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The influences of poly(lactic-co-glycolic acid) (PLGA) coating on the biodegradability, bioactivity, and biocompatibility of calcium silicate bioceramics

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Abstract Calcium silicate ($CaSiO_3$) bioceramics and polyesters have complementary qualities as potential bone substituted materials. In this study, sintered $CaSiO_3$ bioceramics were prepared and coated with poly(lactic-co-glycolic acid) (PLGA), and the influences of the PLGA coating on the degradation, hydrophilicity, bioactivity, and biocompatibility of $CaSiO_3$ ceramics were investigated. The results showed that the degradation rate was reduced, while hydrophilicity was decreased with the increase of the polymer coating. In addition, the polymer coating resulted in a decrease of the alkaline pH value during the degradation of the ceramics, which indicated an increase of the cell biocompatibility, confirmed by the attachment and proliferation of rMSCs on the surface of the polymer-coated ceramics. Furthermore, the apatite-forming ability of the PLGA-coated $CaSiO_3$ bioceramics was maintained. This study suggested that the coating with PLGA might be a useful method to improve the integrative properties of $CaSiO_3$ bioceramics for applications in bone regeneration and bone tissue engineering.

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Introduction

Ideal biomaterials for tissue regeneration and tissue engineering applications should be biodegradable, bioactive, biocompatible, and mechanically strong [1]. Calcium silicate ($CaSiO_3$) ceramic has been proved to be bioactive, degradable, and hydrophilic [2–6]. The recent study showed that the calcium silicate bioceramic possessed excellent bone regeneration ability and biodegradability [7–11]. However, the high-ionic dissolution of $CaSiO_3$ can lead to high-local pH environment, especially for the porous samples, which can result in an adverse cellular response, and is not suitable for tissue engineering applications [12–14]. This limitation could be mitigated by developing $CaSiO_3$/polymer composites. Poly(lactic-co-glycolic acid) (PLGA) is widely used in biomedical fields as bone fixation materials, sutures, materials drug delivery, and tissue engineering applications because of its tailorable degradation rates, biocompatibility, and formability [15–17]. However, there are some drawbacks for PLGA, such as hydrophobic nature, lack of bioactivity, and the release of acidic degradation by-products which can lead to inflammatory response [18–20]. One approach to solve the drawbacks of PLGA is to combine PLGA with bioactive and alkaline inorganic bioceramics. In the previous studies, a variety of biodegradable polymer/$CaSiO_3$ composite materials had shown the potential applications for bone tissue regeneration and tissue engineering [14, 20–23]. However, most of these composites were prepared by mixing unsintered $CaSiO_3$ powders with polymers and they are mechanically not strong. In addition, the effects of polymer on $CaSiO_3$ ceramics degradation lack-specific study, which are important for the design of new composite biomaterials for bone regeneration and bone tissue regeneration applications.

In order to fabricate the $CaSiO_3$ bioceramics with proper biodegradation, bioactivity, and improved cytocompatibility, the sintered $CaSiO_3$ bioceramics were fabricated and then coated with PLGA in different thickness, and the influences of the PLGA coating on the degradation, in vitro bioactivity, hydrophilicity, and in vitro biocompatibility of $CaSiO_3$ bioceramics were investigated.

Materials and methods

Preparation and characterization of PLGA-coated $CaSiO_3$ bioceramics

Calcium silicate powders were synthesized by chemical precipitation method [8]. In brief, mixing of an aqueous solution of Na_2SiO_3 with $Ca(NO_3)_2$ (mol ratio = 1:1) at room temperature was carried out overnight. Then the resulting calcium silicate precipitates was filtered and washed with deionized water. After being drying at 80 °C overnight and calcined at 800 °C for 2 h, the $CaSiO_3$ powders were obtained. The obtained $CaSiO_3$ powders were uniaxially pressed into disks with the dimension of 10 mm in diameter and 3 mm in thickness under a pressure of 8 MPa. Subsequently, they were pressureless-sintered in air at 1100 °C for 3 h at a heating rate of 2 °C/min. The samples were cooled to room temperature in the furnace.

The PLGA (copolymer ratio of 75:25, viscosity of 1.66) was obtained from Sichuan Dikang Sci. and Tech. Pharmaceutical Co. Ltd. (Chengdu, China). Various amounts (5, 7.5, 10, 12.5% w/v) of PLGA were dissolved in acetone under stirring at room temperature. The sintered $CaSiO_3$ bioceramics were dipped into the solution and then rotated for 5 s to remove the excess solution to obtain a uniform coating film on the surface of the ceramic disks. Then PLGA-coated samples were dried at 60 °C for 1 day to remove the solvent.

X-ray diffraction (XRD; Geigerflex, Rigaku, Japan) with CuKα radiation was used to characterize the phase composition of the samples. The surface morphology and fracture surfaces of the prepared composites were observed by scanning electron microscopy (SEM; JSM-6700F, JEOL, Japan).

In vitro bioactivity

The in vitro bioactivity of the prepared samples was evaluated by examining the bone-like apatite formation ability on the samples in simulated body fluid (SBF), which was prepared as previously described by Kokubo [24] and had similar ion concentrations to those in human blood plasma. In brief, analytical reagent grade NaCl, $NaHCO_3$, KCl, K_2HPO_4, $MgCl_2$, $CaCl_2$, and Na_2SO_4 were dissolved in distilled water and the solution was buffered to pH 7.4 at 37 °C with trishydroxymethyl aminomethane and hydrochloric acid.

The PLGA-coated $CaSiO_3$ bioceramics were immersed in 20 mL SBF for 7 days at 37 °C, and the SBF solutions were replaced daily with fresh solution. After soaking, the samples were removed from the SBF, gently rinsed with deionized water, and dried at 60 °C for 1 day. SEM and Electron dispersive spectrometer (EDS; INCA Energy, Oxford Instruments, UK) were used to examine the formation of bone-like apatite on the surface of the samples.

In vitro degradation

The degradability of the prepared samples was determined by measuring the weight loss percentage of the samples after soaking in 0.05 M Tris-HCl buffer solution. The Tris-HCl buffer solution was prepared by dissolving analytical reagent grade Tris(hydroxymethyl) aminomethane in distilled water and then was buffered to pH 7.4 at 37 °C with hydrochloric acid. The samples were soaked in the Tris-HCl buffer solution for different time periods at 37 °C with the ratio of surface area (cm^2) to solution volume (mL) of 0.1, The Tris-HCl buffer solutions were unchanged during the whole period of degradation test. After various soaking periods, the samples were removed from the Tris-HCl buffer solution, gently rinsed with distilled water followed by drying at 60 °C for 24 h before further characterization. The weight loss percentage was calculated according to following equation:

$$\text{Weight loss}(\%) = ((M_0 - M_t)/M_0) \times 100\%$$

where M_0 and M_t are the masses at the immersion time of 0 and t, respectively. The morphological change of samples after the degradation was observed by SEM (JSM-6700F, JEOL, Japan).

The pH values of the soaking solution were monitored every 2 days during the degradation process using an electrolyte type pH meter (PHS-2C, Jingke Leici Co., Shanghai, China). In this study, three samples from each group were tested to obtain an average degradability and pH values.

Hydrophilicity determination

The hydrophilicity of the disks was evaluated by automatic contact angle meter (SL200B, Solon technology science Co. Ltd, Shanghai, China). The water droplet was 0.5 μL prevent gravitational distortion of the spherical profile. Each determination was obtained by averaging the results of five measurements.

Cell culture and proliferation

Rabbit bone marrow stromal cells (rMSCs) were isolated from tibias of adult New Zealand white rabbits by the process as described previously [25, 26]. In brief, after the bone was excised under sterile condition, the fresh bone marrow was collected aseptically and suspended in cell culture dishes containing 10 mL Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS) plus antibiotics. After 10 days of culture at 37 °C in a humidified atmosphere of 95% air and 5% CO_2, hematopoietic and other floating cells were removed from the dishes by repeated washing with phosphate-buffered saline (PBS). Culture media were refreshed every 2 days. The cells were routinely subcultured by trypsinization, for this investigation only cells between the third and fifth passage were employed.

The rMSCs were seeded on ceramic disks placed in a 48-well culture plate at an initial density of 2.0×10^4 cells/well and cultured in DMEM culture medium supplemented with 10% FCS maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. After 24 h, the disks were removed from the culture wells, rinsed with PBS, and staining with Giemsa solution. The morphology of the attached cells on the samples was observed using an optical microscope (Leica S6D, Germany). After incubation for 1 and 7 days, MTT test was carried out to test cell viability. In brief, 100 μL of 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added into each well. After additional incubation for 4 h, dimethyl sulfoxide (DMSO) was added to stop the reaction between MTT and cells. The optical density (OD) was measured at the wavelength of 490 nm using an enzyme linked immunosorbent assay plate reader (ELx 800, BIOTEK, USA).

Results

Characterization of the CS and PLGA/CS bioceramics

XRD patterns of the sintered $CaSiO_3$ bioceramics are shown in Fig. 1a. It was obvious that only β-$CaSiO_3$ peaks existed. Figure 1b shows the surface morphology of the pure $CaSiO_3$ bioceramics. It could be seen that the grain size was about 1–3 μm and the CS ceramic exhibited a loose and rough surface with irregular pores.

Figure 2 shows the relationship between the polymer coating concentration and the amount of PLGA on the ceramic disks. The weight percentage of PLGA layers on the samples increased with the increase of the PLGA concentration. However, when the concentration increased to 12.5% m/v, the deviation increased significantly, and the coated polymer films became uneven because of the high-viscosity of PLGA solution. Therefore, the samples coated by 0, 5, and 10% m/v PLGA (denoted as CS, 5PLGA/CS, 10PLGA/CS, respectively) were chosen for further investigation of the various properties of the composites.

The SEM micrographs (Fig. 3) of the cross sections of the 5% and 10% PLGA/CS disks show that the PLGA layers adhere to the calcium silicate ceramic closely. With the increase of the coating concentration, the thickness of PLGA films increased correspondingly. The average thicknesses of the PLGA layers were about 9 μm on 5PLGA/CS and 18 μm on 10PLGA/CS, respectively.

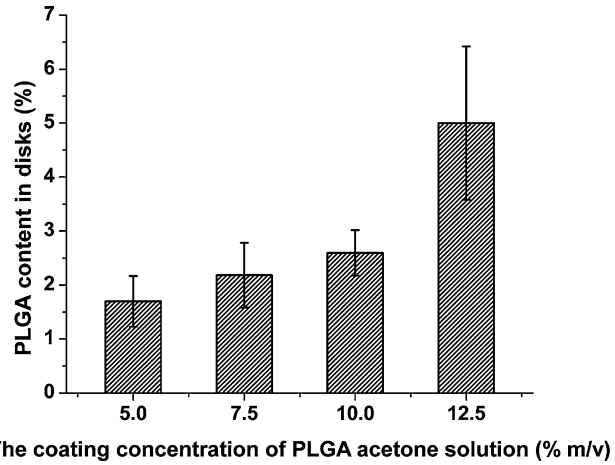

Fig. 2 The effect of coating concentration on the weight percentage of PLGA layers on the prepared composite disks

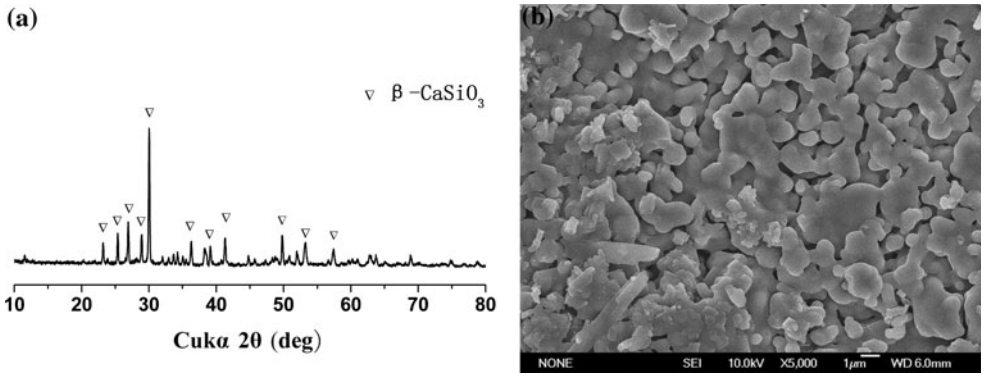

Fig. 1 The XRD patterns (**a**) and SEM micrograph (**b**) of the sintered $CaSiO_3$ bioceramics

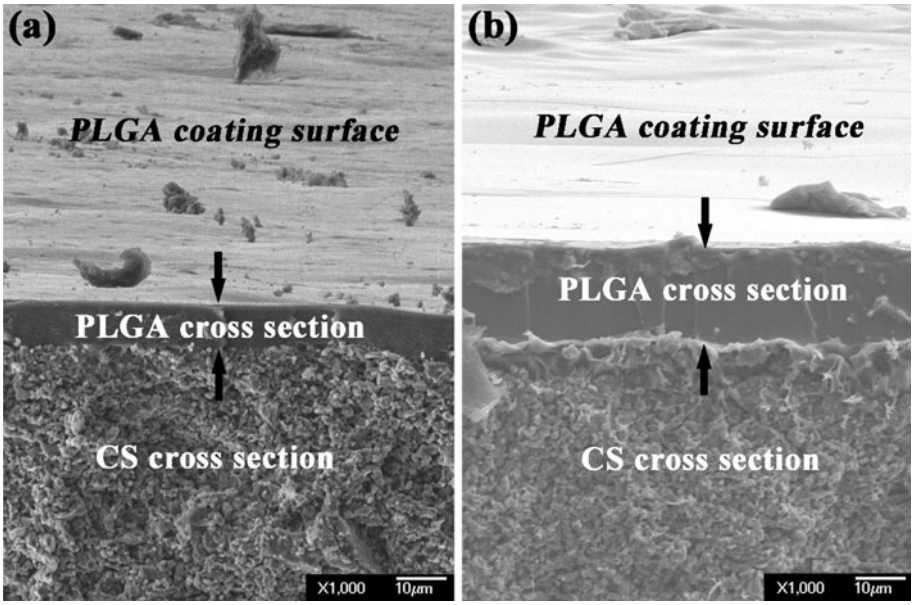

Fig. 3 The SEM microstructure of the cross sections of the PLGA/CS disks: 5PLGA/CS (**a**) and 10PLGA/CS (**b**)

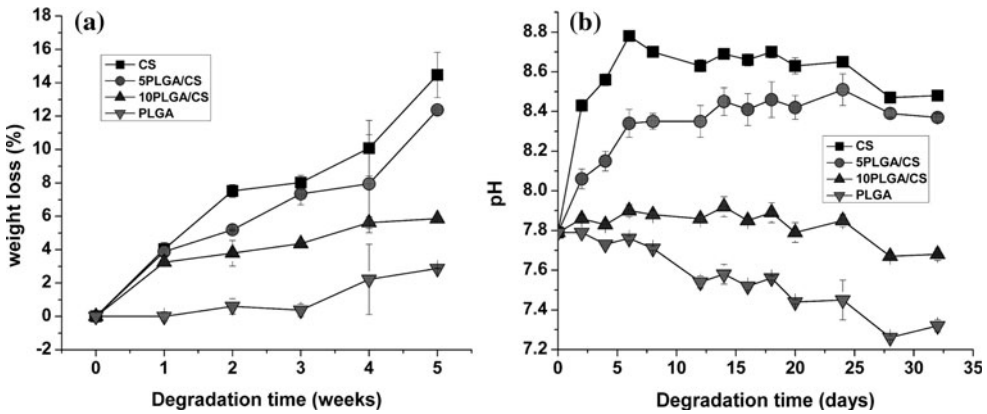

Fig. 4 The weight loss (**a**) and pH change (**b**) during degradation

In vitro degradation

Figure 4a shows weight loss of the disks during degradation after soaking in Tris-HCl buffer solution. It can be seen that the weight loss of all samples increased with the increase of the soaking time. The pure CS showed higher weight loss as compared to the PLGA/CS. As expected, the degradation rate was effectively hindered by PLGA coating, and the degradation rate could be tailored through regulation the thickness of the coated-PLGA films. With the increase of the thickness of the PLGA films, the degradation rate decreased apparently. After soaking in Tris-HCl buffer solution for 5 weeks, the weight loss of CS reached about 14.5%, while the weight loss of 10PLGA/CS was only about 5.9%.

Figure 4b shows the pH value change of the solution during degradation. At the initial stage of the degradation, the pH value of the pure $CaSiO_3$ ceramic increased rapidly, while the pH value of pure PLGA decreased rapidly. As expected, the PLGA film could effectively reduce the change of pH, the pH value of 10PLGA/CS disk stabilized in the range of 7.6–7.9 during the whole degradation period.

As shown in Fig. 5, the surface of the pure CS ceramic is loose and rough (Fig. 5a). After coating with PLGA film, the surface became smooth (Fig. 5c, e). After degradation in Tris-HCl buffer solution for 4 weeks, the PLGA layer of the 5PLGA/CS disk was almost degraded, exposing the rough ceramic substrate with many pores (Fig. 5d). When the thickness of the PLGA layer increased, the PLGA was not completely degraded, and there were a lot of particles on the surface of the 10PLGA/CS disk (Fig. 5f).

Hydrophilicity determination

Table 1 showed the surface water contact angles of the pure $CaSiO_3$, 5PLGA/CS, 10PLGA/CS, and pure PLGA samples. It could be seen that the water contact angle of the

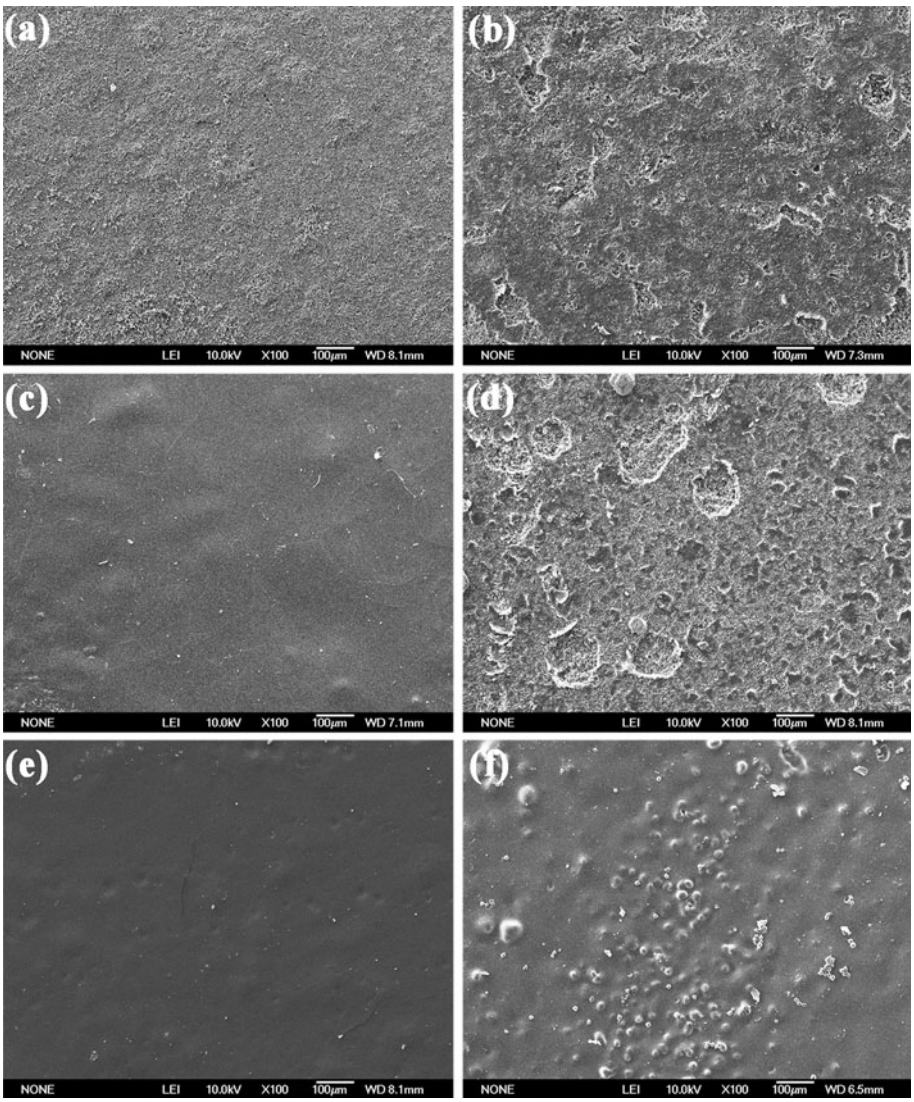

Fig. 5 SEM images of the surface of the ceramic and composite disks before and after degradation. **a** CS before degradation, **b** CS after degradation for 4 weeks, **c** 5PLGA/CS before degradation, **d** 5PLGA/CS after degradation for 4 weeks, **e**10PLGA/CS before degradation, **f** 10PLGA/CS after degradation for 4 weeks

Table 1 Water contact angles of the samples

Samples	Water contact angles (Degree)
$CaSiO_3$	0
5PLGA/CS	37.32 ± 9.5
10PLGA/CS	71.77 ± 2.3
PLGA	77.07 ± 3.5

samples was increased with the increase of the thickness of PLGA films.

Characterization of disks after soaking in SBF

Figure 6 shows the SEM images of pure $CaSiO_3$ (a), 5PLGA/CS (b), and 10PLGA/CS (c) after soaking in SBF

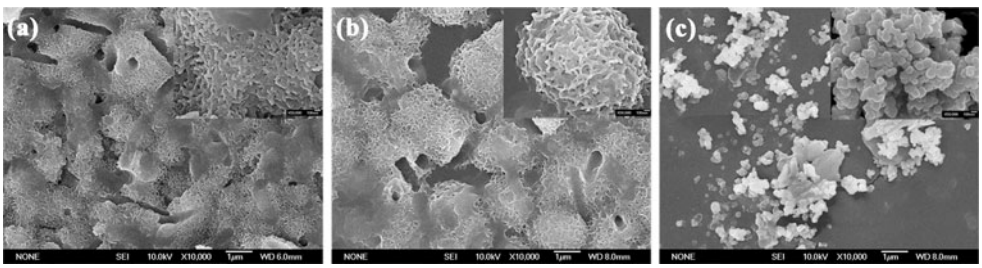

Fig. 6 SEM images of the CS and PLGA/CS disks after soaking in SBF for 7 days: CS (**a**); 5PLGA/CS (**b**); 10PLGA/CS (**c**)

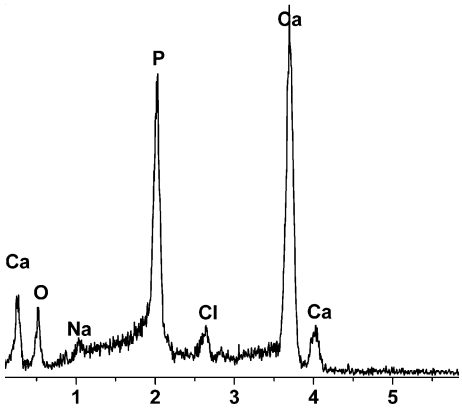

Fig. 7 EDS analysis of the surface of the 5PLGA/CS after soaking in SBF for 7 days

for 7 days. It was obvious that some deposits were formed on the surface of the samples after soaking, and the quantity of the deposited particles decreased with the increase of the PLGA coating thickness. The high-magnification images (inserts) showed that the deposits were composed of crystals in typically worm-like morphology of bone-like apatite on the surface of CS and 5PLGA/CS disks, while the precipitates on the surface of 10PLGA/CS were in microsphere-like morphology. The EDS analysis further confirmed that the Ca/P molar ratio of the newly formed worm-like crystals was 1.63 (Fig. 7), which was close to that of the hydroxyapatite.

Viability of rMSCs on the ceramics

Figure 8 illustrates the morphology of the rMSCs adhering on the CS, 5PLGA/CS, and 10PLGA/CS disks after incubation for 24 h. It can be see that the rMSCs on the CS disks do not show any sign of spreading but rather a round morphology (Fig. 8a). The morphology of the rMSCs on the 5PLGA/CS is similar with those on the CS, but there are a small amount of rMSCs spreading on the edge of the 5PLGA/CS disks (Fig. 8b). However, the rMSCs adhered and spread well on the 10PLGA/CS disks after 24 h of culture (Fig. 8c).

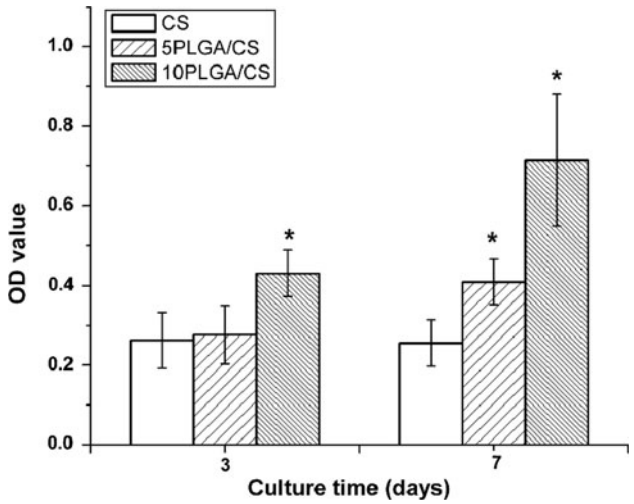

Fig. 9 rMSCs viability on the CS, 5PLGA/CS, and 10PLGA/CS disks. *$p < 0.05$ (compared to the pure CS)

Figure 9 showed the results of the MTT assay of the rMSC on the CS, 5PLGA/CS, 10PLGA/CS disks after 3 and 7 days culture. It is clear to see that the OD values increased apparently with the increase of the culture periods and the thickness of the PLGA coating films.

Discussion

The balance of the biodegradability and biocompatibility or bioactivity of a bone grafting material is of central importance for its application in bone repair. The $CaSiO_3$ ceramics have good bioactivity and hydrophilicity, but the high-degradation rate and resulted high pH value limit their biological applications. Since the surface properties play an important role in determining the properties of biomaterials, the biodegradability and biocompatibility of $CaSiO_3$ ceramics can be improved via surface modification. This study showed that the PLGA coating has significant influence on the degradation and biocompatibility of $CaSiO_3$ bioceramics. The pure $CaSiO_3$ ceramics are rough and have many micropores which facilitate its dissolution. When the $CaSiO_3$ ceramics were coated with PLGA films,

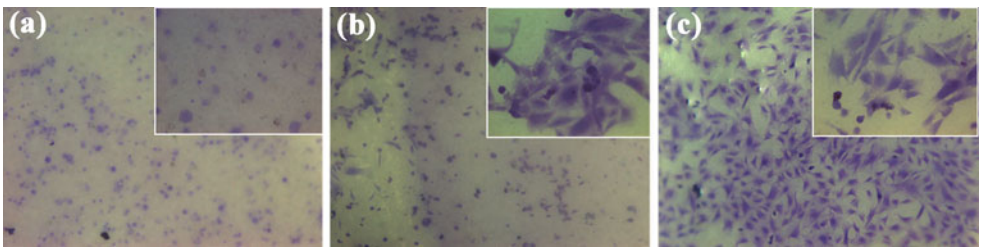

Fig. 8 Optical microscopic images of rMSCs attaching on the samples after 24 h of culture: CS (**a**); 5PLGA/CS (**b**); 10PLGA/CS (**c**). Original magnifications were 50× (inserts were ×200)

the rough surface of the $CaSiO_3$ ceramics was covered, which reduced the diffusion of the $CaSiO_3$ degradation products. Therefore, the degradation rate of the $CaSiO_3$ ceramics was effectively reduced by PLGA coating.

Furthermore, the degradation of pure $CaSiO_3$ ceramics often results in an increase of the environmental pH because of the release of alkaline ions during the dissolution of the ceramics [27], which may affect its biocompatibility. It is known that, the acidic degradation compounds will be released during the degradation of PLGA, which is assumed to be able to neutralize the alkaline ion products of $CaSiO_3$ bioceramics and reduce the pH of the environment. In addition, the PLGA film on the surface of the ceramics will also reduce the release rate of the alkaline ion products of $CaSiO_3$, which can further reduce the pH value. These results confirmed the assumption and showed that the PLGA coating effectively reduced the pH value during degradation and improved the biocompatibility of the $CaSiO_3$ bioceramics by controlling the amount of the coating materials.

It is known that the cell morphology and proliferation can be influenced by the quality of material surfaces and the cell-material interactions [28]. Previous researches have suggested that degradation products and pH value of the implant materials had multiple effects on cells metabolism and function [29]. The high pH value will affect the cell growth and proliferation, thus the rMSCs on the pure CS ceramic with a loose surface do not spread and proliferate well. The cell culture experiments demonstrated that the $CaSiO_3$ bioceramic coated with PLGA provided a more biocompatible surface for rMSCs adhesion, spreading and proliferation as compared to the pure $CaSiO_3$. Since the degradation rate and cell biocompatibility of the biomaterials are critical for designing bone tissue engineering materials, this result also suggests that the coating of $CaSiO_3$ bioceramics with PLGA might be an effective way to prepare biocompatible materials for bone tissue engineering applications, and the cell biocompatibility can be easily adjusted by controlling the composition, amount, thickness, and molecular weight of the polymer materials.

It is reported that the silicate-based bioactive glasses and ceramics can induce formation of bone-like apatite on the surface of the materials in simulated body fluid and in vivo when implanted in animal which reflect the potential of the materials to bond with bone tissue [30–32]. The pure $CaSiO_3$ bioceramics have this ability [3, 11]. So it is worth to evaluate whether the PLGA coating will affect the apatite inducing ability of $CaSiO_3$ bioceramic. These results demonstrated that the PLGA-coated $CaSiO_3$ bioceramics still have the ability to induce the formation of the bone-like apatite, and this activity was dependent on the thickness of the coating. In fact, when the PLGA-coated ceramics were immersed in SBF, polymer coatings started to degrade and certain parts of the $CaSiO_3$ bioceramic were exposed to the SBF solution, which provide the nucleation sites for apatite formation. It is understandable that the thinner coating will result in a more rapid exposure of ceramics during the degradation of polymer coating. Therefore, the ability of the coated ceramics to induce apatite formation can be adjusted by controlling the thickness of the polymer coating.

Conclusion

In this study, the sintered $CaSiO_3$ bioceramics were coated with PLGA to modify the properties of the ceramics. The results indicated that the polymer coating layer had significant influences on the biodegradability, hydrophilicity, bioactivity, and biocompatibility of the $CaSiO_3$ bioceramics. The degradation rate of the ceramics was reduced, since the coating hindered the dissolution of the ceramics, while the apatite-forming ability of the PLGA-coated $CaSiO_3$ bioceramics was maintained. In addition, the biocompatibility of the ceramics was increased by the polymer coating because of the neutralization of the alkaline degradation products of the $CaSiO_3$ bioceramics by the acidic degradation products of the PLGA. The 10% w/v PLGA-coated $CaSiO_3$ bioceramic showed the best cell biocompatibility, which was confirmed by the rMSCs attachment and proliferation. These results suggested that the coating with PLGA on $CaSiO_3$ bioceramics could be used to control the integrative properties of $CaSiO_3$ bioceramics and the PLGA-coated ceramics may be used for bone regeneration and bone tissue engineering.

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References

1. Hench LL, Polak JM (2002) Science 295:1014
2. Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T (1990) J Biomed Mater Res 24:721
3. De Aza PN, Guitian F, De Aza S (1994) Scripta Metall Mater 31:1001
4. Siriphannon P, Hayashi S, Yasumori A, Okada K (1999) J Mater Res 14:529
5. Siriphannon P, Kameshima Y, Yasumori A, Okada K, Hayashi S (2000) J Mater Res 52:30
6. Siriphannon P, Kameshima Y, Yasumori A, Okada K, Hayashi S (2002) J Eur Ceram Soc 22:511
7. Lin KL, Chang J, Wang ZJ (2005) Inorg Mater 20:692
8. Lin KL, Ni SY, Zhai WY, Chang J, Qian WJ, Zeng Y (2005) Ceram Intern 31:323
9. Ni SY, Chang J, Chou L (2006) J Biomed Mater Res A 76:196

10. Ni SY, Chang J, Chou L, Zhai WY (2007) J Biomed Mater Res B 80:174
11. Xu SF, Lin KL, Wang Z, Chang J, Wang L, Lu JX, Ning CQ (2008) Biomaterials 29:2588
12. El-Ghannam A, Ducheyne P, Shapiro IM (1997) Biomaterials 18:295
13. Silver IA, Deas J, Erecinska M (2001) Biomaterials 22:175
14. Wu CT, Ramaswamy Y, Boughton P, Zreiqat H (2008) Acta Biomater 4:343
15. Middleton JC, Tipton AJ (2000) Biomaterials 21:2335
16. Huang YC, Connell M, Park Y, Mooney DJ, Rice KG (2003) J Biomed Mater Res A 67A:1384
17. Yang ZJ, Best SM, Cameron RE (2009) Adv Mater 21:3900
18. Therin M, Christel P, Li SM, Garreau H, Vert M (1992) Biomaterials 13:594
19. Bostman O, Pihlajamaki H (2000) Biomaterials 21:2615
20. Li H, Chang J (2004) J Mater Sci Mater Med 15:1089
21. Li H, Chang J (2004) Biomaterials 25:5473
22. Li X, Chang J (2005) J Mater Sci Mater Med 16:361
23. Wei J, Heo SJ, Kim DH, Kim SE, Hyun YT, Shin JW (2008) J R Soc Interface 5:617
24. Kokubo T, Takadama H (2006) Biomaterials 27:2907
25. Lecoeur L, Ouhayoun JP (1997) Biomaterials 18:989
26. Zhang ML, Zhai WY, Lin KL, Pan HB, Lu W, Chang J (2010) J Biomed Mater Res B 93B:252
27. Li H, Chang J (2005) Polym Degrad Stabil 87:301
28. Amaral IR, Cordeiro AL, Sampaio P, Barbosa MA (2007) J Biomat Sci-Polym E 18:469
29. Silver IA, Deas J, Erecinska M (2001) Biomaterials 22:175
30. Pereira MM, Clark AE, Hench LL (1994) J Biomed Mater Res 28:693
31. Mami M, Lucas-Girot A, Oudadesse H, Dorbez-Sridi R, Mezahi F, Dietrich E (2008) Appl Surf Sci 254:7386
32. Fujimura K, Bessho K, Okubo Y, Segami N, Iizuka T (2003) Clin. Oral Impl Res 14:659