

***In vitro* Dissolution of Plasma Sprayed HA Coatings of Varying Crystallinity per ASTM F1926**

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Statement of Purpose: Plasma sprayed hydroxyapatite (PSHA) coating is widely used on metal implant surfaces to improve implant fixation. However, variation in process parameters results in a broad spectrum of HA coating crystallinity due to generation of amorphous calcium phosphate (ACP). Effect of HA coating crystallinity on fixation is unclear; *in vivo* animal studies of low and high crystallinity HA coatings have shown inconsistent results. The inconsistency could be attributed to opposite effects of ACP dissolution on biological response and mechanical integrity (adhesion) of HA coatings to metal substrate. Dissolution of PSHA coatings has been characterized before under different testing parameters such as solution compositions, level of supersaturation, and powder-to-solution ratios. The dissolution results may not be directly comparable among different studies. To address this issue, an accepted standard such as ASTM F1926 could be employed to standardize dissolution tests so that the results are comparable. In this study, dissolution rates of plasma sprayed HA coatings of 15% to 74% crystallinity were compared to fully crystalline HA and octacalcium phosphate (OCP) per ASTM F1926-08 [1].

Methods: HA coating powders of various crystallinities were collected by scraping coated flat grit-blasted surface. HA coatings, Low (~15%), Medium (Med, ~60%) to Medium High (M-H, ~74%) crystallinity, were compared to NIST SRM 2910a HA (NIST). OCP was prepared using the method as described by LeGeros [2]. Dissolution test solutions (0.08mM CaCl₂, 0.05mM K₃PO₄, and 0.08mM NaCl) were prepared at pH 7.4 using 1M Tris-HCl and at pH 5.5 using 0.5M MES (2-(N-morpholino) ethanesulfonic acid) buffer solutions in accordance with ASTM F1926. A Ca²⁺ ion selective electrode (9720BNWP, Thermo Scientific) was used to measure Ca²⁺ release every three minutes for 24 hours. Ca probe was calibrated using 1, 5 and 10 ppm Ca standards at 37 ± 0.5 °C. 4.5 mg of HA powders were added directly to 450 ml of test solution in a 1L double glass incubator for a 0.01 mg/ml ratio. All dissolution tests were done at 37.0 ± 0.5 °C. Theoretical calculations of supersaturation ratio (SR) were made with Chemist software (v1.0.3, Micromath) using thermodynamic constants at 37 °C published by NIST [3]. An X-ray diffractometer (45 V and 40 mA, Philips Analytical) with programmed divergence slit was employed to collect diffraction patterns of Ca-P powders from 3° to 60° (2θ). **Results:** X-ray diffraction (XRD) patterns of HA coatings (not shown) reveal the relative amount of ACP to HA decreases dramatically from low crystallinity HA coating to medium crystallinity HA. Both XRD patterns of NIST 2910a and OCP agree with standard XRD patterns from JCPDS. Atomic absorption (Ca) and UV-vis (P) shows Ca/P ratios of HA and OCP are 1.67 ± 0.04 and 1.33 ± 0.02, respectively. Table 1 shows the percentage of mass dissolved calculated for NIST, HA coatings, and OCP

assuming stoichiometry. OCP lost 87-90% of total mass at both pH 7.4 and pH 5.5. HA showed little mass loss in pH 7.4 but was roughly half dissolved at pH 5.5 over 24 hrs. With respect to HA coatings, total mass loss and initial dissolution rate (15 minutes) increased with decreasing crystallinity of HA coatings at either test solution pH. Interestingly, mass loss of the same HA coatings did not always increase at lower test solution pH. Lower pH did not increase mass loss of Low and Med HA coatings but it did increase for M-H HA coatings. HA coatings were composed of ACP and crystalline HA, the latter (crystalline HA) shows unambiguously higher dissolution rate at lower pH. A possible explanation for the lack of further increase of dissolution of Low and Med crystallinity HA coatings could be less dissolution of ACP at lower pH. Table 2 describes the SR of HA and OCP in blood plasma and ASTM F1926 specified solutions. Physiological body fluid (pH 7.4) has much higher SR (> x15) than does ASTM pH 7.4 condition for both HA and OCP. SR in local inflammatory condition (pH 4-5) of body fluid is more closely represented by ASTM pH 5.5 condition.

Table 1: pH 7.4 and pH 5.5 Dissolution Results [mean (std)]

Tris pH 7.4	Ca Release (ppm)	Mass Loss (%)	Dissolution Rates (ppm/hr)		
			15min	60min	24hr
OCP	3.31 (0.21)	87 (9)	4.63 (1.38)	1.15 (0.60)	0.09
Low	3.92 (0.31)	98 (8)	5.77 (1.13)	2.30 (0.48)	0.00
Med	2.45 (0.40)	61 (10)	5.57 (2.73)	1.03 (0.17)	0.01
M-H	0.61 (0.08)	15 (2)	0.50 (0.15)	0.17 (0.06)	0.00
NIST	0.12 (0.04)	3 (1)	0.20 (0.32)	0.10 (0.06)	0.00

MES pH 5.5	Ca Release (ppm)	Mass Loss (%)	Dissolution Rates (ppm/hr)		
			15min	60min	24hr
OCP	3.16 (0.40)	90 (9)	4.64 (0.93)	1.29 (0.16)	0.02
Low	3.28 (0.46)	82 (12)	3.58 (1.34)	1.49 (0.24)	0.05
Med	2.35 (0.28)	59 (7)	2.68 (1.09)	1.17 (0.43)	0.01
M-H	1.65 (0.30)	41 (8)	1.59 (0.54)	0.63 (0.13)	0.03
NIST	2.12 (0.59)	53 (15)	2.36 (0.21)	1.02 (0.11)	0.01

Table 2: Supersaturation Ratios (SRs)

Test Solution SR Initial	ASTM		Blood plasma	
	pH 7.4 Tris	pH 5.5 MES	pH 7.4	pH 4.5
HA	0.80	0.07	13.59	0.14
OCP	0.12	0.02	2.02	0.06

Conclusions: In either pH 7.4 or pH 5.5 test solutions, mass loss was positively correlated with ACP content. While lower pH of solution caused higher dissolution (mass loss and initial dissolution rates) in higher crystalline coatings (M-H) and NIST HA, lower pH did not cause higher mass loss in ACP rich coatings (Low and Med). OCP dissolution behavior was similar to low crystallinity HA coating at both pH conditions.

References: (1) ASTM F1926-08. 01 Apr 09; (2) LeGeros RZ. *Calcif Tissue Int* .1985; 37:194-197; (3) Markovic et al. *Mineral Scale Formation and Inhibition* (Ed. Z. Amjad), 1995: 271-282.