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# Dexamethasone-loaded PLGA Microspheres in Calcium Phosphate Cements for Bone Regeneration: Physicochemical Characterization

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**Objectives** Bone regeneration is one of the most challenging issues in medicine, for which researchers have been seeking new practical strategies. Calcium phosphate cements (CPCs) are proper candidates for bone scaffolds due to their high biocompatibility, self-setting features, and similar mineral content to the bone.
**Methods** The present study aimed to fabricate composite CPC/ dexamethasone-loaded PLGA microsphere scaffolds, which significantly affected tissue remodeling, and to determine their potential for bone regeneration purposes. The SEM images were used to study the microstructure of the fabricated scaffolds and to investigate the distribution of PLGA in CPC. Moreover, FTIR analysis was performed to determine the chemical components of the fabricated scaffold and to approve the presence of dexamethasone and PLGA in the composite scaffolds. Next, ultraviolet spectroscopy was used to determine the amount of dexamethasone released over time.
**Results** The FTIR results confirmed the presence of dexamethasone in the scaffold. Moreover, the cement/dexamethasone-loaded PLGA scaffold had a lower drug release compared to pure PLGA. Besides, a higher level of PLGA loading led to an increase in the drug release rate.
**Conclusion** According to the results, different weight percentages of dexamethasone-loaded PLGA microspheres incorporated into CPC showed differences in the release time.
**Keywords** Scaffold; Dexamethasone; Microspheres; Calcium phosphate; Bone cements; Tissue engineering

## Introduction

Bones have significant functions in the human body, such as providing support for internal organs, enabling the body to move, and haematopoiesis. [1-3] Today, bone lesions are one of the most common clinical challenges worldwide. The number of these lesions, such as bone defects, which sometimes cannot self-repair, is increasing day by day. [4, 5] In the past decades, synthesis and development of biomaterials, which can be used for bone regeneration, have gained considerable attention to settle this issue. [6] The selected biomaterials for bone tissue engineering should have several characteristics, including biocompatibility, suitable pore size, and suitable porosity, which provide the proper conditions for nutrient transportation and cell growth and promote bone repair and regeneration processes. [7]

The history of progress in the development of biomaterials for bone regeneration indicates three generations of biomaterials. The first generation of biomaterials, such as metals and alloys (e.g., titanium and stainless steel), polymers (e.g., polylactic acid), and ceramics (e.g., alumina), are made in a way that they have similar physical properties with a replaced tissue; they also exhibit the least toxic response to the host. The second generation of biomaterials are polymers (collagen and polyesters) and calcium phosphates (natural or derived from algae or bovine bone), which can be obtained as natural or synthesized materials and calcium sulfates. The third generation of biomaterials incorporates instructive cues into materials to exhibit suitable cellular responses.[8]

Calcium phosphate cements (CPCs) as one of the second-generation biomaterials, discovered in 1980 by Brown and Chow. [9, 10] CPCs have various characteristics, such as biocompatibility, bioactivity, and osteoconduction, which make them ideal for bone repair and regeneration. [11-16] When CPCs are implanted in the body, by releasing calcium and phosphorus ions, the number of osteoblasts and osteoclast activities regulate and promote the bone regeneration process. [12] Besides the abovementioned characteristic, the main advantage of CPCs is their ability to harden in vivo at body temperature, which can prepare them for bone regeneration, as well as drug delivery applications. [7]

Although CPCs have many remarkable characteristics, they have some shortcomings, such as low mechanical strength, low porosity, and low degradation, with direct effects on tissue generation and biodegradability. To overcome these issues, researchers have focused on these challenges and proposed different strategies. [17-19] In a study by Zuo et al., to resolve these shortcomings, CPC-polylactic acid (PLA) and CPC-polycaprolactone (PCL) composites were prepared, and their mechanical properties and porosity were evaluated. Although the addition of PLA and PCL to CPC did not increase its mechanical properties, the porosity increased. [15] In another study, Barralet et al. used frozen sodium phosphate buffer solution particles to create macroporous CPCs. They mixed frozen sodium phosphate solution particles in CPC powder and compressed the mixture under a pressure of 106 MPa; therefore, sodium

phosphate solution particles melted, and a macroporous CPC was created. [20]

To overcome the low degradation rate, researchers have employed different strategies. In this regard, Habraken et al. investigated the effect of poly(lactic-co-glycolic acid) (PLGA) microsphere on the degradation properties of CPC and concluded that a higher amount of PLGA was associated with a higher degradation rate of CPCs. [21] In another study by Teliang Lu et al., the effects of different morphologies of PLGA particles, including PLGA microspheres, porous PLGA microspheres, and a dense irregular shape on the mechanical properties, porosity, setting time, degradation rate, setting time, in vitro degradation, and cytocompatibility of CPCs were examined. [22] They found that CPCs loaded with dense spherical and irregular PLGA had a suitable setting time and a more acceptable compressive strength. They also demonstrated that irregular PLGA had a faster in vitro degradation rate. In another study by Li et al., the drug release rate of vancomycin hydrochloride-loaded PLGA with different concentrations of silk fibroin (SF) coating was studied. [23] They found that the in vitro release of drug-loaded microspheres was initially high, and drug-loaded microspheres coated with SF could reduce the initial release rate.

PLGA is a safe polymer, which has been widely used in the human body due to its biocompatibility and biodegradability. [24, 25] Some researchers have employed PLGA in bone regeneration to create macroporous CPCs and facilitate drug delivery simultaneously. For example, Li et al. used PLGA and calcium phosphate composites for bone tissue engineering. They loaded the PLGA microsphere with alendronate, which prevented bone loss, increased the bone mass, and reduced bone fractures. The fabricated composite showed a suitable pore size, porosity, and compressive strength for bone generation. [26]

In another study, Plachokova et al. prepared a composite, consisting of CPC and TGF-β1-loaded PLGA. The effect of growth factor (TGF-β1) on bone formation was also investigated, and it was observed that TGF-β1 had a significant effect on bone formation. [27] Additionally, Roozbahani et al. evaluated the effect of bicarbonate ($NaHCO_3$) on the mechanical, physical, and biological properties of CPC-laponite nanoplate (LAP) composites, loaded with dexamethasone. They showed that this composite reduced the setting time of CPC; also, the presence of dexamethasone-LAP could enhance MG63 cell proliferation. [6]

The literature review suggests that in previous studies, dexamethasone was not considered a suitable component to stimulate osteoblast differentiation of stem cells and promote tissue remodeling. Therefore, in this paper, a CPC-PLGA microsphere composite scaffold loaded with dexamethasone was fabricated, and its potential for drug delivery purposes and bone tissue scaffolding was investigated. Scanning electron microscopy (SEM) was employed to investigate the microstructure of the fabricated scaffold and to evaluate the distribution of PLGA in CPCs. Moreover, Fourier transform infrared spectroscopy (FTIR) was used to specify the chemical components of the fabricated scaffold. Subsequently, the ultraviolet spectroscopy technique was utilized to determine the intensity and amount of released dexamethasone in CPCs.

## Methods and Materials

CPC, phosphate buffer saline (PBS), dexamethasone, and poly(lactide-co-glycolide) 50:50 (RG 504H) were purchased from Sigma-Aldrich© (USA). Acetic acid, chloroform, acetone, and polyvinyl alcohol (PVA) were also purchased from Merck© (Germany).

Preparation of PLGA microspheres containing dexamethasone

To prepare PLGA microspheres without dexamethasone loading, the double emulsion solvent evaporation method (water/oil/water) was employed. Briefly, to prepare the oil phase, 100 mg of PLGA was solved in 500 µL of chloroform solvent. Next, for preparing the first water phase solvent, 5 mL of a %2 (w/v) solution of PVA/distilled deionized water ($ddH_2O$) was prepared. Subsequently, the oil phase was slowly added to the first water phase. The obtained solution was homogenized for less than two minutes and mixed with 30 mL of the second water phase, that is, 0.2% (w/v) PVA/$ddH_2O$, and placed under a hood on a magnetic stirrer at 500 rpm for three hours to remove chloroform.

Next, using a centrifuge at 10,000 rpm for 10 minutes, the microspheres were separated from the solution. To separate polyvinyl alcohol particles, the microspheres were washed in three stages. In all three stages, the isolated microspheres were washed with deionized water and centrifuged. In the next step, the samples were placed in a freezer at -20°C for 24 hours and finally, placed in a freeze dryer (Christ Alpha 1-4; Germany) for 48 hours.

To prepare microspheres loaded with dexamethasone, the drug should be added to the oil phase. Therefore, in the oil phase preparation, a specific amount of dexamethasone powder was added to the polymer solution. The remaining preparation steps were performed similarly to the previous description.

Preparation of loaded CPC with dexamethasone

CPCs were prepared by combining the powder and liquid phase at a ratio of (p/l)=3. The solid phase consisted of tetracalcium phosphate (TTCP) and dicalcium phosphate dihydrate (DCPD), and the liquid phase consisted of a 3% wt solution of sodium dihydrogen phosphate ($NaH_2PO_4$)

(SDP). The solid phase was slowly added to the liquid phase, and the obtained composition was added to the mold. After CPC preparation, to prepare the cement containing drug-loaded microspheres, the microspheres were mixed with cement powder; the microspheres were added at 30% and 50% of cement weight. Figure 1 shows a schematic representation of the constitutive elements of the composite CPC-PLGA scaffold, loaded with dexamethasone, and its application in bone regeneration.

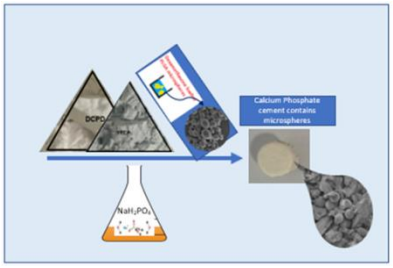


**Figure 1:** The schematic image of CPC-PLGA composite.

SEM analysis

The SEM analysis is a technique by which high-quality images can be acquired by imaging the sample with a beam of electrons. The SEM analysis was used to evaluate the morphology and microstructure of PLGA, cement, and composite scaffolds.

FTIR analysis

The FTIR analysis is one of the most potent methods for determining the chemical properties of materials. This method was used to analyze the chemical structure of the fabricated scaffolds.

Dexamethasone Release

Ultraviolet spectroscopy was used to evaluate the concentration of released dexamethasone encapsulated in PLGA microspheres and to determine the amount of drug extracted from CPC. The in vitro release profile was obtained by incubating 16 mg of dexamethasone-loaded PLGA microspheres in 3 mL of PBS at 37°C via shaking (away from light). Every two days, the sample was centrifuged, and the UV absorbance of the supernatant was measured at 237 nm. The loading of dexamethasone in the PLGA microspheres was determined using a UV spectrophotometer. Briefly, 16 mg of dexamethasone-loaded PLGA microspheres was hydrolyzed in 3 mL of 5% (w/v) sodium chloride (SDS) in 0.1 M sodium hydroxide (NaOH) via overnight stirring. The hydrolyzed sample was then centrifuged, and the UV absorbance of the supernatant was measured at 237 nm against a blank, consisting of empty PLGA microspheres prepared under the same conditions. The loading percentage of dexamethasone was calculated by comparing the UV absorbance of the unknown sample with a standard curve generated under the same conditions. The loading percentage (w/w) was defined as the amount of dexamethasone entrapped per dry weight of microspheres:

$$\text{Loading efficiency (\%)} = \frac{M\ actual}{M\ theoretical} \times 100$$

where *M actual* is the actual amount of dexamethasone in each microsphere, determined in the abovementioned experiment, and *M theoretical* is the theoretical amount of dexamethasone in each microsphere calculated by the quantity added in the fabrication process.

Statistical Analysis

The results of dexamethasone release were analyzed by SPSS and level of significance (P value) was set at 0.05.

## Results

Morphological analysis

Figure 2 presents the SEM images of cement at the magnification of 200x and 1kx. As shown in Figure 2, the particle size of cement varied from 0.5 µm to 25 µm, and there were micron-sized pores on the fracture surface of hardened cement. Additionally, needle-shaped crystals were formed in cement. Also, SEM images were utilized to study the morphology and size of microspheres. The SEM images of a PLGA microsphere are illustrated in Figure 3. As shown in Figure 3, the microspheres were completely spherical with a smooth surface without porosity. The average diameter of microspheres was 2-10 µm.

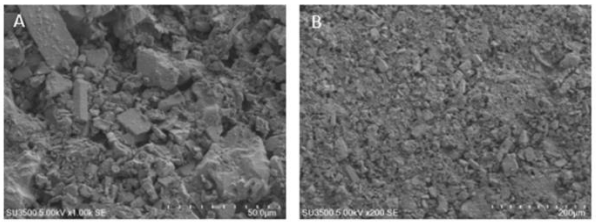


**Figure 2:** The SEM images of a cement sample at magnification of (A) 1.00 kx and (B) 200 x.

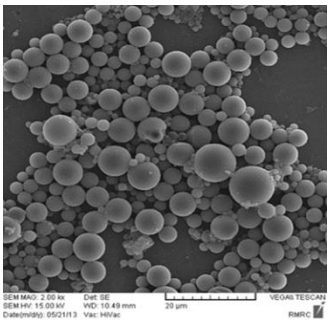

**Figure 3:** The SEM image of a PLGA microsphere at magnification of (A) 1.00 kx and (B) 3.00 k.

The SEM images of 30% and 50% microsphere/cement composite scaffold are illustrated in Figure 4. Most PLGA microspheres were tightly connected and covered with a layer of CPC. Additionally, Figure 4 shows that the amount of microspheres had an inverse relationship with the cohesion of the structure; in other words, by

increasing the percentage of microspheres, less coherence was observed in the structure. Figure 5 shows the SEM images of 30% and 50% microsphere/cement composite scaffold containing dexamethasone at variable magnifications. As shown in Figure 5, the presence of dexamethasone in the microspheres had little effect on the morphology of the composite. It should be noted that PLGA microspheres were almost uniformly distributed in the cement structure.

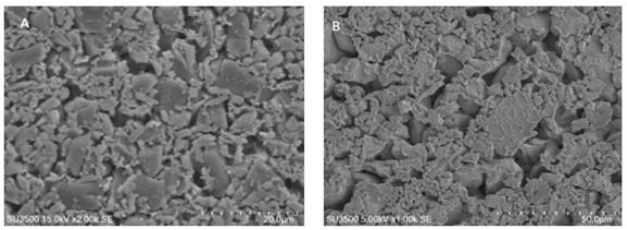

**Figure 4:** (A) The SEM image of 30% microsphere/cement composite scaffold at 2.00 kx magnification. (B) The SEM image of 50% microsphere/cement at 1.00 kx magnification.

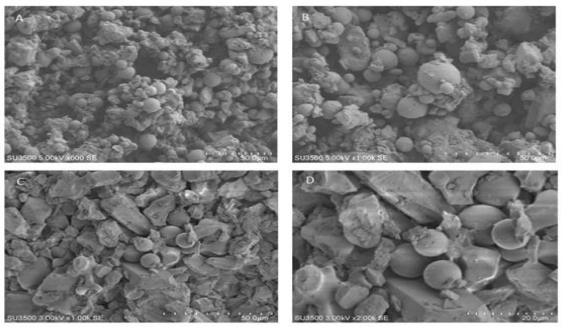

**Figure 5:** The SEM images of (A) 30% microsphere/cement composite scaffold containing dexamethasone at 600x magnification, (B) 30% microsphere/cement composite scaffold containing dexamethasone at 1.00 kx magnification, (C) 50% microsphere/cement composite scaffold containing dexamethasone at 1.00 kx magnification, and (D) 50% microsphere/cement composite scaffold containing dexamethasone at 2.00 kx magnification.

FTIR spectrum analysis

The indicative peaks on infrared spectroscopy are shown in Table 1. According to Table 1 and the FTIR spectra for the scaffolds, the spectra of cement, PLGA, dexamethasone, and their complexes were interpreted. Figure 6 indicates the FTIR spectrum diagram of pure cement and composite scaffolds. As shown in Figure 6, when the microsphere contained the drug, the peak of OH band in dexamethasone overlapped the OH band in CPC at 3540 $cm^{-1}$ and returned to its previous state. The presence of C=O bond in cement, PLGA, and dexamethasone was confirmed, and its peak was observed around 1652 $cm^{-1}$ in the cement sample; the peak was also visible in the composite scaffold (cement and microsphere) and was found to be sharper due to the overlap (at 1758 $cm^{-1}$); these changes were more clearly visible in the composite sample containing the drug, and the peak sharpness was seen at 1758 $cm^{-1}$.

Moreover, the very sharp peak at 237 $cm^{-1}$ of CH band, which indicated the presence of PLGA in the composite, changed when the microsphere contained the drug. Also, in the composite $PO_4^{3-}$, a band was observed around 1126, 1062, and 989 $cm^{-1}$, which indicates the presence of cement in the composition. In drug-containing composites, these peaks were displaced due to mixing and overlap. However, the test conditions (e.g., manufacturing method, duration, and speed of magnet rotation) might lead to imperceptible movements of the peaks.

**Table 1-** Indicative peaks in infrared spectroscopy ($cm^{-1}$)

| | OH | $CO_3^{2-}$ | $PO_4^{3-}$ | $HPO_4^{2-}$ | CH | CF |
|---|---|---|---|---|---|---|
| Cement | 3572 | 1479 | 1035<br>602<br>1100 | 870<br>910 | | |
| PLGA | | 1695<br>1753<br>1706 | | | 294<br>292<br>285 | |
| Dexamethasone | 3390 | 1662<br>1621 | | | | 1268 |

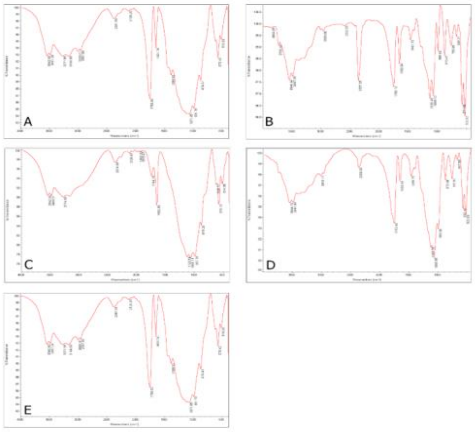

**Figure 6:** (A) The FTIR spectrum diagram of cement samples. (B) The FTIR spectrum diagram of 30% microsphere/cement. (C) The FTIR spectrum diagram of 50% microsphere/cement. (D) The FTIR spectrum diagram of 30% microsphere/cement containing dexamethasone. (E) The FTIR spectrum diagram of 50% microsphere/cement containing dexamethasone.

Dexamethasone release

To utilize microspheres and their composites for drug delivery purposes, their potential in releasing targeted drugs should be investigated. Figure 7 shows the absorption of dexamethasone in terms of wavelength. Dexamethasone had the highest absorption at 237 nm and was used in the following analysis. Also, to determine the relationship between absorbance and drug concentration, the standard curve of dexamethasone at 237 nm was plotted, and its behavior was formulated as Equation 1:

$$y = 406.022x + 0.02351$$

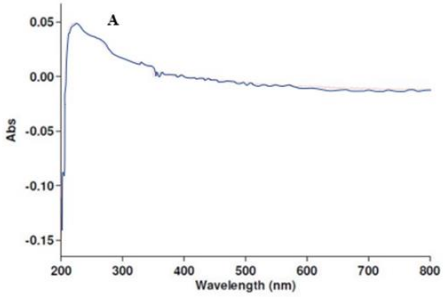


**Figure 7:** Absorption curve of dexamethasone at different wavelengths.

where $y$ represents the drug concentration, and $x$ denotes absorbance; by determining the amount of absorption, the drug concentration can be measured. According to the standard equation, the released drug from PLGA is shown in Figure 8 in terms of time (days). A significant amount of drug was released in the first 24 hours, and about 10% of dexamethasone was released. Also, by the ninth day, the trend of drug release was incremental, and at the end of the ninth day, approximately 25% of dexamethasone was released, which is due to the release of drug from the surface layer of microspheres. After the ninth day, the rate of release was reduced. It is predicted that over time, the slope of the chart would approach zero, which shows that PLGA can facilitate a controllable process for releasing the drug.

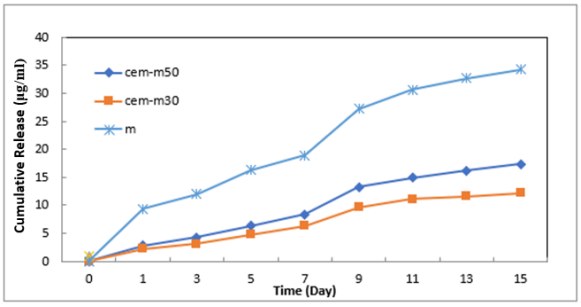


**Figure 8:** Cumulative release curve of dexamethasone from PLGA microspheres, 30% microsphere/cement composite, and 50% microsphere/cement composite at 237 nm.

The drug release of the cement/PLGA scaffold loaded with dexamethasone (30 wt%) is shown in Figure 8. The composite scaffold released about 2% of drug on the first day, which is a small amount compared to PLGA. Similar to PLGA, release of dexamethasone loaded in the composite scaffold showed an incremental trend by the ninth day. On the ninth day, almost 12% of dexamethasone release was observed, after which it became slower and more stable. In other words, loading of microspheres inside cement led to a reduction in the drug release rate. The cumulative release of drug-time curve for the composite scaffold (50 wt%) is shown in Figure 8. Similar to the 30 wt% scaffold, the 50 wt% scaffold released a small amount of drug compared to PLGA. However, it released a larger amount of the drug compared to the 30 wt% scaffold, although their trends were similar.

## Discussion

CPC, as one of the most important biomaterials, is widely used by researchers for bone tissue repair purposes. Besides the application of CPCs in tissue repair, they have been used as drug delivery systems. However, researchers face some limitations in using CPCs, including low degradation, which is because of their small pore size. [26] Consequently, they have continuously attempted to propose new methods which can overcome these limitations. In this paper, the potential of composite CPC/PLGA scaffold loaded with dexamethasone was evaluated for bone tissue engineering.

The SEM results showed that PLGA particles were spherical; in previous research, a similar geometric shape was observed for PLGA. [28, 29] Additionally, the effect of PLGA microspheres loaded with dexamethasone on the promotion of bone formation has been studied in previous research. A study [30] showed that dexamethasone-loaded alginate graphene oxide microspheres could be used as bone scaffolds. The results showed that the presence of dexamethasone increased cell proliferation with the induction of apatite formation. The effect of dexamethasone on promoting bone regeneration has been also reported by many researchers [31], which suggests the importance of designing drug-delivery systems for dexamethasone.

The results of the current study showed that since PLGA particles are inserted into CPCs, the pore size of CPCs is increased, which helps promote CPC degradation. Previous research confirms faster degradation of CPC-PLGA bone scaffold. In a study by Claire et al. [32], in vivo biological response of CPC-PLGA showed the significant degradation rate of CPC-PLGA bone scaffold. The results of FTIR study confirmed the presence of dexamethasone in the fabricated scaffold. Moreover, ultraviolet spectroscopy was used to investigate the drug delivery potential of the fabricated scaffold. The pure PLGA showed significant drug release on the first day, and after nine days, about 25% of dexamethasone was released. Previous studies have confirmed this trend, where a remarkable drug release was followed by a descending in the slope of drug release curve. [33]

In composite scaffolds with different concentrations of PLGA, since CPCs surround PLGA, the drug release rate is different from that of pure PLGA. Therefore, on the first days, about 2% of dexamethasone was released, and after nine days, it reached 12%. According to the results, different values of PLGA could lead to different drug release rates, which might help researchers use this drug

delivery system for patient-specific applications. Besides the double emulsion solvent evaporation method, there are other approaches that can generate microspheres loaded with a drug, including the microfluidic-based method. The outcomes of this method were similar to the results of our approach. In a study by Su et al. [34], PLGA microspheres loaded with bicalutamide (BCS class II) were generated with a microfluidic-based approach, and the drug release curve was similar to that of the present study. On the first days, the rate of drug release was incremental, while it decreased after several days; this finding is completely consistent with the current results. However, these two methods have several differences in terms of the microsphere size and uniformity, as the microfluidic-based approach can generate more uniform microspheres.

## Conclusion

In this study, CPCs loaded with PLGA microspheres containing dexamethasone were investigated. According to the morphological results of SEM images, the presence of dexamethasone had little effects on the morphology of the composite. To examine the presence of CPC, PLGA, and dexamethasone, FTIR analysis was performed, which indicated their presence in the composition. Considering the drug release rate, dexamethasone showed a stable release in the composite, which could be used for bone tissue engineering applications, although further in vitro studies are required. The results of the present study revealed that encapsulation of dexamethasone in PLGA microspheres could help release the loaded factors during a critical period. Overall, different weight percentages are associated with different release behaviors, creating patient-specific potentials for bone regeneration challenges.

## Conflict of Interest

No Conflict of Interest Declared ■

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