



In Situ Potentiostatic Deposition of Calcium Phosphate with Gentamicin-Loaded Chitosan Nanoparticles on Titanium Alloy Surfaces



Midhun B. Thomas^{a,1}, Noah Metoki^{a,1}, Daniel Mandler^{b,**}, Noam Eliaz^{a,*}

^a Biomaterials and Corrosion Lab, Department of Materials Science and Engineering, Tel-Aviv University, Ramat Aviv 6997801, Israel

^b Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel

ARTICLE INFO

Article history:

Received 24 July 2016

Received in revised form 25 October 2016

Accepted 28 October 2016

Available online 29 October 2016

Keywords:

calcium phosphate
drug release
electrodeposition
gentamicin antibiotics
in-situ deposition

ABSTRACT

Bone implants must be biocompatible and are usually built to promote osseointegration, e.g. by application of plasma spray calcium phosphate (CaP) coating. The risk of infection and biofilm formation on implant surfaces is a well-known problem. The combination of electrochemically deposited CaP coating with antibiotics may offer significant benefits. Here, we demonstrate an innovative *in situ* electrodeposition of gentamicin encapsulated in chitosan nanoparticles along with CaP. The deposition of the coating was observed and studied at several temperatures. A high drug loading into the coating and a controlled release of the drug over two days were demonstrated.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Dental and orthopedic hip implants have been in vogue in the past few decades. One of the major concerns is the risk of infection [1], which is estimated in the range of 0.5–5.0% for total hip arthroplasty [2]. Similarly, in the field of dentistry, a condition well known as “peri-implantitis” is found to be common [3]. It is mainly caused by infection around the implant, which leads to loss of supporting circumferential bone, causing its failure [3]. Most infections are due to contaminations adhering to the implant surface during surgery and involve the formation of a biofilm [4]. These bacteria usually form a biofilm that covers the implant surface. The biofilm protects the bacteria from environmental attacks and systemic antibiotic [5]. Most of the infections develop from an early contamination that occurs during the operation or in the first few days after surgery. Events such as these, which become symptomatic or anyway manifest shortly following

surgery, within 3 months of implantation, have been referred to as “early” infections by Trampuz et al. [6].

Gentamicin is an antibiotic of the aminoglycoside family, which is extensively used in this context. Therefore, antibacterial implants containing gentamicin have been prevalently studied by numerous research groups using a variety of coatings and loading methods [7–9]. For example, Lucke et al. [7] studied gentamicin-loaded poly(D,L-lactide) coating on metallic implant in a rat model. Price et al. [8] used polylactic-co-glycolic acid copolymer as a biodegradable carrier for gentamicin. Pishbin et al. [9] studied the electrophoretic deposition of chitosan-bioactive glass composite containing gentamicin. The coating eluted antibiotics for sixty days, 40% of which during the first five days. The topic has also been reviewed by Hayes et al. and Van de Belt et al. [10,11].

In the past two decades, since their food and drug administration (FDA) approval, calcium phosphate (CaP) bioceramics have been used prevalently as coatings for orthopedic implants interacting with bone tissue [12,13]. These are preferred due to their high biocompatibility and osseointegration properties [12]. Most commonly, CaP coatings have been applied by plasma spray technology [14,15]. However, the resulting coatings suffer from several key drawbacks [16]. One prominent drawback is the inability to incorporate organic compounds, such as antibiotics, during the coating process due to the extremely high processing temperatures [17]. Therefore, in order to incorporate antibiotics into such coatings, a post treatment has been implemented, usually by physical absorption [17]. This is also common for other

Abbreviations: FDA, U.S. Food and Drug Administration; CaP, calcium phosphate; HA₂P, hydroxyapatite; ED, electrodeposition; DCPD, dibasic calcium phosphate dehydrate (Brushite); OCP, octacalcium phosphate; NPs, nanoparticles; TPP, pentasodium tripolyphosphate hexahydrate; PBS, phosphate buffered saline; DI, dionized; SCE, saturated calomel electrode; ESEM, environmental scanning electron microscope; XRD, X-ray diffraction; TCP, tricalcium phosphate.

* Corresponding author. Tel.: +972-3-6407384, Fax: +972-3-6406648.

** Corresponding author. Tel.: +972-2-6585831, Fax: +972-2-6585319.

E-mail addresses: daniel.mandler@mail.huji.ac.il (D. Mandler), neliaz@tau.ac.il (N. Eliaz).

¹ M.B.T. and N.M. contributed equally to this work.

methods of CaP deposition [18,19]. For example, Rajesh et al. [19] studied pulsed laser deposition of hydroxyapatite (HAp) on titania nanotubes containing gentamicin. The drug was inserted through dipping and vacuum drying after the formation of the coating.

Physical absorption of antibiotics and other molecules onto the surface of CaPs limits the amount loaded and release kinetics. Antibiotic loading by a dipping method leads to a burst release of the antibiotics, such that more than 80–90% of the antibiotics are released from the CaP coating within the first 60 minutes [20]. Therefore, it is better to develop a method whereby the coating is carried out at significantly lower temperature and the drug is added continuously during the deposition.

Nowadays, an emerging technique slowly making its way into the industry is electrodeposition (ED) of CaP [14–16,21]. The most documented procedures for ED of CaP have involved relatively high temperatures (70–95 °C) in an attempt to improve the uniformity of the coating and the bonding between the CaP and the substrate [22,23]. Furthermore, fairly high current densities (namely, 10–120 A cm⁻²) have been applied. Several studies have been carried out in order to lower the deposition temperature and currents [24–27]. For example, Kuo and Yen [26] demonstrated the formation of a uniform HAp coating on pure Ti substrate at pH 4.11 and 25 °C, while using current densities higher than 10 mA cm⁻². At lower current densities (1–5 mA cm⁻²), dibasic calcium phosphate dihydrate (DCPD, CaHPO₄ × 2H₂O) was formed. Most recently, Metoki et al. [21] demonstrated the formation of HAp and octacalcium phosphate (OCP) phases on titanium, using near-physiological bath conditions (pH 7.4, 37 °C) and low current densities (0.05 mA cm⁻²).

However and to the best of our knowledge, only one study on the *in situ* electrophoretic deposition of CaP **with antibiotics** has been reported by Altomare et al. [28]. The latter describes applying fairly high cathodic current (20 mA cm⁻²) for 240 seconds at room temperature [28]. The authors report an effective incorporation of gentamicin without any major impact to the morphology, density and structure of the brushite coating [28]. Furthermore, they indicated that the coating showed antibacterial efficacy on different streptococcal strains, using an inhibition zone [28]. Yet, the amount of antibiotics present in the coating was not tested, and no release studies were made. Here, we present an *in situ* method of CaP ED with gentamicin-loaded chitosan nanoparticles (NPs) at low potential and current and different temperatures, which yielded high drug load. The coating produced was ~2 μm thick, and contained up to 42 wt % of antibiotics, depending on the temperature of deposition.

2. Experimental

2.1. Materials

Gentamicin sulfate, dextran sulfate, low molecular weight chitosan (serial# 448869), acetic acid (analytical grade), pentasodium tripolyphosphate hexahydrate (TPP), hydrofluoric acid (40%), nitric acid (65%), sodium hydroxide (analytical grade), calcium nitrate (99%), ammonium dihydrogen phosphate (98%), O-phthalaldehyde, sodium borate, 2-mercaptoethanol and phosphate buffered saline (PBS, serial# 79383) were purchased from Sigma-Aldrich. Isopropanol (analytical grade) and hydrochloric acid (analytical grade) were purchased from Bio-Lab. Ti-6Al-4V ELI grade (ASTM F136-02a) rods, 5 mm long and 9.5 mm in diameter, were produced by Dynamet Inc. (Washington, PA) and supplied by Barmil (Petach-Tikva, Israel). The disks served as the working electrode. Deionized (DI) water (>18 MΩ cm) was used for all the experiments.

2.2. Instrumentation

The NPs were collected by ultracentrifugation, which was carried out by Beckman Coulter optima le70 ultracentrifuge, operating with a SW41 rotor. The retrieval was performed at 25,000 rpm for 30 min at 4 °C. The NPs were characterized using Zetasizer Nano Z DLS (particle diameter range: 3.8 nm–100 μm) to determine both size and zeta potential. Electrochemical measurements were conducted with Bio-Logic SAS galvanostat/potentiostat model VSP operating in potentiostatic mode. A three electrode cell was employed with saturated calomel electrode (SCE) as reference electrode, a graphite rod as a counter electrode and titanium substrate as the working electrode. The surface morphology of the coating was characterized using ESEM (Quanta 200 FEG, FEI). The weight of the coating was determined by measuring the sample before and after deposition using Sartorius Basic scale, sensitive up to 1 × 10⁻⁴ g. The thickness of the coating was measured on a metallographic cross-section using ESEM. The amount of gentamicin in the NPs was analyzed with a UV-vis spectrophotometer (Varian, Cary 5000 UV vis NIR, wavelength range: 175–3300 nm) at λ = 333 nm. pH of the solution was tested using InoLab pH/Oxi Level 3 meter. Grazing incidence x-ray diffraction (XRD) scans were acquired using a Scintag powder diffractometer within the range of 2θ = 0–60° at a scan rate of 0.05 deg s⁻¹. They allowed determining the phase content in the coating.

2.3. Procedures

2.3.1. Preparation of the NPs

The preparation of the gentamicin-loaded chitosan NPs was as described by Lu et al. [30]. The process is described in Fig. 1. 10 mL of gentamicin sulfate (0.2% w/v) was mixed with 10 mL of dextran sulfate at 2 mg mL⁻¹ for 30 s. At the same time, 20 mL of low molecular weight chitosan solution (0.2% w/v) was prepared in 0.1 M aqueous acetic acid. After the chitosan solution was prepared, the gentamicin-dextran sulfate mixture was added drop wise while magnetically stirring at 1000 rpm for 1 min. This was followed by drop wise addition of 10 mL (0.8% w/v) of TPP under the same conditions. The drug-loaded nanoparticles were formed immediately and the stirring was continued for 5 min. The particles were collected by ultracentrifugation. Following, the supernatant was collected and the precipitate was suspended in DI water.

2.3.2. Pre-treatment of the substrates

In order to facilitate coating, the titanium substrates were initially subjected to pre-treatment using hydrofluoric acid and sodium hydroxide, as described earlier [21]. In short, the sample was polished with P600, etched with HF/HNO₃ and grit blasted with alumina (59–68 μm). The substrate was subsequently suspended in 5 M sodium hydroxide for 24 h.

2.3.3. Co-electrodeposition of CaP and NPs

The three-electrode cell was filled with 30 mL of sample solution which contained 0.61 mM Ca(NO₃)₂ and 0.36 mM NH₄H₂PO₄ together with 1 mg mL⁻¹ of the gentamicin/chitosan NPs. The pH of the solution was adjusted to 5 using 1 M sodium hydroxide. Potentiostatic deposition was performed at -1.4 V vs. SCE for 2 h while stirring the solution at 200 rpm during deposition. The experiment was carried out at three different temperatures - 37, 60 and 90 °C - and the cell temperature was kept constant by a Lauda Ecoline E-220T thermostatic bath (Lauda, Königshofen, Germany).

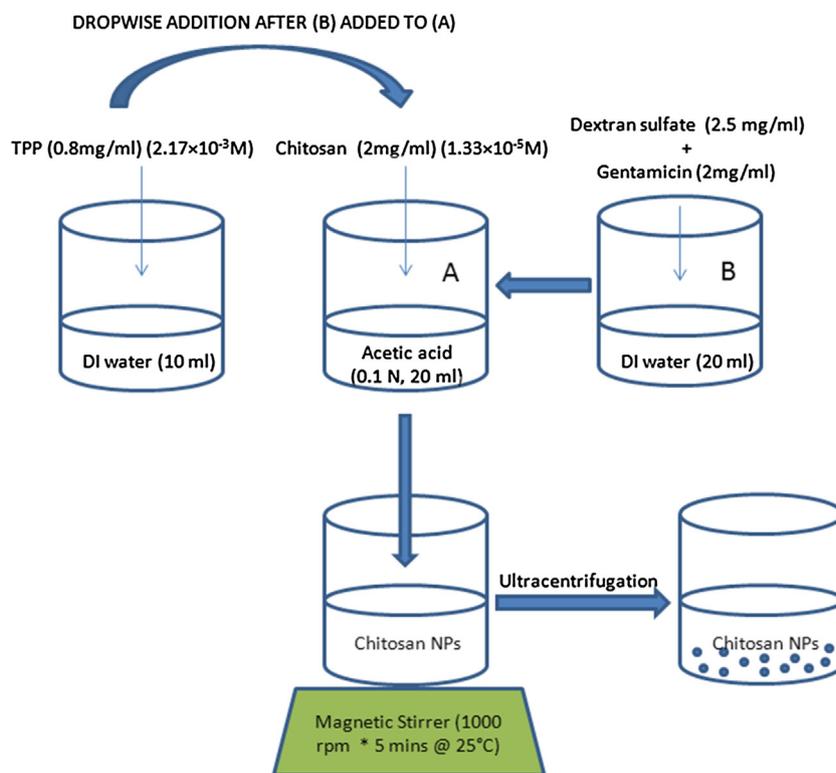


Fig. 1. Schematic of the preparation of the drug-loaded chitosan nanoparticles.

2.4. Evaluating the amount of gentamicin

The amount of gentamicin in the NPs and the surfactant were analyzed in the following manner: 200 μL of sample solution was mixed with 200 μL isopropanol and 200 μL of the reagent (preparation of the reagent is described elsewhere [30]). After incubation for 45 min at 4 $^{\circ}\text{C}$, the samples were transferred into a semi-micro quartz cuvette (with a width of 4 mm) and analyzed using a calibration curve. Similarly, the amount of gentamicin in the coating at different temperatures was tested by dissolving the coating in 1 mL of 0.5 M HCl for 30 min. In all tests, in order to ensure that chitosan is removed, 500 μL of the sample was mixed with 500 μL of borate buffer (pH 4) and centrifuged at 4000 rpm for 40 min. Subsequently, 200 μL of the solution was removed and the procedure was followed as mentioned above.

2.5. Drug release tests

Release studies were carried out in PBS over a period of 29 days. The electrodeposited samples were vertically suspended in 2 mL of PBS. The samples were stored in an incubator at 37 $^{\circ}\text{C}$ and 5% CO_2 . At the stipulated time points, the PBS was replaced by fresh PBS and analyzed spectroscopically.

3. Results and discussion

The objective is to introduce antibiotics into electrochemically deposited CaP. As a model drug, we chose gentamicin. Our approach involves the electrochemical deposition of CaP from its ionic constituents in the presence of chitosan-based nanoparticles loaded with gentamicin. Therefore, we first synthesized and characterized the chitosan/gentamicin NPs followed by their co-deposition *in situ* with the CaP and finally examined the release of the drug.

3.1. Preparation and examination of the NPs

The chitosan/gentamicin NPs were prepared as described previously by Lu et al. [29]. The amount of gentamicin in the NPs was determined as 69 ± 3 wt.%, which is slightly higher than the values reported by Lu et al. (63.1 ± 1.5 wt.%). The effects of pH, and the ionic species, i.e. calcium and phosphate, in the suspension on the size of the NPs and their ζ -potential were examined, as shown in Fig. 2 and Fig. 3. The pK of chitosan is ~ 6.3 and therefore, as expected, the ζ -potential is positive at $\text{pH} < \text{pK}$ and diminishes at higher pH [31]. Hence, at $\text{pH} > \text{pK}$ aggregation commences as can be seen in the particle size. In acidic pH, the NPs have high zeta potential and low diameter, indicating stability in the suspension. These results imply that precipitation of the NPs is possible from acidic solution in an electrochemical system based on applying

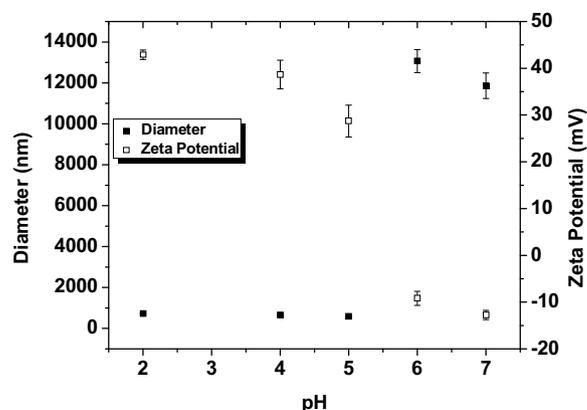


Fig. 2. Effect of pH on size and zeta potential of drug-loaded particles in suspension (1 mg mL^{-1}).

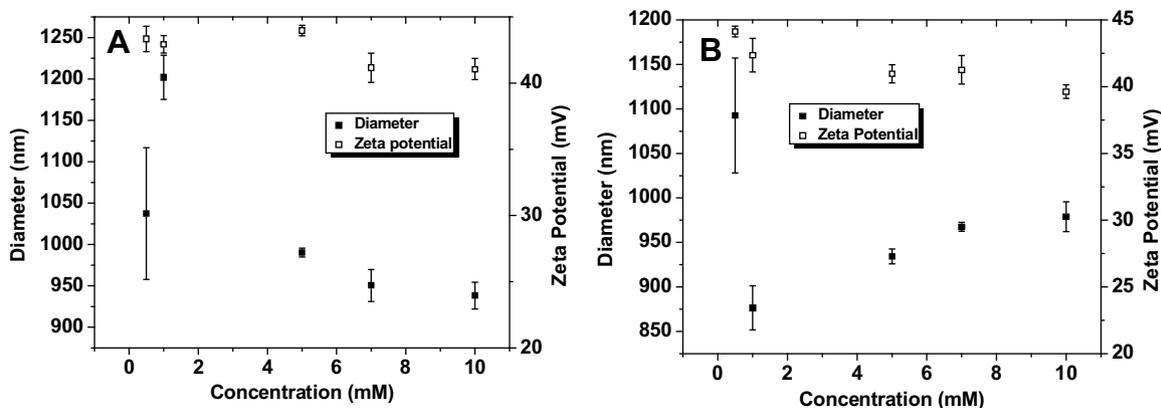


Fig. 3. A) Effect of calcium concentration in solution on drug-loaded particles in suspension (1 mg mL^{-1}) with regard to diameter, and zeta potential. B) The effects of calcium phosphate on drug-loaded NPs in suspension (1 mg mL^{-1}) on the diameter of NPs and the zeta potential.

Table 1

The amount of gentamicin in the coating, the thickness and the chemical composition of the coating as a function of bath temperature.

Temperature [°C]	Amt. of deposited antibiotics [$\mu\text{g}/\text{cm}^2$]	wt% of gentamicin in the coating	Coating thickness [μm]	Ca/P atomic ratio
37	92 ± 15	12.5	2.5 ± 1.9	1.51
60	99 ± 14	32.0	2.5 ± 1.1	1.59
90	97 ± 5	41.8	2.1 ± 1.1	1.50

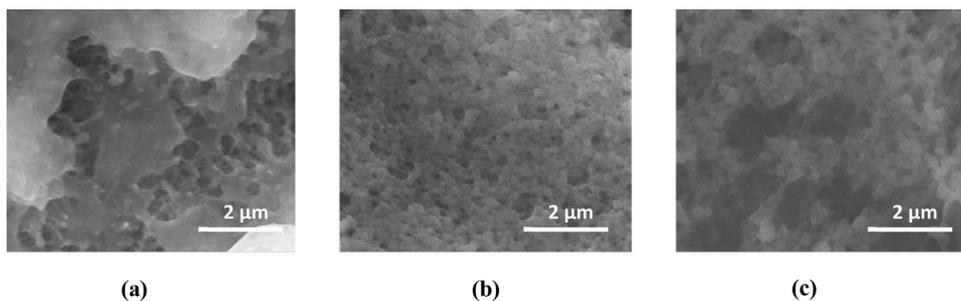


Fig. 4. SEM images of coatings electrodeposited at (a) 37°C , (b) 60°C and (c) 90°C . All scale bars equal $2.0 \mu\text{m}$.

negative potentials that cause the reduction of water and the elevation of pH in the vicinity of the working electrode. This fits extremely well to the electrochemical deposition of CaP, which is also driven by increasing the pH electrochemically [15,16,21]. Hence, we expect that upon electrochemically reducing water in a suspension containing both chitosan/gentamicin NPs and ionic species of phosphate and calcium, codeposition of the two will take place.

Yet, we had to verify that neither calcium, nor calcium phosphate have an effect on the ζ -potential and aggregation of the NPs. Fig. 3a shows that calcium ions (added as $\text{Ca}(\text{NO}_3)_2$) have no effect on the ζ -potential and NPs size (examined up to 10 mM). Fig. 3b shows that the addition of calcium phosphate (up to 10 mM) has only a minor effect on aggregation. It is important to note that the ratio of calcium to phosphate was maintained at 1.67 at all concentrations. The suspension remained stable at all concentrations, and the NPs were only slightly enlarged as compared with their original diameter of 600 nm. These results were very encouraging, as they indicated that neither calcium nor calcium phosphate affect the stability of the chitosan/gentamicin NPs.

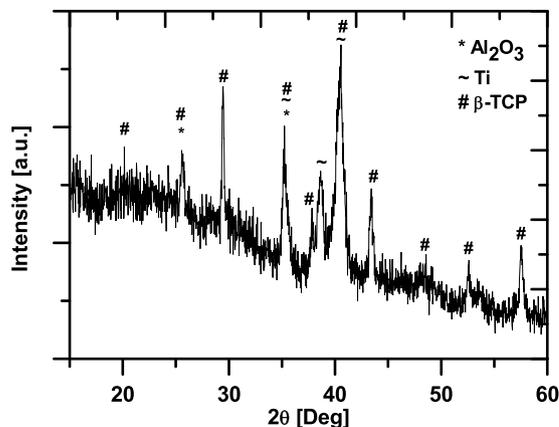


Fig. 5. XRD spectra in grazing incidence of CaP coating co-deposited with chitosan/gentamicin NPs at 60°C .

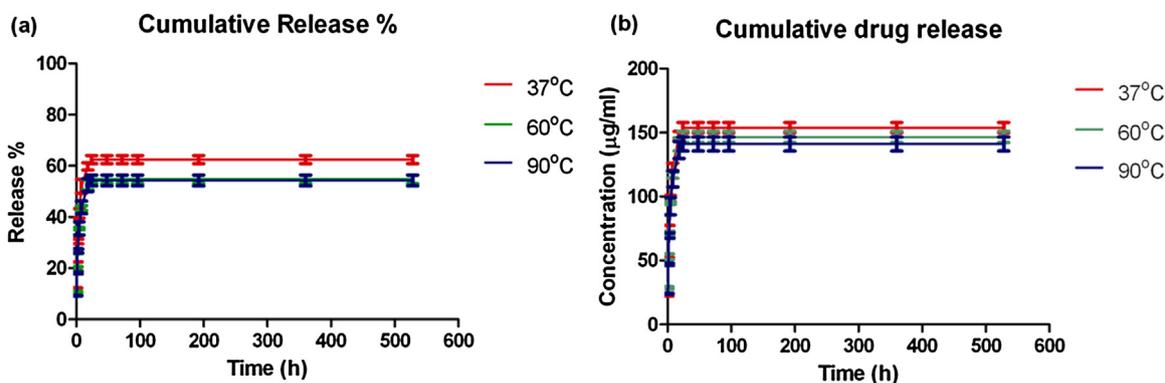


Fig. 6. Cumulative release profile (a) and concentration release profile (b) of gentamicin deposited *in situ* with CaP at three different temperatures ($n=3$).

3.2. ED of NPs on the substrates

The co-deposition was examined in a solution containing 0.61 mM calcium, 0.32 mM phosphate and 1 mg mL⁻¹ NPs. These concentrations were chosen in order to ensure HAP and OCP supersaturation in solution, as shown previously [16,21]. The deposition was studied at 37, 60 and 90 °C and the effect of the deposition temperature on the controlled release of the drug, was also examined. Deposition was carried out at -1.4 V vs. SCE for 2 hours [21]. Table 1 presents the amount of gentamicin deposited per unit area, its weight percent in the coating, the coating thickness, and the calcium to phosphate atomic ratio (EDS).

While the amount deposited per unit surface area was similar (t -test statistical analysis showed no difference between the groups), the weight percent of gentamicin in the coating changed dramatically, increasing with temperature. Previously reported loading of gentamicin in CaP was typically low, achieving only up to 30 wt% [30,32,33]. Simon et al. [32] showed a mixture of CaP and gentamicin powders, forming a cement with 2.5 wt. % antibiotics. In addition, when CaP is coated; the loading was not reported to be very high. Gbureck et al. [33] showed a 3D-printed CaP with biodegradable polymer coating dipped in gentamicin solution and vacuumed. The coating contained 5.7–71 µg cm⁻² of gentamicin. Both Taha et al. [34] and Rajesh et al. [19] first coated titanium substrate with HAP, and then dipped the coated substrate in gentamicin solution, achieving up to 800 µg cm⁻² antibiotics in the coating. In the present research we present, for the first time, an *in situ* preparation of CaP coating containing high amount of gentamicin.

As seen in Table 1, The Ca/P ratio based on EDS analysis was close to that of HAP, 1.67. However, EDS data might be ambiguous when it comes to identification of different CaP phases [35]. The thickness and amount of gentamicin per unit of surface area remained similar at all three studied temperatures. This may indicate that, the wt. % of CaP decreases with temperature that implies on increasing porosity. Fig. 4 presents SEM images of the coatings formed at different temperatures. The coating is fairly uniform and follows the texture of the pre-treated surface. Yet, it can be seen that the surface morphology slightly alters with temperature, which is likely to affect cell proliferation and bone ingrowth *in vivo*. It is important to note that the surface morphology is markedly different than that seen before [15,21]. This may be attributed to the co-deposition of the NPs on the electrode surface, altering the local pH, which has been shown to significantly affect the electrodeposition process of CaP [15,16,21,23,35].

In order to evaluate the phase content in the coating, XRD was employed. Fig. 5 shows the grazing incidence XRD spectra of the

CaP coating formed at 60 °C containing chitosan/gentamicin NPs. It is worth mentioning that the NPs were first analyzed as powder, which resulted in neither reflections, nor an indication of an amorphous substance. The spectrum in Fig. 5 indicates on the presence of the substrate, titanium (JCPD card 04-002-2539), the alumina used as surface treatment (JCPD card 00-010-0173), and β-tricalcium phosphate (β-TCP, JCPD card 00-009-0169). Moreover, it is evident that the material contains also an amorphous phase, which is attributed to amorphous CaP formed on the surface. Our previous studies reported on either HAP or both OCP and HAP phases in our coatings [15,21]. The presence of amorphous CaP and β-TCP alludes to the effect of the chitosan/gentamicin NPs on the formation of CaP on the Ti. Nonetheless, both phases are regularly used in industry as cements, due to high solubility properties [36]. The presence of both amorphous calcium phosphate and β-TCP may facilitate the quick dissolution of the coating, allowing the drug to elute, as well as play a role in the formation of new bone on the surface.

3.3. Release study

Fig. 6 shows the cumulative release profile measured up to 8 days, in terms of both concentration and release percent. For all samples, a high initial burst in the first day is followed by a slower release. The coating deposition temperature did not have any significant effect on the curve shape. Nevertheless, it can be seen that the total amount released from the sample coated at 37 °C is somewhat higher than from samples coated at 60 °C and 90 °C.

The release of antibiotics levels off after two days. Further sampling of the solution did not reveal any increase of the concentration in the solution, indicating that ~40% of antibiotics is still encapsulated in the coating. Since the antibiotic is highly soluble, it can be hypothesized that either the drug encapsulated is not exposed to the aqueous environment or there is a diffusion barrier inside the coating. This encapsulation may be beneficial if the barriers can be removed during the late stages of implantation, allowing the rest of the drug to elute.

4. Conclusions

In this paper, an *in situ* electrodeposition of gentamicin along with CaP was demonstrated. The weight percent of the drug in the coating was high and demonstrated a controlled release over two days. Increasing the bath temperature did not result in an increase of the amount of gentamicin being deposited. Yet, it changed the internal pore size inside the coating, which may influence the *in vivo* behavior of the coating. The coating consisted of both amorphous CaP and β-TCP. Approximately 40% of the antibiotics

remained encapsulated in the coating. This encapsulation may be beneficial if the barrier can be removed during the late stages of implantation, allowing the rest of the drug to elute.

FUNDING SOURCES

The work was funded by the Israel Ministry of Economy under Kamin grant 52694-5.

ACKNOWLEDGEMENTS

We thank Dr. Yuri Rosenberg from the Wolfson Applied Materials Research Centre at Tel Aviv University for his assistance in the XRD analysis.

References

- [1] K. Yuan, K.C. Chen, Y.J. Chan, C.C. Tsai, H.H. Chen, C.C. Shih, Dental implant failure associated with bacterial infection and long-term bisphosphonate usage: a case report, *Implant Dent.* 21 (2012) 3–7.
- [2] A. Virk, D.R. Osmon, Prosthetic joint infections, *Curr. Treat. Options Infect. Dis.* 3 (2001) 287–300.
- [3] A. Mombelli, Microbiology and antimicrobial therapy of peri-implantitis, *Periodontol.* 28 (2000) 177–189.
- [4] N. Davis, A. Curry, A.K. Gambhir, H. Panigrahi, C.R. Walker, E.G. Wilkins, M.A. Worsley, P.R. Kay, Intraoperative bacterial contamination in operations for joint replacement, *J. Bone Joint Surg. Br.* 81 (1999) 886–889.
- [5] Z.U. Isaklar, R.O. Darouiche, G.C. Landon, T. Beck, Efficacy of antibiotics alone for orthopedic device-related infections, *Clin. Orthop.* 332 (1996) 184–189.
- [6] A. Trampuz, W. Zimmerli, Prosthetic joint infections: update in diagnosis and treatment, *Swiss Med. Wkly.* 135 (2005) 243–251.
- [7] M. Lucke, G. Schmidmaier, S. Sadoni, B. Wildemann, R. Schiller, N.P. Haas, M. Raschke, Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats, *Bone* 32 (2003) 521–531.
- [8] J.S. Price, A.F. Tencer, D.M. Arm, G.A. Bohach, Controlled release of antibiotics from coated orthopedic implants, *J. Biomed. Mater. Res.* 30 (1996) 281–286.
- [9] F. Pishbin, V. Mouriño, S. Flor, S. Kreppel, V. Salih, M.P. Ryan, A.R. Boccaccini, Electrophoretic deposition of gentamicin-loaded bioactive glass/chitosan composite coating for orthopedic implants, *ACS Appl. Mater. Interfaces* 6 (2014) 8796–8806.
- [10] G. Hayes, T. Gibson, A review of local antibiotic implants and applications to veterinary orthopedic surgery, *Vet. Comp. Orthop Traumatol.* 26 (2013) 251–259.
- [11] H. van de Belt, D. Neut, W. Schenk, J.R. van Horn, H.C. van der Mei, H.J. Busscher, Infection of orthopedic implants and the use of antibiotic-loaded bone cements, *Acta Orthop. Scand.* 72 (2001) 557–571.
- [12] L. Le Guehennec, A. Soueidan, P. Layrolle, Y. Amouriq, Surface treatments of titanium dental implants for rapid osseointegration, *Dent. Mater.* 23 (2007) 844–854.
- [13] Y.Z. Yang, K.H. Kim, J.L. Ong, Review on calcium phosphate coatings produced using a sputtering process – an alternative to plasma spraying, *Biomaterials* 26 (2005) 327–337.
- [14] H. Wang, N. Eliaz, Z. Xiang, H.P. Hsu, M. Spector, L.W. Hobbs, Early bone apposition in vivo on plasma-sprayed and electrochemically deposited hydroxyapatite coating on titanium alloy, *Biomaterials* 27 (2006) 4192–4203.
- [15] D. Lakstein, W. Kopelovitch, Z. Barkay, M. Bahaa, D. Hendel, N. Eliaz, Enhanced osseointegration of grit-blasted: NaOH-treated and electrochemically hydroxyapatite-coated Ti-6Al-4V implants in rabbits, *Acta Biomaterialia* 5 (2009) 2258–2269.
- [16] N. Eliaz, T.M. Sridhar, Electrocrystallization of hydroxyapatite and its dependence on solution conditions, *Crys. Growth Des.* 8 (2008) 3965–3977.
- [17] S.B. Goodman, Z. Yao, M. Keeney, F. Yang, The future of biologic for orthopedic Implants, *Biomaterials* 34 (2013) 3174–3183.
- [18] M. Stinger, J. Bezemer, K. de Groot, P. Layrolle, Incorporation of different antibiotics into carbonated hydroxyapatite coatings on titanium implants, release and antibiotic efficacy, *J. Control. Release* 99 (2004) 127–137.
- [19] P. Rajesh, N. Mohan, Y. Yokogawa, H. Varma, Pulsed laser deposition of hydroxyapatite on nanostructured titanium towards drug eluting implants, *Mater. Sci. Eng. C Mater. Biol. Appl.* 33 (2013) 2899–2904.
- [20] H. Gautier, G. Daculsi, C. Merle, Association of vancomycin and calcium phosphate by dynamic compaction: in vitro characterization and microbiological activity, *Biomaterials* 22 (2001) 2481–2487.
- [21] N. Metoki, L. Leifenberg-Kuznits, W. Kopelovich, L. Burstein, M. Gozin, N. Eliaz, Hydroxyapatite coating electrodeposited at near-physiological conditions, *Mater. Lett.* 119 (2014) 24–27.
- [22] M.A. Lopez-Heredia, P. Weiss, P. Layrolle, An electrodeposition method of calcium phosphate coating on titanium alloy, *J. Mater. Sci. Mater. Med.* 18 (2007) 381–390.
- [23] N. Eliaz, T.M. Sridhar, Y. Rosenberg, Electrodeposition of hydroxyapatite on titanium implants, in: G. Lütjering, J. Albecht (Eds.), *Proceedings of the 10th World Conference on Titanium*, vol. 5, Wiley-VCH, Weinheim, Germany, 2004, pp. 3299–3306.
- [24] S. Rössler, A. Sewing, M. Stölzel, R. Born, D. Scharnweber, M. Dard, H. Worch, Electrochemically assisted deposition of thin calcium phosphate coatings at near-physiological pH and temperature, *J. Biomed. Mater. Res. A* 64 (2003) 655–663.
- [25] S.H. Wang, W.J. Shih, W.L. Li, M.H. Hon, M.C. Wang, Morphology of the calcium phosphate coatings deposited on the Ti-6Al-4V substrate by an electrolytic method under 80 Torr, *J. Eur. Cera. Soc.* 25 (2005) 3287–3292.
- [26] M.C. Kuo, S.K. Yen, The process of electrochemical deposited hydroxyapatite coatings on biomedical titanium at room temperature, *Mater. Sci. Eng. C Mater. Biol. Appl.* 20 (2002) 153–160.
- [27] R. Narayanan, T.Y. Kwon, K.H. Kim, Direct nanocrystalline hydroxyapatite formation on titanium from ultrasonated electrochemical bath at physiological pH, *Mater. Sci. Eng. C Mater. Biol. Appl.* 28 (2008) 1265–1270.
- [28] L. Altomare, L. Visai, N. Bloise, C.R. Arciola, L. Ulivi, G. Canadiani, A. Cigada, R. Chiesa, L. De Nardo, Electrochemically deposited gentamicin-loaded calcium phosphate coating for bone tissue integration, *Int. J. Artif. Organs* 35 (2012) 876–883.
- [29] E. Lu, S. Franzblau, H. Onyuksel, C. Popescu, Preparation of aminoglycoside-loaded chitosan nanoparticles using dextran sulphate as a concentration, *J. Microencapsul.* 26 (2009) 346–354.
- [30] S.S. Sampath, D.H. Robinson, Comparison of new and existing spectrophotometric methods of analysis of tobramycin and other aminoglycosides, *J. Pharm. Sci.* 79 (1990) 428–431.
- [31] T. Danieli, D. Mandler, Local surface patterning by chitosan-stabilized gold nanoparticles using the direct mode of scanning electrochemical microscopy (SECM), *J. Solid State Electrochem.* 17 (2013) 2989–2997.
- [32] D. Simon, S. Manuel, H. Varma, Novel nonporous bioceramic spheres for drug delivery application: a preliminary in vitro investigation, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 115 (2013) e7–e14.
- [33] F. Chai, J.C. Hornez, N. Blanchemian, C. Neut, M. Descamps, H.F. Hildebrand, Antibacterial activation of hydroxyapatite (HA) with controlled porosity by different antibiotics, *Biomol. Eng.* 24 (2007) 510–514.
- [34] M. Taha, F. Chai, N. Blanchemian, M. Goube, B. Martel, H.F. Hildebrand, Validating the poly-cyclodextrins based local drug delivery system on plasma-sprayed hydroxyapatite coated orthopedic implant with toluidine blue O, *Mater. Sci. Eng. C Mater. Biol. Appl.* 33 (2013) 2639–2647.
- [35] N. Eliaz, W. Kopelovitch, L. Burstein, E. Kobayashi, T. Hanawa, Electrochemical processes of nucleation and growth of calcium phosphate on titanium supported by real-time quartz crystal microbalance measurements and XPS Analysis, *J. Biomed. Mater. Res. A* 89 (2009) 270–280.
- [36] M. Navarro, A. Michiardi, O. Castaño, J.A. Planell, Biomaterials in orthopaedics, *J. R. Soc. Interface* 5 (2008) 1137–1158.