Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Minerals Substituted Hydroxyapatite Deposited on TiO_2 Nanoporous Modulate the Directional Growth and Activity of Osteoblastic Cells†

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Results and Discussion

Ions released test

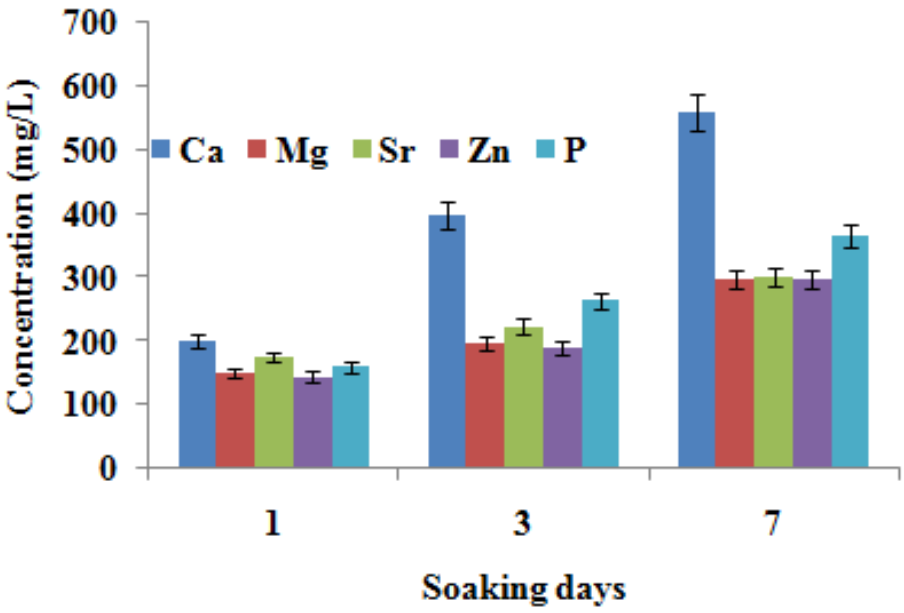

Figure. S1 Ca, Mg, Sr, Zn and P concentrations in SBF solutions after soaking the M-HAP/TiO_2 (at 80^{o}C) for various day periods.

Adhesion strength

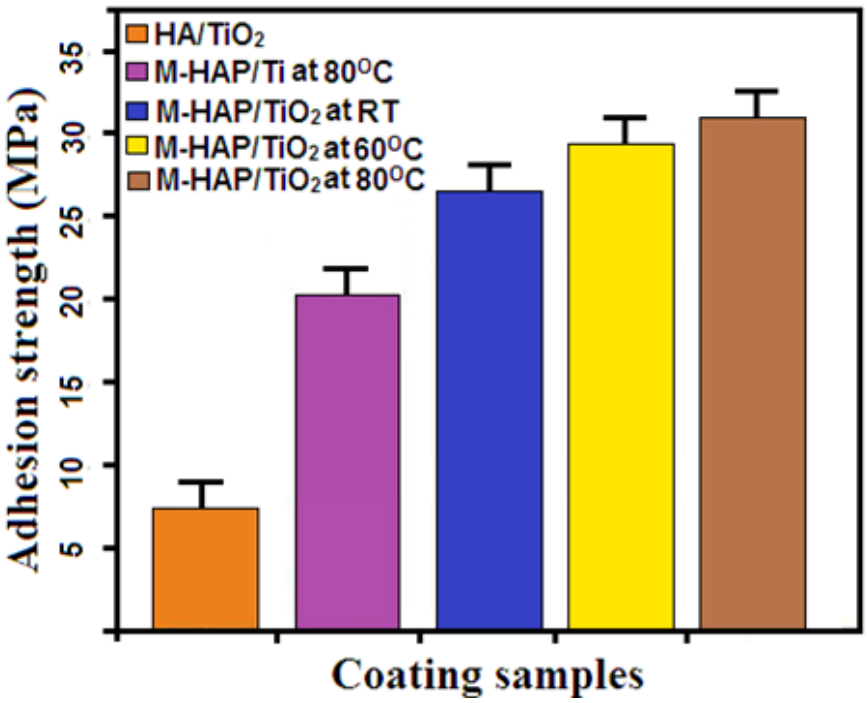

Figure. S2 Adhesion strength of coated samples.

Antimicrobial activity

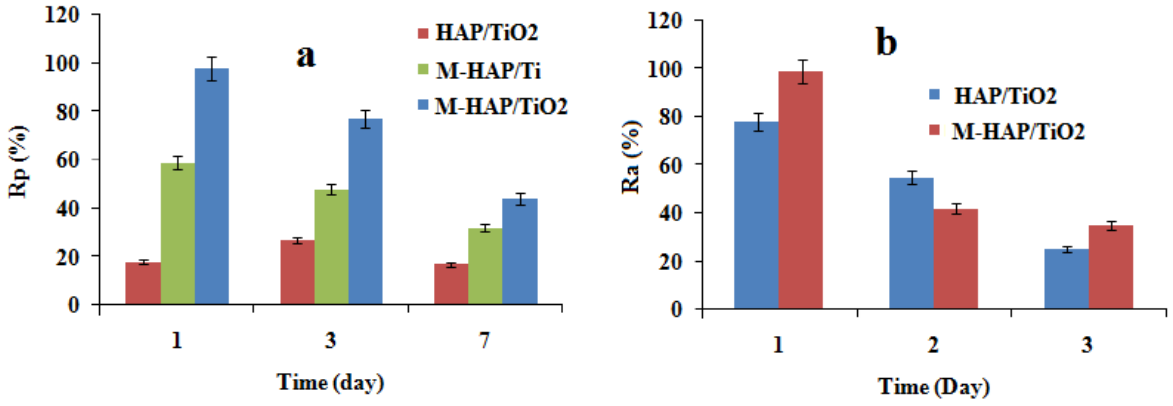

Figure. S3 Antimicrobial activity of M-HAP coatings against (a) E. coli and (b) S.aureus (A= HAP deposition at 80 ^{o}C; B= M-HAP deposition at RT and C= M-HAP deposition at 80 ^{o}C)

1. Experimental Section

1.1. Preparation of nano M-HAP and HAP

From the start, 0.5 wt. % of serine and 0.294 M of $CaCl_2.4H_2O$ (Sigma Aldrich), 0.042 M $SrCl_2.6H_2O$ (Sigma Aldrich), 0.042 M $MgCl_2.6H_2O$ (Sigma Aldrich) and 0.042 M $ZnCl_2.6H_2O$ (Sigma Aldrich) were taken in glass beaker and stirred with vigorously a magnetic stirrer for 30 minutes to make sure the mutual interaction and the self-assembly process was completed. The solution was adjusted to pH 9.5 using NH_4OH (Sigma Aldrich). At that point 0.25 M $(NH_4)_2HPO_4$ (Sigma Aldrich) was added dropwise to the mixture under stirred conditioned 6h and formed a white precipitate. The mixture was placed in a microwave oven and irradiated for 120 seconds at 740W (A CEM Discover microwave synthesizer (Model No: 908010) operating

at 180/264 V and 50/60 Hz with microwave power maximum level of 300 W and microwave frequency of 2455 MHz was employed for the microwave assisted experiments done in this work). Then, the resultant powder was thermally treated at 900°C for 2 h in a muffle furnace to obtain nano minerals substituted bioceramic. The pure HAP was also prepared by the same process yet without Sr, Mg and Zn sources.

2. Characterization

2.1. HRTEM analysis

The morphology and size of the as-prepared nanoparticles were characterized using high resolution transmission electron microscopy (HRTEM JEM-2010, JEOL). The nanoparticles for HRTEM analysis were dispersed in acetone by sonication and loaded on a carbon coated copper mesh.

2.2. Protein adsorption assay

A 1 ml droplet of least crucial medium (a-MEM) (Hyclone) containing 10% fetal calf serum (FCS, Hyclone) was pipetted onto every example set in 24-well plates. After brooding at $37^{O}C$ for 2 h, the examples were put in new 24-well plates and washed with PBS three times. An aliquot of 500μL of 1% sodium dodecyl sulfate (SDS) arrangement was added to these wells, which were shaken at 200 rpm for 1 h to segregate proteins from the surfaces of nano ceramic on titanium implants. A Micro BCA protein test pack (Boster) was utilized to gauge the protein fixations in the SDS arrangements.

3. Results and Discussion

3.1. HRTEM analysis of the synthesized HAP and M-HAP nanoparticles

The size and morphology of the synthesized HAP and M-HAP nanoparticles were further assessed by HRTEM and the picture is demonstrated in Figure. S4. As can be visually perceived from the micrograph it is clear that the HAP and M-HAP demonstrated the particles with the size

extended from100 to150 nm. EDAX examination obviously demonstrated the vicinity of Ca, P, O, Sr, Mg and Zn in the HAP [1]. Subsequently, EDAX examination affirmed that, no unclean components were discovered in the structure.

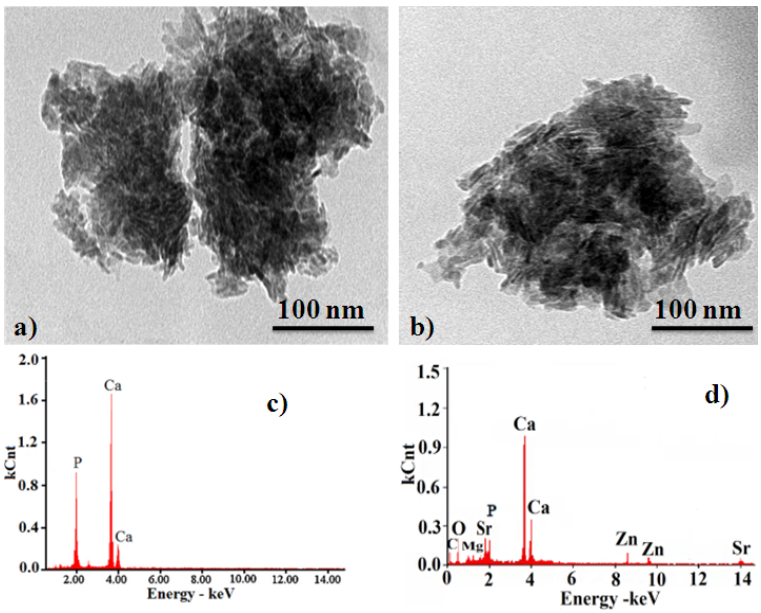

Figure. S4 TEM images of (a) HAP and (b) M-HAP.

Protein adsorption

The control of protein/surface collaborations by outside jolts is often needed in bio-applications [2]. Protein adsorption information from the culture medium with 10% FCS after 2 h of brooding is shown in Figure. S5. The measure of protein on the M-HAP/Ti, TiO_2 and HAP/TiO_2 specimen was short of what that on the M-HAP/TiO_2 covering at condition RT, 60 and 80^{o}C specimens, resulting about enhanced osseointegration between bone and implant.

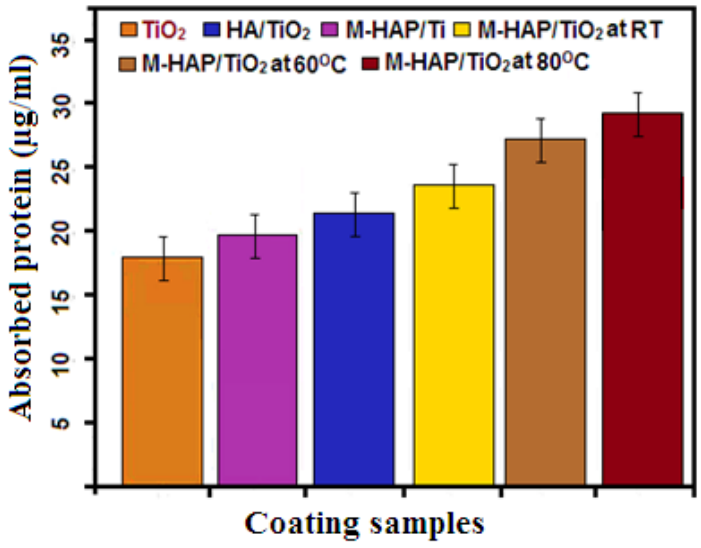

Figure. S5 Measure of adsorbed protein on distinctive specimens after 2 h of incubation

REFERENCES

1 S.C. Cox, P. Jamshidi, L.M. Grover, and K.K. Mallick, Mater Sci Eng C Mater Biol Appl. 2014, 35, 106.

2 W.Yang, Z. Tang, Y. Luan, W. Liu, D. Li and H.Chen, ACS Appl. Mater. Interfaces s 2013, 5, 3816−3823.