Published in final edited form as:

Mater Sci Eng C Mater Biol Appl. 2013 October ; 33(7): 3576–3582. doi:10.1016/j.msec.2013.04.001.

Tricalcium Phosphate and Tricalcium Phosphate/ Polycaprolactone Particulate Composite for Controlled Release of Protein

Sahar Vahabzadeh, Joe Edgington, and Susmita Bose*

W. M. Keck Biomedical Materials Research Laboratory, School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164, USA

Abstract

β -Tricalcium phosphate (β -TCP) with three different particle size ranges was used to study the effects of particle size and surface area on protein adsorption and release. Polycaprolactone (PCL) coating was applied on the particle systems to investigate its effect on particulate systems properties from both structural and application aspects. The maximum loading of 27 mg/g was achieved for 100 nm particles. Bovine serum albumin (BSA) loading amount was controlled by varying the BSA loading solution concentration, as well as the sample powder's surface area. Increasing the surface area of the delivery powder significantly increased loading and release yield. Unlike the samples with low surface area, the lowest particle size samples showed sigmoidal release profile. This indicated that release was governed by different mechanism for particles with different sizes. While the majority of samples showed no more than 50% release, the 550nm particles demonstrated 100% release. PCL-coating showed no significant ability to attenuate burst release in PBS. However, it led to a steadier release profile as compared to the bare TCP particles. FTIR analysis also proved that the secondary structure of BSA did not change significantly during the adsorption; however, minor denaturation was found during the release.

The same results were found when PCL coating was applied on the TCP particles. We envision potential use of TCP and TCP+PCL systems in bone growth factor or orthopedic drug delivery applications in future bone tissue engineering application.

Keywords

Tricalcium phosphate; Polycaprolactone coating; Particle surface area; Bovine serum albumin release; Denaturation

1. Introduction

Calcium phosphates (CaP's) including powder, cements, as well as composites with polymers have been attracted much attention for use in drug delivery systems (DDS)[1]. Calcium phosphate nanoparticles have been used to deliver a multitude of drugs and therapeutic agents, including chemotherapy drugs and proteins [2,3]. Some studies have revealed that nanoparticles can improve the resistance of therapeutic agents to enzymatic

© 2013 Elsevier B.V. All rights reserved.

*Corresponding author: sbosed@wsu.edu, Tel. 509-335-7461, Fax. 509-335-4662.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

attack, as well as transporting the agents in a controlled manner via minimally-invasive procedures [4,5]. The natural incidence of CaP's in the body is one of its major benefits when compared to synthetic drug delivery systems. CaP nanoparticles have other advantages over polymeric nanoparticulate delivery systems, including low susceptibility to immune response, low toxicity, and resistance to lipases and bile salts[6]. CaP ceramics have a variety of dissolution properties that arise as a consequence of their chemistry and crystallinity, which has provided them with much attention as drug delivery vehicles. CaPs are significant part of tissues such as bone and tooth enamel. It is usually in the form of amorphous calcium phosphate (ACP), as well as crystalline hydroxyapatite (HAp). Among the various calcium phosphate (CaPs) ceramics β -tricalcium phosphate [β -TCP, $\text{Ca}_3(\text{PO}_4)_2$] is most commonly used because of its osteogenic property, phase stability and the ability to form strong bonds with the host bone tissues. Furthermore, β -TCP has time-dependent mechanical and dissolution properties that make it an ideal material for DDS applications, in which TCP dissolves, and can subsequently release drugs in a controlled and sustained manner [7]. However, various studies have shown that the uncontrolled release of bovin serum albumin (BSA) during the initial time is a primary concern in using these compositions. This burst release behavior can be retarded by surface modification of nanoparticles[2].

Polycaprolactone (PCL) is a polymer which is widely used as scaffolds for bone tissue engineering applications due to its promising mechanical properties and biodegradability[8]. However, its low bioactivity, hydrophobicity and the possible low protein adsorption cause poor osseointegration. Higher bioactivity of PCL is achieved by integration of compounds such as CaP ceramics[9]. Several works have shown that β -TCP+PCL nanocomposites can be considered as suitable candidates for tissue engineering applications [9,10]. These composites have been also used as suitable delivery vehicles [11]. However, almost all reports are based on using the scaffolds.

This work presents application of TCP and TCP+PCL particulate systems as BSA delivery vehicles. Effects of particle size and surface area of TCP, initial BSA solution concentration, and surface modification of TCP using PCL coatings on the loading and release behavior of BSA were investigated. To the best of our knowledge, there is no report on investigation of above parameters in such systems. Moreover, BSA structural changes will be also studied. Our hypothesis is that the adsorption and release of any drug or protein, in this case the model protein BSA can be highly controlled by changing above parameters. In addition, controlled release as opposed to burst release of from nanoparticles is also expected.

2. Materials and methods

2.1. Raw materials and powders

High purity β -TCP powder was purchased from Berkeley Advanced Biomaterials Inc. (Berkeley, CA, USA) with two different average particle sizes of 100 ± 14 nm and 550 ± 29 nm. For the third set of samples, β -TCP with particle size of 1850 ± 163 nm was synthesized by heating a mixture of monetite (CaHPO_4 , DCPA) and calcium carbonate (CaCO_3 , CC) to 1050°C for 24 h, followed by quenching to room temperature in a desiccator [12].

Polycaprolactone pellets (PCL, MW 10,000, Aldrich, St. Louis, MO) were also used to form coating on the three different β -TCP powders. Acetone (J.T. Baker, Phillipsburg, NJ) and deionized water were also used during the synthesis process.

2.2. PCL coating

In order to apply PCL coatings, polycaprolactone pellets were dissolved in acetone at a concentration of 5% w/v. 5 g of each set of β -TCP samples with different particle size were

placed separately into the 25 ml of PCL solution for 5 min under constant stirring, and subsequently centrifuged to separate phases. 5g The powder was then washed twice with deionized water after the PCL had been allowed to set for 15 min at room temperature. Finally, the powder was dried for 48 h at room temperature.

2.3. Powder characterization

The morphology of the powders was studied using a field emission scanning electron microscope (FESEM, Hitachi s-570, Japan). X-ray diffraction (XRD) method with scan rate of $0.1^{\circ} \text{ sec}^{-1}$ was used for phase analysis of the β -TCP, by a Siemens D-500 Diffractometer system operating at 35.0 kV and 30.0 mA, which used a Cu K α radiation with a Ni filter. Fourier transform infrared (FTIR) spectra of samples were obtained using an ATR-FTIR spectrophotometer (Nicolet6700 FTIR, Madison, WI, USA) in the 400–4000 cm^{-1} wave number range.

The specific average surface area of the powders was determined using a Micromeritics Tristar 3000 surface area analyzer (Micromeritics, Norcross, GA), which uses the Brunauer, Emmett and Teller (BET) method for determining surface area.

2.4. Protein Loading on TCP and TCP/PCL samples

Bovine serum albumin (BSA, A7030, Sigma, St. Louis, MO) was used as a model protein in this study. 1g of TCP powders or TCP+PCL particulate composites were placed in 10 ml of the solutions with 3 different BSA concentrations of 1, 3, and 5 mg/ml in deionized water, and shaken for 12 h, found as equilibrium time for adsorption, in an orbital shaker set to 200 rpm at room temperature. TCP and TCP+PCL samples loaded by initial BSA concentration of $\times$ mg/ml are named as $\times$ bare and $\times$ comp, respectively. After loading, the powder was separated from the aqueous phase by centrifuging at 4000 rpm for 10 min, followed by drying at room temperature for 48 h. The amount of adsorbed BSA per unit mass of powders was measured using Micro BCA protein assay kit (Sigma, bicinchoninic acid protein assay kit, #23225). UV-Vis spectroscopy (Biotek) was used for the characterization of absorbance peaks at 562 nm to determine the BSA concentration through the use of a predetermined standard concentration-intensity calibration curve.

2.5. In vitro protein release

250 mg of BSA-loaded TCP and TCP+PCL particulate materials was added to 20 mL of phosphate buffer solution at pH7.2. The particles were dispersed by shaking them on an orbital shaker at a rate of 200 rpm at 37 °C. The released amount of BSA in the supernatant was measured by BCA protein assay after 1, 3, 6, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, and 216 h. At each data point, sample vials were centrifuged, and 0.4 ml of supernatant was removed. The amount of released BSA was measured using the same method for the measurement of the adsorbed BSA.

2.6. BSA secondary structure

BSA secondary structures were analyzed using the released protein to investigate the percentage of denaturation after adsorption and release on powders with particle size of 550 nm. Gaussian curve fitting using the Origin software was performed on the amide I band region of IR spectra. The areas of assigned bands and the fraction of the total area in the region were calculated, which then presented as the fraction of each subunit.

Surface area, protein loading, in vitro release and BSA secondary structure were analyzed in triplicates of samples. Measurements on each were also performed in three independent samples.

3. Results

3.1. Powder characterization

Figure 1 shows the XRD patterns of the uncoated TCP powders, both commercial and synthesized. They show the presence of only pure β -TCP (JCPDS No. 09-169) with no alpha phase (JCPDS No. 09-0348). The XRD peaks are sharp, which indicates that the β -TCP powders are highly crystalline.

FTIR spectra of the PCL and the powders are shown in Fig. 2. The intense peak at 1725 cm^{-1} is the ester band of PCL, which has been conserved in the TCP + PCL particles. This indicates that PCL-coating has been achieved. Fig. 3 shows the FTIR spectra of the BSA-loaded powders. There are two bands at ranges of $1600\text{--}1700\text{ cm}^{-1}$ and $1500\text{--}1600\text{ cm}^{-1}$. The first one is the amide I band attributed to C=O stretching, and the second one is the amide II band due to bending and stretching mode of N-H and C-N vibrations [11]. These peaks are present in the loaded samples indicating successful adsorption of BSA onto the delivery vehicles. However, the Amide II band cannot be resolved in the 100 nm particles, due to overlap with a broad carbonate peak.

Fig. 4 presents the specific average surface area of the bare and coated particulate samples. In uncoated samples, this value was 1.04, 2.74, and $22.54\text{ m}^2/\text{g}$ for the 1850, 550, and 100 nm particles, respectively. PCL coating did not change the surface area significantly and this parameter was 1.03, 2.49, and $22.40\text{ m}^2/\text{g}$ for the 1850, 550, and 100 nm TCP+PCL composites.

Fig 5a shows the FESEM surface morphology of the bare 550 nm powder, which indicates a surface that is smoother than the 100 nm particles shown in Fig 5b. The 1850 nm bare particles, shown in Fig 5c, appear to be much larger, and much smoother than the other two sample sets. The surface morphologies of the PCL-coated samples, figures 5d-f, appear almost unchanged when compared to the bare samples.

3.2. Protein loading

Fig 6 shows the amount of adsorbed BSA on different powder systems as a function of initial BSA concentration. There is a distinct increasing trend in the loading behavior as the concentration of the BSA loading solution is increased. In bare 100 nm particles, the increase in BSA initial concentration from 1mg/ml to 3 mg/ml results in significant higher adsorption, around 250%. However, as the loading solution concentration is increased from 3mg/ml to 5mg/ml, there was only a 6.4% increase in loading. In general, the PCL-coated samples exhibited lower adsorption at all loading concentrations. The bare 100 nm particles showed the highest amount of BSA adsorption of 27.6 mg/g , when loaded in the 5mg/ml BSA loading solution. In the same initial BSA concentration, loaded protein amounts on two other samples with the particle sizes of 550 nm and 1850 nm were 13.3 and 11.9 mg/g , respectively. However, the 1850 nm particles loaded in the 1mg/ml BSA loading solution had negligible protein adsorption. Comparing fig. 6 and table 1, although higher initial BSA concentration results in an increase of BSA adsorption, the yield percent (absorbed protein/initial amount of protein) decreased.

3.3. Protein release

The release profiles for the 550 nm particle systems, loaded at the various initial concentrations, are presented in Fig. 7. All samples exhibited a burst release during the initial 6 h time period, which was followed by a continuous BSA release until the conclusion of the experiment. The amount of BSA released during this burst release period ranged from 20% to 45% release. While the PCL-coating did not significantly attenuate the burst release

of BSA, it did provide for a much steadier release of BSA after the initial burst release (fig. 7b). In general, all the 550 nm samples showed a final release in the range of 42%-70% at the end of the 216 h study, except for the 1 bare sample which showed BSA release of 100%.

Figs 8a and b show the release profiles of BSA from the 100 nm particle systems. The 100 nm particles also exhibited an initial period of burst release, which occurred during the first hour of the release, and ranged from 1.5 to 6.5% release. Then, there was a gradual release until 50 h, followed by much more rapid release up to the 120 h, and finally the release plateau of 15-43% for different conditions was achieved. It can be concluded that for 100 nm particle systems, the 1 bare sample has the highest percentage release, while the 3 comp sample has the lowest. In particles with 100 nm size, PCL coating also provided slower BSA release comparing to the bare systems (fig. 8b)

The release profiles for the 1850 nm particle systems are presented in fig. 9. As no BSA was loaded on 1 bare and 1 comp samples, there was no release from these samples. The remaining compositions all exhibited a burst release during the first 1 hour, and showed a sustained release thereafter. The particulate composite loaded in the 3 mg/ml BSA loading solution showed the highest overall release, with a total of 53%. However, unlike the composite vehicles from the other particle sizes, this release was not steady. The 3 bare, 5 bare and 5 comp samples showed very low overall release, with a range of 10-17%, the majority of which was released during the initial burst release. In contrast to other particle size ranges of 100 and 550 nm, 1850 nm coated particles had higher BSA released rather than the bare system.

3. 4. Secondary structure of BSA

Table 1 shows the compositional analysis of pure BSA, BSA loaded on TCP and TCP+PCL systems, as well as the released BSA after 3 and 7 days. The band position of 1655 cm^{-1} is assigned to α - helix and the bands at 1625 and 1635 cm^{-1} are related to β - sheet structure [2,11]. During the release, the value for β - sheet increases from $34\pm 1\%$ for loaded BSA to $39\pm 1\%$ after 7 day release, for TCP samples. However, α - helix amount decreases from $38\pm 1\%$ to $24\pm 1\%$ by increasing the release time for the same particles, which shows slight denaturation of BSA during release. However, TCP- PCL particles shows less change in secondary structure of BSA.

4. Discussion

Surface area, initial protein concentration and surface modification of particles are the most important parameters which can affect the loading and release behavior of any particulate drug or protein delivery system. In this study, the influences of TCP powder surface area and BSA loading solution concentration were investigated on BSA loading and release. Initial burst release behavior of BSA from β -TCP particle has been reported in literature [13]. PCL coated BSA particle was investigated to study the effect of coating on BSA release kinetics. Initial burst release is one of the major concerns in using CaP ceramics. Changes in dissolution rate of these delivery systems, using the surface modification can retard it and change the release trend.

XRD analysis of the three TCP samples indicated that only β -phase peaks were present. This was the desired phase due to its lower dissolution rate as compared to α -TCP [14]. The FTIR spectra of PCL showed the sharp C=O peak at 1725 cm^{-1} [15], which was also present in the coated samples, indicating that the PCL coating onto TCP particle was successful. Comparing the FTIR spectra of all particulate systems before and after BSA loading, there was no extra peak in β -TCP and β -TCP+PCL groups due to chemical

interaction between particles and protein. This is the sign of physical adsorption of BSA on particulate systems [16].

Controlling the loading amount of protein onto a delivery vehicle is of primary concern, as the protein delivery amount must meet the prescription, as dictated by age, weight, and stage of disease [17]. The two approaches of changing the surface area and initial BSA concentration in solution showed significant effects on controlling the loading and release. Increasing the surface area of powders by decrease in particle size not only resulted in higher BSA adsorption, but also enhanced the adsorption yield. These results are in accordance with those of Santos et al. who showed that nanotopography of CaP plays significant role on protein adsorption [18]. The second approach was changing the initial BSA concentration. Generally, increasing this parameter led to higher protein adsorption. The only exception in current work was 100 nm PCL coated particles which showed less BSA adsorption with increase the protein concentration from 3 mg/ml to 5 mg/ml. Our hypothesis is occurrence of equilibrium in adsorption and desorption of protein. Usually, higher initial concentration of protein/drug results in higher adsorption. On the other hand, regarding the mixing time, high surface area and high concentration of BSA, desorption of BSA could have occurred which gives less adsorption. Furthermore, as shown in table 1 the yield percentage of loading decreased at higher concentration. This shows that determining the appropriate BSA initial concentration in a defined particulate system is really important to achieve the desired efficiency [19]. Moreover, this trend might be associated to a non-monolayer formation of BSA on β -TCP particles. Variety of possible interactions between the protein and the particles is due to its specific molecular shape and surface charge and heterogeneity. The discontinuous trend in protein adsorption with regard to changes in initial BSA concentration observed in present work may be related to the dependence of adsorbed protein orientation at interface on the initial concentration, as a result of non-spherical shape of BSA [20]. Comparing the bare particles with the coated ones, it is also worth noting that, while the PCL-coating used to control the release, this coating led to a slight decrease in the surface area. In addition, BSA adsorption is lower in almost all coated samples compared to the bare particles. It may be attributed to lower surface area and changes in surface chemistry. The isoelectric point of BSA is 4.7, thus at the working pH of 7.2 in present work the interaction of BSA with nanoparticles is via its COO^- groups. As shown in fig. 10, COO^- binds to Ca^{2+} ions of CaP nanoparticles through electrostatic interactions [2]. However, due to hydrophobic nature of PCL, its interaction with BSA is not strong [21] and might be via the protein hydrophobic residues [22].

In addition to have reasonable efficiency of protein adsorption, it is necessary for particulate drug delivery vehicles to have the desired release kinetics. In an ideal situation, the protein should be released in a continuous fashion during the bone-growth and recovery process. The primary forces acting in the adsorption process of BSA onto the pure TCP and particulate composites are the Van der Waals and electrostatic interactions [23]. These bonding forces are relatively weak, so the protein release is rather sudden, and occurs within the initial hours of release. However, the general release profile is different for samples with different particle size. At low particle size of 100 nm, the sigmoidal release trend with initial burst release of 2-7% and final release of 10-40% was obtained for different conditions. This type of release behavior, known as “pulsatile” release system, is a characteristic of energy dependent release process. Same release patterns are reported in different types of drugs and biomolecule delivery systems [24,25]. However, for larger particles of 550 nm, the burst release time increased from 1 h to 6 h, followed by continuous protein release up to 30-100% release. For larger particles of 1850 nm, the burst release time was almost the same as 100 nm particles, and little release of 2-10% occurred after that. The burst release is due to the weak electrostatic attraction between protein molecules and nanoparticle surface. As the layer number of adsorbed protein increased, this interaction becomes weaker and the

initial release occurs due to the loosely bound distant protein layers release [2]. Although the release mechanisms need to be further understood, the current results show that the mechanisms are highly dependent on the particle size/ surface area, regardless of the adsorbed protein amount [24]. In addition, PCL-coating had no significant effect on the burst release in any of the used TCP materials. However, after the initial burst release, the release of BSA from the larger powder of 550 nm and 1850 nm was much steadier in the PCL-coated materials, as compared to the bare TCP (figures 7&9).

In protein or growth factor delivery applications, denaturation of protein structure is another primary concern. In case of BSA, amide I and amide II bands are generally used in the study of its structure [26,27]. The adsorption of BSA onto the powders was confirmed by the presence of C=O stretching mode of amide I band at 1625 cm^{-1} , and the N-H bending mode of amide II band at 1500 cm^{-1} . Moreover, 14% decrease in α -helix content indicates minor distortion in BSA structure during the release. This distortion appears due to the unidirectional electrostatic interaction between Ca^{2+} and COO^- [28].

5. Conclusions

In order to achieve the delivery of protein in a sustained and controlled manner, β -TCP was used in various particle sizes, and subsequently coated with PCL. BSA was used as a model protein, and loaded onto the TCP and TCP+PCL particulate composites via surface adsorption. BSA loading efficiency was controlled by varying its initial concentration, as well as the powder surface area. Increasing the surface area of the delivery powder significantly resulted in higher protein adsorption and sigmoidal release profile. This indicates that the release mechanism depends on particle surface area. PCL-coating showed no significant ability to retard burst release in PBS. However, PCL-coating led to a steadier release profile as compared to the bare TCP particles. This study demonstrated that TCP +PCL particulate composites have the potential to be used in bone-tissue engineering applications, and should be studied further in order to optimize loading and release.

Acknowledgments

Authors acknowledge financial support from the National Institute of Health (NIH-RO1-EB-007351).

References

1. Verron E, Khairoun I, Guicheux J, Bouler J-M. Calcium phosphate biomaterials as bone drug delivery systems: a review. *Drug Discov Today*. 2010; 15:547–552. [PubMed: 20546919]
2. Tarafder S, Banerjee S, Bandyopadhyay A, Bose S. Electrically polarized biphasic calcium phosphates: adsorption and release of bovine serum albumin. *Langmuir*. 2010; 26:16625–16629. [PubMed: 20939493]
3. Morgan TT, Muddana HS, E I, Altino lu S, Rouse M, Tabakovi A, Tabouillot T, Russin TJ, Shanmugavelandy SS, Butler PJ, Eklund PC, Yun JK, Kester M, Adair JH. Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. *Nano Lett*. 2008; 8:4108–4115. [PubMed: 19367837]
4. Tabakovic A, Kester M, Adair JH. Calcium phosphate-based composite nanoparticles in bioimaging and therapeutic delivery applications. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2011; 4:96–112. [PubMed: 21965173]
5. Krebs MD, Salter E, Chen E, Sutter KA, Alsberg E. Calcium phosphate-DNA nanoparticle gene delivery from alginate hydrogels induces in vivo osteogenesis. *J Biomed Mater Res Part A*. 2010; 92A:1131–1138.
6. Dasgupta S, Banerjee SS, Bandyopadhyay A, Bose S. Zn- and Mg-doped hydroxyapatite nanoparticles for controlled release of protein. *Langmuir*. 2010; 26:4958–4964. [PubMed: 20131882]

7. Metsger DS, Rieger MR, Foreman DW. Mechanical properties of sintered hydroxyapatite and tricalcium phosphate ceramic. *J Mater Sci - Mater Med*. 1999; 10:9–17. [PubMed: 15347989]
8. Kim S-J, Kim M-R, Oh J-S, Han I, Shin S-W. Effects of polycaprolactone-tricalcium phosphate, recombinant human bone morphogenetic protein-2 and dog mesenchymal stem cells on bone formation: pilot study in dogs. *Yonsei med j*. 2009; 50:825–831. [PubMed: 20046425]
9. Zhou Y, Hutmacher DW, Varawan S-L, Lim TM. In vitro bone engineering based on polycaprolactone and polycaprolactone tricalcium phosphate composites. *Poly Int*. 2007; 56:333–342.
10. Erisken C, Kalyon DM, Wang H. Functionally graded electrospun polycaprolactone and β -tricalcium phosphate nanocomposites for tissue engineering applications. *Biomaterials*. 2008; 29:4065–4073. [PubMed: 18649939]
11. Xue W, Bandyopadhyay A, Bose S. Polycaprolactone coated porous tricalcium phosphate scaffolds for controlled release of protein for tissue engineering. *J Biomed Mater Res Part B*. 2009; 91B:831–838.
12. Gbureck U, Grolms O, Barralet JE, Grover LM, Thull R. Mechanical activation and cement formation of [beta]-tricalcium phosphate. *Biomaterials*. 2003; 24:4123–4131. [PubMed: 12853242]
13. Ziegler J, Mayr-Wohlfart U, Kessler S, Breitig D, Günther K-P. Adsorption and release properties of growth factors from biodegradable implants. *J Biomed Mater Res*. 2002; 59:422–428. [PubMed: 11774299]
14. Radin SR, Ducheyne P. The effect of calcium phosphate ceramic composition and structure on in vitro behavior. II. Precipitation. *J Biomed Mater Res*. 1993; 27:35–45. [PubMed: 8380597]
15. Avella M, Errico ME, Laurienzo P, Martuscelli E, Raimo M, Rimedio R. Preparation and characterisation of compatibilised polycaprolactone/starch composites. *Polymer*. 2000; 41:3875–3881.
16. Vasilescu C, Drob P, Vasilescu E, Demetrescu I, Ionita D, Prodana M, Drob SI. Characterisation and corrosion resistance of the electrodeposited hydroxyapatite and bovine serum albumin/hydroxyapatite films on Ti–6Al–4V–1Zr alloy surface. *Corros Sci*. 2011; 53:992–999.
17. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J R Soc Interface*. 2011; 8:153–170. [PubMed: 20719768]
18. dos Santos EA, Farina M, Soares GA, Anselme K. Chemical and topographical influence of hydroxyapatite and β -tricalcium phosphate surfaces on human osteoblastic cell behavior. *J Biomed Mater Res Part A*. 2008; 89A:510–520.
19. Yu X, Qu H, Knecht DA, Wei M. Incorporation of bovine serum albumin into biomimetic coatings on titanium with high loading efficacy and its release behavior. *J J Mater Sci - Mater Med*. 2009; 20:287–294.
20. Mavropoulos E, Costa AM, Costa LT, Achete CA, Mello A, Granjeiro JM, Rossi AM. Adsorption and bioactivity studies of albumin onto hydroxyapatite surface. *Colloids and Surfaces B: Biointerfaces*. 2011; 83:1–9.
21. Yang Y-Y, Chung T-S, Ng NP. Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. *Biomaterials*. 2001; 22:231–241. [PubMed: 11197498]
22. Li X, Chen M, Yang W, Zhou Z, Liu L, Zhang Q. Interaction of bovine serum albumin with self-assembled nanoparticles of 6-O-cholesterol modified chitosan. *Colloids Surf B*. 2012; 92:136–141.
23. Hartmann M. Ordered mesoporous materials for bioadsorption and biocatalysis. *Chem Mater*. 2005; 17:4577–4593.
24. Mendez J, Monteagudo A, Griebenow K. Stimulus-responsive controlled release system by covalent immobilization of an enzyme into mesoporous silica nanoparticles. *Bioconjug Chem*. 2012; 23:698–704. [PubMed: 22375899]
25. Singh, Anamika; Dubey, Harikesh; Shukla, Indu; Dharmchand P, Singh. Pulsatile Drug Delivery System: an Approach of Medication according to Circadian Rhythm. *Journal of Applied Pharmaceutical Science*. 2012; 02:166–176.

26. Carrasquillo KG, et al. Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly(lactide-co-glycolide) microspheres results in release of native protein. *J Controlled Release*. 2001; 76:199–208.
27. Wang F, Yang Z, Zhou Y, Weng S, Zhang L, Wu J. Influence of metal ions on phosphatidylcholine-bovine serum albumin model membrane, an FTIR study. *J Mol Struct*. 2006; 794:1–11.
28. Dasgupta S, Bandyopadhyay A, Bose S. Reverse micelle-mediated synthesis of calcium phosphate nanocarriers for controlled release of bovine serum albumin. *Acta Biomater*. 2009; 5:3112–3121. [PubMed: 19435617]

Highlights

- BSA adsorption and release values are highly dependent on particle size and initial protein concentration.
- BSA release mechanism is governed by particle size.
- PCL coating cannot retard burst release effect.
- Insignificant protein denaturation occurs using TCP/ TCP+PCL systems.

Figure 1.
XRD patterns of powders with different size of: a) 100, b) 550, and c) 1850 nm.

Figure 2.

FT-IR spectra of: a) TCP 100 nm, b) TCP+PCL 100 nm, c) TCP 550 nm, d) TCP+PCL 550 nm, e) TCP 1850 nm, f) TCP+PCL 1850 nm, and g) PCL samples.

Figure 3. FT-IR spectra of BSA-loaded samples of: a) TCP 100 nm, b) TCP+PCL 100 nm, c) TCP 550 nm, d) TCP+PCL 550 nm, e) TCP 1850 nm, f) TCP+PCL 1850 nm, and g) BSA.

Figure 4.
Surface area values of TCP and TCP+PCL samples(error bars show the standard deviation).

Figure 5.

SEM morphologies of bare powders and particulate composites at 20,000X magnification (a) 550nm TCP, (b) 100nm TCP, (c) 1850nm TCP, (d) 550nm PCL+TCP, (e) 100nm PCL+TCP, (f) 1850nm PCL+TCP.

Figure 6. Loaded BSA as a function of initial loading concentration (error bars show the standard deviation).

Figure 7.
BSA release profiles from 550nm particles, a) bare and b) PCL coated (error bars show the standard deviation).

Figure 8. BSA release profiles from 100nm particles, a) bare and b) PCL coated (error bars show the standard deviation).

Figure 9. BSA release profiles from 1850nm particles, a) bare and b) PCL coated (error bars show the standard deviation).

Fig. 10.
Schematic presentation of BSA adsorption on a) TCP and b) PCL coated samples.

Table 1

Yield % of different particulate systems.

Particulate systems	Bare 100 nm		PCL coated 100 nm		Bare 550 nm		PCL coated 550 nm		Bare 1850 nm		PCL coated 1850 nm	
	1	3	5	1	3	5	1	3	5	1	3	5
Initial BSA concentration (mg/ml)												
Yield %	77.8	86.0	55.2	89.3	77.1	39.9	52.6	38.16	26.58	-	49.4	23.78
								33.8	22.76	-	30.4	23.52

Table 2

Secondary structure of pure, loaded, and released BSA.

		α - helix ($\pm 1\%$)	β - sheet ($\pm 1\%$)
TCP system	Pure BSA	41%	31%
	Loaded BSA	38 %	34%
	Released after 3 days	30%	37%
	Released after 7 days	24%	39%
TCP/PCL system	Loaded BSA	39%	33%
	Released after 3 days	36%	34%
	Released after 7 days	32%	37%