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Correlation between degradation and compressive strength of an injectable macroporous calcium phosphate cement

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ABSTRACT

Variation of compressive strength of a macroporous calcium phosphate cement (CPC) was investigated in the present study. Poly (lactide-co-glycolide acid) (PLGA) microspheres was incorporated with the CPC powder. Sodium citrate solution was used as the cement liquid phase. The formation of macropores and the decline in mechanical strength of the paste was investigated. The results showed that the macroporous CPC exhibited a good property of injectability but the self-setting time was slightly prolonged. With the degradation of PLGA microspheres, the weight loss increased and the average values of compressive strength of the paste was reduced dramatically. The formulation of negative correlation between compressive and weight loss was established as following: $y_2 = -0.794y_1 + 38.278$. In conclusion, the present macroporous CPC showed that excellent injectability accompanied with the prolonged setting time, the degradation of microspheres and compressive strength of CPC are also in negative correlation. Regulate the acceleration of degeneration to a proper extents perhaps is another method to enhance the compressive strength in macroporous CPCs.

1. Introduction

The first CPC was reported in 1986 by Brown and Chow [1], it consisted of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) [1]. Besides excellent osteoconductivity and bone replacement capability [2–4], calcium phosphate cements (CPC) can also be molded and set in situ to provide intimate adaptation to the contours of defect surfaces [1,5–7], so it is highly promising for a wide range of clinical applications. However, the currently used CPC still has some limitations due to its poor initial mechanical properties, low biodegradation rate in vivo and relatively long setting time [8,9]. As a fact, in clinical applications, bone implants are required to provide adequate short- and long-term mechanical support in the defect site, as this is crucial for bone replacement [10,11]. So it is natural that the use of CPC was "limited to the reconstruction of non-stress-bearing bone" [10,11], and "clinical usage was limited by . . . brittleness . . ." [4].

In order to relieve the drawbacks of CPC, many CPC composites were formulated and studied. With all these efforts, the injectability has been enhanced [12,13], setting time has been shortened [14,15], and different kinds of degradable CPCs have been fabricated [16,17]. It has been confirmed that pore sizes of at least 100 μm were required for significant bone ingrowth [18]. In addition, after macroporous materials were implanted in vivo, the strength significantly increased once new bone started to grow into the macropores [19,20]. The emerged phenomenon draws widespread interest in macrospores from researchers. Macropores have been built into biomaterials for bone repair [21–23], they can facilitate both bony ingrowth and implant fixation [24–26]. Hitherto, various macroporous CPCs have been fabricate and exhibited some good properties [27–30], but almost all these macropores have slightly or even severely degraded the CPC strength at least in vitro [27–31].

Notwithstanding all the advances that have been made in the field of CPC, stress-bearing bones are still restricted zones for CPC for clinical application. Many methods have been tried in an attempt to enhance the compressive strength of CPCs, but unfortunately, the optical strength could only be able to increase a little and most improved strength was still less than 15 MPa [32–35]. Only recently, it was reported that the compressive strength of the CPC-fibre implant was able to increase to 33 MPa [36]. When chitosan was used to strengthen CPC and control protein release, in another investigation, it was found that flexural strength of CPC containing 100 ng/mL of protein could be improved to 19.8 ± 1.4 MPa with 15% chitosan [36,37].

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As mentioned above, CPC is brittle and macropores will further decrease its strength. Why so many researchers still concentrate their attention on macroporous CPCs? Is the problem of low strength not as serious as mentioned in that article [11]? No, certainly not. In fact, the theoretic basis of previous studies on macroporous CPCs is just "the strength significantly increased once new bone started to grow into the macropores" [19,20]. Therefore, authors thought it is in the early stage of implantation when the macroporous implant is in the most need of strength [8,19,20,35]. However, in that paper [38], the formation of new bone was found at 4 weeks after implantation, furthermore, at eight weeks after implantation, the total amount of new bone formation had not increased compared to the fourth week, the highest compressive strength in the Ø300 μm sized cylindrical-type was only 15.8 MPa. That is to say, the ingrowth of new bone neither starts very early nor progresses very rapidly, the increased strength was yet not very high.

As of now, the crucial problem of low strength of CPC has not been resolved, which on the contrary has resulted in a confusion of research. On the one hand, we want to promote the properties of CPC by the macropores, on the other hand, macropores actually decrease the strength in most cases. Hence, the most imperative issue is to grasp the relationship between macropores and compressive strength. In present study, authors investigated the common properties of setting time and injectability of CPC, the correlation between degradation and compressive strength in our macroporous CPC was also established.

2. Experimental procedures

2.1. Materials and preparation

The CPC powder used in this study was prepared by mixing partially crystallized calcium phosphate (PCCP) and dicalcium phosphate anhydrous (DCPA) at a mass ratio of 1:1, as described in our previous work [39,40]. The precursor of PCCP was synthesized from an aqueous solution of $Ca(NO_3)_2{\cdot}4H_2O$ (0.36 mol l^{-1}) and $(NH_4)_2HPO_4{\cdot}12H_2O$ (0.15 mol l^{-1}) by chemical precipitation method in our laboratory. Then the deposit was centrifugally separated, freeze-dried and calcined at 450 °C for 2 h in a furnace to attain PCCP. The PCCP powders were milled in a planetary ball mill using ZrO_2 balls at 400 rpm for 2 h. DCPA of analytical grade purity was commercially obtained from Shanghai No. 4 Reagent & H.V. Chemical Co. Inc., China. Sodium citrate was obtained from Tianjin Y.H. Chemical Reagent Co. Inc., China.

PLGA (50/50 lactide to glycolide ratio, molecular weight = 30,000) was purchased from Jinan M.K. Biotechnology Co., Ltd., China. PLGA microspheres were made by a solvent evaporation method. PLGA (1.5 g) was dissolved in 10 ml methylene chloride (CH_2Cl_2) to form a homogeneous solution, which was subsequently poured into 5 ml of 0.5% methyl cellulose (M20, Sinopharm Chemical Reagent Co., Ltd.) solution. The solution was stirred at 450 rpm with an overhead stirrer for 8 h at room temperature, allowing the solvent to evaporate. After stirring, the solution was allowed to stand for 4 h and the liquid was decanted. The microspheres were then washed three times with deionized water, centrifuged and lyophilized. Microspheres with diameters less than 80 μm were separated by sieving for use in this study.

The PLGA microspheres were uniformly mixed with CPC powder at a PLGA to CPC weight ratio of 30/70. Sodium citrate solution (15%) was used as the liquid phase in this study. Then the PLGA/CPC mixture was homogeneously mixed with sodium citrate solution at a liquid to CPC ratio of 0.4 ml g^{-1}. All processes were carried out at 25 ± 2 °C and 50–60% humidity.

2.2. Setting time measurements

The setting time of CPC containing and not containing PLGA microspheres was measured according to ASTM C191-03 [41]. The samples were tested using a Vicat apparatus which had a movable rod of 300 ± 0.5 g mass and a removable needle of 1 ± 0.05 mm diameter, fixed at the end of the rod. The Vicat needle was carefully lowered vertically onto the surface of the newly shaped cement samples and kept there for 5 s, applying an equivalent static pressure of 3.7 MPa. The indentation was repeated at intervals of 30 s until the cement was hardened. The initial setting time was calculated as the difference between the time when the needle penetrated 25 mm into the cement paste and the time of the initial contact between the powder and the liquid phase. The final set occurred when there was no visible penetration. The setting times of the cements were measured in a humidity chamber at 37 °C and >90% humidity and in normal laboratory atmosphere (25 ± 2 °C and 50–60% humidity), respectively. Each measurement was performed six times and the average value was calculated.

2.3. Injectability tests

The injectability of solo CPC and the CPC containing PLGA microspheres was tested with a syringe of 14.5 mm inner diameter, which was fitted with a needle of 1.6 mm inner diameter. After mixing the PLGA/CPC mixture with sodium citrate solution of 20 wt.% for 1 min, the as-prepared paste was poured into the syringe. A 5 kg compressive load was then mounted vertically on the top of the plunger for 2 min. The entire process totally took about 4 min, which was much shorter than the initial and final setting time. The mass of the paste before and after injecting was measured and the injectability was calculated according to Eq. (1). Each test was performed six times and the average value was calculated

$$\text{Injectability } (\%) = \frac{\text{Mass expelled from the syringe}}{\text{Total mass before injecting}} \quad (1)$$

2.4. Mechanical properties tests

The compressive strength of the specimens was measured using a universal material testing machine (Instron 5567, Instron Co., USA) at a crosshead speed of 0.5 mm min^{-1}. Steel cylindrical molds with an inner diameter of 6 mm and a height of 12 mm were used to prepare specimens for compressive tests. After pouring the cement into the steel molds, the cement was pressed by a steel column with a diameter of 5.6 mm under a stress of about 0.7 MPa for 5 s. Then the specimens were immediately remolded and stored in an incubator at 37 °C with 97% humidity for 24 h. After having been immersed in the phosphate buffer solution (PBS) at 37 °C in the humidor for 7, 14, 28, 42, 56 and 70 days, CPC containing PLGA microspheres specimens were salvaged out and measured. Each measurement was performed six times and the average value was calculated.

2.5. In vitro degradation

The specimens were weighed and then immersed in phosphate buffer solution at 37 °C for 7, 14, 28, 42 and 56 days at a liquid to initially weighed solid ratio of 50 ml g^{-1}. The immersion solution was refreshed every 7 days. For each data point, weight loss, compressive strength and microstructure of specimens were analyzed. The weight loss (WL) was calculated as follows:

$$\text{WL}(\%) = \frac{W_0 - W_d}{W_0} \times 100 \quad (2)$$

where W_0 is the initial weight of the specimen and W_d is the weight of the specimen dried after different degradation time. Each measurement was performed six times and the average value was calculated.

After measuremnet, each specimen was broken in two pieces by hand to make fracture surface. The morphoplogy of the surface was analyzed by scanning electron microscpy (SEM) using a JEOL scanning electron microscope (JSM-6400F) at 10.0 kV. Samples were coated with silver under vacuum by a SPI sputter coating unit. The dynamic morphological change of samples with time was observed.

2.6. Statistical analysis

Quantitative data are presented as mean ± standard deviation and statistical analysis was performed using a one-way analysis of variance (one-way ANOVA). A comparison between two means was made using the Tukey's test, with statistical significance set at $p < 0.05$.

3. Results and discussion

3.1. Setting time

The initial and final setting times of CPC containing and not containing PLGA microspheres with sodium citrate at room temperature (25 °C) and body temperature (37 °C) are presented in Table 1. The addition of PLGA microspheres prolonged the setting time of CPC. At 25 °C, the initial and final setting times of the paste without microspheres were 15.30 min and 37.98 min and were increased respectively to 20.95 min and 45.16 min when microspheres were incorporated. At 37 °C, the initial and final setting times were increased from 13.06 min and 30.52 min to 17.79 min and 38.51 min respectively. The statistic difference between two groups was significant. Setting time was increased upon addition of the PLGA microspheres, which is in agreement with one previous study [42]. When gelatin microspheres were incorporated into CPC, the same result could be obtained [43]. The parameter

Table 1
Initial and final setting time of CPC containing and not containing PLGA microspheres at 25 °C and 37 °C. Data are presented as mean ± standard deviation ($n = 6$).

Group	25 °C		37 °C	
	Initial setting time	Final setting time	Initial setting time	Final setting time
CPC	15.30 ± 2.20	37.98 ± 4.01	13.06 ± 1.72	30.52 ± 7.15
CPC/PLGA	20.95 ± 2.05	45.16 ± 6.62	17.79 ± 2.12	38.51 ± 5.47
t	−5.09	−3.35	−3.24	−2.78
p	0.004	0.020	0.023	0.039

that influenced the setting of PLGA/CPC perhaps is the large volume of microspheres that hampers the entanglement of calcium phosphate crystals (setting process), this possible reason is inferred from another previous study [44]. In present study, CPC was comprised of nanogranules, when PLGA microspheres were mixed, the average volume of composites increased enormously even before the microspheres swelling.

3.2. Injectability and compressive strength

The effect of PLGA microspheres on the injectability and compressive strength of the CPC is illustrated in Table 2. The injectability of the paste increased from 40.1 to 67.63% when microspheres were incorporated. The addition of microshperes significantly improved the injectability of CPC (*t*-test; $p = 0.002$). Meanwhile, The compressive strength of the cement, as measured after 24 h of hardening, was decreased from 39.00 MPa to 31.98 MPa with an addition of 30% PLGA microspheres ($p = 0.174$).

Incorporated PLGA microspheres absorb some of the liquid and thus increase the viscosity of the paste, and microspheres with a relatively large diameter increase the tendency of clogging at the syringe opening [42]. In previous studies, it had been showed that with PLGA microspheres [42] and gelatin microspheres [43], extra aqueous phase was present to improve the injectability of the composite (PLGA) or as a result of the preparation method (pre-swollen gelatin microspheres). On the other hand, Sodium citrate solution was used as the liquid phase in this study, which could be attributed to the adsorption of citrate ions with high zeta-potentials at the solid-to-liquid interface. The increase in the zeta-potential led to a much lower viscosity of the cement paste due to a decrease in the attractive interparticulate forces by electrostatic mutual repulsion [45].

The compressive strength decreased in present study, but when compared with control CPC, there was no significant statistical difference between them. It was reported that when degradable poly(trimethylene carbonate) (PTMC) was incorporated with CPC, the compressive strength decreased [46]. As the macropore percentage of the scaffold increased, the compressive strength of CPC decreased, as the weight ratio of hydroxyapatite to tricalcium (HA/TCP) in the scaffold increased, the compressive strength first increased and then decreased but the dissolving rate uniformly decreased [47]. When macroporous CPCs were prepared using a porogen, it was also found that the presence of a porogen in a CPC led to significant decreases in both its initial setting time and compressive strength [48]. All these results, as well as present result, indicate the degradable microspheres could improve the injectability but decrease the initial compressive strength. How to balance the two contradicts still be a problem to settle immediately.

Table 2
Injectability and compressive strength of CPC and PLGA/CPC. Data are presented as mean ± standard deviation ($n = 6$).

Group	Injectability (%)	Compressive strength (MPa)
CPC	40.10 ± 11.84	39.00 ± 7.01
CPC/PLGA	67.63 ± 17.34	31.98 ± 3.56
t	−5.87	1.887
p	0.002	0.118

3.3. In vitro degradation behavior

Weight loss of CPC containing PLGA microspheres is presented in Fig. 1. With the prolongation of immersion time, the weight loss of specimens containing PLGA microspheres significantly increased (one-way ANOVA, $p = 0.001$), and expect for those immersed for 14 days and 28 days (one-way ANOVA, $p = 0.240$), the difference of weight loss between each interval was significant. However, the weight loss of control CPC only increased a little (one-way ANOVA, $p = 0.468$). The results indicated that the weight loss of specimens mainly resulted from the degeneration of PLGA microspheres, when immersed in PBS. After immersion in PBS for 56 days, the weight loss of CPC containing PLGA microspheres reached 30.62%, while that of control cement only reached 7.17%. The results suggested that even without degradable microspheres, the cement also could lose weight. However, the main weight loss resulted from the degradation of microspheres.

The compressive strength of CPC containing and not containing PLGA microspheres decreased significantly with increasing immersion time (one-way ANOVA, $p = 0.001$), as shown in Fig. 2. Compressive strength of specimens containing microspheres decreased from 31.98 MPa before immersion to 11.42 MPa after immersion in PBS for 56 days, however, the difference of compressive strength between each interval was not always significant, only during the first two weeks (one-way ANOVA, $p = 0.028$ and 0.011 respectively) and the third two weeks (one-way ANOVA, $p = 0.007$) did the compressive strength decrease dramatically. The decline in compressive strength, from 39.00 MPa to 33.26 MPa, also presented in the specimens without microspheres (one-way ANOVA, $p = 0.041$), however, the compressive strength of these specimens only decreased moderately, between each interval, the difference was not significant (one-way ANOVA, $p > 0.05$). The results above

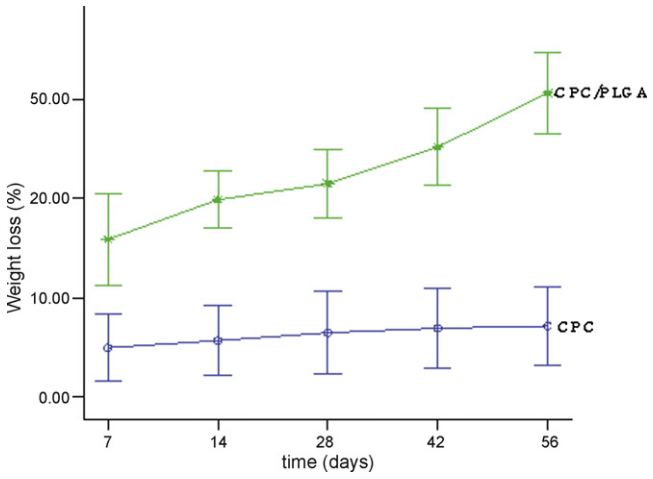

Fig. 1. Weight loss of CPC containing and not containing PLGA microspheres at different immersion time. Data are presented as mean ± standard deviation ($n = 6$).

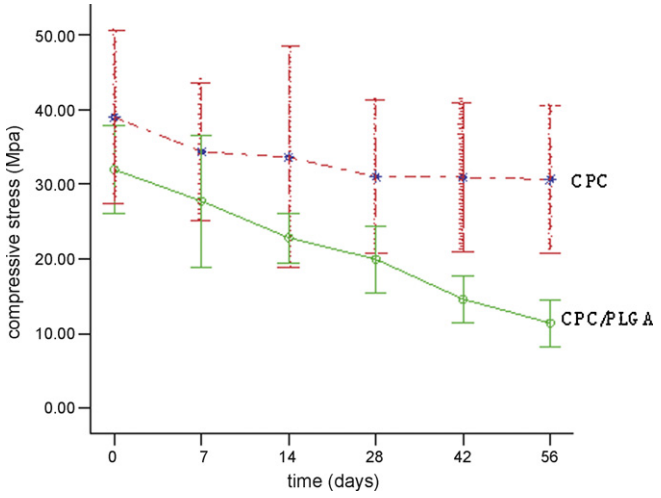

Fig. 2. Compressive strength of CPC containing and not containing PLGA microspheres at different immersion time. Data are presented as mean ± standard deviation ($n = 6$).

in this paragraph suggest that the microspheres perhaps should be responsible for the decline in compressive strength of CPC.

The morphologies of CPC containing and not containing PLGA microspheres immersed in PBS for different times are presented in Fig. 3. CPC without microspheres is composed of lots of microparticles, which were not adherent tightly with each other (Fig. 3A). In the specimens with microspheres immersed in PBS for 7 days (Fig. 3B), the PLGA microspheres were intact and tightly embedded in the CPC matrix. After immersion in PBS for 14 days, surface erosion of PLGA microspheres can be seen in Fig. 3C. Bulk erosion of PLGA microspheres became visible in the specimen immersed in PBS for 28 days, as shown in Fig. 3D. In the specimen immersed in PBS for 42 days (Fig. 3E), there were many macropores generated in situ by the degradation of PLGA microspheres, with some PLGA membrane covering the inside of the macropores. After immersion in PBS for 56 days, the PLGA microspheres in CPC were almost completely degraded, leaving a macroporous structure, as shown in Fig. 3F. The sizes of most macropores were in the range of 50–80 μm, in accordance with the sizes of the incorporated PLGA microspheres.

These results showed that the gradual decreased compressive strength accompanied with weight loss, and the main weight loss resulted from the degradation of PLGA microspheres. It was reported that there is a general conflict between macroporosity and mechanical strength in CPC [29,49,50]. For example, the

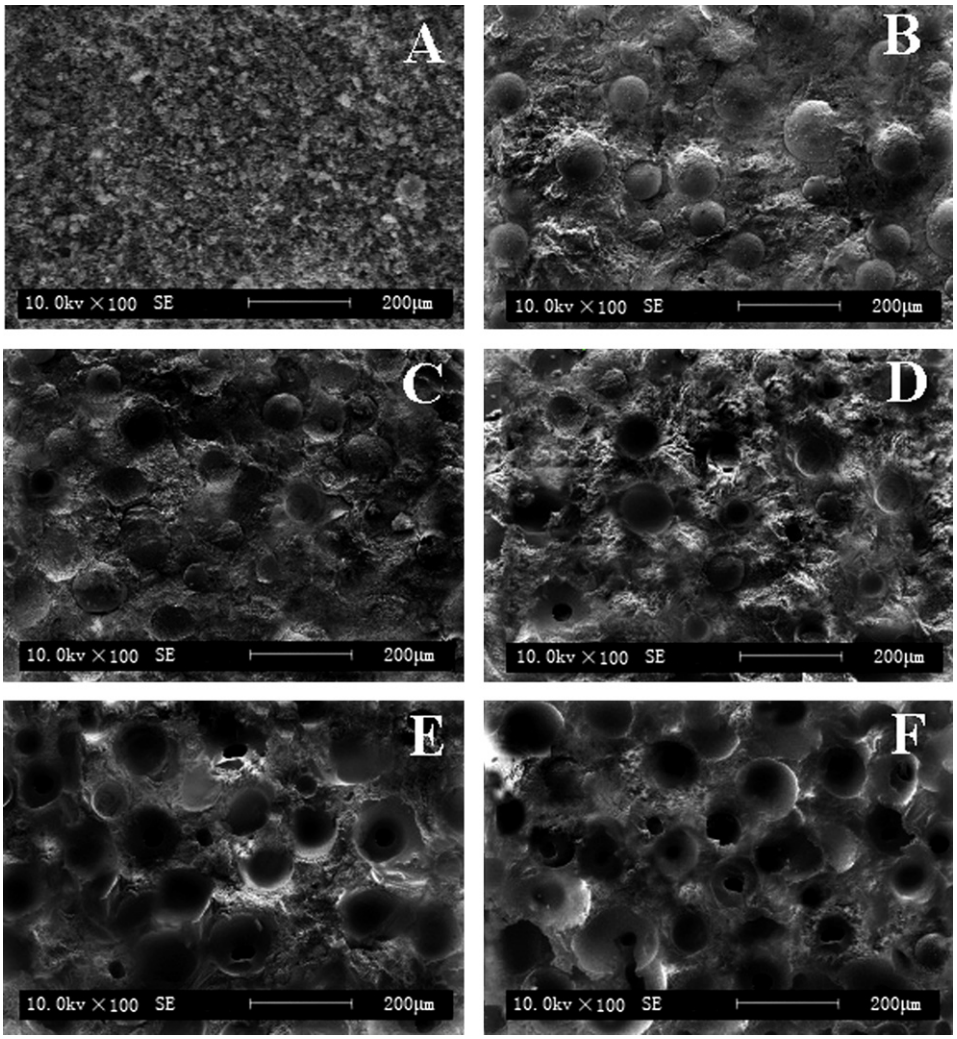

Fig. 3. SEM morphologies of CPC (a) and CPC/PLGA compounds after immersion in phosphate buffer solution for 7 days (b), 14 days (c), 28 days (d), 42 days (e) and 56 days (f).

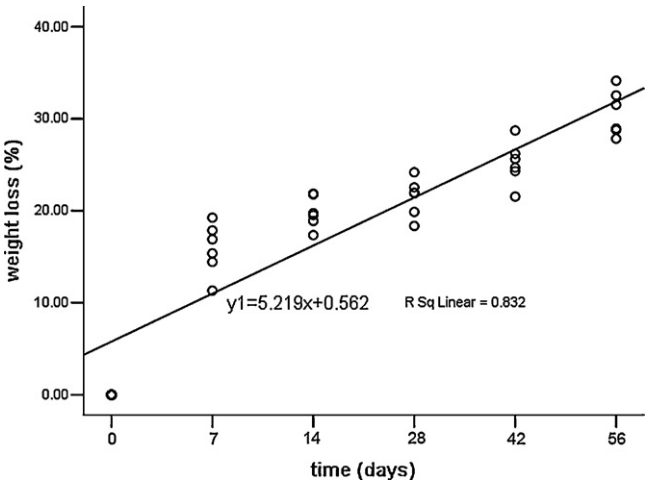

Fig. 4. Correlation between time and weight loss of PLGA/CPC.

compressive strength of CPC without macropores was shown to be approximately 37 MPa, which decreased to 2.9 MPa with 29% macropores and further decreased to 0.4 MPa with 40% macropores [51]. Here we found that during the first weeks after immersion, the extent of decrease in compressive strength between CPC and PLGA/CPC was similar, and during this period, the PLGA microspheres were intact (Fig. 3B). After that, the PLGA microspheres began to degrade (Fig. 3C), the weight loss of PLGA/CPC increased quickly, also the compressive strength decreased drastically. These relationships between the morphologies and the figures give us a hint that if the degradation was slowed, perhaps the early strength might maintain longer. After the PLGA/CPC specimens were immersed in PBS for 28 days, degradation only appeared in superficial microshperes and thus the swallows were shallow (Fig. 3D). During the period from 14 days to 28 days, both the increase in weight loss and the decline in compressive strength was only a little. However, since the degradation of almost all microspheres began, the strength decreased drastically again after immersion for 48 days (Fig. 3E). This phenomenon indicated that both the acceleration and extent of degradation in microspheres have an effect on the decline in compressive strength of PLGA/CPC.

Variations of weight loss and compressive strength in PLGA/CPC are showed in the figures (Figs. 4 and 5). We can find clearly that weight loss and time are in positive correlation, the compressive strength and time are in negative correlation. From the formulations (3) and (4):

$$y_1 = 5.219x + 0.562 \quad (3)$$

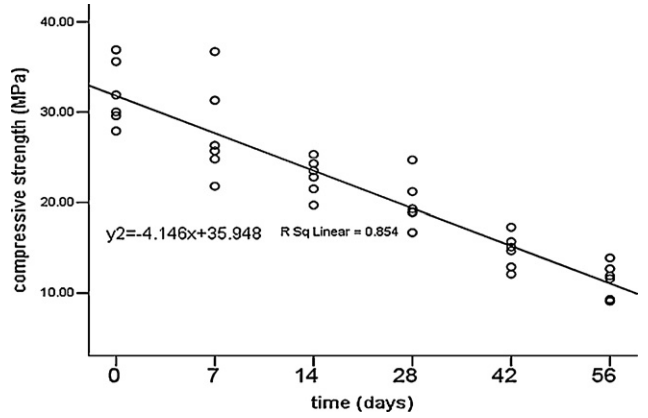

Fig. 5. Correlation between time and compressive strength of PLGA/CPC.

$$y_2 = -4.146x + 35.948 \quad (4)$$

the following formulation (5) can be inferred:

$$y_2 = -0.794y_1 + 38.278 \quad (5)$$

where y_1 is weight loss, y_2 is compressive strength. The formulations (3) and (4) notify us that after immersion in PBS for different time, CPCs have different values of weight loss and compressive strength. So when compressive strength is investigated, comparison should be carried out at the same time after immersion. The negative correlation between weight loss and compressive strength suggests that if the acceleration or extent of degradation of microshperes could be regulated to a proper decreased level, the compressive strength should be enhanced.

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