Supporting Information

Interfacial Reaction-Driven Formation of Silica-Carbonate Biomorphs with Subcellular Topographic Features and Their Biological Activity

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Experimental Section

Coating fabrication: High temperature solid reaction was used to synthesize the $Sr_2ZnSi_2O_7$ (hereafter, referred to as SZnS). An atmosphere plasma spraying system (APS, Sulzer Metco, Switzerland) was applied to deposit the SZnS coating on biomedical grade Ti alloy plates (Ti-6Al-4V, Baoji Junhang Metal Material Co., Ltd.) .

Biomorp formation: a. Incubation in cell culture medium, coatings were incubated in cell culture medium with/without 10%FBS at 37^{o}C for 5 hours without extra CO_2 supplementation. *b. Autoclave sterilization process:* Coating samples were put in a small glass bottle with a volume of 10ml containing 3ml buffers containing 4.2mM CO_3^{2-}. The mouths of the bottle were covered by autoclave tapes. The autoclave parameters used for normal sterilization of biological samples are utilized without any modification. After autoclave, the samples were aseptically washed with sterilized water and dried in an aseptic environment. For comparison, some coating samples subjected to UV irradiation for 1h for sterilization were used as control samples.

Characterization： Field emission scanning electron microscopy (Zeiss Ultra Plus FE-SEM) was used to observe the surface topography of the coatings. Raman spectroscopy (Renishaw inVia Raman Microscope), and Thin film X-ray diffraction (Shimadzu 6000 with Cu Kα radiation (λ=1.5418 Å)) were applied to analyze the phase composition of the coating and those coatings subjected to post-treatments with a grazing angle of 1^{o}C. High resolution transmission electron microscope (HRTEM) images and energy dispersion X-Ray spectroscopy (EDS) were also used to further characterize the crystalline structure and chemical composition of the crystals. A JEOL JEM-2100F TEM (EM-20014, UHR, 200 kV, Japan) equipped with a digital camera of type F-216 (TVIPS, Germany), a high angle annular dark field (HAADF) detector and an INCA X-SIGHT EDX system (OXFORD, UK) was used.

Cell culture experiments: MC3T3-E1 cells were used to evaluate the biocompatibility and bioactivity of the produced films. Cell culture, cell seeding on the films and the observation of cell morphology using SEM were performed as previously described [1]. The ALP activity and collagen production were evaluated in the osteoblasts cultured on the coating surface covered by biomorph crystals and the as-sprayed one. The procedures for these two assays were provided in Supplementary materials.

ALP activity measurement: StemTAG^{TM} Alkaline Phosphatase Activity Assay Kit (Colorimetric) (CELL BIOLABS, INC.) was used to evaluate the alkaline phosphatase (ALP) activity of osteoblasts. Cells were seeded with a density of 4×10^4 cells/mL. After culturing

for 14 days, cells were washed twice with cold PBS, and then 150µl cell lysis buffer was added to each well, followed by 10 mins incubation at 4^{o}C. Cell lysis buffer was collected and centrifuged at 13,400 rpm for 10min. The supernatant was collected for protein and ALP analysis. The protein assay and the ALP activity measurement were performed according to the protocols provided by the supplier. The optical density was measured using a plate reader, the wavelengths used for protein and ALP assays were 550nm and 405nm, respectively. The ALP activity was normalized to the intracellular total protein content.

Collagen Assay: For collagen assay, the cells incubated for 14 days were fixed with 4% paraformaldehyde and incubated for 1h with Picro-Sirius Red solution (1g/L direct red 80 in saturated picric acid). To remove thoroughly the unattached dyes, 0.5% acetic acid was used to wash the cells after incubation with picro-sirius red, and then washed with distilled water for several times before quantitative measurement. For collagen quantitative measurement, 0.1M NaOH was used to re-solubilize the dye binding to collagen and the concentration of the dissolved dye was evaluated by the optical density measured at 550 nm using a spectrophotometer. To rule out the possibility that the coating could adsorb the dye, coating samples without cells were incubated with the dye and subjected to the exactly same treatments as those used for the coating with cells. Results show that only a tiny amount of dye left on the coating surface, rendering no significant interference in the collagen assay.

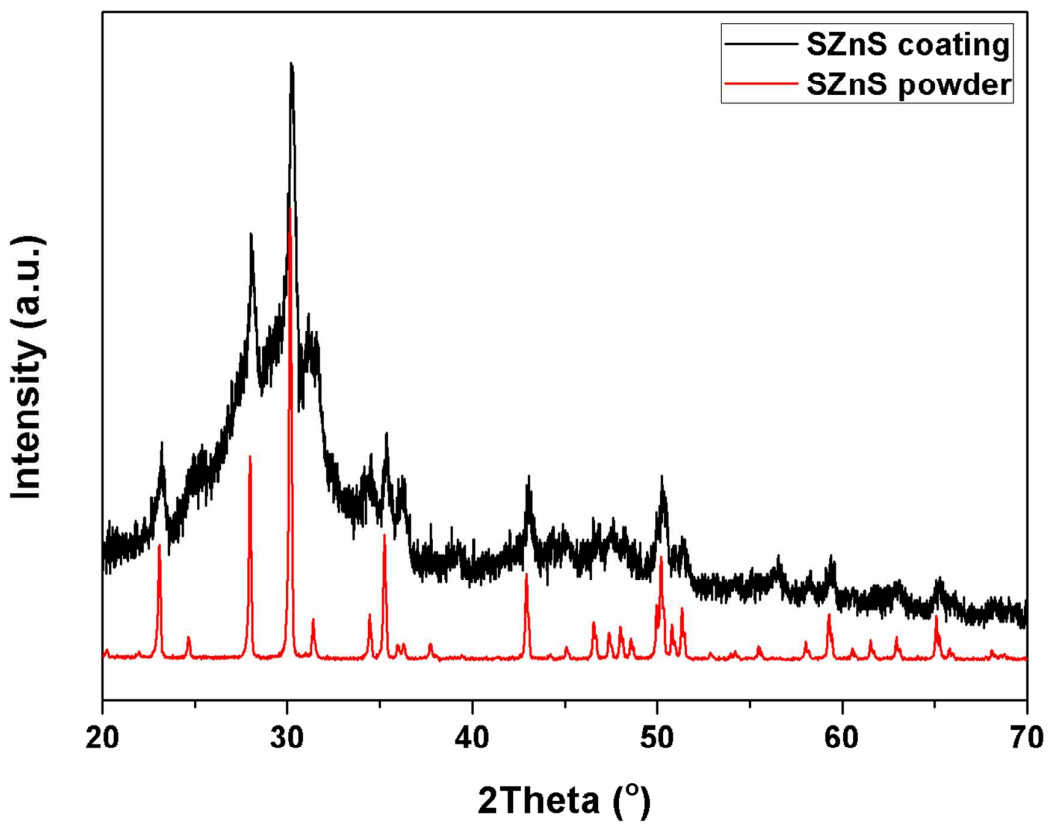

Figure S1. XRD patterns of the as-sprayed SZnS coating and feedstock powders.

The phase composition of the as-sprayed coatings and the feedstock powders were analyzed by XRD. All the peaks present in the XRD patterns of as-sprayed coatings and the feedstock powders can be assigned to $Sr_2ZnSi_2O_7$ (PDF No. 39-0235). The diffraction peaks of the powders are sharp indicating the high degree of crystallization. By contrast, the as-sprayed SZnS coating is less crystallized (glassy), implied by the broadening of the diffraction peaks and appearance of the hump peak at around $25^o - 35^o$ of 2theta.

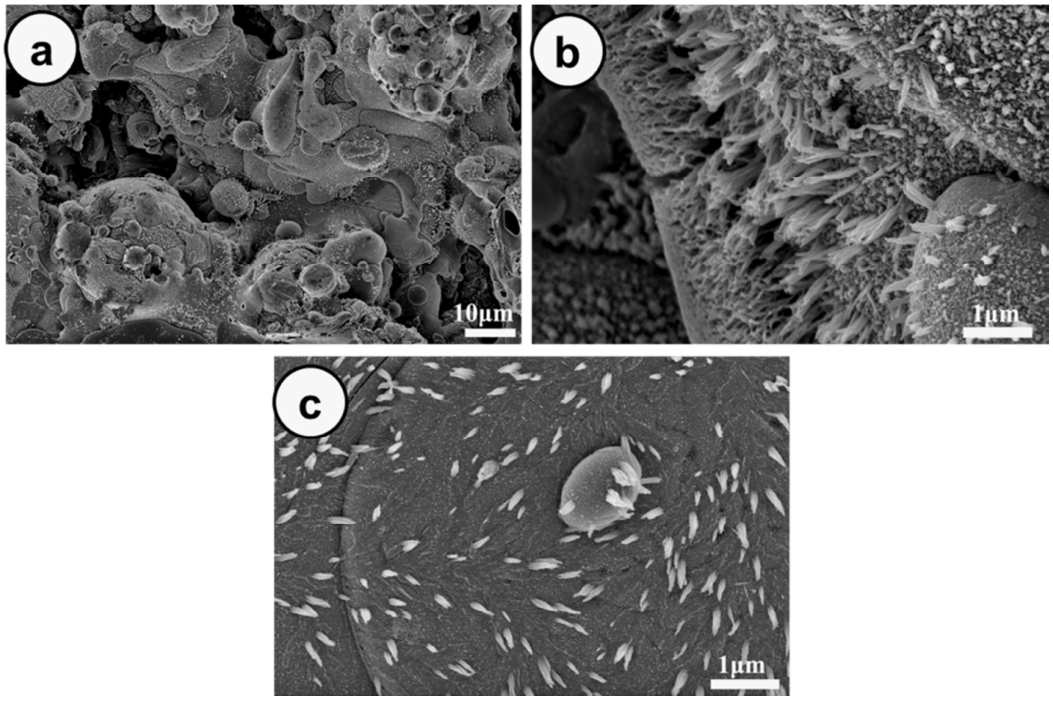

Figure S2. SEM images of the as-sprayed SZnS coating under a lower magnification (a) and higher magnifications (b and c).

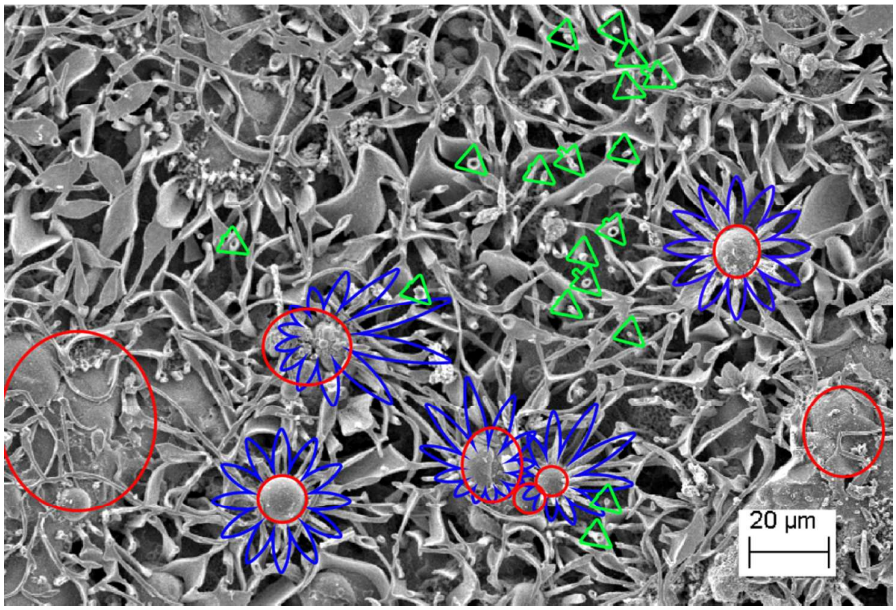

Figure S3. SEM images of the surface biomorph crystals formed on the SZnS coating surface after autoclave sterilization. It can be seen that crystals in the low-lying area grow vertically (marked by green triangles) while those on the upland island (ridges and islands) grow laterally forming a flower-like shapes (marked in blue circles). It is noted that seldom can any crystals be found on the "hilltop" (Red circle).

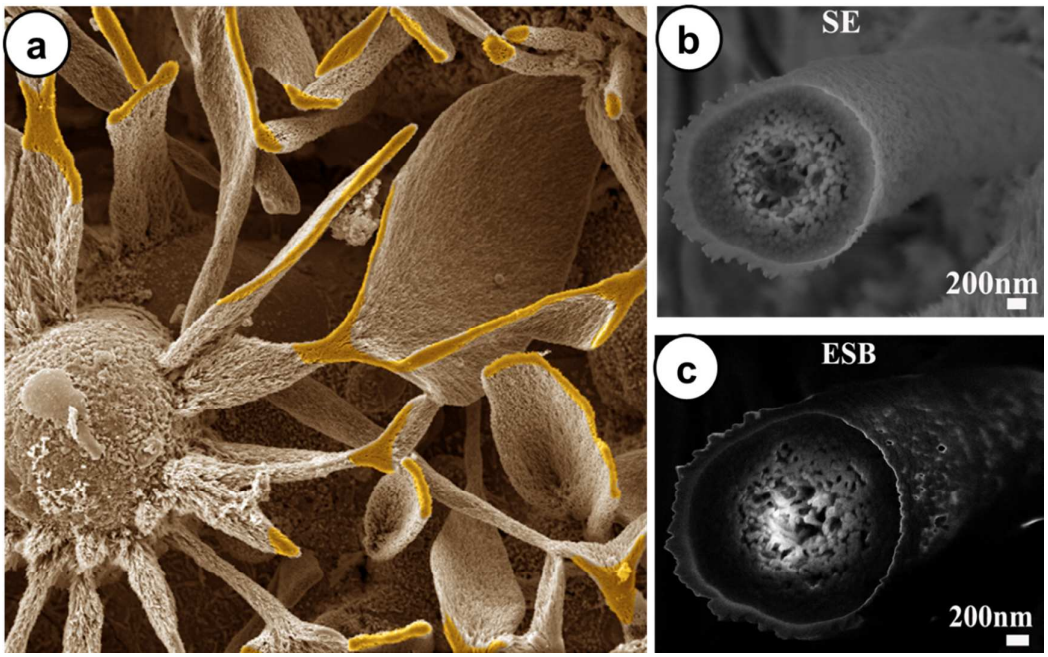

Figure S4. SEM image showing the morphology of the biomorph crystals (a), second electron image (a) and back scattering electron image of a selected stem-like crystal (c).

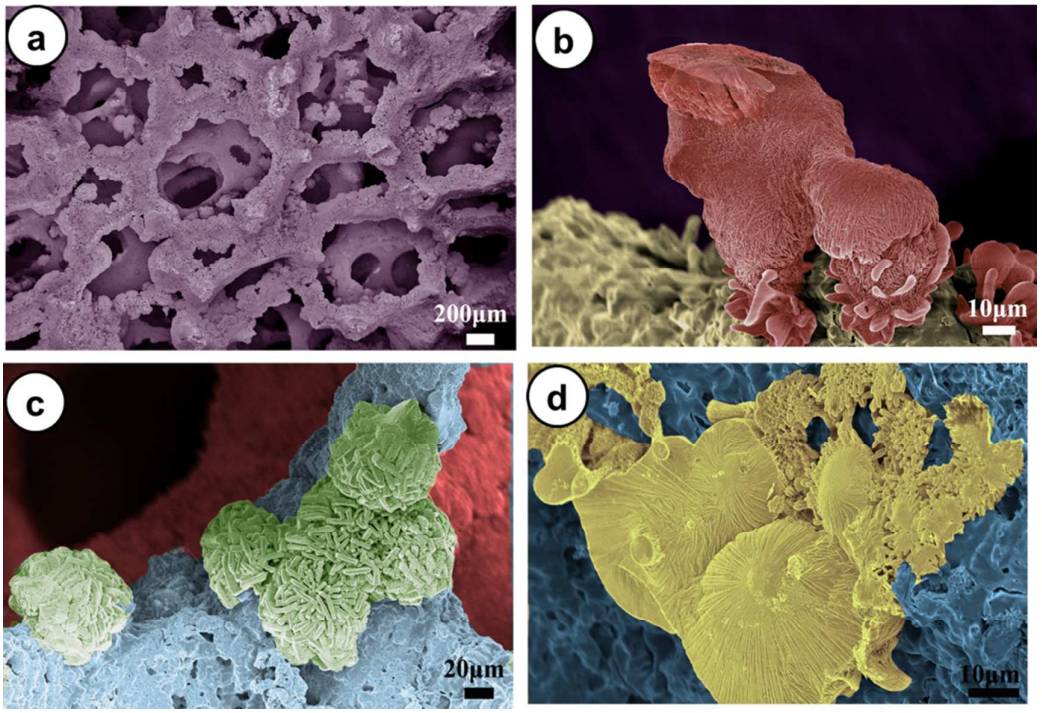

Figure S5. SEM images of the morphology of the SZnS porous scaffold after incubation for 24hours with osteoblasts (a). (b, c and d): some typical morphology of the crystals formed on the surface.

References

1. Yate, L.; Coy, L. E.; Gregurec, D.; Aperador, W.; Moya, S. E.; Wang, G. Nb-C Nanocomposite Films with Enhanced Biocompatibility and Mechanical Properties for Hard-Tissue Implant Applications. *ACS Appl. Mater. Interfaces* **2015**, *7* (11), 6351–6358.