Strontium-calcium phosphate hybrid cement with enhanced osteogenic and angiogenic properties for vascularised bone regeneration—supplementary information

1. Materials and methods

1.1. Three-point flexural test

A three-point flexural test was performed to measure the bending strength of cements. According to the ASTM C1421-10, the specimens were fabricated using a polyethylene mould. After solidified for three days, specimens were polished and placed on two supporting pins with a span length of 30 mm, the bending test was applied incrementally at a speed of 0.5 mm/min at the mid-span. The bending strength was calculated from the load–displacement curve:

bending strength: $S = 3F_{max}L/2bh^2$

Where F_{max} is the maximum load on the load–displacement curve, L is the flexure span, b is the specimen width and h is the specimen thickness.

1.2. Cell scanning electron microscopy

The micro morphology of human umbilical vein endothelial cells (HUVECs) adhering on the surface of bone cement disc was observed by Scanning Electron Microscopy (SEM, Quanta 250, FEI, USA). After culturing for 72 h, the discs attaching with cells were dehydrated and dehumidified, following by gold sputter coating procedure for 45 s. After that, the morphology was observed by SEM at an accelerating voltage of 20 kV.

1.3. Alkaline Phosphatase (ALP) staining

ALP staining was conducted using Alkaline Phosphatase Staining kit (Beyotime, China) according to the manufacturer's protocol. Briefly, MC3T3-E1 cells were cultured for osteogenic induction in the 24-well plates (2×10^4 cells per well), after 7 and 14 days of culture, cells were fixed in a 4% paraformaldehyde solution for 15 min. Then, cells were stained with ALP staining solution (prepared by dissolving one SIGMA FASTBCIP/NBT tablet in 10 mL of water) for 30 min and imaged using an Inversion Microscope.

1.4. Cell scratch assay

Cell scratch assay was performed to detect the migration ability of HUVECs under conditioned medium. HUVECs were cultured in the 6-well plates (10⁵ cells per well) using standard growth medium, a uniform and straight scratch was made after cell completely overgrow. To exclude the influence of cell proliferation, replaced original culture medium with conditioned medium or standard medium without serum. At 0, 12 and 24 h, obtained images using an Inversion Microscope and calculated the distance of cell migration by ImageJ.

2. Figures



Figure S1. Bending strength of cements. (* p < 0.05; ** p < 0.01).





Figure S2. The XRD patterns of CPHC and CPC.



Figure S3. Sr²⁺ release profile of Sr-CPHC in cell culture media.



Figure S4. OD values (450nm) of CCK-8 assay on HUVECs at 1 day and 3 days. (NS, no significant difference).



Figure S5. Live/dead staining at 1 day for MC3T3-E1 cells.



Figure S6. SEM micrographs of cells on different cements.



Figure S7. ALP staining after osteogenic-induction for 7 days and 14 days.



Figure S8. (A) Cell scratch assay of HUVECs culturing in medium without serum for 0, 12, and 24 h. (B) The distance of migration in cell scratch assay quantifying by ImageJ. (* p < 0.05; ** p < 0.01).



Figure S9. (A) 3D-reconstruction by Micro-CT after implantation for 4 W. (B, C) Micro-CT analysis of Tb. N (B) and Tb. Sp (C). (* p < 0.05; ** p < 0.01; *** p < 0.001).