Electronic supplementary information to

ARTICLE

Acemannan coated, cobalt-doped biphasic calcium phosphate nanoparticles for immunomodulation regulated bone regeneration

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Deepa Negi,[a] Kumari Bhavya,[a] Durba Pal,[a] and Yashveer Singh[a,b]*

[a]*Department of Biomedical Engineering, Indian Institute of Technology Ropar, Rupnagar-140 001, Punjab, India.*
[b]*Department of Chemistry, Indian Institute of Technology Ropar, Rupnagar-140 001, Punjab, India.*
Email: yash@iitrpr.ac.in

Electronic supplementary information to

ARTICLE

Table of contents **Page #**

Abbreviations

Ace: acemannan.

BCP: biphasic calcium phosphate nanoparticles.

CoBCP: cobalt-doped biphasic calcium phosphate nanoparticles.

RAW264.7: murine leukemic monocyte macrophage cell line.

Quantification of biphasic calcium phosphate nanoparticles (BCP)

Content of HAP and β-tricalcium phosphate in biphasic calcium phosphate nanoparticles

The percentage of hydroxyapatite (HAP) and β-tricalcium phosphate (β-TCP) was estimated by X-ray diffraction (XRD) technique. The XRD of biphasic calcium phosphate nanoparticles was scanned from 2° to 90°. The ratio was determined by using the ratio of intensities of the most intense diffraction peak of hydroxyapatite phase and that of β-TCP. XRD pattern of BCP indexes most intense peaks corresponds to HAP at 32.2° (816) and β-TCP at 25.8° (315). The ratio was calculated and the percentage of HAP in biphasic calcium phosphate and β-TCP were found to be 70.6% and 29.40%.

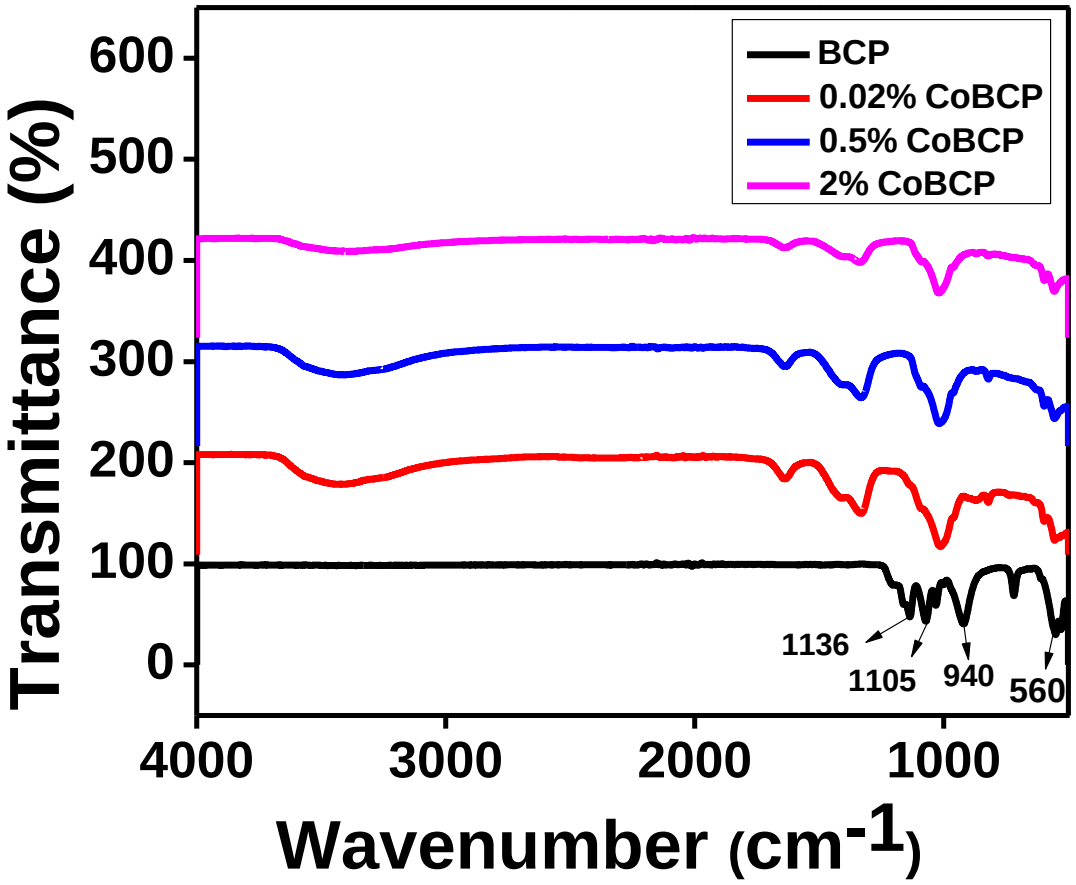

Fig. S1 FTIR spectra of BCP, 0.02% CoBCP, 0.5% CoBCP, and 2% CoBCP.

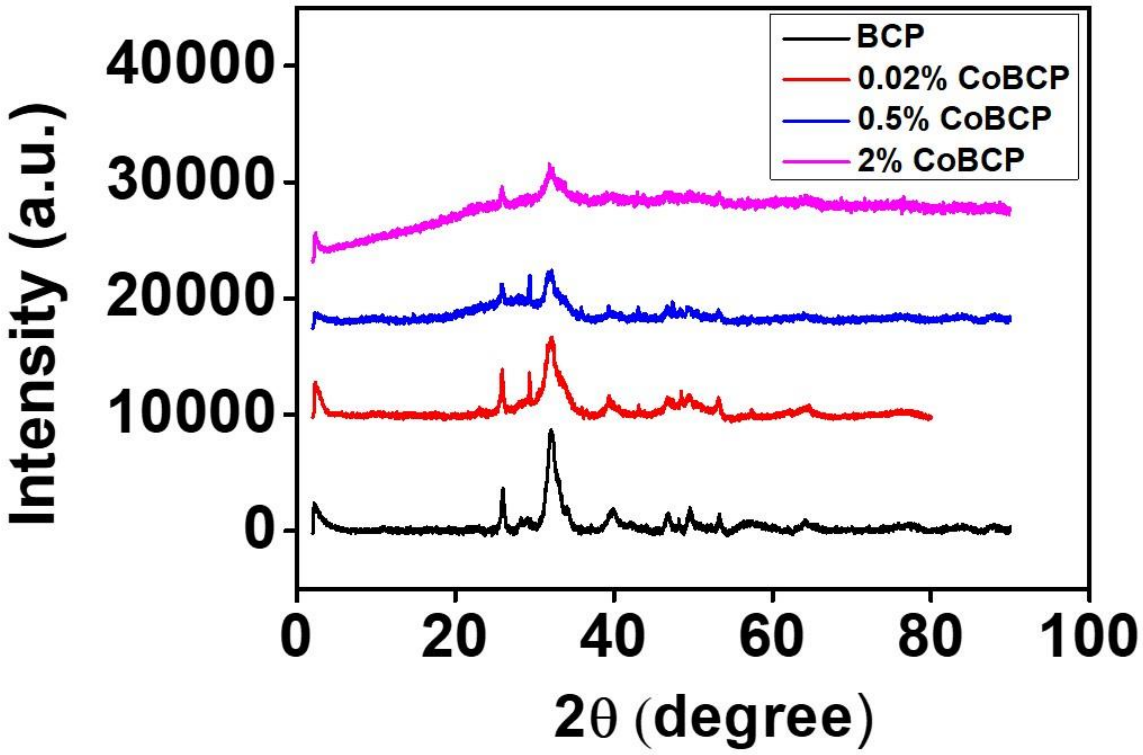

Fig. S2 XRD spectra of BCP, 0.02% CoBCP, 0.5% CoBCP, and 2% CoBCP.

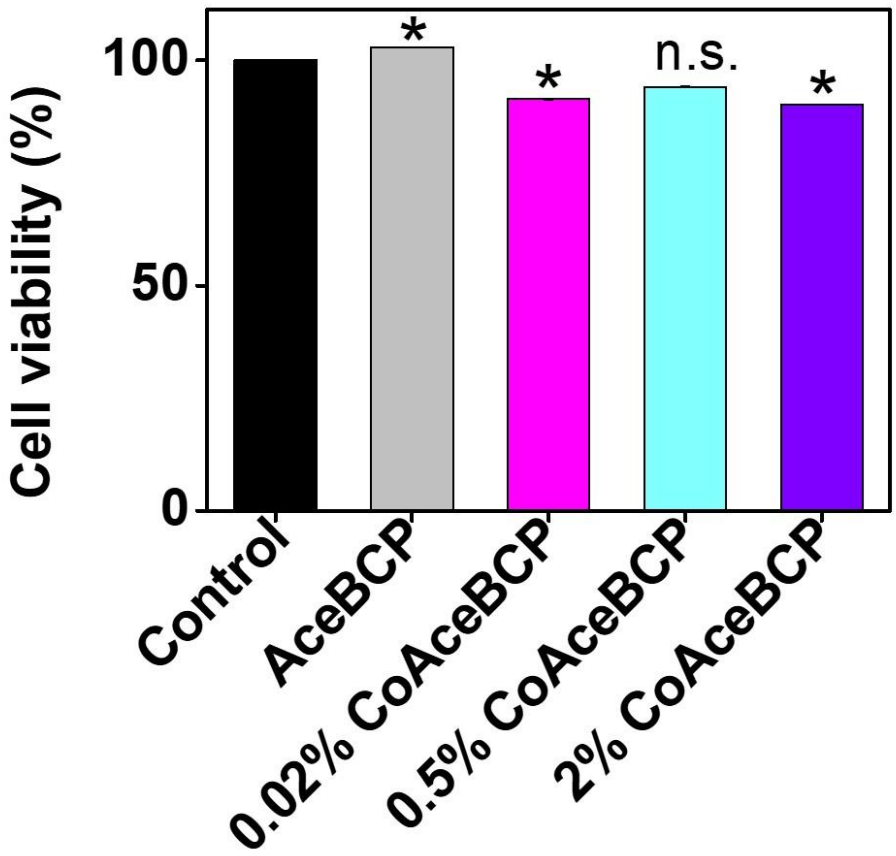

Fig. S3 Viability of RAW264.7 cells in presence of nanoparticles using MTT assay. **p < 0.005 and *p < 0.05 denotes significant difference and n.s. corresponds to non-significant data (n = 3).

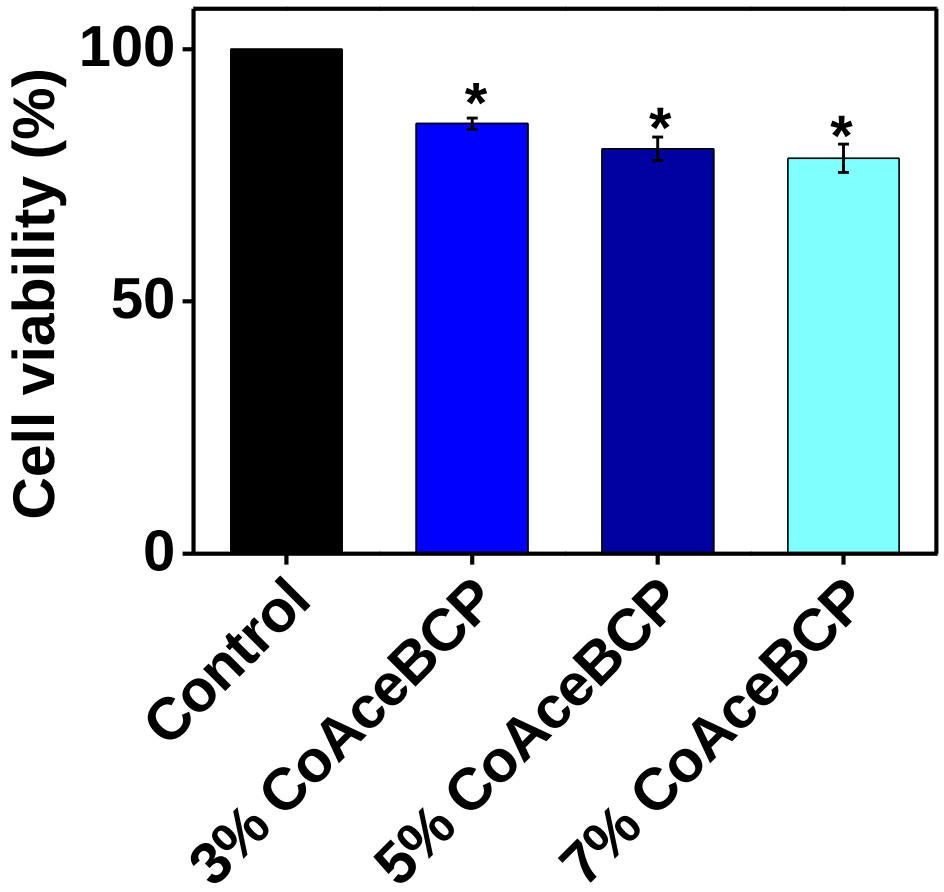

Fig. S4 Cell viability of MC3T3-EI cells in presence of 3%, 5%, and 7% acemannan coated, cobalt-doped biphasic calcium phosphate nanoparticles.

Table S1 List of antibodies used along with their details.

No.	Antibody	Dilution	Company	Catalog No.
1	iNOS	1:400 for IF	CST	13120
2	Arginase-1	1:50 for IF	CST	AM4302
3	Anti-rabbit IgG (Alexa Fluor 488 conjugated)	5 µg/ml for ICC	Invitrogen	A-11034
4	TruStainFcX™ (anti-mouse CD16/32	0.1 µg/million cells for FC	BioLegend	101319
5	FITC anti-mouse CD86	5 µl/million cells for FC	BioLegend	105005
6	APC anti-mouse CD206	5 µl/million cells for FC	BioLegend	141707
7	Anti-rabbit IgG (Alexa Fluor 568 conjugated)	2 µg/ml for ICC	Invitrogen	A-1101
8	HRP-conjugated anti-mouse IgG antibody	1:20000 for WB	Sigma-Aldrich	A9044
9	HRP-conjugated anti-rabbit IgG antibody	1:20000 for WB	Sigma-Aldrich	A9169

Table S2 List of primers and their sequences.

Mouse Primers			
No.	**Gene**	**F.P. (5'-3')**	**R.P. (5'-3')**
1	*β-Actin*	GTACTCTGTGTGGATCGGTGG	AGGGTGTAAAACGCAGCTCAG
2	*CD163*	TGCTCAGGAAACCAATCCCA	ACCTCCACTCTTCCAGCG
3	*CD206*	TTCAGCTATTGGACGCGAGG	GAATCTGACACCCAGCGGAA
4	*CD68*	GGACTACATGGCGGTGGAAT	TGGTCACGGTTGCAAGAGAA
5	*iNOS*	CTTGGTGAAGGGACTGAGCTG	CGTTCTCCGTTCTCTTGCAGT