Supporting Information

Surface modification of strontium-doped porous bioactive ceramic scaffolds via poly(DOPA) coating and immobilizing silk fibroin for excellent angiogenic and osteogenic property

Xu Wang[#], Zhipeng Gu[#], Li Li, Bo Jiang, Xixun Yu[*]

Supplementary Methods

Preparation of SF and SCPP

Bombyx morisilk were treated twice with 0.5% (w/w) $NaHCO_3$ solution at 70 °C with consistant agitate for 1 h and then rinsed with 70 °C distilled water to remove sericin and then dried it at 60 °C. Degummed silk was dissolved in a mix solvent system of $CaCl_2/CH_3CH_2OH/H_2O$ (mole ratio, 1:2:8) at 80 °C for 2 h and filtered to get a SF solution. The SF solution was dialysis and then lyophilized. Firstly, calcium carbonate and strontium carbonate (the molar ratio of them was 92:8 to ensure ultimately obtained 8% SCPP) were added into 85% phosphoric acid slowly with constant stirring. After reacted at room temperature for 8h, the solvent were removed by reduced pressure distillation. Subsequently, obtained precipitates were calcined at 500 ℃ for 10h to form SCPP and heated to1200 ℃ with a 15 ℃/min heating rate to melt. Then, cooled it down on frozen distilled water and yielded powders in a size range of 75 μm through ball-milling treatment. Mixing the powders with a little stearic acid and 3wt% poly (vinyl alcohol) and pressed them into a disk, while the mass ratio of strontium-doped calcium polyphosphate and stearic acid was 3:2 to make sure scaffolds possessed applicable porosity and pore size. After sintering under high temperature, porous SCPP cylinders with 10 mm diameter and 2 mm thickness could be obtained.

Supplementary Results

Supplementary Figure 1

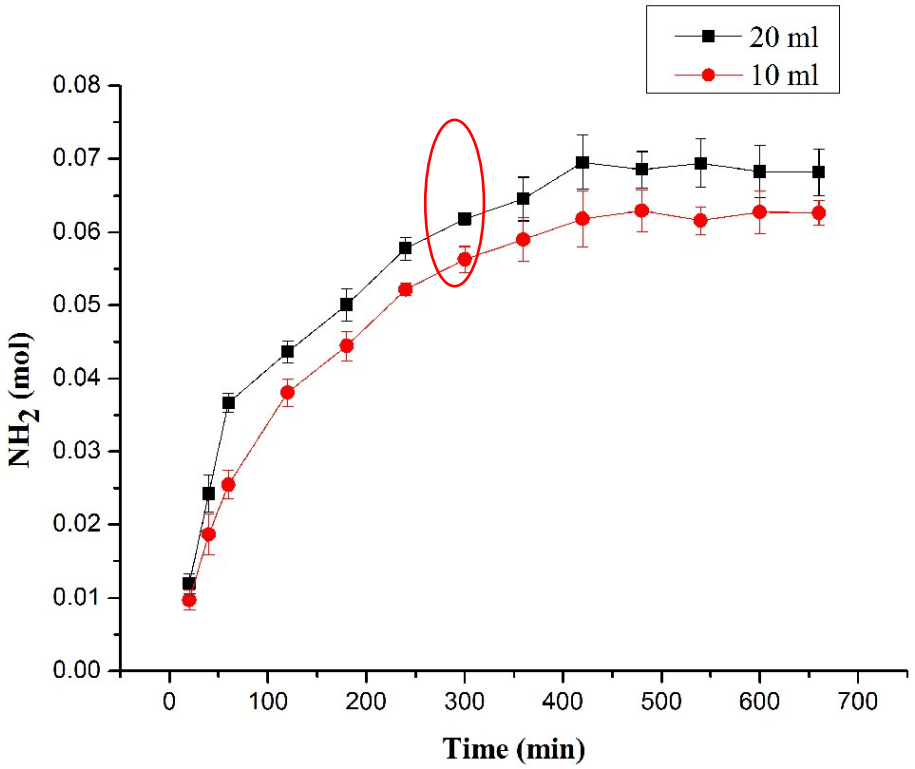

Figure S1. The change of amino concentration contained in scaffolds treated with different volume of dopamine solution

The amount of amino groups contained in scaffolds increased with elapsing of reaction time or DOPA solution doubled. The amount of SF introduced into scaffolds was in direct proportion to amino groups from DOPA. In this experiment, we used ninhydrin method to determine the optimal concentration of DOPA solution and reaction time (ensure the amount of amino groups reach the peak). After heating with ninhydrin (110 °C) for 20 min, the amino amount, which was directly proportional to optical absorbance of solution, was recorded.[1-3] Since the initial concentration of DOPA was defined at 2.0 mg ml^{-1}, the amount of DOPA introduced could only be control by reaction time and solution volume used. The maximal value was reached in 20ml solution after 7 h, which was selected as the favorable reaction condition.

Supplementary Figure 2

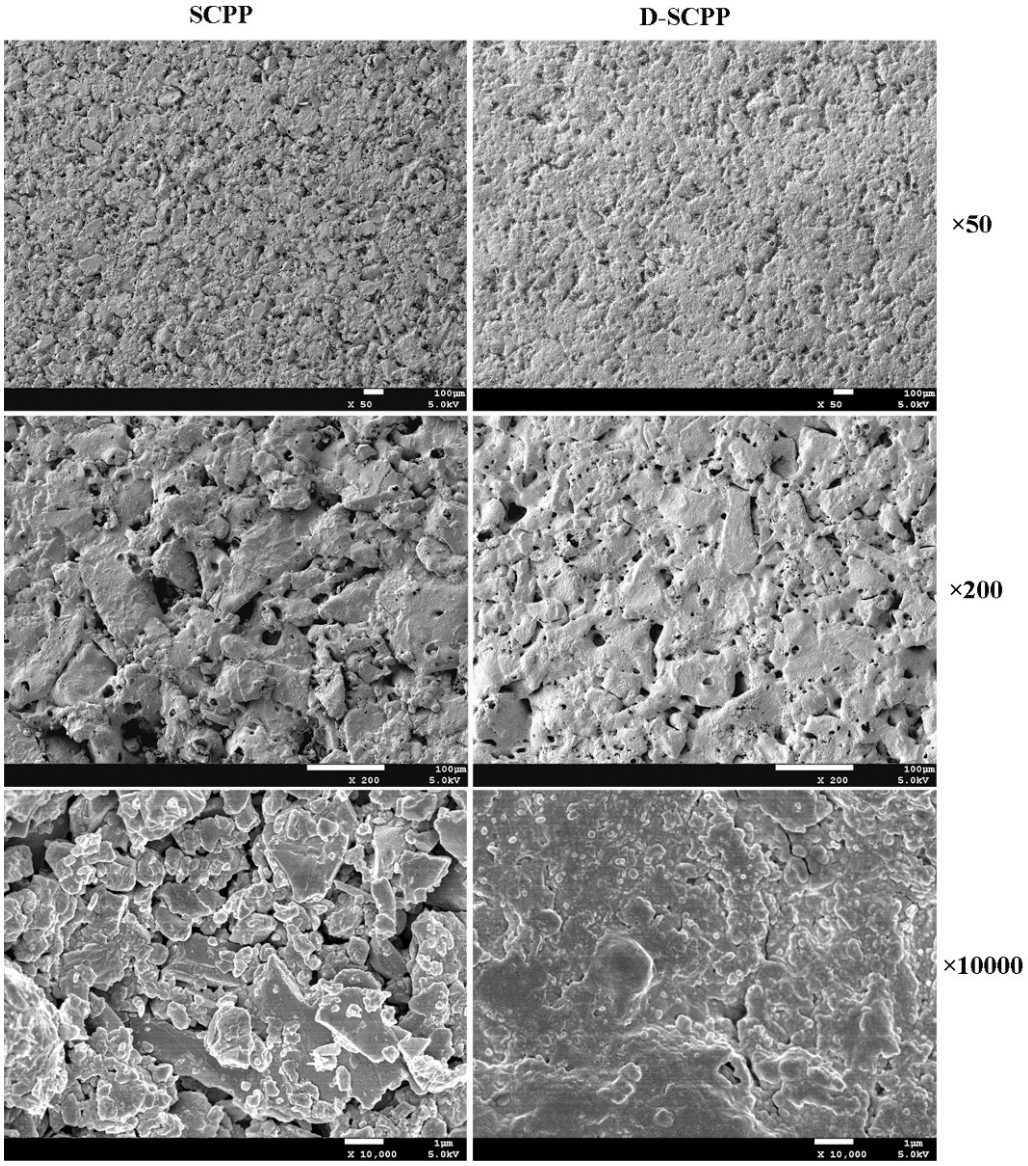

Figure S2. SEM images of the scaffolds-surface before(left) and after(right) coating with DOPA

After DOPA coating, the surface of scaffold turned smoother and there were less pores on it. That might be attributed to following reason: since self-polymerized crosslinking structure of DOPA, a thin polymer layer formed and tightly adhered to the surface of scaffolds for self-polymerized crosslinking structure of DOPA. SEM observations certified DOPA's successful coating, which was in according with the result in XPS.

Supplementary Figure 3

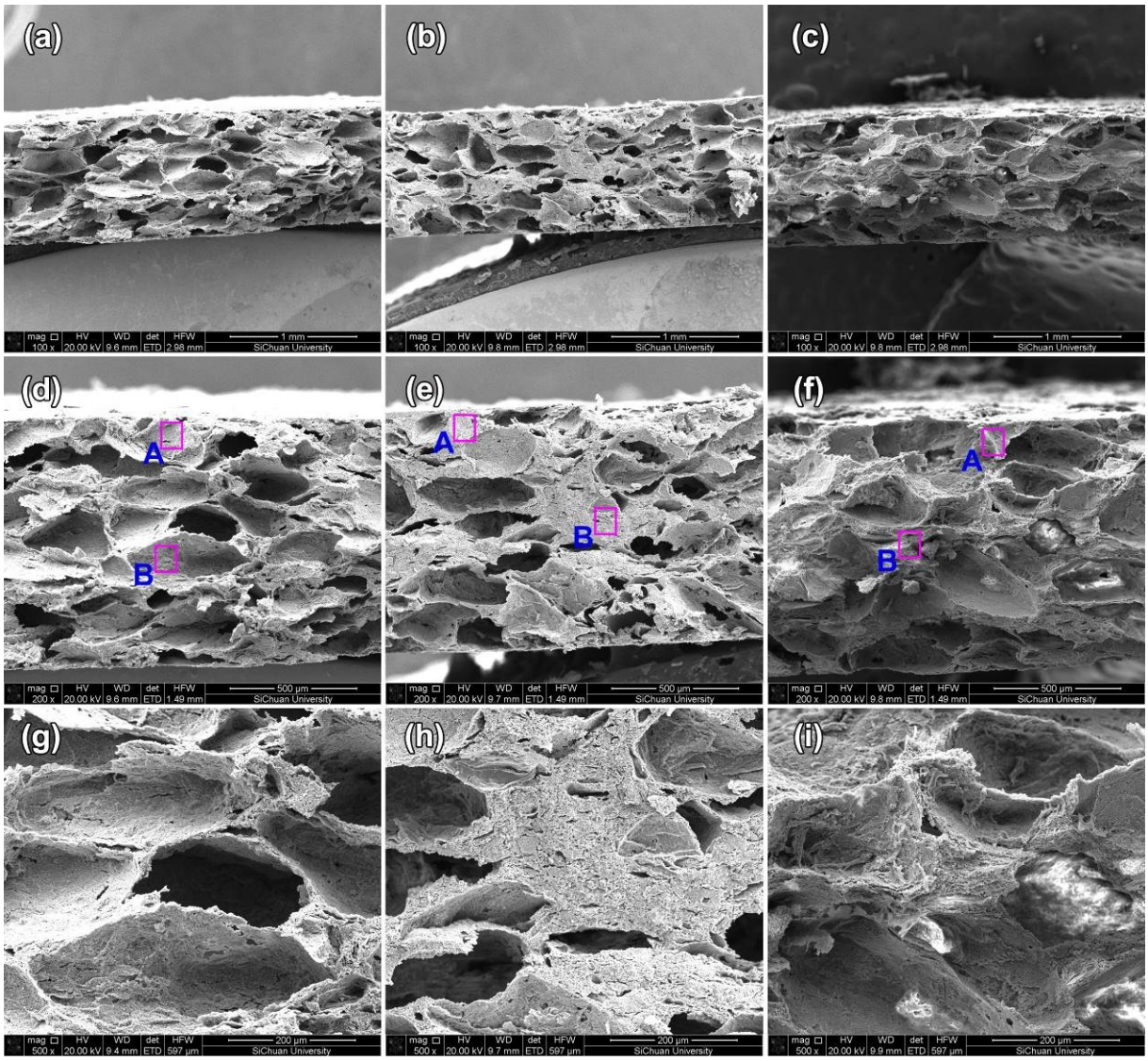

Fig. S3 SEM images of scaffolds impacted surface and their EDS analysis. SCPP scaffold of (a) 100×, (b) 200×, (c) 500×; D-SCPP scaffold of (d) 100×, (e) 200×, (f) 500×; SCPP/D/SF scaffold of (g) 100×, (h) 200×, (i) 500×. Rectangular areas marked as A and B are positions exterior and interior of scaffold chosen for EDS analysis.

Supplementary Table 1

Table S1 Nitrogen content from EDS results corresponding to Fig. S3

Scalfolds	Wt %	
	A	B
SCPP[a]	---	---

D-SCPP	7.21	5.24
SCPP/D/SF	8.76	8.57

EDS result were exhibited in Tab. 1. After DOPA coating nitrogen element were successfully introduced. However, as exhibited in Fig. S3, there were no obvious difference of chemical compositions between interior and exterior of scaffold. Similar phenomenon was also observed in SCPP/D/SF group. Therefore, the chemical compositions of modified and unmodified SCPP scaffold were nearly homogeneous and the dramatically reduction of cells number inside SCPP/D/SF scaffold may mainly be caused by lacking of oxygen and other nutrient substance.

Supplementary Figure 4

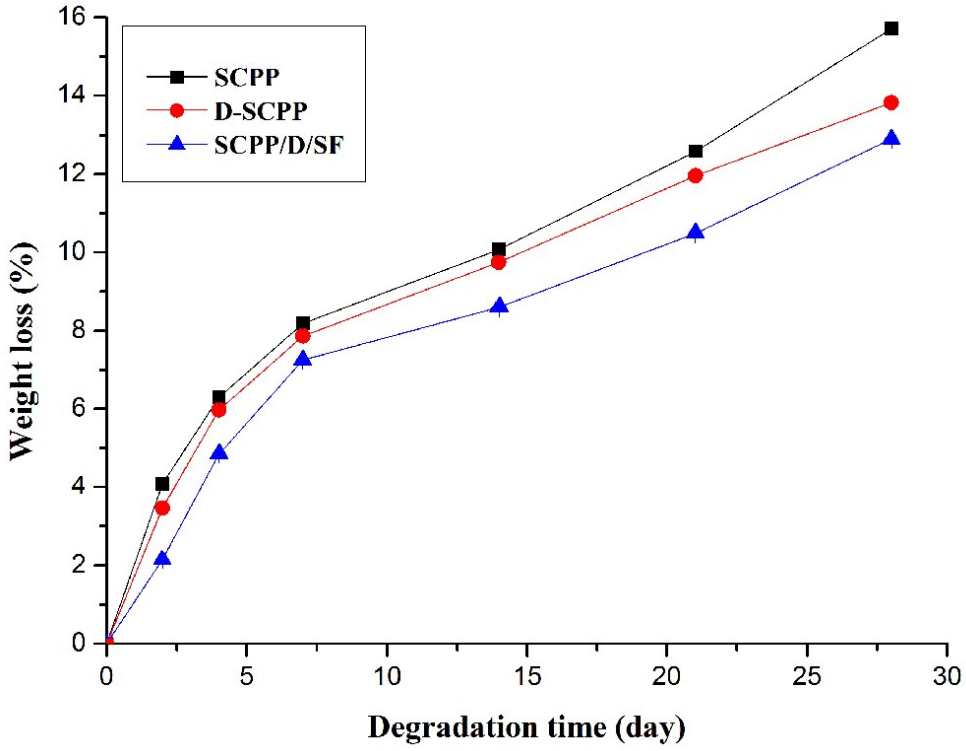

Figure S4. The curve of weight loss of scaffods

DOPA-SF coating can protect the scaffolds from immediate degradation over a period of

time. As can be seen from figure, during the same degradation period, there were no obvious differences on weight loss between SCPP group and purely DOPA coating modified group (D-SCPP group). However, after DOPA-SF coating, the weight loss of scaffolds decreased significantly.

Supplementary Figure 5

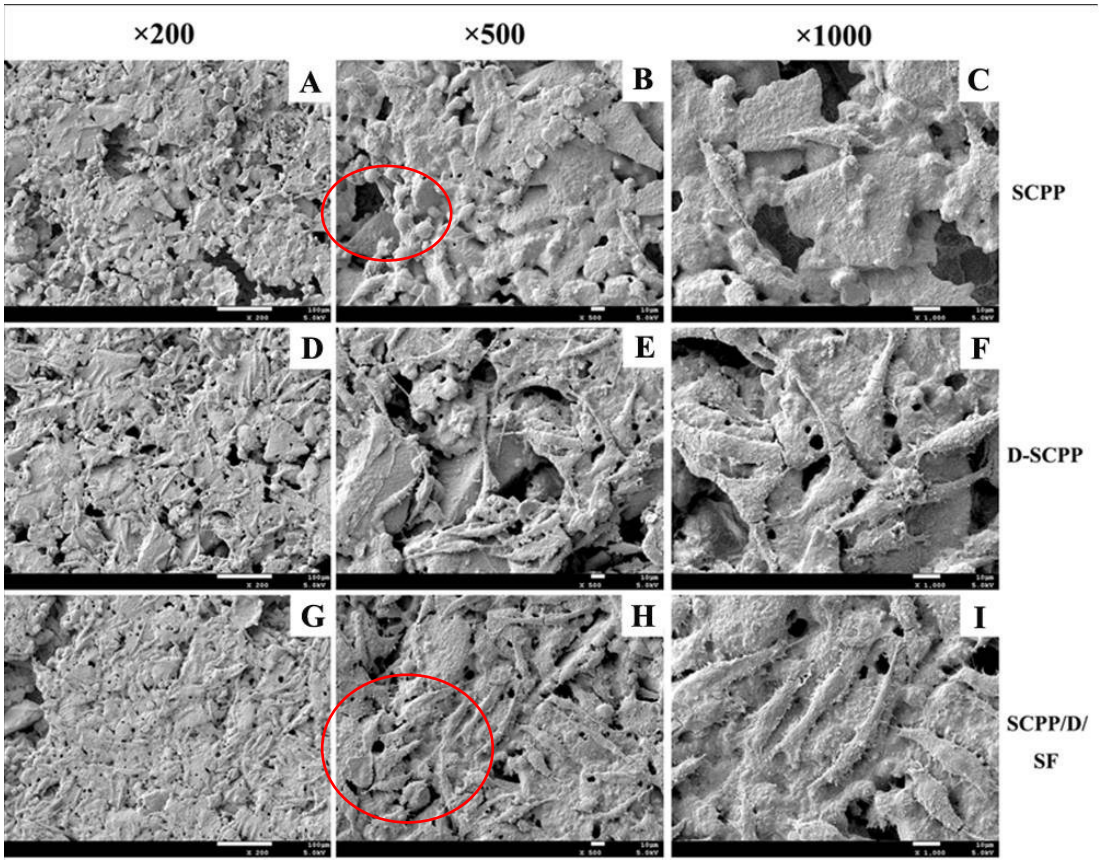

Figure S5. SEM images of MG63 culturing on scaffolds for 4 day

SEM observation presented the morphology of MG63 growing on scaffolds on the 4th day. DOPA-SF modification not only promoted cell proliferation significantly, but also made MG63 tightly attached to the scaffold. On the SCPP/D/SF, cells adhered and grew better with a characteristic spindle-like morphology. While on SCPP or D/SCPP scaffolds, the morphology of some cells were rounded, which were the characteristics of inactive cells. Its result was in according with that in CLSM, which could further certify the promotion for cells

proliferation and adhesion by SCPP/D/SF.

Supplementary Figure 6

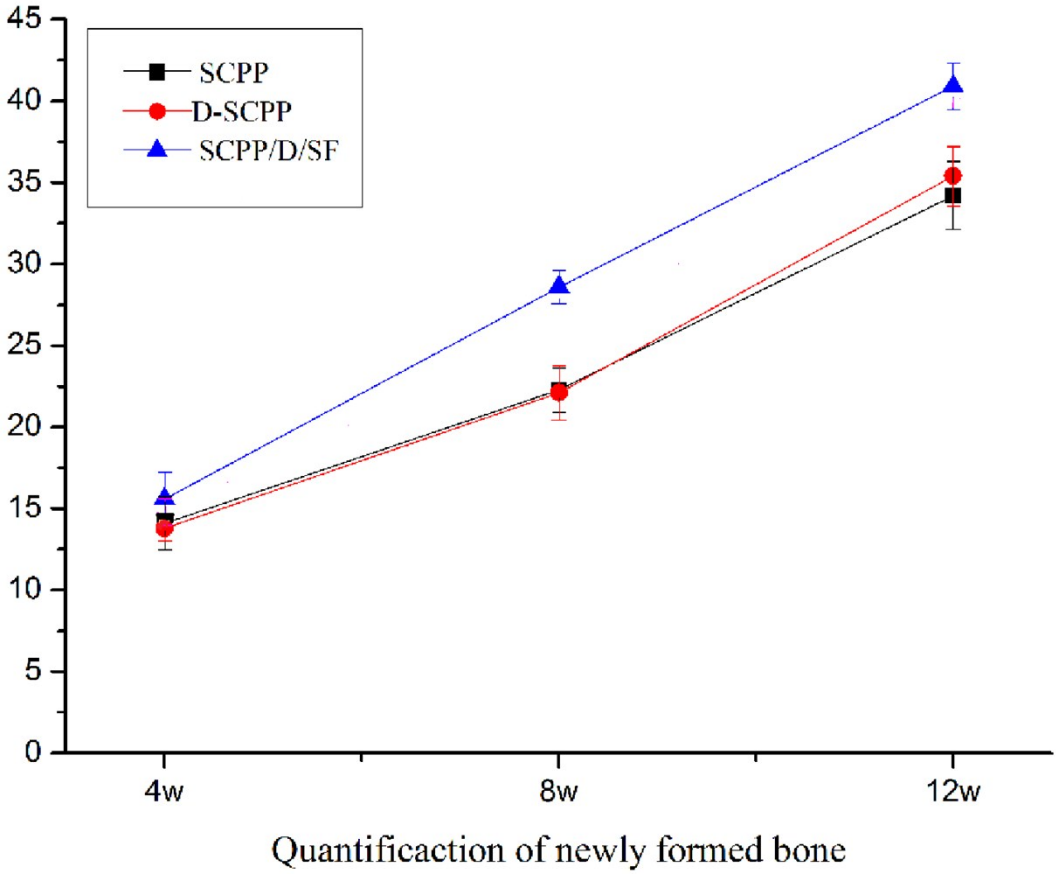

Figure S6. Newly formed bone quantification of scaffolds postoperatively in 4, 8 and 12 weeks

Histological observation and immunohistological assessment had proved that DOPA-SF coating could enhance VEGF and bFGF secretion from host cells, possbily leading to faster process of osteogenesis and vascularization. The figure presented the results of bone mineral density test which was aimed to determine the accurate quantification of newly formed bone. It proved that SCPP/D/SF could promote the formation of new bone effectively.

Supplementary Figure 7

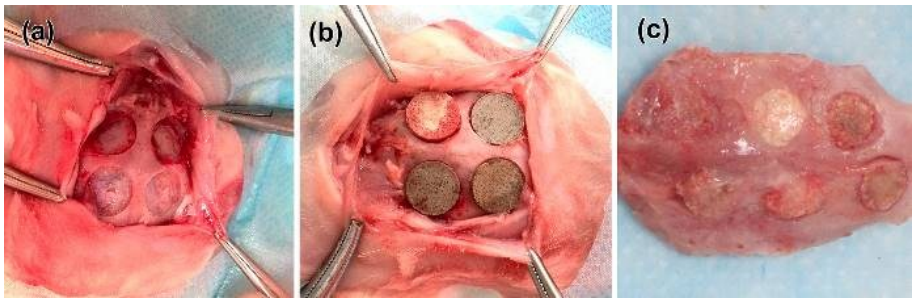

Fig. S7. Photographs of three stages of implantation process: (a) Defection created on the crinial bone of rabbit; (b) implanted materials; (c) crinial bone after 12 weeks of reparing.

Supplementary References

[1] A. F. S. A. Habeeb. , Anal. Biochem. 1966, 14, 328

[2] S. Lie., J. Inst. Brew. 1973, 79, 37

[3] E. Poboży, W. Czarkowska, M. Trojanowicz.. J. Biochem. Bioph. Methods, 2006, 67, 37