coatings

Supplementary Materials: Zn-Doped CaP-Based Coatings on Ti–6Al–4V and Ti–6Al–7Nb Alloys Prepared by Magnetron Sputtering: Controllable Biodegradation, Bacteriostatic, and Osteogenic Activities

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Citation: Prosolov, K.A.; Mitrichenko, D.V.; Prosolov, A.B.; Nikolaeva, O.O.; Lastovka, V.V.; Belyavskaya, O.A.; Chebodaeva, V.A.; Glukhov, I.A.; Litvinova, L.S.; Shupletsova, V.V.; et al. Zn-Doped CaP-Based Coatings on Ti–6Al–4V and Ti–6Al–7Nb Alloys Prepared by Magnetron Sputtering: Controllable Biodegradation, Bacteriostatic, and Osteogenic Activities. *Coatings* **2021**, *11*, 809. https://doi.org/10.3390/coatings11070809

Academic Editors: Seunghan Oh and Elvira De Giglio

Received: 12 April 2021
Accepted: 29 June 2021
Published: 3 July 2021

Additional analysis of statistical power and sample size for results after coating deposition presented in Table 2:

Type of coating/substrate	Statistical power calculated	Real sample size	Sample size required
Zn-CaP/Ti–6Al–7Nb	0.99	6	5
Zn-CaP/Ti–6Al–4V	0.99	6	3
CaP/Ti–6Al–7Nb	0.99	6	6
CaP/Ti–6Al–4V	0.99	6	3

Additional analysis of statistical power and sample size for results presented in Table 4

Type of Coating/Substrate	Statistical Power (SP) Calculated	Real Sample Sizes in Coated Substrates	Sample Size Required
Zn-CaP/Ti–6Al–4V vs. CaP/Ti–6Al–4V	0.91	8	7
Zn-CaP/Ti–6Al–7Nb vs. Zn-CaP/Ti–6Al–4V	0.99	8	6
CaP/Ti–6Al–7Nb vs. CaP/Ti–6Al–4V	0.83	8	8

(a)

(b)

(c)

Figure S1. Surface condition of test samples after 5 weeks of in vitro immersion in 0.9% NaCl solution: (**a**) Ti-6Al-4V substrate; (**b**) CaP coating; (**c**) Zn-CaP coating. A site of visible coating biodegradation is shown by black arrow. .

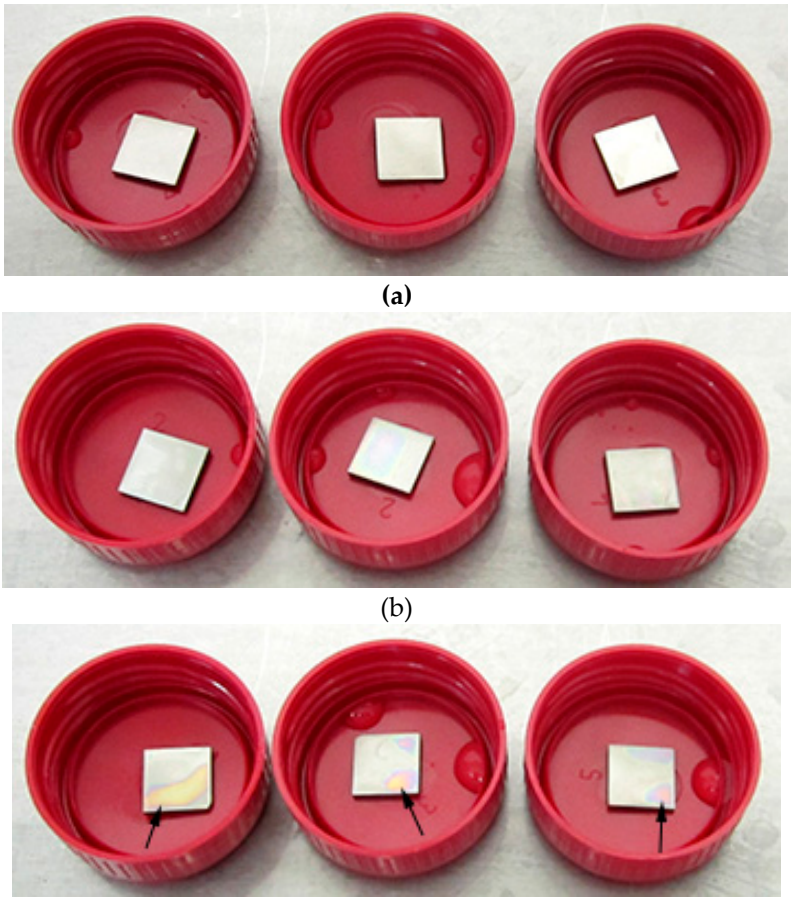

Figure S2. Surface condition of test samples after 5 weeks of in vitro immersion in 0.9% NaCl solution: (**a**) Ti-6Al-7Nb substrate; (**b**) CaP coating; (**c**) Zn-CaP coating. Sites of visible coating biodegradation are shown by black arrows. .

Table S1. Viability of Jurkat T cells after different culture periods in the presence of one-sided CaP coatings on Ti-based alloys; Me (Q1–Q3).

Groups, n=3		**Cell Number, %**					
		Live cells	Apoptotic-like cells	Dead cells	Live cells	Apoptotic-like cells	Dead cells
		24 h			48 h		
Control (without test samples)		97.45 (97.17-97.90)	0.47 (0.37-0.84)	1.78 (1.11-2.17)	93.26# (92.64-93.54) P_T	3.15# (3.04-3.30) P_T	3.59# (3.22-4.03) P_T
Ti-6Al-4V alloy							
1	Uncoated metal substrate	95.30 (94.39-95.62)	0.82 (0.67-0.89)	3.91* (3.31-4.73)	93.83 (93.29-93.87) P_T	2.86# (2.54-2.89) P_T	3.22 (3.09-3.96)
2	CaP coating	94.38* (94.30-96.00)	1.14 (0.63-1.35)	4.28* (3.30-4.48)	93.40 (93.14-93.57) P_T	3.05# (2.75-3.20) P_T	3.42 (3.36-3.84)
3	Zn-CaP coating	96.85 (95.62-99.88)	0.67 (0.49-1.09)	2.17 (1.51-3.74)	91.79# (91.04-92.69) P1,P2; P_T	3.56*# (3.34-3.77) P1; P_T	4.75# (3.76-5.37) P1
Ti-6Al-7Nb alloy							

4	Uncoated metal substrate	91.99* (90.79-94.31) P1	2.03* (1.73-2.56) P1	5.81* (3.96-6.35)	90.14* (89.33-91.08) P1; P_T	4.55*# (4.21-4.78) P1; P_T	5.32* (4.72-5.89) P1
5	CaP coating	95.72 (94.43-97.33) P4	0,93 (0,64-1,44)	3,27* (2,56-3,89) P4	91,58*# (90,66-92,52) P2; P_T	3,75*# (3,33-3,89) P4; P_T	4,68*# (4,16-5,46) P2; P_T
6	Zn-CaP coating	93.29* (91.10-94.47) P5	2.38* (1.79-2.87) P5	4.80* (3.73-6.18)	89.16*# (87.66-89.30) P3,P5; P_T	4.62*# (4.47-4.74) P3,P5; P_T	6.28* (6.18-7.40) P3,P5; P_T

n - the number of test samples (wells) tested; Statistical differences (P<0.05) are shown according to the Mann-Whitney test: *) - with control; #) - with corresponding values of 24-h culture; P1-P5 - with corresponding test groups. P_T means statistical differences (P<0.05) with corresponding values of 24-h culture according to the Wilcoxon criterion for dependent samples. Duplicate probes for each test sample were measured. Each control well was done in quadruplicate.

Table S2. Results of Zn^{2+} concentration (mg/dm^3; ppm) in extracts determined by stripping voltammetry during 5-week in vitro immersion of one-sided CaP coatings on Ti-based alloys in 0.9% NaCl solution; Me(Q1–Q3).

		Weeks of Immersion							
Groups, n=3		1		2		3		5	
		Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb
1	Control (without test samples)	0.023 (0.018-0.026) n_1=24							
2	CaP coating	0.020 (0.019-0.022) n_1=9	0.019 (0.014-0.020) n_1=9	0.021 (0.019-0.030) n_1=9	0.025 (0.024-0.031) n_1=9	0.024 (0.023-0.031) n_1=9	0.021 (0.015-0.036) n_1=9	0.032 (0.024-0.033) n_1=6	0.027 (0.023-0.036) n_1=6
3	Zn-CaP coating	0.019 (0.015-0.020) n_1=9	0.035* (0.029-0.036) n_1=9 P1=0.0007 P2=0.0003 P3<0.05	0.061* (0.058-0.063) n_1=9 P1=0.000001 P2=0.0000001	0.037* (0.031-0.043) n_1=9 P1=0.008 P2=0.03 P3<0.05	0.041* (0.032-0.046) n_1=9 P1=0.000004 P2=0.00004	0.044* (0.043-0.054) n_1=9 P1=0.000008 P2=0.0000001	0.069* (0.068-0.070) n_1=9 P1=0.0000001 P2=0.0000001	0.050* (0.046-0.053) n_1=9 P1=0.0000001 P2=0.00003

n—the number of test samples (wells) tested; n_1 - the number of probes measured; *) - Statistical differences (P1, P2) with corresponding groups and between Zn-CaP coating on diverse substrates (P3) are shown according to the Mann-Whitney test. Small variation of surface areas of CaP coatings (Table 1) were not taken into account.

Table S3. Results of analyte concentration (mM) and pH value in extracts determined by ion-selecTable 5. week.in vitro immersion of one-sided CaP coatings on Ti-based alloys in 0.9% NaCl solution; Me(Q1–Q3).

	1 week							
Groups, n=3	**pH**		**[Ca] total**		**[Ca^{2+}]**		**[PO_4^{3-}]**	
	Ti-6Al-4V	**Ti-6Al-7Nb**	**Ti-6Al-4V**	**Ti-6Al-7Nb**	**Ti-6Al-4V**	**Ti-6Al-7Nb**	**Ti-6Al-4V**	**Ti-6Al-7Nb**
Control (without test samples)	7.29 (7.27-7.30)		0.01 (0-0.01)		0.09 (0.08-0.11)		0 (0-0.04)	
Uncoated metal substrate	7.28 (7.28-7.28)	7.25* (7.24-7.25)	0.01 (0.01-0.01)	0.01 (0.01-0.02)	0.07 (0.07-0.09)	0.09 (0.09-0.09)	0.06 (0.04-0.07)	0.04 (0.03-0.05)

CaP coating	7.46*# (7.32-7.46)	7.46*# (7.43-7.46)	0.82*# (0.30-1.12)	0.96*# (0.90-1.11)	1.04*# (0.49-1.36)	1.21*# (1.17-1.41)	0.40*# (0.22-0.47)	0.35*# (0.32-0.46)
Zn-CaP coating	7.42*# (7.41-7.46)	7.51*# (7.47-7.52)	0.91*# (0.80-0.93)	1.11*# (1.06-1.13)	1.16*# (0.88-1.22)	1.44*# (1.33-1.45)	0.27*# (0.22-0.28)	0.31*# (0.30-0.40)
Groups, n=3	**2 weeks**							
	pH		[Ca] total		$[Ca^{2+}]$		$[PO_4^{3-}]$	
	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb
Control (without test samples)	7.29 (7.29-7.36)		0.01 (0.01-0.02)		0.09 (0.08-0.11)		0.04 (0-0.06)	
Uncoated metal substrate	7.28 (7.28-7.30)	7.29 (7.26-7.31)	0.01 (0-0.01)	0.02 (0.02-0.03)	0.09 (0.08-0.10)	0.11 (0.09-0.14)	0.07 (0.06-0.08)	0.06 (0.06-0.07)
CaP coating	7.29 (7.29-7.41)	7.40*# (7.40-7.42)	0,29*# (0.29-0.77)	0.60*# (0.58-0.72)	0.46*# (0.46-0.98)	0.82*# (0.81-0.95)	0.35*# (0.32-0.36)	0.47*# (0.46-0.48)
Zn-CaP coating	7.30 (7.28-7.31)	7.38*# (7.38-7.41)	0,23*# (0.18-0.27)	0.65*# (0.62-0.74)	0.39*# (0.34-0.46)	0.87*# (0.86-0.96)	0.29*# (0.23-0.35)	0.49*# (0.48-0.54)
Groups, n=3	**3 weeks**							
	pH		[Ca] total		$[Ca^{2+}]$		$[PO_4^{3-}]$	
	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb
Control (without test samples)	7.29 (7.29-7.30)		0.01 (0-0.01)		0.09 (0.08-0.10)		0.03 (0-0.08)	
Uncoated metal substrate	7.29 (7.29-7.31)	7.27 (7.27-7.29)	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.10 (0.09-0.11)	0.10 (0.09-0.12)	0.04 (0.03-0.05)	0.04 (0-0.05)
CaP coating	7.27*# (7.26-7.28)	7.30 (7.28-7.32)	0.03*# (0.03-0.10)	0.20*# (0.19-0.22)	0.13*# (0.12-0.24)	0.36*# (0.36-0.41)	0.10 (0.08-0.16)	0.25*# (0.23-0.30)
Zn-CaP coating	7.27# (7.26-7.29)	7.27 (7.27-7.29)	0.07*# (0.05-0.10)	0.16*# (0.15-0.16)	0.21*# (0.17-0.24)	0.31*# (0.31-0.32)	0.15*# (0.12-0.20)	0.22*# (0.20-0.23)
Groups, n=3	**4 weeks**							
	pH		**[Ca] total**		**$[Ca^{2+}]$**		**$[PO_4^{3-}]$**	
	Ti-6Al-4V	**Ti-6Al-7Nb**	**Ti-6Al-4V**	**Ti-6Al-7Nb**	**Ti-6Al-4V**	**Ti-6Al-7Nb**	**Ti-6Al-4V**	**Ti-6Al-7Nb**
Control (without test samples)	7.28 (7.27-7.29)		0.01 (0.01-0.02)		0.11 (0.11-0.11)		0,06 (0-0,06)	
Uncoated metal substrate	7.28 (7.24-7.29)	7.28 (7.26-7.29)	0.01 (0.01-0.01)	0.02 (0.01-0.02)	0.09 (0.08-0.09)	0.10 (0.08-0.10)	0.05 (0.04-0.06)	0.06 (0.04-0.08)

CaP coating	7.27 (7.21-7.28)	7.21*# (7.18-7.25)	0.01 (0.01-0.03)	0.06*# (0.05-0.06)	0.11 (0.09-0.15)	0.13*# (0.13-0.16)	0.04 (0.03-0.08)	0.13 (0.06-0.14)
Zn-CaP coating	7.25 (7.24-7.29)	7.22*# (7.21-7.23)	0.03# (0.02-0.03)	0.05*# (0.04-0.05)	0.12# (0.10-0.13)	0.14*# (0.12-0.15)	0.06 (0.05-0.20)	0.10*# (0.09-0.12)

	5 weeks							
Groups, n=3	pH		[Ca] total		[Ca^{2+}]		[PO_4^{3-}]	
	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb	Ti-6Al-4V	Ti-6Al-7Nb
Control (without test samples)	7.30 (7.30-7.30)		0.02 (0.01-0.03)		0.11 (0.11-0.13)		0 (0-0.02)	
Uncoated metal substrate	7.30 (7.29-7.31)	7.27* (7.25-7.28)	0.01 (0.01-0.01)	0.01 (0.01-0.01)	0.09 (0.08-0.11)	0.07* (0.06-0.08)	0.01 (0-0.02)	0 (0-0.01)
CaP coating	7.29* (7.27-7.29)	7.25* (7.23-7.26)	0.03 (0.01-0.04)	0.03# (0.03-0.04)	0.12 (0.09-0.16)	0.10 (0.10-0.12)	0 (0-0.03)	0.03 (0-0.03)
Zn-CaP coating	7.30 (7.25-7.30)	7.26* (7.25-7.26)	0.01 (0.01-0.02)	0.02# (0.02-0.03)	0.10* (0.07-0.10)	0.10*# (0.09-0.10)	0.04 (0-0.05)	0.02 (0-0.06)

n—the number of test samples (wells) tested; *) - Statistical differences ($P < 0.05$) with control group; #) Statistical differences with corresponding uncoated metal substrate are shown according to the Mann-Whitney test. Duplicate probes for each test sample were measured. Small variation of surface areas of CaP coatings (Table 1) were not taken into account.