Electronic Supplementary Material (ESI) for Environmental Science: Advances.

Electronic Supplementary Information

Hemocompatibility of biogenic phosphorus nano-agromaterials at environmentally relevant and supra-environmental concentrations for occupational exposure

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1. Phosphorus – based nanomaterials (P-Based NMs) used in the study: origin/synthesis, characterizations and properties.

We used four different types of nanohydroxyapatites (nHAPs) and one nanophosphorus (nP) variant derived from rock phosphate (RP). These nanomaterials (NMs) were thoroughly characterized using different physicochemical techniques (**ESI table 1**). The NMs were characterized in their pristine form. 1mg.mL^{-1} of each of these NMs were prepared in de-ionized water and characterized for shape and size using Scanning Electron Microscope (EVO, MA10, Carl Zeiss, Oberkochen, Germany) and TEM (Tecnai G2 30-U twin microscope, FEI, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at voltage of 200 kV. To measure the hydrodynamic diameter and zeta potential, NMs were freshly prepared in de-ionized water (1mg.mL^{-1}) and sonicated for 30 min before being measured by dynamic light scattering (DLS) (Zetasizer Nano-ZS, Malvern, UK). The elemental composition for Ca and P was determined from AAS (iCE 3000 AA05123903 v1.30, ThermoFisher Scientific, USA) and ICP/MS (ICP-MS 7900 with UHMI, Agilent technologies, California, USA). For functional group identification, sample analysis was performed on Thermofisher FTIR spectrometer with FIR attachment at ATR mode (Nicolet iS50 FTIR Tri-detector, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at room temperature in the range of 4000-400 cm^{-1} with 100 scans per sample. The synthesized particles were also characterized by X-ray diffraction (XRD). Powder XRD spectra were recorded at room temperature, using Bruker, D8 discover high resolution X-ray diffractometer (Bruker, Billerica, Massachusetts, USA) with Cu Kα = 1.5406 Å, 3 kW as radiation source operating at 40 kV and 40 mA. The diffraction patterns were collected over a 2θ range from 20° to 90° with an incremental step size of 0.02° using flat plane geometry. The acquisition time was set at 2 seconds for each scan. The heat capacity and thermal stability of nP and nHAP (biologically synthesized and commercially available) were assessed by differential scanning calorimetry (DSC-60,

Shimadzu, Kyoto, Japan) where 2 mg of dried sample was first crimped in the aluminum pan and the sealed pan was then loaded to DSC analyzer for analysis from 25°C - 550°C.

ESI Table 1. Phosphorus – based nanomaterials (P-Based NMs) used in the study: origin/synthesis, characterizations and properties.

	nHAP_B[1]	**nHAP_C**	**nHAP_Sigma**	**nHAP_SRL**	**nP**
Origin/Synthesis	**Biosynthesis**	**Chemical synthesis**	**Commercial (SigmaAldrich)**	**Commercial (SRL Pvt. Ltd.)**	**Biosynthesis**
Shape	**Platelet**	**Rod**	**Sphere**	**Needle**	**Dots**
Size by electron microscope [nm ± S.D.]	35.74 ± 11.65	L = 83.92 ± 17.98 W = 26.85 ± 3.74	33.9 ± 8.6	L = 64.64 ± 4.33 W = 14.01 ± 1.26	~5-10 nm
Hydrodynamic size [nm ± S.D.]	325.8 ± 37.1	756.2 ± 28.8	874.3 ± 51.5	892.8 ± 21.1	798.5 ± 23.36
Zeta potential [mV ± S.D.]	−31.3 ± 3.5	−45.2 ± 1.7	−10.3 ± 0.3	−9.7 ± 0.9	-11.61 ± 0.01
Ca to P ratio	~1.6	~1.6	~1.6	~1.6	~3.8
FTIR peaks	Stretching and bending peaks for PO_4^{3-} and CO_3^{2-}. Absorption peak for CO_3^{2-}.				
XRD peaks	apatite	apatite	apatite	apatite	Tri calcium phosphate and calcium penta-phosphate
DSC peaks (25°C - 550°C)	No peak	No peak	No peak	No peak	451.66 with heat: -49.4mJ

2. Statistical analysis of blood contact properties: hemolysis, blood cell aggregation assay and plasma coagulation time

The overall effect on hemolysis, blood cell aggregation and plasma coagulation time after exposure to biologically synthesized nHAP (nHAP_B), chemically synthesized nHAP (nHAP_C), SigmaAldrich nHAP (nHAP_Sigma), SRL nHAP (nHAP_SRL), nanophosporous (nP), rock phosphate bulk control (RP), and calcium phosphate bulk control (Ca_3PO_4), were subjected to ANOVA to check if the overall variance was significant. This would not

explicitly tell where the significant differences lie and was thus, then followed by a Tukey post hoc multiple comparison test, to identify the treatment groups with significant difference in mean values. A p-value less than 0.05 was considered as statistically significant.

	nHAP_B (Platelet)	nHAP_C (Rod)	nHAP_ Sigma (Spherical)	nHAP_SRL (Needle)	nP (Dots)	Ca_3PO_4 (Bulk material)	RP (Bulk material)
nHAP_B (Platelet)	-						
nHAP_C (Rod)	<0.0001	-					
nHAP_Sigma (Spherical)	0.0479	<0.0001	-				
nHAP_SRL (Needle)	<0.0001	<0.0001	<0.0001	-			
nP (Dots)	<0.0001	<0.0001	0.0002	<0.0001	-		
Ca_3PO_4(Bulk material)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	
RP (Bulk material)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

p>0.05
p<0.05
p<0.01
p<0.001
p<0.0001

ESI Figure 1. P-value orthogonal matrix evaluated using Tukey's multiple comparisons test between different NMs and bulk materials for the effects of their concentration on hemolysis. The colour gradient (figure inset) denotes different levels of significance.

	nHAP_B (Platelet)	nHAP_C (Rod)	nHAP_ Sigma (Spherical)	nHAP_SRL (Needle)	nP (Dots)	Ca_3PO_4 (Bulk material)	RP (Bulk material)
nHAP_B (Platelet)	-						
nHAP_C (Rod)	>0.9999	-					
nHAP_Sigma (Spherical)	>0.9999	>0.9999	-				
nHAP_SRL (Needle)	>0.9999	>0.9999	>0.9999	-			
nP (Dots)	>0.9999	>0.9999	>0.9999	>0.9999	-		
Ca_3PO_4(Bulk material)	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	-	
RP (Bulk material)	0.9998	0.9998	0.9998	0.9998	0.9998	0.9998	-

p>0.05
p<0.05
p<0.01
p<0.001
p<0.0001

ESI Figure 2. P-value orthogonal matrix evaluated using Tukey's multiple comparisons test between different NMs and bulk materials for the effects of their concentration on blood cell aggregation. The colour gradient (figure inset) denotes different levels of significance.

	nHAP_B (Platelet)	nHAP_C (Rod)	nHAP_ Sigma (Spherical)	nHAP_SRL (Needle)	nP (Dots)	Ca_3PO_4 (Bulk material)	RP (Bulk material)
nHAP_B (Platelet)	-						
nHAP_C (Rod)	<0.0001	-					
nHAP_Sigma (Spherical)	<0.0001	<0.0001	-				
nHAP_SRL (Needle)	<0.0001	<0.0001	<0.0001	-			
nP (Dots)	<0.0001	<0.0001	>0.9999	<0.0001	-		
Ca_3PO_4(Bulk material)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	
RP (Bulk material)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

p>0.05
p<0.05
p<0.01
p<0.001
p<0.0001

ESI Figure 3. P-value orthogonal matrix evaluated using Tukey's multiple comparisons test between different NMs and bulk materials for the effects of their concentration on plasma coagulation time. The colour gradient (figure inset) denotes different levels of significance.

ESI Table 2. Statistical significance values between protein content determined from different washes (W1, W2 and W3) and the hard corona (HC). The p values for each of the comparison was determined by Tukey's multiple comparison test.

Tukey's multiple comparisons test	Statistical significance summary	P value
nHAP_B		
W1 vs. W2	Not significant	0.0949
W1 vs. W3	****	<0.0001
W1 vs. HC	****	<0.0001
W2 vs. W3	***	0.0008
W2 vs. HC	****	<0.0001
W3 vs. HC	Not significant	0.5530
nHAP_C		
W1 vs. W2	Not significant	0.0982
W1 vs. W3	***	0.0002
W1 vs. HC	****	<0.0001
W2 vs. W3	Not significant	0.1290

W2 vs. HC	****	<0.0001
W3 vs. HC	*	0.0329
nHAP_Sigma		
W1 vs. W2	Not significant	0.0517
W1 vs. W3	****	<0.0001
W1 vs. HC	****	<0.0001
W2 vs. W3	****	<0.0001
W2 vs. HC	****	<0.0001
W3 vs. HC	Not significant	0.9612
nHAP_SRL		
W1 vs. W2	****	<0.0001
W1 vs. W3	****	<0.0001
W1 vs. HC	****	<0.0001
W2 vs. W3	***	0.0009
W2 vs. HC	****	<0.0001
W3 vs. HC	Not significant	0.6044
nP		
W1 vs. W2	Not significant	0.0699
W1 vs. W3	****	<0.0001
W1 vs. HC	****	<0.0001
W2 vs. W3	*	0.0204
W2 vs. HC	**	0.0013
W3 vs. HC	Not significant	0.7586

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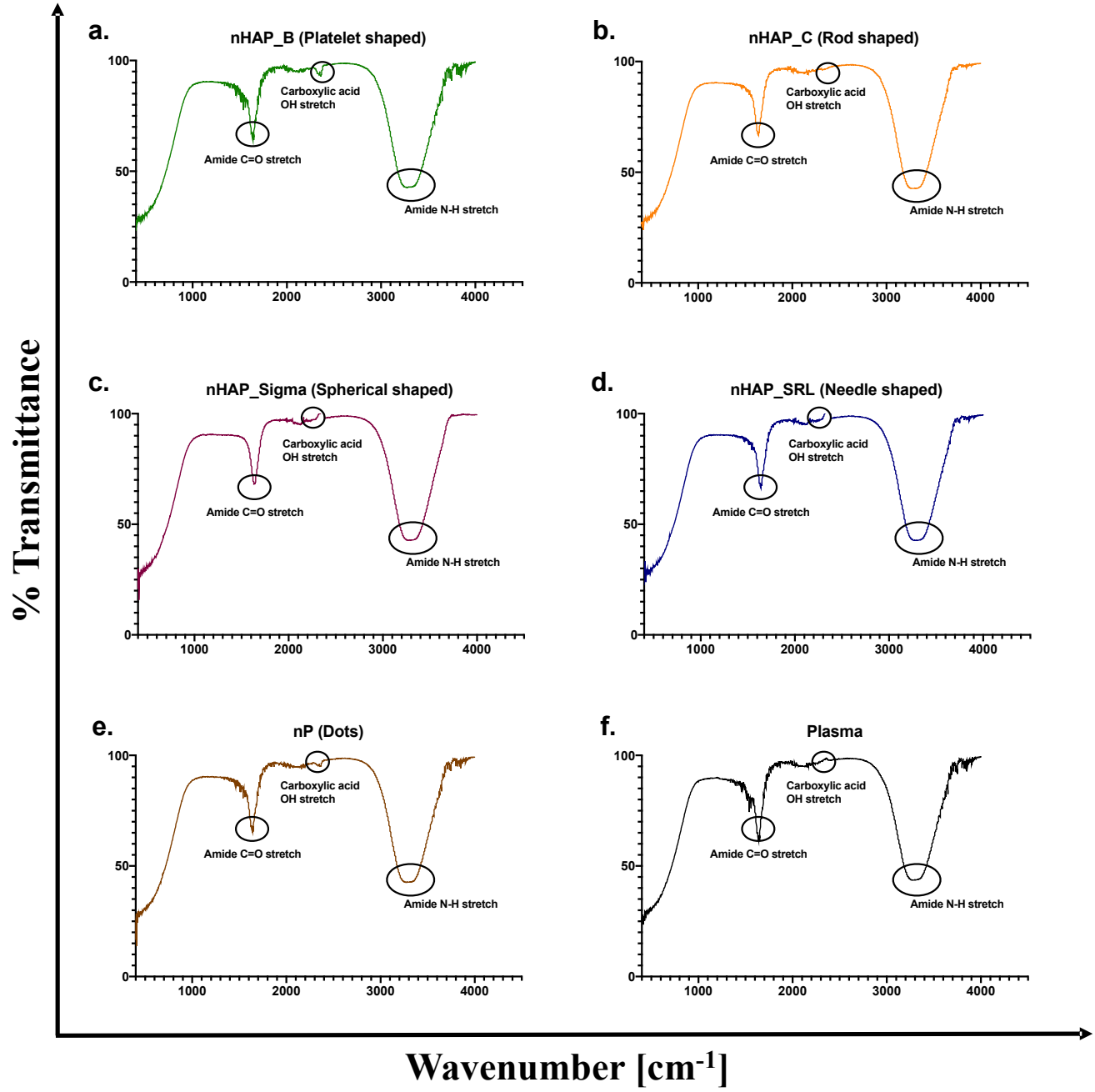

ESI Figure 4. FTIR spectra for hard corona on a. biologically synthesized nHAP (nHAP_B), b. chemically synthesized nHAP (nHAP_C), c. SigmaAldrich nHAP (nHAP_Sigma), d. SRL nHAP (nHAP_SRL), e. nanophosporous (nP) and f. only plasma. Peaks at wavenumbers 1650, 2360 - 2370 and 3270 – 3380 cm^{-1} denote presence of amide C=O stretch, carboxylic acid OH stretch and amide N-H stretch respectively.

Reference:

1. Priyam, A.; Das, R. K.; Schultz, A.; Singh, P. P., A new method for biological synthesis of agriculturally relevant nanohydroxyapatite with elucidated effects on soil bacteria. *Scientific reports* **2019,** *9* (1), 1-14.