Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information for

Strontium folic acid derivative functionalized titanium surfaces for enhanced osteogenic differentiation of mesenchymal stem cells *in vitro* and bone formation *in vivo*

Kui Xu,[a,b] Weizhen Chen,[a] Caiyun Mu,[a] Yonglin Yu[a] and Kaiyong Cai*[a]

[a]Key Laboratory of Biorheological Science and Technology, Ministry of Education College of Bioengineering, Chongqing University, Chongqing 400044, P. R. China

[b]Biomedical Engineering Research Center, Medical School of Ningbo University, Ningbo, Zhejiang 315211, P. R. China

*Corresponding author: Prof. Kaiyong Cai

College of Bioengineering

Chongqing University

Chongqing 400044

P. R. China

Tel: +86-23-65102507

Fax: +86-23-65102877

E-mail: kaiyong_cai@cqu.edu.cn

List of Contents

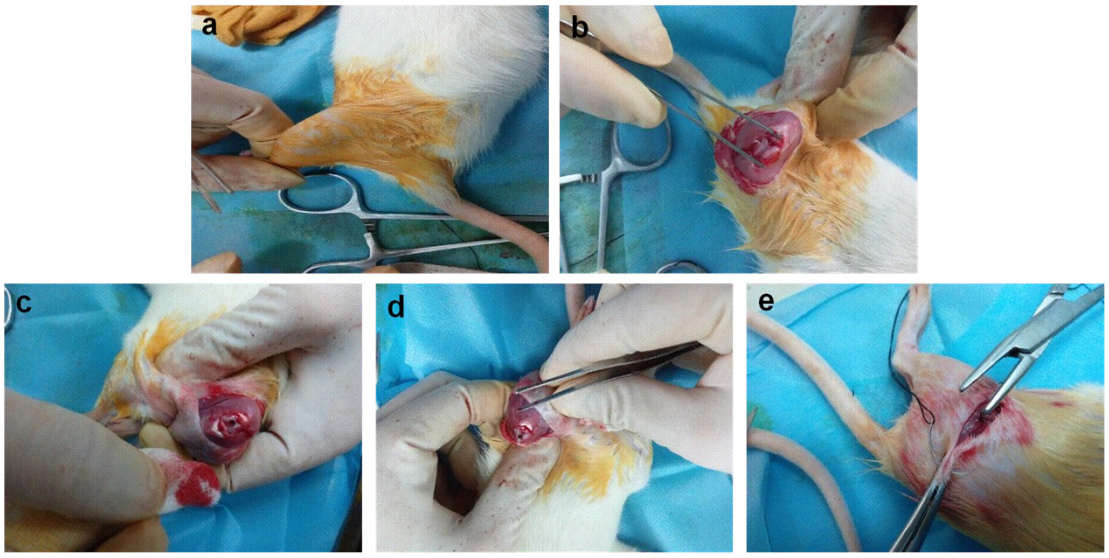


Fig. S1 Surgical procedure: (a) the upper legs of SD rats were shaved and disinfected with iodophor; (b) the skin and periosteum were separated, then the femurs were exposed; (c) a hole was made on the planar surface of femur with a tryphine drill; (d) an implant was gently inserted into the hole, and the end of samples should keep parallel with bone surface; (e) the wound bed was closed in layers with sutures and disinfection treatment was performed.

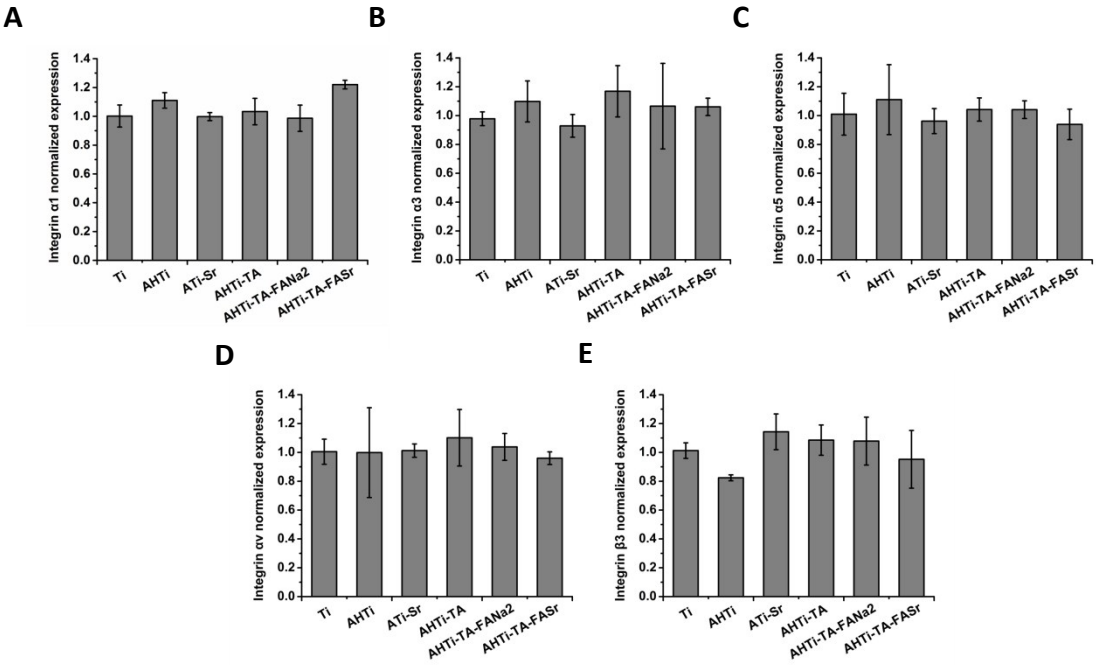


Fig. S2 Relative mRNA expression of cell adhesion related proteins when the cells were seeded onto the substrates for 24 h: (A) Integrin α1, (B) Integrin α3, (C) Integrin α5, (D) Integrin αv and (E) Integrin β3, respectively. The total RNA of each group was extracted when the cells were collected from 5 samples of this group. Each qRT-PCR was performed in triplicate for qRT-PCR yield validation. And 2 repeating runs were carried out. The value was normalized to β-actin. Error bars represent mean ± SD for n = 6, *p < 0.05.

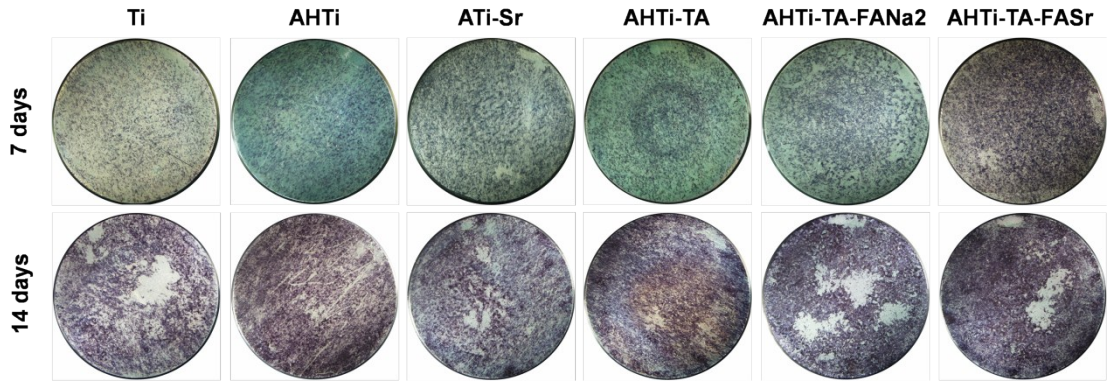


Fig. S3 Representative ALP staining of MSCs cultured onto the different Ti substrates after incubation for 7 and 14 days.

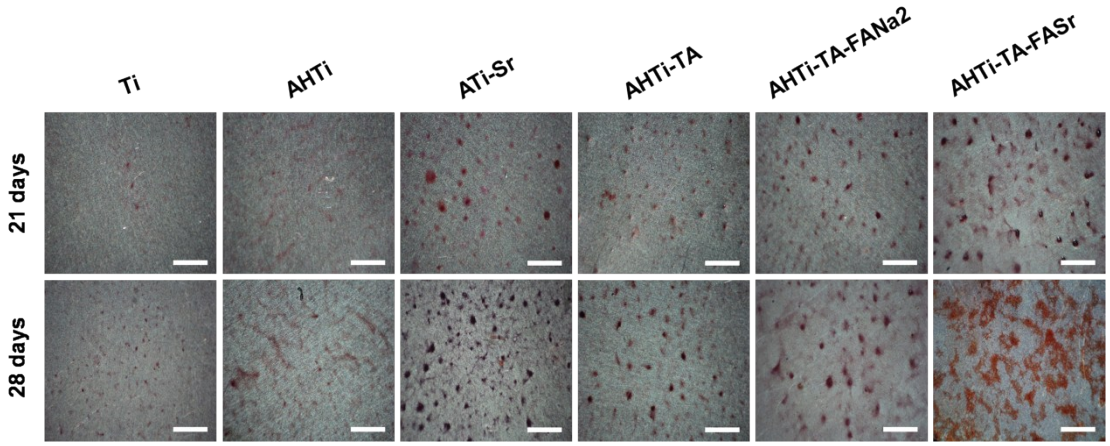


Fig. S4 Representative optical images of ECM mineralization of MSCs adhered to the different Ti substrates for 3 and 4 weeks (scale bar: 1 mm).

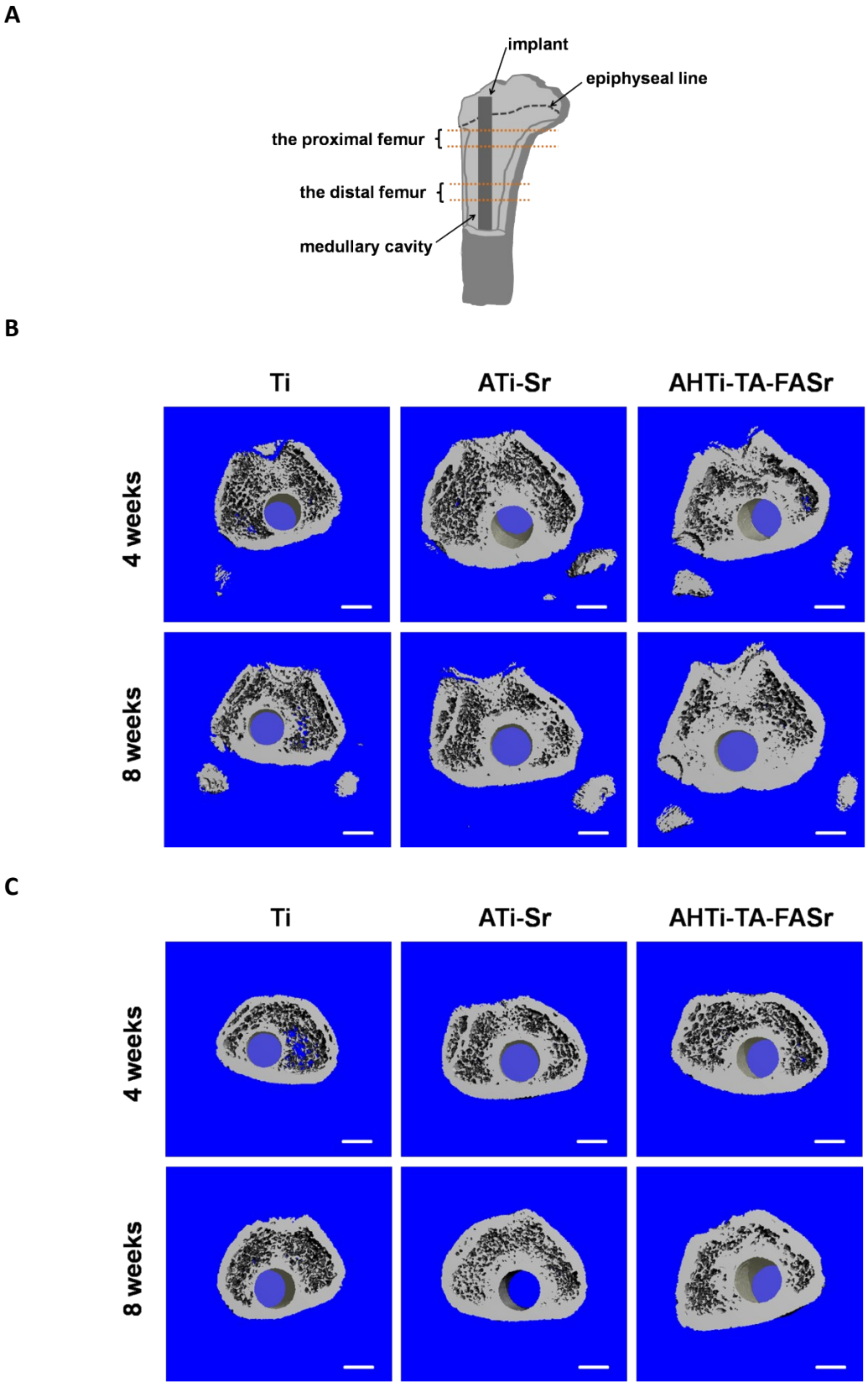
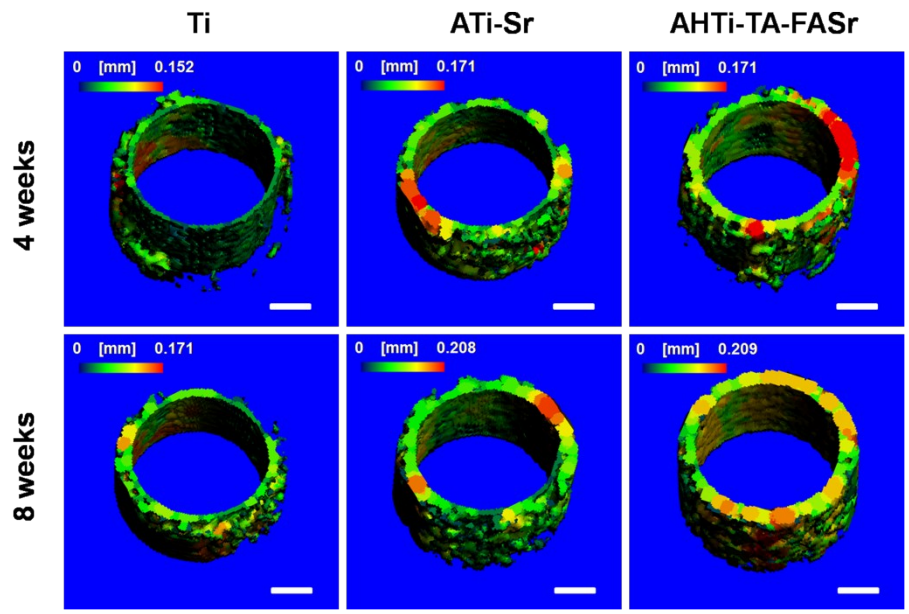


Fig. S5 Transverse cutting 3D micro-CT reconstruction. (A) Illustrarion of the choice of transverse cutting position. All the selected regions were located below the

epiphyseal line. 100 slices were analyzed by the affiliated software of viva-CT 40. (B) The across cutting 3D micro-CT reconstruction images of the proximal femur with implants (close to epiphyseal line) after implantation for 4 and 8 weeks (scale bar: 1 mm). (C) The across cutting 3D micro-CT reconstruction images of the distal femur with implants (in the medullary cavity) after implantation for 4 and 8 weeks (scale bar: 1 mm).

A



B

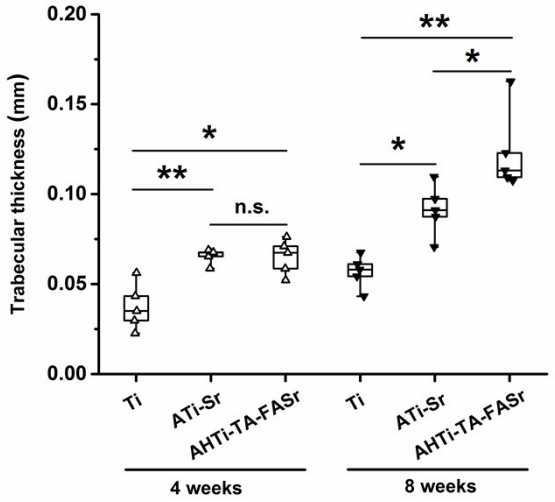
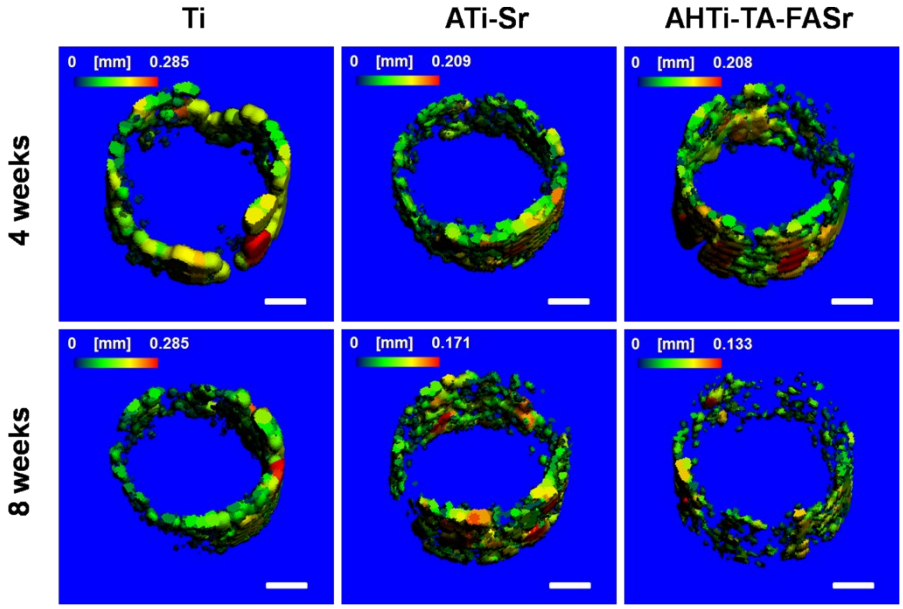


Fig. S6 Trabecular thickness analysis. (A) Trabecular thickness of Ti, ATi-Sr and AHTi-TA-FASr after implantation for 4 and 8 weeks (scale bar, 200 μm). (B) Quantitative analysis of the newly formed bone was performed for trabecular thickness. The above data were obtained according to the Micro-CT analysis. All data were shown by mean (bar), 25th and 75th quartiles (box) with whiskers extending 1.5 interquartile ranges (IQR) beyond them. The confidence levels were set as 95% and 99%, n = 5, *$p < 0.05$, **$p < 0.01$.

A



B

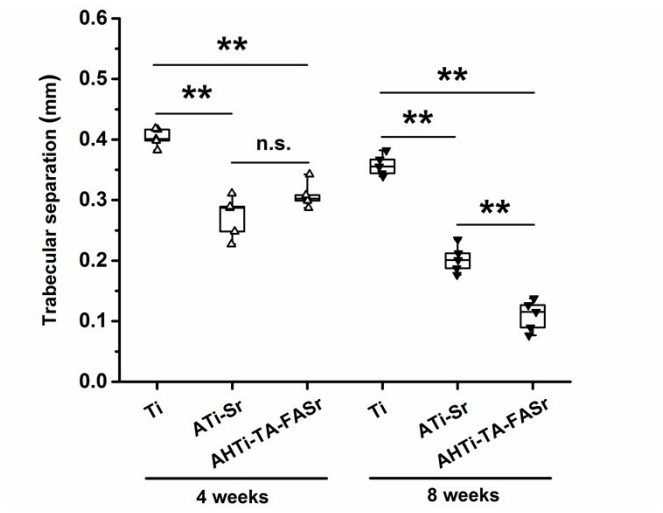


Fig. S7 Trabecular separation analysis. (A) Micro-CT reconstructed images of trabecular separation for Ti, ATi-Sr and AHTi-TA-FASr after implantation for 4 and 8 weeks (scale bar: 200 μm); (B) Quantitative analysis of trabecular separation for different Ti implants. All data were shown by mean (bar), 25th and 75th quartiles (box) with whiskers extending 1.5 interquartile ranges (IQR) beyond them. The confidence levels were set as 95% and 99%, n = 5, **p < 0.01.