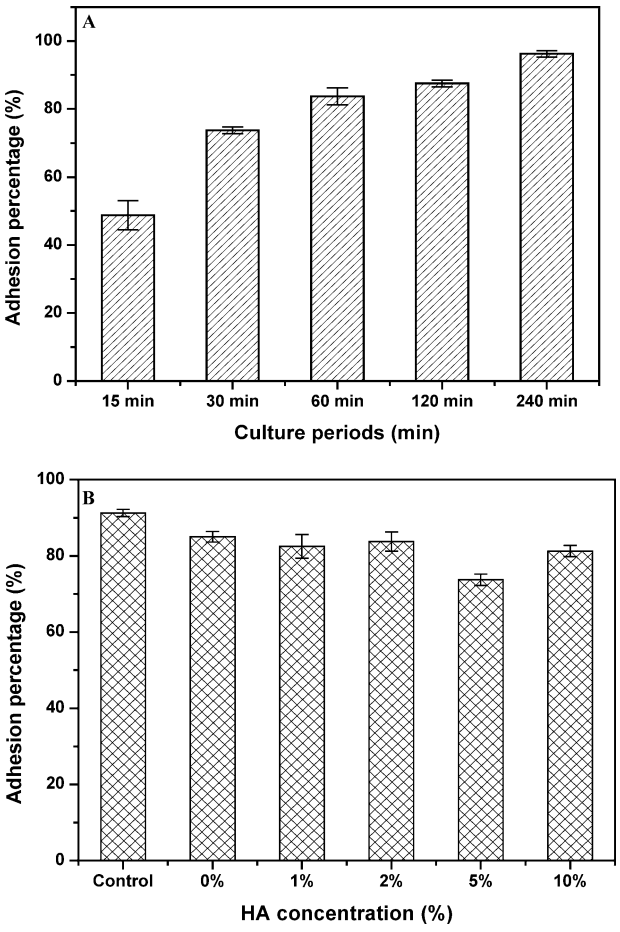



**Fig. 5** The adhere efficiency of 3T3 fibroblasts on the surface of the scaffold. (A) adhesion percentage with different time (PLGA/HA 2wt% blend scaffold); (B) adhesion percentage of different scaffold for 60 min

### 3.2 Evaluation of hydrophilicity

Surface hydrophilicity is an important factor for cells adhesion. In order to evaluate the hydrophilicity of PLGA/HA blend scaffolds, their surfaces were characterized by the measurement of water contact angle. The introduction of hydrophilic HA into PLGA copolymer obviously enhanced its hydrophilicity in comparison with pure PLGA, as shown in Fig. 2. The water contact angle can be used as an indicator for the hydrophilicity of the surface. From the literature, the optimal contact angle for cells adhesion is about 70° [33]. The contact angles of pure PLGA and hyaluronic acid were about 110° and 54°, respectively, and they decreased with respect to time due to gravity. The initial contact angles of different samples decreased with increasing hyaluronic acid content (Fig. 2). This was due to the inclusion of HA into the scaffold, leading to an increase of hydrophilicity of the scaffold. The contact angle at any position of each sample was almost the same, indicating a good mixing for the blend. The increased hydrophilicity of the scaffold may help the diffusion of nutrient into the scaffold, which is obviously dependent on the content of hyaluronic acid.

### 3.3 Surface morphologies

PLGA/HA blend scaffolds with different HA compositions were fabricated by salt-leaching and freeze-drying methods. After the solvent was removed by freeze- drying method, then the scaffold was immersed in deionized water to wash off the salt and the macro pores will be formed in the interior and on the surface of the scaffold. Surface and cross sectional morphologies of the scaffolds are shown in Fig. 3. It can be seen that the surface has highly porous structure and it was well interconnected throughout the scaffold. The pore size was measured in the range of 50–200 $\mu$m. It was larger than the cells size(about 10 $\mu$m). Therefore, there is still enough space for nutrient and carbon dioxide, and these spaces can be utilized to diffuse various ingredients in the scaffold and to supply the needs of the cells.

### 3.4 *In-vitro* cells compatibility

The introduction of HA into the PLGA copolymer was envisioned to enhance the hydrophilicity and can be used to help the cells to adhere on the scaffold. For this reason, the cells were directly seeded on the scaffolds. In this research, mouse 3T3 fibroblasts were used to test the cell affinity. Scaffolds made of PLGA/HA blend with different composition (0, 1, 2, 5, 10% HA) will be seeded with $1 \times 10^5$ cells for each sample. The results indicated that cells on the control system reached up to $3.4 \times 10^5$ cells (Fig. 4) while cells on the scaffolds with different HA composition (0, 1, 2, 5,

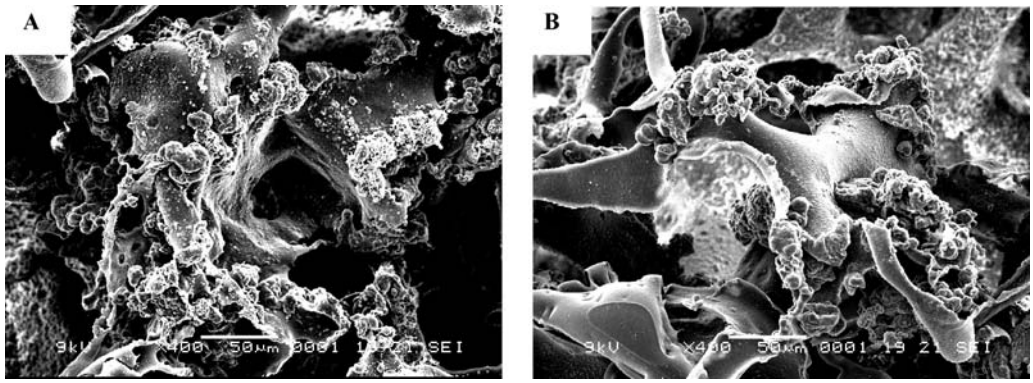



**Fig. 6** The morphologies of mouse 3T3 fibroblasts following cells culture on porous scaffold over a cultivation period of 3 weeks. (A) PLGA/HA (2 wt%) blend scaffold surface; (B) cross-section of PLGA/HA(2 wt%) blend scaffold

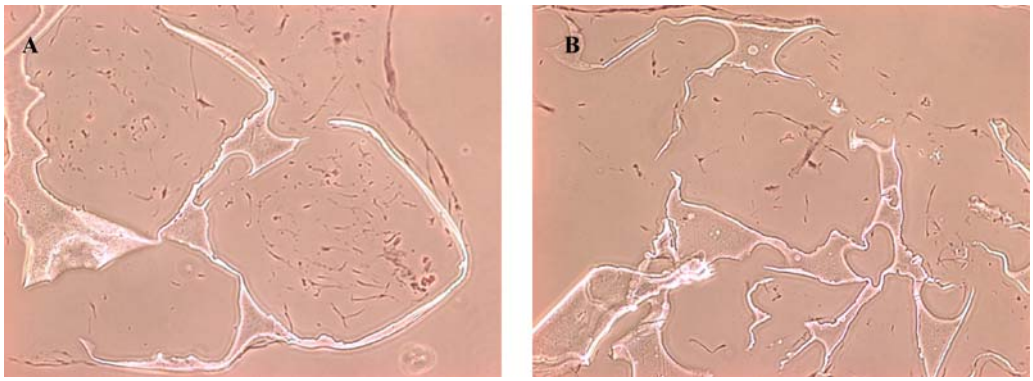



**Fig. 7** H&E staining of cells on the PLGA/HA(2%) blend scaffold after fixed by formalin. (A) Surface; (B) cross section

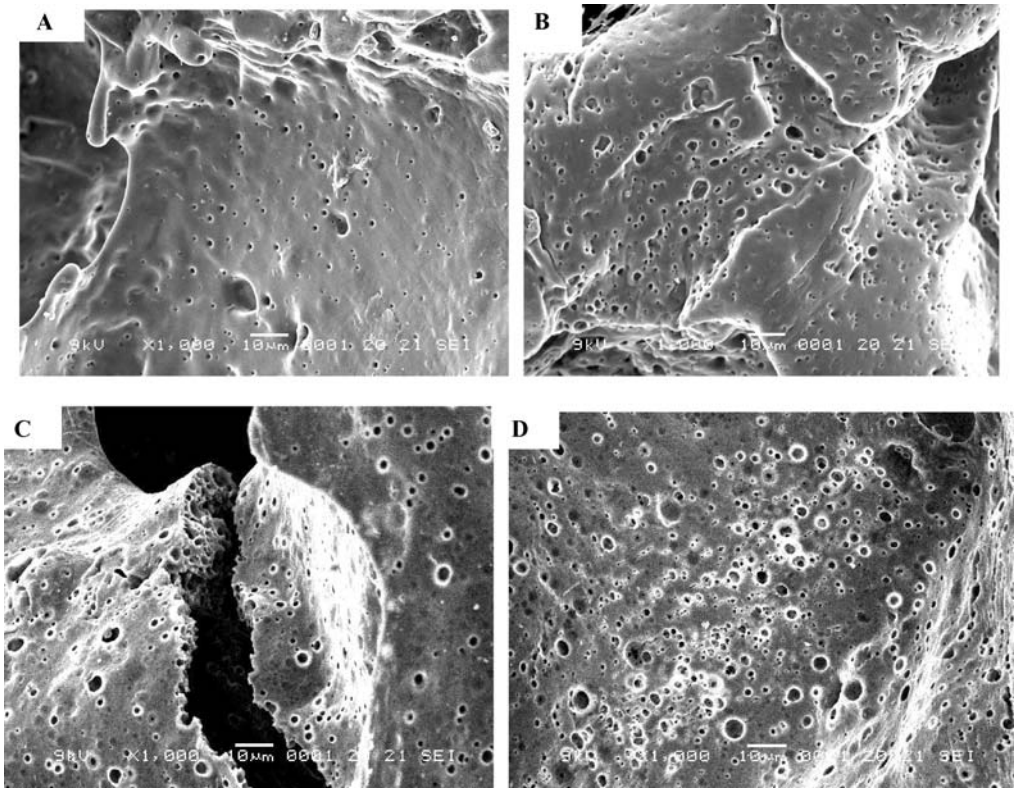



**Fig. 8** SEM images of PLGA/HA (2 wt%) blend scaffold degraded for different time intervals. (A) day 0; (B) day 14; (C) day 28; (d) day 46

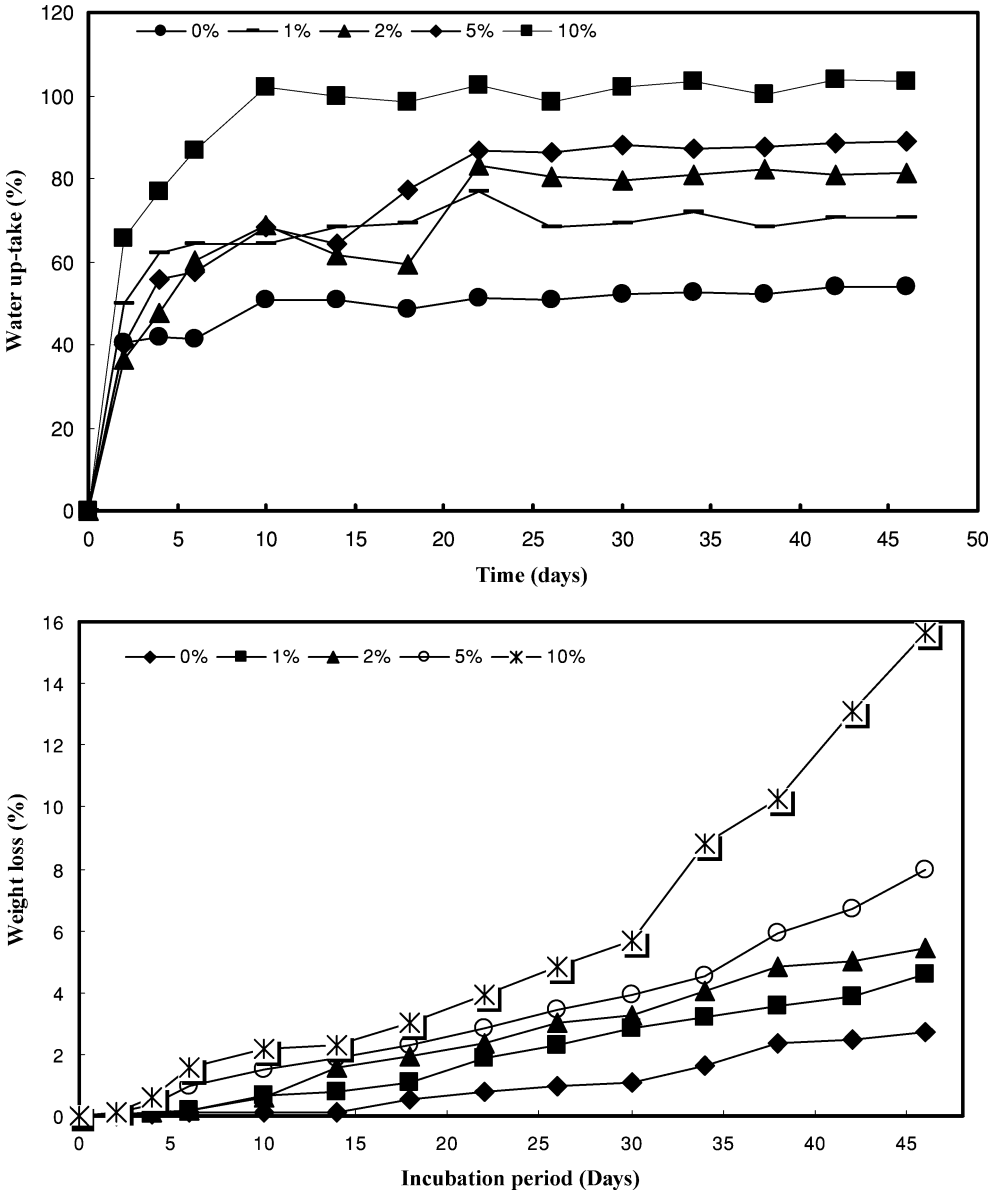



**Fig. 9** Water-uptake and weight loss of the PLGA/HA blend scaffold that were subjected to the hydrolytic degradation

10%) were 2.6, 2.69, 2.85, 2.42, $2.38 \times 10^5$ cells, respectively. Scaffolds containing HA have higher cell survivability than the pure PLGA scaffold while the scaffold containing 2% HA has the highest cells survivability. Although HA shows a strong affinity to cells receptor, only limited amount of HA is favorable for cell aggregation, signifying that an excess of HA will block cross-bridges between cells [34, 35]. Therefore, 2% HA may be a suitable content in the blend scaffold. The 2% HA composition scaffold will be further tested for the cells adhesion rate as well as the cells adhesion efficiency.

The incorporation of HA into PLGA will have better cell affinity because most animal cells possess a polysaccharide-rich outer termed a glycocalyx [24]. To demonstrate this, cells were seeded directly on films of PLGA/HA blend and pure PLGA. The cell attachment efficiency on blend materials and PLGA were shown in Fig. 5A. Figure 5A shows the adhesion result after $1 \times 10^5$ cell/ml was seeded on PLGA/HA 2 wt% blend scaffold. After seeding for 15 mins, about 44% of the cells were adhered on the polymer films. With the increase of culture time, the adhesion percentage also increased. After 60 mins, most of the cells have adhered to the surface (about 80%). The adhesion percentage will reach to approximately 90% after 240 mins. Therefore, 60 mins will be chosen as the culture time to determine the adhesion percentage for scaffolds containing different HA composition (Fig. 5B). On each sample, there was about 80% of the cell adhered on the surface and no significant difference was observed.

The interaction between the 3T3 fibroblast and the scaffold was observed via SEM. It was observed that the cells adhered to the surface of PLGA/HA blend scaffold could maintain their shapes after 2 weeks incubation, as shown in Fig. 6. Some cells spread on the surface and others aggregated to form spheral structure of which they were ascribed to the PLGA crystals. The morphology of the crystal region was much dense which can retard the water near the surface [36]. From our results, the 3T3 fibroblast not only can proliferate on the surface, but it also can migrate into the pores of the scaffold, as confirmed in H&E stain after fixed by formalin (shown in Fig.7). The distribution of the fibroblast on the scaffold was not even, in fact, some tended to aggregate together on the surface. Thehydrophilicity of PLGA scaffold was increased by the addition of HA with which it may facilitate the migration of cells into the scaffold [36]. The pore size for the scaffold was around 50–250 $\mu$m. After the incubation of cells, the scaffolds with pores still have enough space and it became beneficial for transportation of the nutrient into the internal of scaffold to support the proliferation of cells.

### 3.5 *In-vitro* degradation behavior

#### *3.5.1 Surface morphologies*

Figure 8 shows the changes of the scaffold during the degradation. The surface morphologies were observed. After the degradation of scaffold for a certain period of time, a microporous structure can be produced. After 28 days for *in vitro* degradation, the rough and micro-porous state of the surface was evidenced and there exists no dense structure. The degradation behavior may presumably be caused by the initial swelling effect of the medium absorption. As the time increases, the pore size became larger and rougher (46 days) than the previous scaffold. After about 60 days, the scaffold disintegrated gradually.

#### *3.5.2 Water uptake & weight loss*

Biodegradable materials were degraded gradually with time. As the polymer scaffold degraded, the water was diffused into the pores of the materials. When the scaffold was immersed in the PBS buffer solution, it was weighted at different time interval. Figure 9 shows that the mass loss and water uptake profiles of the scaffolds during the degradation intervals. The PLGA/HA blend scaffold with different HA composition is shown to contain different amount of water. The scaffold with higher HA composition may have higher water uptake, and higher water uptake leads to greater weight loss which results in more rapid degradation [37]. This event may be attributed to the hydrophilicity of hyaluronic acid, which leads to a higher water uptake. About 20 days later, the water uptake of all scaffolds reached a equilibrium state. The maximum water absorption can reach the values of around 98% for scaffold containing 10 wt% HA. The scaffold with more hyaluronic acid composition suffers from greater weight loss because the higher water uptake causes faster hydrolysis of the scaffold. Furthermore, it was observed that the surface of the scaffold tends to degrade faster and form abundant micro-porous structure (as shown in Fig. 8) and it had a very rough, wrinkled surface. These porous structures may lead the water diffusion into the pores due to the effect of capillarity. This phenomenon is good for the nutrients to diffuse into the internal of the scaffold.

## 4 Conclusions

The PLGA/HA blend scaffolds were prepared by particle leaching and freeze drying techniques. The porosity of the scaffolds was about 85% with open pores and internal connecting pores. The mechanical properties of PLGA/HA blend scaffold with 2% HA were good enough to be used in cells culture and its hydrophilic surface also provides good environment for cells adhesion and growth. The SEM and H&E stained observation showed that cells not only grew on the surface but can be migrated into the internal of the scaffold. The degradation behavior of the scaffold was also affected by the composition of HA. The rate of hydrolysis of the PLGA/HA scaffold will be faster than that of the virgin PLGA scaffold with the same molecular weight. In addition, the incorporation of HA into the hydrophobic PLGA is indeed helpful in improving the properties of the scaffold and in enhancing the biocompatibility. These features make the PLGA/HA blend a good candidate to be used as the material for scaffold in tissue engineering.

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## References

1. A. HATEFI and B. AMSDEN, *J. Controlled Release* **80** (2002) 9.
2. G. WINZENBURG, C. SCHMIDT, S. FUCHS and T. KISSEL. *Advanced Drug delivery reviews* **56** (2004) 1453.
3. B. JEONG, Y. K. CHOI, Y. H. BAE, G. ZENTER and S. W. KIM, *J. Controlled Release* **62** (1999) 109.
4. RAJEER A. JAIN, *Biomaterials* **21** (2000) 2475.
5. G. CACCIOLA, G. W. M. PETERS and F. P. T. BAAIJENS, *J. Biomechanics* **33** (2000) 653.
6. Y. NOISHIKI, *Mater. Sci. Eng. C* **6** (1998) 227.
7. J. DE HART, G. CACCIOLA, P. J. G. SCHREURS and G. W. M. PETER, *J. Biomechanics* **21** (1998) 629.

8. M.-F. GUIDOIN, Y. MAROIS, J. BEJUI, N. PODDEVIN, M. W. KING and R. GUIDOIN, *Biomaterials* **21** (2000) 2461.
9. J. C. MIDDLETON and ARTHUR J. TIPTON, *Biomaterials* **21** (2000) 2335.
10. P. M. KAUFMANN, S. HEIMRATH, B. S. KIM and D. J. CELL, *Transplantation* **6** (1997) 463.
11. Y. I. JEONG, J. G. SONG, S. S. KANG, H. H. RYU, Y. H. LEE, C. CHOI, B. A. SHIN, K. K. KIM, K. Y. AHN and S. JUNG, *Int. J. Pharmaceutics* **259** (2003) 79.
12. Y. TABATA, *Drug discovery today* **6** (2001) 483.
13. G. BEUMER, C. VAN BLITTERSWIJK and M. PONEC, *J. Biomed. Matter Res.* **28** (1994) 545.
14. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529.
15. S. RALF and S. P. HOERSTRUP, *The Annals of Thoracic Surgery* **70** (2000) 140.
16. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529.
17. M. OKADA, *Prog. Polym. Sci.* **27** (2002) 87.
18. A. GONZALEZ, M. IRIARTE, P. J. IRIONDO and J. J. IRUIN, *Polymer* **44** (2003) 7701.
19. J. E. OH, Y. S. NAM, K. H. LEE and T. G. PARK, *J. Controlled Release* **57** (1999) 269.
20. F.-L. MI, Y.-M. LIN, Y.-B. WU, S.-S. SHYU and Y.-H. TSAI, *Biomaterials* **23** (2002) 3257.
21. M. S. WIDMER, P. K. GUPTA, L. LU, R. K. MESZLENYI, G. R. D. EVANS, K. BRANDT, T. SAVEL, A. GURLEK, C. W. PATRICK, A. G. MIKOS, *Biomaterials* **19** (1998) 1945.
22. V. R. SINHA and R. KUMRIA, *Int. Pharmaceutics* **224** (2001) 19.
23. J. ABURTO, I. ALRIC, S. THIEBAUD, E. BORREDON, D. BIKIARIS, J. PRINOS and C. PANAYIOTOU, *J. Appl. Polym. Sci.* **74** (1999) 1440.
24. Q. CAI, Y. WAN, J BEI and S. WANG, *Biomaterials* **24** (2003) 3555.
25. U. EDLUND and A.-C. ALBERTSSON, *Advanced Drug Delivery Reviews* **55** (2003) 585.
26. R. D. SONWALKAR, C. C. CHEN and L.-K. JU, *Bioresource Technology* **87** (2003) 69.
27. C. WANG, H. LI and X. ZHAO, *Biomaterials* **25** (2004) 5797.
28. J. K. KIM, D.-J. PARK, M.-S. LEE and K. J. IHN, *Polymer* **42** (2001) 7429.
29. S. LI and M. VERT, *Macromolecules* **36** (2003) 8008.
30. J. S. MAO, H. F. LIU, Y. J. YIN and K. D. YAO, *Biomaterials* **24** (2003) 1621.
31. Y. LUO, K. R. KIRKER and G. D. PRESTWICH, *J. Controlled Release* **69** (2000) 169.
32. M. G. CASCONE, B. SIM and SANDRA DOWNES, *Biomaterials* **16** (1995) 569.
33. T. GROTH and G. ALTANKOV In: *Cell-surface interactions and the tissue compatibility of biomedical materials*, (DC: IOS Press, Washington, 1998) p. 12.
34. B. P. TOOLE, In: *Cell Biology of Extracellular Matrix*. (Hay E. ed., New York) (1981) p. 259.
35. B. P. TOOLE In: *Cell Biology of Extracellular Matrix*, (Hay E. ed., New York) (1991) p. 305.
36. Y. WAN, W. CHEN, J. YANG, J. BEI and S. WANG, *Biomaterials* **24** (2003) 2195.
37. M. E. GOMES, A. S. RIBEIRO, P. B. MALAFAYA, R. L. REIS and A. M. CUNHA, *Biomaterials* **22** (2001) 883.