ChemPubSoc Europe

DOI: 10.1002/slct.201601954

Chemistry SELECT Full Papers

Materials Science inc. Nanomaterials & Polymers

Quickly Manufactured, Drug Eluting, Calcium Phosphate Composite Coating

Midhun Ben Thomas[+],[a] Noah Metoki[+],[a] Ori Geuli,[b] Orna Sharabani-Yosef,[c] Tal Zada,[b] Meital Reches,[b] Daniel Mandler,*[b] and Noam Eliaz*[a]

Calcium phosphate (CaP) ceramics have been prevalently used as coatings for implants because of their excellent osteoconductive and bioactive properties. Yet, bone regeneration procedures might have complications such as bacterial infection, local inflammation, bone destruction, and impaired bone healing. Here, we present a novel in situ electrodeposition of CaP with chitosan nanoparticles containing antibiotics. The deposition was shown to be fast and efficient. The deposited layer of octacalcium phosphate (OCP) and monotite contained a large amount of gentamicin, which was released gradually over a period of 15 days. These phases may be beneficial for bone growth, as OCP has higher solubility than the stoichiometric hydroxyapatite (HAp) and is commonly considered as a precursor to HAp, while monotite has even faster resorbability. In addition, both the cytotoxicity and biomineralization of the coating were studied, and the coating was proven to be non-cytotoxic and highly biomimetic.

Introduction

Coating of bone implants by calcium phosphate (CaP) ceramic has many advantages.[1–3] Yet, these coatings do not necessarily provide a solution to complications such as bacterial infection, local inflammation, bone destruction, and impaired bone healing.[4,5] Therefore, introducing a drug into the coating is an appealing option. The common method for coating with CaP today is by plasma spraying.[6–8] The intense heat of this coating process does not permit the introduction of a drug in the course of deposition.[9]

During the past two decades, numerous studies demonstrated the incorporation of several antibiotics in various CaP coating approaches.[10–14] For example, Luginbuehl *et al.*[12] added tetracycline antibiotics into different polymer solutions and sprayed them onto tricalcium phosphate (TCP) coated surfaces. They showed cumulative release of antibiotics over an extended period (up to 70 days). Radin *et al.*[14] loaded CaP coatings with Vancomycin by immersion. This loading showed effective release and inhibition for the first 24 hours. Baro *et al.*[13] mixed gentamicin with poly(lactic acid) (PLA) and CaP paste to form a CaP powder containing antibiotics. They pressed the powder onto implants surfaces, which resulted in long release durations (up to 12 weeks). Stigter *et al.*[10] incorporated a variety of antibiotics into biomimetically prepared carbonated hydroxyapatite (HAp) coating using an immersion technique. They showed that some antibiotics were better incorporated, depending on their chemical structure based on release studies. Moreover, they showed that the release rate differed between the antibiotics, reaching only one-day release for gentamicin.

Electrodeposition (ED) is an alternative technique.[3,7,8,15–21] It allows forming the coating at lower temperature, higher degree of crystallinity, and control over the deposited phases.[3,16] A few publications have shown the combination of electrodeposited CaP coating with antibacterial agents. For example, Bir *et al.*[22] and Huang *et al.*[23] electrodeposited HAp doped with antibacterial ions. The coating demonstrated antimicrobial activity, with no adverse effect on osteoblast cytotoxicity. Karthika *et al.*[24] developed a HAp/gelatin nanocomposite coating on titanium substrate. They showed that the composite was antimicrobial. Moreover, cell proliferation assay demonstrated the viability of fibroblast stem cells on the coating. Fu *et al.*[25] demonstrated a dual step deposition, whereby they first deposited HAp, and then streptomycin on top of the coated layer. It was shown that the loading was more substantial than physical adsorption of the drug. Moreover, they tested for cumulative release, and found that 80% of the drug was released during the first 24 hours.

ED preformed at low temperatures and sufficiently low currents may also provide a possibility for in situ deposition, which is not a viable possibility during plasma coating prevalent today. Recently,[26] we have presented an in situ method of CaP ED with gentamicin-loaded chitosan nano-

[a] *Dr. M. B. Thomas,[+] N. Metoki,[+] Prof. N. Eliaz*
Biomaterials and Corrosion Lab, Department of Materials Science and Engineering
Tel-Aviv University
Ramat Aviv 6997801, Israel
E-mail: neliaz@tauex.tau.ac.il
[b] *O. Geuli, T. Zada, Dr. M. Reches, Prof. D. Mandler*
Institute of Chemistry
The Hebrew University of Jerusalem
Jerusalem 9190401, Israel
E-mail: daniel.madler@mail.huji.ac.il
[c] *Dr. O. Sharabani-Yosef*
Department of Biomedical Engineering, Faculty of Engineering
Tel Aviv University
Ramat Aviv 6997801, Israel
[+] *These authors contributed equally to this work*

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/slct.201601954

particles (NPs) at low potential (−1.4 V *vs.* SCE) and different temperatures, which yielded drug load of 12−42 wt.%. This coating eluded gentamicin for two days. While this has been an important step towards a good drug eluting coating, the antibiotic release was too short. A local inhibition release profile should exhibit a high initial burst in order to respond to the elevated risk of infection post-surgery, yet, it must also follow a sustained release for inhibiting the occurrence of latent infection.[27] Moreover, the amount of antibiotic released was 40% of the drug loaded, and the deposition time was relatively long (2 h). This prolonged deposition time does not suit the industry, and no mechanism was discussed for the partial release. Another study reported[28] in situ deposition of CaP with antibiotics, whereby deposited CaP loaded with gentamicin was formed electrochemically by applying a current density of 20 mA/cm^2 for 240 s. Further studies of this coating showed antibacterial efficacy on different streptococcal strains, yet, no release studies were performed.

Here, we present a substantial improvement of an electrochemically deposited CaP/gentamicin coating on Ti alloy. The coating, made of CaP containing gentamicin-loaded chitosan NPs, was formed galvanostatically at low currents (0.6 mA/cm^2) and within a shorter time (30 min). Subsequently, the antibiotic was released over 15 days. Finally, both the cytotoxicity and biomineralization of the coating were studied

Results and Discussion

The Advantage of Galvanostatic Co–deposition

The co-electrodeposition of CaP and chitosan-loaded gentamicin NPs has previously been examined.[26] In short, the substrate is immersed in an ionic solution of calcium and phosphate ions along with gentamicin/chitosan NPs. While applying the current to the substrate a reduction reaction of water near the surface of the substrate occurs, leading to a local elevation of pH. This pH elevation leads to the deprotonation of the phosphoric acid in solution which is then free to precipitate with the calcium ions, as was previously explained by Eliaz *et al.*.[3,7,8,15–21] The chemical reactions that may occur after deprotonation include the precipitation of various CaP phases, such as that of OCP, as described in equation 1:

$$4\ (Ca^{2+}) + 3\ (HPO_4{}^{2-}) + 2.5 \cdot H_2O \rightarrow OCP + 2H^+ \qquad (1)$$

Moreover, the changes in pH also lead to the precipitation of the gentamicin/chitosan NPs. It was previously noted by Thomas *et al.* that the NPs have a diameter of 600 nm in pH < 5, and their zeta potential varies from 40 mV at pH < 5 to 10 mV at pH > 5.[26] ED takes advantage of the ability to elevate the pH on the implant surface by reducing water, which causes the irreversible deposition of the CaP/chitosan/gentamicin composite. The amount of gentamicin in the NPs was 69 ± 3 wt. %. ED was carried out under potentiostatic conditions, i.e. under constant potential.[26] The pH on the surface depends on the rate of water reduction. Therefore, to fix the pH on the surface and thus, better control deposition, galvanostatic conditions, i.e. constant current, are favored.

The Microstructure, Chemical Composition and Thickness of the Coating

Figure 1 A shows a SEM image of CaP/chitosan/gentamicin coating carried out at 0.6 mA/cm^2. The coating is fairly uniform,

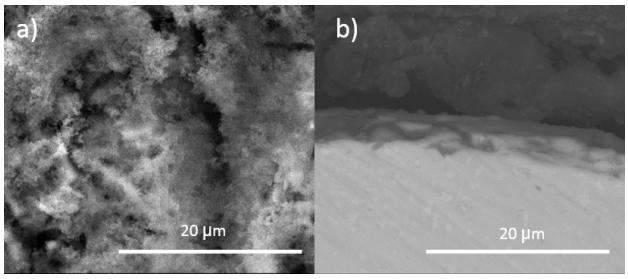

Figure 1. ESEM image of the in situ electrodeposited CaP coating. A) Coating surface morphology, B) Metallographic cross-section. The coating was prepared by applying 0.6 mA/cm^2 for 30 minutes.

and is characterized by granular morphology along with the appearance of some cracks. Similar cracking was previously reported to form during the chemical pretreatment of the Ti alloy in NaOH.[8] The surface morphology is significantly different from that of CaP electrodeposits reported by Eliaz *et al.* at different temperatures.[3,7,8,16–20] This can be attributed to the significant influence of many parameters, such as bath composition, pH, applied potential (or current density), and temperature (which were all altered here) on crystal growth reactions and electrodeposit properties. Energy-dispersive X-ray spectroscopy (EDS) data shows that the Ca/P ratio is 0.98 ± 0.1, indicating a low calcium content phase. Yet, it should be noted that EDS analysis is often unreliable for analysis of CaP coatings,[3,15,16] in contrast to advanced X-ray photoelectron spectroscopy (XPS) and XRD analyses. EDS also reveals the presence of sulfur and carbon in the coating. This indicates a successful encapsulation of the NPs, which are composed of chitosan, gentamicin, and dextran sulfate. The thickness of the coating, shown in Figure 1B, was measured in the SEM on a metallographic cross-section, and was ca. 2.4 ± 1.0 µm.

Coating Phase Identification

Further XPS and XRD analyses were conducted as a means of determining its phase content. XPS analysis (not shown here) showed a Ca/P ratio of 1.11. Yet, the phase content could not be determined unambiguously due to an abundance of oxygen in the coating whose origin is chitosan and gentamicin. The XRD pattern was thus examined for the CaP phases, as shown in Figure 2. The reflections were assigned to monotite and OCP phases, as well as to the titanium substrate (reflections confirmed with JCPDS 04–003-557 for titanium, 13–0391 for OCP, and 09–0080 for monotite). This is in agreement with EDS

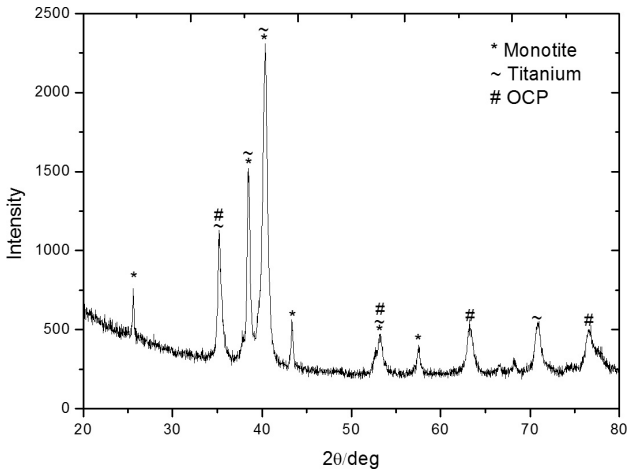

Figure 2. XRD pattern showing dual-phase OCP and monotite coating formed on Ti-6Al-4 V substrate.

and XPS results, and confirms that the coating is composed of two CaP phases that are depleted in calcium compared to the stoichiometric HAp. While HAp is the phase of CaP that is closest to the inorganic bone composition, the formation of monotite and OCP is well fitted with our goal- the release of antibiotics into the body and initiation and bone building adjacent to the surface. Compared to HAp which has very low solubility (-log K_{SP} = 116.8), OCP and especially monotite are much more soluble (-log K_{SP} = 6.90 for monotite and -log K_{SP} = 96.6 for OCP).[1] The high solubility of monotite will allow a faster dissolution of the coating, better releasing the encapsulated drug. Moreover, OCP is known as precursor phase for HAp, having a similar crystallographic structure.[29] OCP also exhibits desirable properties, such as osteoconductivity and osteoinductivity that support bone regeneration, and is approved by the US FDA for clinical use.[30]

The Amount of Gentamicin in the Coating

To determine the amount of gentamicin in the coating, it was dissolved in HCl, and the amount of gentamicin in the solution was measured. It was found that the solution contained 219 μg/mL, which translates into 82 μg/cm^2 on the implant surface. Furthermore, measuring the weight of the coating revealed that 44 wt.% of it was gentamicin. The excess of gentamicin on the surface as well as its weight percent are higher in comparison with different loading systems reported in literature.[31–36] For example, Simon *et al.*[33] showed a mixture of CaP and gentamicin powders, creating a cement that contained 2.5 wt.%. Gbureck *et al.*[34] showed a printed CaP with biodegradable polymer dipped in gentamicin solution and vacuumed. The coating contained 5.7-71 μg/cm^2 of gentamicin. All surveyed literature except for two studies reported low loading values of antibiotics[37,38] Both Taha *et al.*[38] and Rajesh *et al.*[37] first coated titanium substrate with HAp, and then dipped the coated substrate in gentamicin solution, to achieve up to 800 μg/cm^2 of the drug in the coating. Herein, for the first time, an in situ preparation of CaP coating containing gentamicin is presented with high amount of drug content.

Gentamicin Release Profile from the Coating

Cumulative release of gentamicin from the coated specimen was tested for 45 days, and is presented in Figure 3. Gentamicin

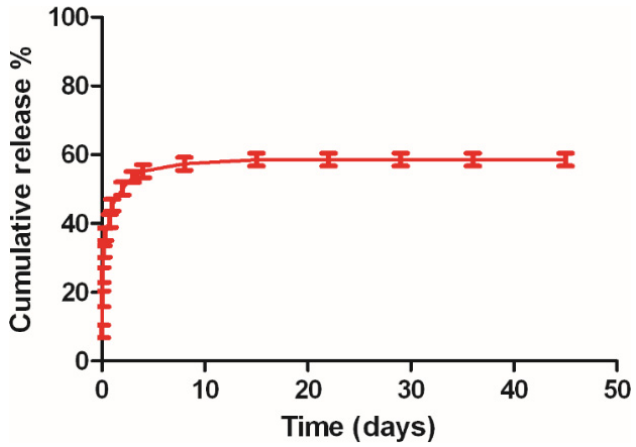

Figure 3. Gentamicin cumulative release profile over 45 days from an electrochemically deposited coating of CaP with NPs pre-loaded with gentamicin.

was released in an exponential manner, which means that the rate decreases with time. Such release profile is typical of diffusion-controlled systems.[39] A large burst was observed during the first day of release, followed by a slower release over the next fourteen days. A total of 59% of the encapsulated antibiotic was released during that time. The initial release of gentamicin from the coating was relatively fast, mainly due to its extremely hydrophilic nature. The rate of release decreases with time since the drug has a progressively longer diffusion path. The drug stopped eluting into solution after 15 days. During the entire experiment, the amount released was above the minimal inhibitory concentration (MIC) of the drug for *S. aureus* and *Pseudomonas aeruginosa*, as reported before.[27,39] This prolonged release is significantly different from that measured by Thomas *et al.*,[26] which could be attributed to the difference in solubility of the CaP phases at near-physiological conditions.[1]

The abrupt stop in drug release after fifteen days is surprising, because the antibiotic is highly soluble. This can be explained by one of the following: 1) There is a physical diffusion barrier inside the coating; 2) The residual drug is chemically bound, and cannot be released. To investigate the underlying reason for the residual gentamicin in the coating, it is essential to have a clear idea as to how the gentamicin-loaded chitosan NPs are chemically structured. The synthesis of the NPs involves first mixing the gentamicin with dextran sulfate. Hence, the positively charged amino group of gentamicin is likely to associate with the negatively charged sulfate (SO_4^{2-}) group of dextran sulfate, as shown before,[40] creating a

gentamicin/dextran sulfate complex that is negatively charged. The next step comprised the addition of chitosan, which is positively charged, and binds to the excess of sulfate groups, forming particles that are positively charged. Finally, the addition of pentasodium tripolyphosphate hexahydrate (TPP), according to a previous report,[40] determines the final particle size and stability; however, its addition maintains the positively charge of the NPs. This suggests that the gentamicin is probably embedded deep inside these NPs. Indeed, we found that no further release of gentamicin from the samples used in the above experiments, namely, after releasing 60% of the drug, was detected after placing the samples in phosphate-buffered saline (PBS, pH = 9) for one week. As the pH > pK_B of chitosan, we speculate that the rest of the gentamicin was physically bound inside the particles, and could not be released upon deprotonation of the chitosan.[41] Following that, the dissolution of the remaining coating in HCl was carried out, and tested for gentamicin. The results showed that gentamicin was released from the remaining coating. These tests suggest that some of the drug remains physically encapsulated after 15 days of release. This encapsulation is beneficial at in vivo conditions for a possible secondary drug release. In the human body, the cells have enzymes such as chitosananse that disintegrate the chitosan and may reveal the drug.[42]

Cytotoxicity

In vitro cytotoxicity tests were performed using hFOB osteoprogenitor human cells in order to demonstrate that the antibiotic incorporation does not show cytotoxic attributes. Figure 4a

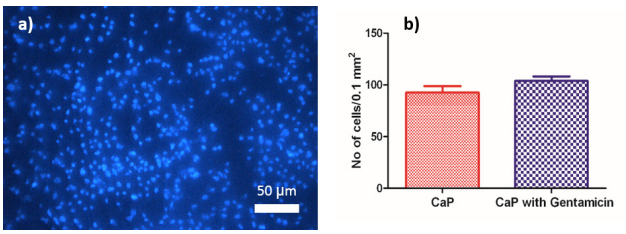

Figure 4. a) Fluorescence microscope image of DAPI-stained nucleus of osteoprogenitor human cells on the co-deposited CaP surface. b) Cell count histograms presenting mean and standard deviation (n = 3).

shows a fluorescence microscope image that illustrates the abundance of cells on the gentamicin co-deposited samples (n = 3). The cells are homogeneously distributed on the sample. Figure 4b shows the cell count histogram, presenting mean and standard deviation on two different substrates: CaP co-deposited with gentamicin NPs and electrodeposited CaP under similar conditions. It can be seen that the two histograms are almost similar. Two tailed t-test showed no significant difference between the two groups (P = 0.199), meaning that the amount of cells growing on both substrates was similar. This indicates that the antibiotic encapsulated in the coating was not cytotoxic to the cells.

In–Vitro Biomineralization

Figure 5 shows the in situ coated sample after biomineralization assay (n = 3). The samples were immersed in concentrated

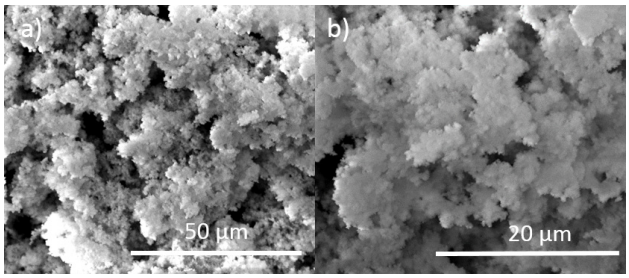

Figure 5. ESEM images (at different magnifications) of the in situ coated sample after biomineralization assay. The biomineralization was tested in SBF X5 for seven days (n = 3).

simulated body fluid (SBF) solution for seven days. Clearly, the coating shows high bioactivity. The biomimetically deposited layer was uniform, with heterogeneous distribution of granular crystal agglomerations on the surface. The surface morphology is vastly different from that seen in Figure 1, indicating a new growth of CaP layer. EDS analysis showed high signals of calcium and phosphate, indicating a thick coating. In addition, the biomimetic coating contained chloride and sulfur on the surface, confirming that dextran sulfate is still encapsulated in the coating.

Bacterial inhibition assay

Samples were tested for antibacterial activity against *Pseudomonas aeruginosa*, which is the most frequent gram-negative etiologic agent associated with infections of foreign body implants, and is susceptible to gentamicin treatment.[43] The samples were placed in PBS buffer for 5 days and left to elute antibiotics. The resulting solution was then tested against the bacteria. Additionally, as a positive control, drops of pure gentamicin (200 µg/mL) were placed in the same manner on the agar plates. A negative control was also examined in the form of pure PBS solution, in order to demonstrate that the buffer does not have antibacterial traits. Inhibition zones were observed where the solutions were placed (Figure 6). On substrates that were used as negative control, zones of inhibition could not be detected. The average radius of inhibition zones derived from the gentamicin release to the buffer, was 0.7 ± 0.4 cm. The average radius of inhibition zones in the case of pure gentamicin was 0.95 ± 0.04 cm. The difference in the size of the inhibition zone is probably due to the difference in the gentamicin concentration. In addition, in the case of gentamicin release from the substrate, there is a dependence in various factors, such as temperature, diffusion coefficient, etc. In the case of pure gentamicin, the zone of inhibition will be repeated in different experiments. This is probably also the reason for the difference in the standard

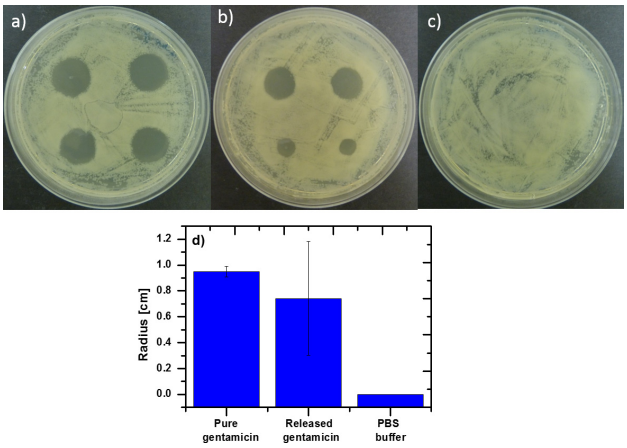

Figure 6. a-c) Image of diffusion tests performed with *Pseudomonas aeruginosa* (a) Pure gentamicin, (b) Gentamicin released to the solution, (c) PBS buffer. d) Average and standard deviation of the inhibition zones of the diffusion tests of the PBS solution surrounding the samples after 5 days, PBS buffer as a negative control, and pure gentamicin solution as a positive control (n = 10).

deviation. In the case of pure gentamicin the standard deviation is small in contrast to the gentamicin release from the substrates.

Conclusions

In this study, a galvanostatic in situ electrodeposition at low currents (0.6 mA/cm^2) of CaP with gentamicin-loaded chitosan NPs is demonstrated. This co-deposition technique is a massive improvement on the techniques present in the market today, which are based on inserting the antibiotics after the CaP coating, which is usually plasma sprayed. The deposited layer is found to consist of human osteoblast cells, and biomineralization monotite and OCP phases, along with large amounts of gentamicin. These phases may be beneficial for bone growth, as OCP has higher solubility than the stoichiometric HAp and is commonly considered as a precursor to HAp, while monotite has even faster resorbability. Gentamicin was released for 15 days, which accounts for 59% of the encapsulated drug. Moreover, the coating is non-cytotoxic to the assay, supporting its biocompatibility.

Supporting Information Summary

Supporting Information contains the Experimental Section.

Acknowledgements

M.B.T. and N.M. contributed equally to this work. This study was financially supported by a Kamin grant 52694–5 from the Israel Ministry of Economy. The partial support by the Focal Technology Area through. The authors thank Dr. Yuri Rosenberg and Dr. Larisa Burstein from the Wolfson Applied Materials Resegarch Centre at Tel-Aviv University for their XRD and XPS characterization service, respectively. We thank also Mario Levinshtein for his technical assistance.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Calcium phosphate · Cytotoxicity · Drug eluting · Gentamicin · *In situ* deposition

[1] S. V. Dorozhkin, Self Setting *Int. J. Mater. Chem.* **2011**, *1*, 1–48.
[2] R. J. B. Sakkers, R. A. J. Dalmeyer, R. Brand, P. M. Rozing, C. A. van Blitterswijk, *J. Biomed. Mater. Res.* **1997**, *36*, 265–273.
[3] N. Eliaz, *Isr. J. Chem.* **2008**, *48*, 159–168.
[4] T. Miclau, A. H. Schmidt, J. C. Wenke, L. X. Webb, J. M. Harro, R. Prabhakara, M. E. Shirtliff, *J. Orthop. Trauma* **2010**, *24*, 583–586.
[5] J. Esteban, J. Cordero-Ampuero, *Expert Opin. Pharmacother.* **2011**, *12*, 899–912.
[6] Y. Z. Yang, K. H. Kim, J. L. Ong, *Biomaterials* **2005**, *26*, 327–337.
[7] N. Eliaz, O. Ritman-Hertz, D. Aronov, E. Weinberg, Y. Shenhar, G. Rosenman, M. Weinreb, E. Ron, *J. Mater. Sci. Mater. Med.* **2011**, *22*, 1741–1752.
[8] N. Eliaz, S. Shmueli, I. Shur, D. Benayahu, D. Aronov, G. Rosenman, *Acta Biomater.* **2009**, *5*, 3178–3191.
[9] S. B. Goodman, Z. Yao, M. Keeney, F. Yang, *Biomaterials* **2013**, *34*, 3174–3183.
[10] M. Stinger, J. Bezemer, K. de-Groot, P. Layroll, *J. Controlled Release* **2004**, *99*, 127–137.
[11] M. Stinger, K. de-Groot, P. Layrolle, *Biomaterials* **2002**, *23*, 4143–4153.
[12] V. Luginbuehl, K. Ruffieux, C. Hess, D. Reichardt, B. von Rechenberg, K. Nuss, *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2010**, *92B*, 341–352.
[13] M. Baro, E. Sánchez, A. Delgado, A. Perera, C. Évora, *J. Controlled Release* **2002**, *83*, 353–364.
[14] S. Radin, J. T. Campbell, P. Ducheyne, J. M. Cuckler, *Biomaterials* **1997**, *18*, 777–782.
[15] D. Lakstein, W. Kopelovitch, Z. Barkay, M. Bahaa, D. Hendel, N. Eliaz, *Acta Biomater.* **2009**, *5*, 2258–2269.
[16] N. Metoki, L. Leifenberg-Kuznits, W. Kopelovich, L. Burstein, M. Gozin, N. Eliaz, *Mater. Lett.* **2014**, *119*, 24–27.
[17] H. Wang, N. Eliaz, Z. Xiang, H. P. Hsu, M. Spector, L. W. Hobbs, *Biomaterials* **2006**, *27*, 4192–4203.
[18] N. Eliaz, T. M. Sridhar, U. Kamachi Mudali, R. Baldev, *Surface Eng.* **2005**, *21*, 238–242.
[19] T. M. Sridhar, N. Eliaz, U. Kamachi Mudali, R. Baldev. *Corr. Rev.* **2002**, *20*, 255–293.
[20] N. Eliaz, M. Eliyahu, *J. Biomed. Mater. Res., Part A* **2007**, *80*, 621–634.
[21] N. Eliaz, T. M. Sridhar, *Cryst. Growth Des.* **2008**, *8*, 3965–3977.
[22] F. Bir, H. Khireddine, A. Touati, D. Sidane, S. Yala, H. Oudadesse, *Appl. Surf. Sci.* **2012**, *258*, 7021–7030.
[23] Y. Huang, X. Zhang, H. Mao, T. Li, R. Zhao, Y. Yan, X. Pang, *RSC Adv.* **2015**, *5*, 17076–17086.
[24] A. Karthika, L. Kavitha, M. Surendiran, S. Kannan, D. Gopi, *RSC Adv.* **2015**, *5*, 47341–47352.
[25] C. Fu, B. Song, C. Wan, K. Savino, Y. Wang, X. Zhang, M. Z. Yates, *Surf. Coat. Technol.* **2015**, *276*, 618–625.
[26] M. B. Thomas, N. Metoki, D. Mandler, N. Eliaz, Accepted to *Electrochim. Acta*, DOI: 10.1016/j.electacta.2016.10.186.
[27] M. Zilberman, J. J. Elsner, *J. Control. Release* **2008**, *130*, 202–215.
[28] L. Altomare, L. Visai, N. Bloise, C. R. Arciola, L. Ulivi, G. Canadiani, A. Cigada, R. Chiesa, L. De Nardo, *Int. J. Artif. Organs* **2012**, *35(10)*, 876–883.
[29] W. E. Brown, N. Eidelman, B. Tomazic, *Adv. Dent. Res.* **1987**, *1(2)*, 306–313.
[30] N. M. Boroujeni, H. Zhou, T. J. F. Luchini, S. B. Bhaduri, *J. Biomed. Mater. Res.* **2013**, *102(2)*, 260–266.
[31] D. Simon, S. Manuel, H. Varma, *J. Oral Maxillofac. Surg.* **2013**, *115(3)*, e7-e14.
[32] M. Zhang, J. Chang, *Ultrason. Sonochem.* **2010**, *17*, 789–792.
[33] F. Chai, J. C. Hornez, N. Blanchemian, C. Neut, M. Descamps, H. F. Hildebrand, *Biomol. Eng.* **2007**, *24*, 510–514.

[34] U. Gbureck, E. Vorndran, F. A. Müller, J. E. Barralet, *J. Controlled Release* **2007**, *122*, 173–180.
[35] I. Soriano, C. Évora, *J. Controlled Release* **2000**, *68*, 121–134.
[36] J. Schnieders, U. Gbureck, R. Thull, T. Kissel, *Biomaterials* **2006**, *27*, 4239–4249.
[37] P. Rajesh, N. Mohan, Y. Yokogawa, H. Varma, *Mater. Sci. Eng. C Mater. Biol. Appl.* **2013**, *33*, 2899–2904.
[38] M. Taha, F. Chai, N. Blanchemain, M. Goube, B. Martel, H. F. Hildebrand, *Mater. Sci. Eng. C Mater. Biol. Appl.* **2013**, *33*, 2639–2647.
[39] M. Zilberman, A. Kraitzer, *J. Biomed. Mater. Res. A* **2007**, *84 A(2)*, 313–323.
[40] E. Lu, S. Franzblau, H. Onyuksel, C. Popescu, *J. Microencapsulation* **2009**, *26(4)*, 346–354.
[41] T. Danieli, D. Mandler, *J. Solid State Electrochem.* **2013**, *17*, 2989–2997.
[42] J. Hu, G. Zhang, S. Liu, *Chem. Soc. Rev.* **2012**, *41*, 5933–5949.
[43] P. Brouqui, M. C. Rousseau, A. Stein, M. Drancourt, D. Raoult, *Antimicrob. Agents Chemother.* **1995**, *39*, 2423–2425.

Submitted: December 12, 2016
Accepted: January 2, 2017