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Sustained release of OIC-A006 from PLGA microspheres to induce osteogenesis of composite PLGA/ β -TCP scaffolds

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Abstract: In this article, poly(lactic-co-glycolic acid) (PLGA) microspheres containing the drug OIC-A006 were prepared by ice bath extraction and poly(lactic-co-glycolic acid)/ β -tricalcium phosphate (PLGA/ β -TCP) scaffolds were prepared by freeze-drying. Three kinds of scaffold were prepared by adding no drug, direct loading the drug, and loading with drug microspheres. The mechanical strength, degradation, and microstructure of the scaffolds were analyzed. The drug delivery properties of the scaffolds were determined using high-performance liquid chromatography (HPLC). It was found that PLGA/ β -TCP scaffolds with PLGA microspheres that contained the drug OIC-A006 exhibited a biphasic drug release pattern. Sustained release of the drug OIC-A006 was beneficial to cell adhesion and proliferation. Experiments on rats showed that composite PLGA/ β -TCP scaffolds with PLGA microspheres containing the drug OIC-A006 produced good bone formation and bone induction.

Keywords: bone repair materials; controlled drug release; drug-loaded microspheres; freeze-drying technology; OIC-A006.

1 Introduction

The drug OIC-A006, having the same function as bone morphogenetic proteins (BMPs), is a small molecule that activates the BMPs/Smad pathway [1]. It penetrates the cell membrane easily and is then able to induce differentiation

of bone marrow mesenchymal stem cells, stimulate the symbolic gene expression of bone marrow mesenchymal stem cells, and improve the bone mass of the bone tissue. *In vivo*, OIC-A006 could induce the formation of cartilage and bone. It has the same function of ectopic osteogenesis as BMPs [2–4].

The solid or liquid drug has been contained in poly(lactic-co-glycolic acid) (PLGA) microspheres, which used poly lactic acid polymer material as a carrier to form a microspherical structure [5–9]. The diameters of PLGA microspheres are generally 20–500 μ m or even more [8, 10, 11]. As a new drug delivery technology, drug-loaded microspheres could realize the purpose of long-term release by controlling the drug release rate [12, 13]. With respect to bone growth, this could improve the repair effect by controlling the release of OIC-A006.

An ideal scaffold for bone tissue should be non-toxic, bioactive, and biodegradable [14, 15]. Although bone defects are best treated using autologous bone scaffold, sources of autologous bone are limited [16–19], and autologous bone is not available in many cases [20–22]. A single bone repair material is generally insufficient in meeting the requirements of scaffolds for bone tissue engineering. A common and effective measure is to modify biological materials or prepare composite scaffolds by adding other biological materials [23–29].

The purpose of this study was to prepare a composite PLGA/ β -TCP scaffold containing PLGA microspheres loaded with the drug OIC-A006. The microspheres would enable a sustained long-term release by preventing sudden release of the drug. The composite scaffolds improved osteogenesis to achieve good bone repair. The osteogenesis of composite PLGA/ β -TCP scaffolds was tested and verified *in vitro* and *in vivo*.

2 Materials and methods

2.1 Materials

Samples of OIC-A006 were donated by the Ruijin Hospital of Shanghai Jiaotong University. PLGA

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(lactide:glycolide=ratio, 85:15; molecular weight, 40,000–75,000) was obtained from Sigma Aldrich (USA). β -TCP was kindly provided by the Shanghai Tissue Engineering Center (China). Acetone, Dichloromethane (CH_2Cl_2), polyvinyl alcohol (PVA), and phosphate-buffered saline were obtained from Shanghai Clinical Research Center (China).

2.2 Preparation of drug-loaded microspheres

The PLGA microspheres containing OIC-A006 were prepared by ice bath extraction. The drug OIC-A006 was dissolved in dimethyl sulfoxide (DMSO). The PLGA was dissolved in a mixture of CH_2Cl_2 and acetone. The volume ratio of CH_2Cl_2 to acetone was 4:1. The mass ratio of OIC-A006 to PLGA was 7:250. The solution was emulsified ultrasonically. The PVA was dissolved in deionized water (5‰ g/ml) and mixed with the drug emulsion. The solution was then emulsified ultrasonically to prepare a drug-loaded multiple emulsion. The best mass ratio of OIC-A006 and PVA that could be obtained was 1:10.

In an ice bath thermostatic environment (external temperature, $<1^\circ\text{C}$; internal temperature, $<4^\circ\text{C}$), the drug-loaded multiple emulsion was dropped slowly into the PVA dispersion at constant temperature with magnetic stirring (stirring speed, 500 rpm; time, 1 h) to produce drug-loaded PLGA microspheres. After extraction and filtration, the initial drug-loaded microspheres were collected and washed several times with deionized water at constant temperature ($35\text{--}40^\circ\text{C}$) to ensure that the organic solvent was sufficiently volatile. The drug-loaded microspheres were placed in a freeze dryer (LGJ-10D, Beijing Sihuan Technology Co., Ltd, China) and freeze-dried at -42 to -48°C . After the drug-loaded microspheres had been frozen for 3 h, the inside pressure of the freeze dryer was reduced to 30 Pa. After the vacuum had been created once every 2 h, the pressure was reduced to 18–30 Pa. After freeze-drying for 22–24 h, the final sustained release PLGA microspheres containing the drug OIC-A006 were prepared.

2.3 Preparation of PLGA/ β -TCP scaffolds

The PLGA/ β -TCP scaffolds were prepared by freeze-drying. The PLGA was dissolved in CH_2Cl_2 . The β -TCP was dispersed in deionized water. The mass ratio of PLGA to β -TCP was 4:6. Next, the mixed solution was placed in an oscillatory ball mill (Mixer Mill, MM301, Retsch, Germany)

and mixed for 10 min (oscillation frequency, 15 Hz). The prepared composite slurry was infused at low temperature into negative molds (temperatures, -15°C to -30°C ; pressure, $2\text{--}2.5 \times 10^5$ Pa). Negative molds with composite slurry were freeze-dried for 3 h at -42°C ; the pressure inside the freeze dryer dropped to 18 Pa. After creating a vacuum once every 2 h, the degree of the vacuum was maintained at between 18 and 30 Pa. The temperature was maintained at between -42 and -48°C . After lyophilization for 22–24 h, the negative molds were removed. This produced bionic composite bone scaffolds with no drug.

The drug OIC-A006 was dissolved in DMSO and added to the mixed slurry. The mass ratio of OIC-A006 to PLGA was 8.27:1000. The drug-loaded solution was placed in an oscillatory ball mill for 5 min (oscillation frequency, 15 Hz) to produce a mixed slurry. Composite scaffolds directly loaded with the drug OIC-A006 were then prepared from the slurry, using the same method as for the drug-free scaffolds. The drug-loaded microspheres were added to the complex slurry and placed in an oscillatory ball mill for 1 min (oscillation frequency, 15 Hz) to produce a microsphere slurry. The mass ratio of drug-loaded microspheres to PLGA was 28.57:100. Composite scaffolds containing the drug-loaded microspheres were prepared from the slurry, using the same method as for the drug-free scaffolds.

2.4 Measurement of scaffold porosity

Scaffold porosity was measured by Archimedes' method according to an established method. The porosity of the scaffolds was determined according to equation (1):

$$\text{Porosity (\%)} = \frac{W_d - W_w}{W_s - W_w} \times 100\%, \quad (1)$$

where W_w is the mass of wet scaffold (mg), W_d is the mass of dry scaffold (mg), and W_s is the mass of submerged scaffold (mg).

2.5 Morphological characterization

The surface and shape of the OIC-A006-loaded microspheres and scaffolds were observed under a Hitachi SU1510 (Japan) scanning electron microscope (SEM) after gold coating. The microscopic scaffold structure was viewed with an accelerating voltage of 10 kV. However, owing to the poor electrical conductivity of the OIC-A006 microspheres, an accelerating voltage of 15 kV was used to view the microsphere surface.

2.6 Morphological study

The compressive mechanical properties of the scaffolds were tested using a Zwick BZ2.5/TS1S material testing machine (Zwick/Roell Co., Germany). The crosshead speed was 0.15 mm/min at room temperature. Three groups were tested, with three samples per group.

2.7 In-vitro degradation of scaffolds

In vitro degradation of the drug-free scaffold, the drug-loaded scaffold, and the scaffold with drug-loaded microspheres was carried out by incubating the samples in phosphate-buffered saline at 37°C, pH 7.4, under static conditions. Six scaffolds were placed in a 50-ml glass bottle containing 24-ml phosphate-buffered saline. The samples were stored in a thermostatic water bath with stirring (50 rpm) at 37±0.1°C for up to 12 weeks. At weekly intervals, the phosphate-buffered saline solution was replaced with fresh phosphate-buffered saline. The mass loss rate of the scaffolds was determined according to equation (2):

$$\text{Mass loss ratio (\%)} = \frac{M_o - M_t}{M_o} \times 100\%, \quad (2)$$

where M_o is the mass of the original scaffold (mg) and M_t is the mass of dry scaffold measured after lyophilization (mg).

2.8 Determination of drug encapsulation efficiency

The drug encapsulation efficiency of the PLGA OIC-A006 microspheres was determined in triplicate using high-performance liquid chromatography (HPLC, Agilent 1200, Germany). A sample of 10 mg of the PLGA OIC-A006 microspheres was dissolved in 1 ml of DMSO. Then, 5 ml of mobile phase (methanol/triethylamine; v/v, 52/48) was added to extract the OIC-A006. A flowing nitrogen atmosphere was introduced to evaporate the DMSO until a clear solution was obtained for HPLC analysis. A 5 µl aliquot of the sample was injected, and the mobile phase was delivered at a flow rate of 1.0 ml/min. The column effluent was detected at 340 nm using an ultraviolet detector. The calibration curve for the OIC-A006 quantification was linear over the range of standard concentrations, 1–10,000 µg/ml with the regression equation (2):

$$Y = 61606X + 399756, \quad (3)$$

where Y is the peak area and X is the concentration of OIC-A006 (µg/ml) and $R^2 = 0.9995$.

The encapsulation efficiency (η) is expressed by equation (3) [30]:

$$\eta = A/T \times 100\%, \quad (4)$$

where A is the actual drug loading and T is the theoretical drug loading.

2.9 In-vitro drug release

Drug release experiments were performed in triplicate using dialysis and HPLC. Samples of 50 mg PLGA OIC-A006 microspheres were suspended in 8-ml phosphate-buffered solution (pH, 7.4) in dialysis tubes. The tubes were placed in an orbital shaker bath, maintained at 37°C, and shaken horizontally at 100 rpm for 56 days. At particular time intervals, 500 µl solutions were collected periodically with equal amounts of phosphate-buffered saline. The resulting solutions were subjected to HPLC for the analysis of OIC-A006 concentration.

2.10 Culture of bone marrow stromal cells

Cell seeding and observation on the scaffolds was undertaken with the assistance of the Animal Care and Experiment Committee of Shanghai Jiao Tong University School of Medicine (no. SYXK 2008–0050). Bone marrow stromal cells provided by the Animal Care and Experiment Committee of Shanghai Jiao Tong University School of Medicine were seeded onto the drug-free scaffolds, the drug-loaded scaffolds, and the scaffolds with the drug-loaded microspheres at a density of 1×10^7 cells/ml and incubated in Dulbecco's modified Eagle's medium at 37°C in a humidified atmosphere containing 5% CO₂. Finally, osteogenic induction occurred with the osteogenic medium at 37°C for 50 ml/l CO₂.

2.11 Alkaline phosphatase activity

Cell numbers on the scaffolds were determined by alkaline phosphatase activity assays after 1, 3, and 7 days [31]. First, the scaffolds were removed from the culture medium, washed twice with phosphate-buffered saline, and then fixed in paraformaldehyde. Next, they were washed twice with Tris-buffered saline with Tween 20 (20 mM Tris-HCl, 0.15 M NaCl, 0.05% Tween-20, pH 7.4). Finally, they were incubated for 20 min at room temperature in the dark

with BM Purple (Roche, Germany) and washed twice with phosphate-buffered saline after the addition of blot substrate solution.

2.12 *In-vivo* study

The surgical operation and observation of bone growth on scaffolds were assisted by the Animal Care and Experiment Committee of Shanghai Jiao Tong University School of Medicine (no. SYXK 2008–0050). The protocol was approved by the Animal Care and Experiment Committee of the Shanghai Jiao Tong University School of Medicine and University guidelines for the care and use of laboratory animals were followed. Nine mature male Sprague Dawley rats, 24 weeks old, were used as experimental animals. Anesthesia for all animals was induced using 2.5% pentobarbital (30 mg/kg) intra-peritoneal injection. A critical defect with the size of 3 mm in diameter and 3 mm in depth was created in the lower end of each rat's thigh with 5 mm from the humerus. The defects were filled with the scaffolds and the wounds were sutured.

2.13 X-ray analysis

At 8 weeks after the scaffolds had been transplanted into the rats, three rats were chosen. X-radiographs were acquired under anesthesia which was induced for all animals by 2.5% pentobarbital (30 mg/kg).

2.14 Hematoxylin and eosin staining

The rats were treated with 5% sodium pentobarbital anesthesia (60 mg/kg). When they were dead, the rats' humeruses were removed and fixed with 4% formaldehyde solution for 24 h (18–22°C). The decalcified tissues were washed with water for 1–2 h. The tissues were stained with hematoxylin after dewax treatment for 5–10 min. After differentiation with 1% hydrochloric acid alcohol for a few seconds, the tissues were washed with water until fully fixed. Then, eosin staining was observed with neutral rubber.

2.15 Tartrate-resistant acid phosphatase (TRAP) staining experiment

The rats' humeruses were washed three times with phosphate-buffered saline. At room temperature, fixed liquid

was discarded for 30 s, and the humeruses were washed again three times with phosphate-buffered saline. The cell culture medium was then air-dried. TRAP solution, which was heated in a water bath (37°C), was added to the culture wells. Each well contained 250 µl TRAP staining solution. The samples were cultured in a water bath for 1 h at 37°C, after which the TRAP staining solution in the culture wells was discarded. The cells were observed under a fluorescence microscope.

2.16 Statistical analysis

Results are expressed as mean±standard deviation. Statistical significance was determined using analysis of variance (ANOVA). Statistical significance was accepted for $p < 0.05$.

3 Results

3.1 Morphology of microspheres and scaffolds

Figure 1 shows the composite scaffolds with no drugs, with direct drug loading, and with drug-loaded microspheres.

The microspheres and scaffolds were observed with SEM. As shown in Figure 2, the microspheres loaded with OIC-A006 were spherical, smooth, and non-porous. The average diameter of the microspheres was 400 ± 150 µm. It can be seen from the figure that the addition of OIC-A006 had no effect on the PLGA microspheres.

The microstructure of the drug-free scaffold is shown in Figure 3A. The scaffold was porous and lamellar, with

Figure 1: Composite bionic bone scaffolds: (A) without drug, (B) directly loaded with drug, and (C) with drug-loaded microspheres.

Figure 2: Scanning electron micrographs of (A) microsphere without drug and (B) drug-loaded microsphere.

Figure 3: Scanning electron micrographs of (A) scaffold without drug and (B) scaffold with drug-load microspheres.

Figure 4: Compressive strength of β -TCP/PLGA scaffolds. NOS, composite bone scaffolds with no drug; OS, composite scaffolds with direct drug loading; and POS, composite scaffolds with drug-loaded microspheres.

good connectivity, and a pore size between 200 and 550 μm . The microstructure of the composite scaffold with the drug-loaded microspheres is shown in Figure 3B. The structure of the scaffold was flaky and porous, forming a suitable biomimetic scaffold, to which the drug-loaded microspheres easily adhered.

3.2 Mechanical properties

The compressive strength of the scaffolds was recorded as the stress from the test start to the fracture point of the scaffold, which occurred at between 10% and 20% strain. As shown in Figure 4, the drug-free scaffold had a maximum compressive strength of 2.93 MPa. The drug-loaded scaffold had a maximum compressive strength of 2.86 MPa. The scaffold with drug-loaded microspheres had a maximum compressive strength of 1.97 MPa.

3.3 Porosity and scaffold degradation

In this article, three groups of scaffolds were tested, each group comprising three scaffolds. All the scaffolds were fabricated in almost the same way with the same concentration slurry. The results show that the porosity of scaffolds is from 58.88% to 60.15%. The average porosity of the scaffolds ($N=6$) is 59.51%. The average porosity of the scaffolds containing drug-loaded microspheres is high than the no loading. Degradation characteristics of the three scaffold groups were studied. The experiment lasted 12 weeks and samples were taken once a week. The mass loss ratio curve was obtained using equation (2). As seen in Figure 5, the drug-free scaffold was the slowest to degrade, and all scaffolds degraded within 12 weeks. The scaffold with direct drug loading was next slowest to degrading in 11 weeks. The scaffold with the drug-loaded microspheres was the fastest to degrade; it was fully degraded in 8 weeks.

Figure 5: Mass loss ratio curve. NOS, composite bone scaffolds with no drug; OS, composite scaffolds with direct drug loading; POS, composite scaffolds with drug-loaded microspheres.

3.4 Biphasic drug release pattern

Drug encapsulation efficiency plays an important role in drug delivery systems and directly influences the therapeutic effect [32]. In this study, the OIC-A006 encapsulation efficiency of the composite scaffold with drug-loaded microspheres was $72.4 \pm 7.7\%$. In the *in-vitro* osteogenesis assay, 10 mg composite scaffold with drug-loaded microspheres was added to 2 ml osteogenic-induced medium, to produce a theoretically micromolar concentration of OIC-A006 when the scaffold degraded completely.

Sustained OIC-A006 release plays an important role in the process of bone formation.

As shown in Figure 6, the composite scaffold with the drug-loaded microspheres exhibited a biphasic drug release pattern. It can be observed that there was an initial burst release effect, with approximately 40% drug release in the first 3 days. Thereafter, the OIC-A006 release profile displayed a relatively tardy and continuous release, with about 95% of the total OIC-A006 loading amount released into the surrounding medium over the next 56 days.

Figure 6: Cumulative release of OIC-A006 from composite scaffold. OS, composite scaffolds with direct drug loading; POS, composite scaffolds with drug-loaded microspheres.

scaffold. By the seventh day, the alkaline phosphatase activity of the scaffold with the drug-loaded microspheres was higher than the other two scaffolds.

3.5 Alkaline phosphatase activity

The results of the alkaline phosphatase activity assay are shown in Figure 7. The alkaline phosphatase activity of the three groups on the first day was approximately equal. On the third day, the alkaline phosphatase activity of drug-loaded scaffolds was higher than that of the drug-free

3.6 X radiography

Figure 8 shows X radiographs of the rats at the 8th week. The scaffold with the drug-loaded microspheres performed better than the drug-free and drug-loaded scaffolds. The bone defect had been covered with new bone, showing a good repair effect. The other scaffolds had been gradually degraded in the bone defect area and new bone had begun to grow.

Figure 7: ALP expression results of bone marrow stromal cells on fabricated scaffolds at various incubation times.

3.7 Hematoxylin and eosin staining

Bone formation performance in the rat bone defects was analyzed by hematoxylin and eosin staining. As shown in Figure 9, a large number of osteoblasts were gathered in the interior of drug-free scaffold and drug-loaded scaffold, and there was new bone growth in the bone defect. The bone repair for the scaffold with the drug-loaded microspheres was best. The new bone grew into the scaffolds and the scaffold had been degraded. The degradation time of scaffold materials *in vivo* was equal to that observed for the degradation experiment *in vitro*.

3.8 TRAP experiment

In the 8th week, humeruses of the rats were removed for a TRAP experiment. As shown in Figure 10, a large number of osteoclasts were gathered in the interior of the drug-free scaffold and the drug-loaded scaffold. The bone repair effect of the scaffold with drug-loaded microspheres was best. Owing to the large numbers of osteoblasts and osteoclasts, the new bone grew into scaffolds and the scaffold had been degraded.

4 Discussion

4.1 Preparation of drug-loaded microspheres and scaffolds

Experimental studies had shown that more uniform microspheres were obtained for a higher magnetic stirring speed and a longer formation time. A suitable ice bath could be used to improve the rate of formation of the microspheres. The pore size of the scaffold is bigger than the microspheres, and its structure was flaky and porous, which the drug-loaded microspheres easily adhered. SEM observations showed that the scaffolds had good interconnectivity, thus providing adequate space for cell seeding, growth, and proliferation. Moreover, the way that the micropores are distributed on the surface of the drug-loaded scaffolds creates channels through which cells can absorb nutrients and discharge metabolites implanted.

Figure 8: X-radiographs of rats at 8 weeks. Scaffold with (A) no drug, (B) direct drug loading, and (C) drug-loaded microspheres.

Figure 9: H&E staining at 8 weeks for scaffolds with (A) no drug, (B) direct drug loading, and (C) drug-loaded microspheres. The arrows indicate new bone (blue arrows), scaffold remnants (black arrows), blood vessels (blue arrowheads), and bone marrow (black arrowheads) (color figure online).

Figure 10: TRAP staining at 8 weeks for scaffolds with (A) no drug, (B) direct drug loading, and (C) drug-loaded microspheres. The arrows indicate new bone (red arrows), scaffold remnants (black arrows), and bone marrow (red arrowheads) (color figure online).

The results showed that no matter porosity or pore sizes and distribution of scaffolds to match the requirements of bone tissue engineering. In the preparation process, pre-freezing temperature played an important role in determining the pore structure and porosity of the scaffold [33]. It also had some influence on the mechanical strength of the scaffold. Lower pre-freezing temperatures decreased the porosity of the scaffold and increased the strength of the scaffold. Conversely, the higher the pre-freezing temperature, the greater the porosity of the scaffold, and the lower the strength of the scaffold. In this study, to obtain a suitable porosity and mechanical strength, scaffolds were manufactured at a pre-freezing temperature of -42°C .

The adding of the drug decreased the strength of scaffold slightly. Adding of the microspheres decreased the strength of the scaffold to two-thirds of its original strength. The presence of large microspheres that may act as stress concentrators in the porous structure. However, the strength requirements of the scaffold were still satisfied. Under the same conditions, PLGA has a higher degradation rate than β -TCP. In addition, the microspheres are

prepared by PLGA. The incorporation of the microspheres increases the amount of PLGA and the degradation rate of scaffolds. The mass loss ratio curve also reflects this characteristic. The biomimetic composite scaffold with the drug-loaded microspheres (Figure 3B) had an appropriate porosity and provided a suitable environment for the growth of cells and their adhesion and proliferation. The microspheres adhered to the scaffold, which provided an appropriate condition for the sustained release of the drug.

4.2 In-vitro properties

The process of fracture healing can be divided into three phases: the reactive phase, the repair phase, and the remodeling phase [34]. The formation of a fracture callus typically takes 4–8 weeks, depending on the location and type of fracture [35]. The scaffold with drug-loaded microspheres degraded completely in 8 weeks, more quickly than did the drug-free scaffold. Under the same conditions,

PLGA has a higher degradation rate than β -TCP. Moreover, the microspheres are prepared by PLGA. The incorporation of the microspheres increases the amount of PLGA and the degradation rate of scaffolds. The mass loss ratio curve also reflects this characteristic.

The drug OIC-A006, having a similar function to BMP2, plays a role in inducing osteogenesis in bone repair [36]. It penetrates the cell membrane easily and is then able to induce differentiation of bone marrow mesenchymal stem cells, stimulate symbolic gene expression of bone marrow mesenchymal stem cells, and improve the bone mass of the bone tissue. In the early bone repair process, it requires more osteoinductive factor. However, in the later, there is more self-generated osteoinductive protein and the demand of factor become less *in vivo*. The burst release effect was more severe in first 15 days, with approximately 80% drug release. The drug release pattern maintains drug release for the period of bone formation. Compared with drug burst release pattern, it has certain research value. The common scaffold loads drug directly. The scaffold controls drug release by its microscopic structure. In the usual dosing process, the drug would be released suddenly. Our drug-loaded scaffold can realize the sustained release of OIC-A006 by PLGA microspheres to induce osteogenesis.

It was found that adding the drug OIC-A006 increased the activity of alkaline phosphatase significantly, compared with the drug-free scaffold. Owing to the addition of microspheres, the drug was released slowly [37]. After 7 days or longer, alkaline phosphatase activity of scaffold with drug-loaded microspheres was higher. The effect was better.

4.3 *In-vivo* implant test

At the 8th week, X-radiographs of the rats showed that the scaffold with the drug-loaded microspheres had produced better bone growth than the drug-free and drug-loaded scaffolds. The bone defect had been covered with new bone. The PLGA and β -TCP are commonly used biomimetic materials. Two materials are intrinsically safe and non-toxic [11, 37]. At the same time, bone formation performance on the rat bone defect was analyzed by hematoxylin and eosin staining and TRAP staining. A large number of osteoblasts and osteoclasts were gathered in the interior of the drug-free and drug-loaded scaffolds. There was new bone growth into the bone defect. The bone repair effect of the scaffold with drug-loaded microspheres was best. The new bone had grown into the scaffold and the scaffold had been degraded.

The *in-vivo* implant test showed that the scaffold with drug-loaded microspheres had a better effect than the drug-free and drug-loaded scaffolds. Results were similar to the results for alkaline phosphatase activity experiments.

5 Conclusions

The method of preparing the PLGA/ β -TCP composite scaffold using freeze-drying technology worked well. The drug-loaded microspheres prepared using the ice bath extraction method had good drug delivery characteristics and could effectively prevent the sudden release of the drug. Adding the drug OIC-006 provided an additional supportive role in the repair of bone tissue. OIC-006, a small molecule whose function in the bone repair process is similar to BMP2, has lower manufacturing costs and produces a simpler culture environment.

Through the property test of the three scaffold types, it was found that the scaffold with drug-loaded microspheres gave a better overall performance, meeting the strength requirement of a bionic scaffold. The scaffold's degradation rate was similar to the growth rate of new bone. The drug delivery provided by the scaffold was good. Alkaline phosphatase activity analysis revealed that the composite scaffold with drug-loaded microspheres attracted osteoblast, encouraging bone growth. The *in-vivo* implant test showed that the scaffold with drug-loaded microspheres had a better bone repair effect.

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