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Electronic Supplementary Information (ESI) for

Silanized NaCa₂HSi₃O₉ nanorods with controlled pH value on Ti

for improving osteogenesis and angiogenesis in vitro

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For cross-sectional observation, the samples were cut to form a groove in the cross-section, mechanically broken off and then observed by a field emission scanning electron microscope (FESEM, SU6600, Hitachi, Japan).



Figure S1 Cross-sectional SEM morphologies of (a) HT and (b) HT-S coatings, showing their thicknesses of nanorod layers are about 2.5 μm.

In order to observe the morphologies of cells adhered on different surfaces, fluorescence staining of vinculin, actin and cell nucleus was performed with actin cytoskeleton and focal adhesion staining kit (FAK100, Milipore, USA) according the instruction as described elsewhere [1, 2]. The fluorescence-stained cells were observed by a laser confocal microscope (FV1200, Olympus, Japan) with a high-power laser and an oil-immersion objective lens.

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Figure S2 Vinculin (green), actin (red), and cell nucleus (blue) fluorescence images of hFOB1.19

and HUVEC after 1 day of culture on Ti, CA, and HT-S.



Figure S3 Contact angles of Ti and Ti-S; the insets show the digital photograph of water droplets on the corresponding sample. The NH₂ functionalized silane layer decreased the wettability of Ti surface.

The surface roughness measurements of the samples were performed using laser scanning confocal microscope (VK-9710, KEYENCE, Japan), and scanning areas are $200 \,\mu\text{m} \times 283 \,\mu\text{m}$.



Figure S4 Laser confocal images of different surfaces.

The measured Ra (average roughness) values of the CA, HT and HT-S surfaces are 2.82, 2.64 and 2.61 μ m, respectively, showing that different coatings exhibit similar microporous topography and have similar microscale roughnesses.



Figure S5 Alamarblue assays of hFOB1.19 and HUVECs viability on Ti and Ti-S after 1, 3 and 7 days of incubation. Absorbance values have no statistic difference between two surfaces at each

incubation time, suggesting that silane coating has good cytocompatibility as polished Ti surface.

For the observation of coating/substrate interfaces, samples were embedded in clear acrylic resin, and then the cross-section was mechanically polished using silicon carbide paper and polishing cloth with a liquid suspension of 0.05 µm alumina, orderly. The flat surface ultrasonically cleaned with ethanol and distilled water in an ultrasonic cleaner for 10 min, and then observed by a field emission scanning electron microscope (FESEM, SU6600, Hitachi, Japan).



Figure S6 Cross-sectional SEM morphologies of (a) HT and (b) HT-S coatings, showing that the coatings adhered on substrates tightly, and no obvious cracks formed on the interfaces of coatings and Ti substrates.

References

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