Supporting Information

Strontium and Zoledronate Hydroxyapatites Graded Composite Coatings for Bone Prostheses.

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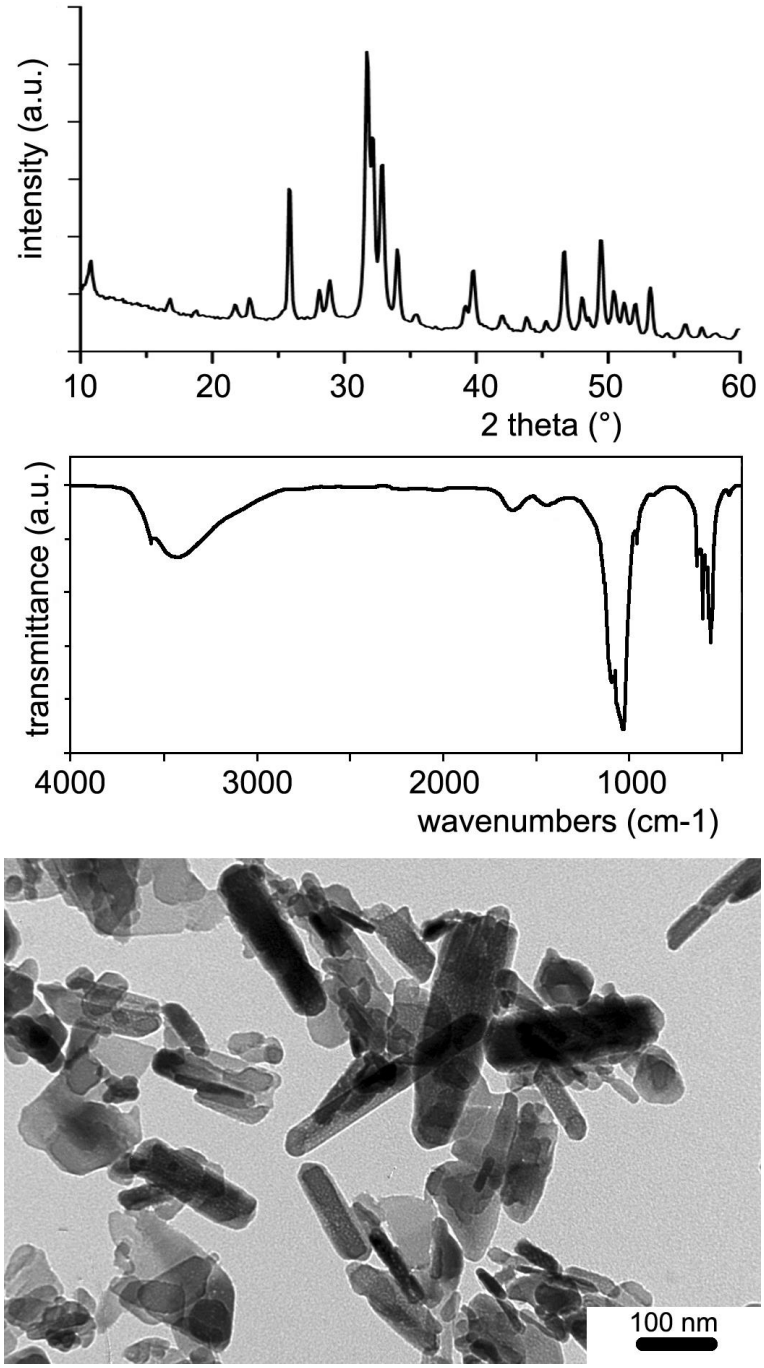

Figure S1 – X-ray diffraction pattern, FT-IR spectrum and TEM image of pure hydroxyapatite powder (HA).

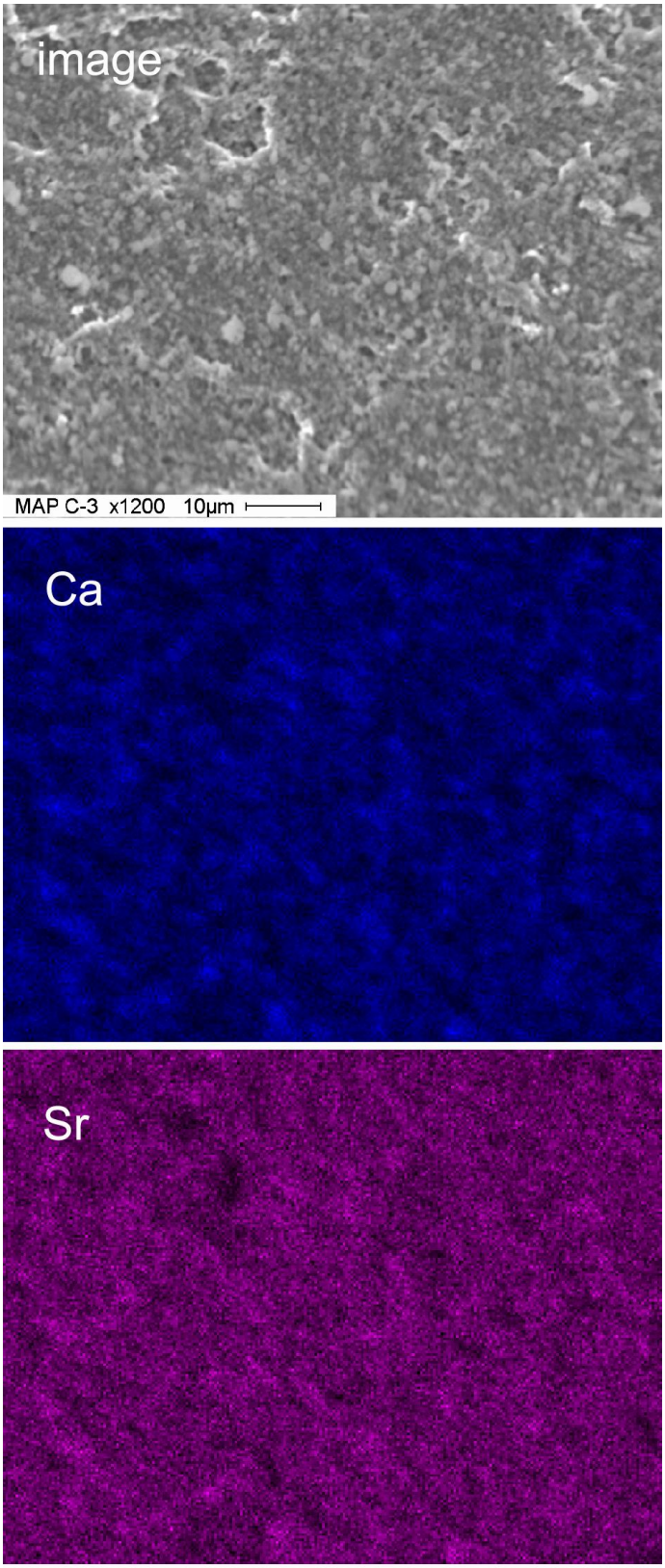

Figure S2 – EDS maps recorded from C-3 sample show the homogeneous distribution of Calcium and Strontium throughout the surface of deposited coating. The image of the area where mapping was performed is also showed and its low resolution is due to the lack of sputter-coating with gold.

Methods for *in-vitro* tests.

Human osteoblasts.

MG-63 human osteoblast-like cells were expanded in DMEM medium (Sigma, UK) supplemented with 10% FCS, and 1% antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin) in standard conditions, at 37°C±0.5 with 95% humidity and 5% CO_2±0.2. Cells were detached from culture flasks by trypsinization, and centrifuged; cell number and viability were checked with trypan blue dye exclusion test for experiment.

Human peripheral blood monocytes.

Peripheral human blood obtained from healthy adult volunteers (Rizzoli Orthopedic Institute Ethic Comittee approval n.33154, 12/09) was used for osteoclast cultures. Density gradient centrifugation was used to separate the mononuclear cells from the other elements of blood. Briefly, a volume of peripheral blood was diluted 1:1 with pre-warmed PBS, carefully layered on an equal volume of Histopaque1077, and was centrifuged with 600g at room temperature for 30min. After centrifugation, the mononuclear cells accumulated at the interface between PBS and Histopaque were collected and washed twice in PBS. Pellet was re-suspended in 1 ml of culture medium (DMEM + 10%FBS); cell number and viability were checked with trypan blue dye exclusion test for experiment.

Osteoblast and osteoclast co-culture.

Monocytes at a concentration of 5×10^5 cells/ml were differentiated in the bottom of 24-well plates in which experimental biomaterials were placed. After 24 hours the non-adherent monocytes were washed off to dispose the culture of contaminating lymphocytes, so that only the adherent monocytes were used for culture. Osteoblasts (2×10^4 cells/ml) were seeded directly on experimental biomaterials and co-cultured with differentiating osteoclasts. Medium was replaced with 50:50 osteoclast differentiation medium (DMEM + 10% FBS, 25ng/ml M-CSF, 30 ng/ml RANKL) and

osteoblast differentiation medium (DMEM + 10% FBS, 10^{-2}M β-Glycerophosphate, 50 μg/ml Ascorbic acid). The same concentration of osteoblasts and osteoclast were seeded in single and in co-culture in empty wells for control of experiment (CTROB, CTROC, CTROB/OC).