Electronic Supplementary Material (ESI) for RSC Advances.

Enhanced healing process of tooth sockets using strontium-doped TiO_2

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Experimental section

Materials

Potassium chloride (KCl, 98%), Strontium nitrate ($Sr(NO_3)_2$, 95%), titanium (IV) isopropoxide (TTIP, AG), hexadecylamine (AG), Dimethylformamide (DMF, AG), and ethanol (AG) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 4,4-methylene diphenyl diisocyanate (MDI, 98%), poly(tetramethyleneoxide) (PTMO, Mn = 1000), anhydrous toluene (99.8%), and 1,4-butylene glycol (AG)were purchased from Acros Organics Co.

Fabrication of Sr-TiO_2 mesoporous nanospheres

The Sr-TiO_2 mesoporous nanospheres were synthesized by a sol-gel strategy and a followed solvothermal process. 0.9 g of KCl was dissolved in 2 mL of deionised water, which was then slowly added to 25 mL of 1 wt% hexadecylamine in ethanol. After addition of 0.1 mmol of $Sr(NO_3)_2$, 2 mL of TTIP was poured into the above solution with continuously stirring for 6 h. The resulted suspension was transferred into the Teflon-lined autoclaves and heated at 180 °C for 12 h. After reaction, the Sr-TiO_2 products were collected, washed, dried, and calcined at 450 °C for 4 h in air. The undoped TiO_2 was prepared using a similar process without the addition of the Sr dopants.

Preparation of NH_2-functionalized Sr-TiO_2 (Sr-TiO_2/MDI) mesoporous nanospheres

3.0 g of MDI was dissolved in 50 mL of anhydrous toluene, which was transferred into a two-necked round-bottomed flask. Subsequently, 1.0 g of Sr-TiO_2 was added to the flask under stirring. The temperature was kept at 60 °C for 6 h under N_2 flow. After reaction, the cooled mixture was filtered and washed with anhydrous toluene to remove excessed MDI and physically adsorbed species. The obtained Sr-TiO_2/MDI product was dried at 120 °C in a vacuum oven.

Synthesis of injectable SPU/Sr-TiO_2/MDI adhesives

Before experiment, the OCN-terminated polymer was firstly prepared by the reaction between MDI (1.5 g) and PTMO (3.0 g) in 15 mL of DMF at 90 °C for 3 h under N_2

flow. As a chain extender, 0.5 mL of 1,4-butylene glycol was then slowly poured into the above mixture under stirring for further 10 minutes. Next, 1.0 g of Sr-TiO_2/MDI was added to the pale yellow viscose solution and stirred for another 2 h to attain the injectable SPU/Sr-TiO_2/MDI adhesives.

Characterization

The morphological information of the Sr-TiO_2 mesoporous nanospheres were studied by transmission electron microscopy (TEM, JEOL JEM 2100 Co., Tokyo, Japan) with an accelerating voltage of 200 keV. The SPU/Sr-TiO_2/MDI adhesives were characterized by scanning electron microscope (SEM, FEI Quanta 200FEG, Oregon, USA) operated at 10 kV. X-ray photoelectron spectroscopy (XPS) technique was performed using Alpha 110 instrument (East Grinstead, UK) with monochromatic Al Kα radiation (hv = 1486.7 eV) and a pass energy of 20 eV. Powder X-ray diffraction (XRD) test were carried out on Philips PW 1710 diffractometer with Cu Kα (λ = 1.5406A) radiation. Raman spectra were acquired on a Raman microscope (LabRAM HR, HORIBA Jobin Yvon Inc.), the used excitation wavelength was 532 nm. UV-vis spectra (UV/vis DRS) were recorded using a Shimadzu spectrophotometer (MPC-2200).

In vitro antibacterial test

The antibacterial activity of SPU/Sr-TiO_2/MDI was estimated with the bacterium strains of E. coli by zone of inhibition method. Firstly, 100 μL of 108CFU/mL bacteria suspension was spread on a LB agar plate. Next, 100 μL of freshly obtained compound was injected into sterile blank disc and placed onto the agar surface. The diameter of zone of inhibition was recorded and analyzed after incubation at 37°C for 24 h.

In vitro cell viability

L02 cells were cultivated in Dulbecco's minimum essential medium containing 10% (v/v) fetal bovine serum, 0.01% ascorbic acid, 10 μg/mL peni-cillin and streptomycin at 37°C in 5% CO_2 and 95% humidity. The culture medium was replaced in every two days. The cells were seeded on a piece of sterile dressing on 96-well plates with 100 μL of the fresh medium. The culture medium was removed from each well after 24 h, followed by the adding of MTT solution (100 μL, 5 mg/mL in fetal bovine serum) and

culturing at 37 °C for another 4 h. Afterward, the 200 μL of dimethyl sulfoxide (DMSO) was introduced to replace the MTT solution. A microplate reader (Tecan i-control multiplate reader, Switzerland) was employed to evaluate the cell viability by measuring the absorbance at 490 nm.

Animal experiments

The in vivo animal experiment was supported by Institute of Radiation Medicine, Chinese Academy of Medical Science (IRM-CAMS) Tianjin, China. The 16 Kunming rats (Male, 10 weeks old with about 200 g body weight) were used for animal studies and randomly divided into two groups (blank SPU and SPU/Sr-TiO_2/MDI). The right mandibular central incisor was removed under general anesthesia with 0.3 ml/kg SU-MIAN-XIN. The tooth sockets were injected with the prepared adhesives for each group. The treated rats were then given ampicillin sodium and fed the soft diet for 3 days. In each group, two rats were randomly selected after 1, 2, 4 and 7 weeks' treatment. The selected rats were continuously perfused with 4% paraformaldehyde through MINI-type infusion pump. The mandibles were separated after complete fixation and treated with 4% paraformaldehyde for 24 h.

Statistical analysis

All the triplicate data in this study have been calculated and analyzed using analysis of variance for presenting a significance level at $p < 0.05$ and standard deviations (±).

Figures

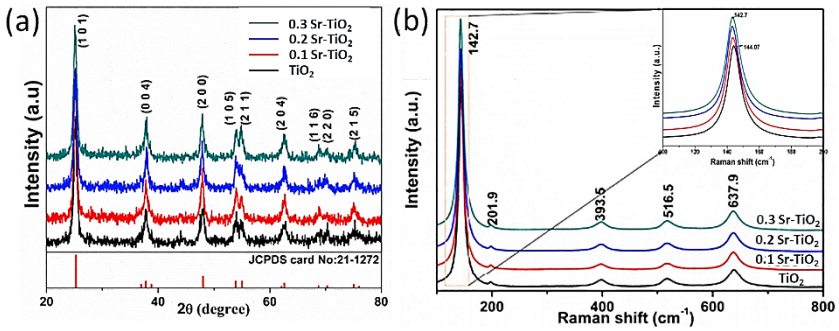

Figure S1 XRD patterns (a) and Raman spectra (b) for TiO_2, 0.1 Sr-TiO_2, 0.2 Sr-TiO_2, and 0.3 Sr-TiO_2 products.

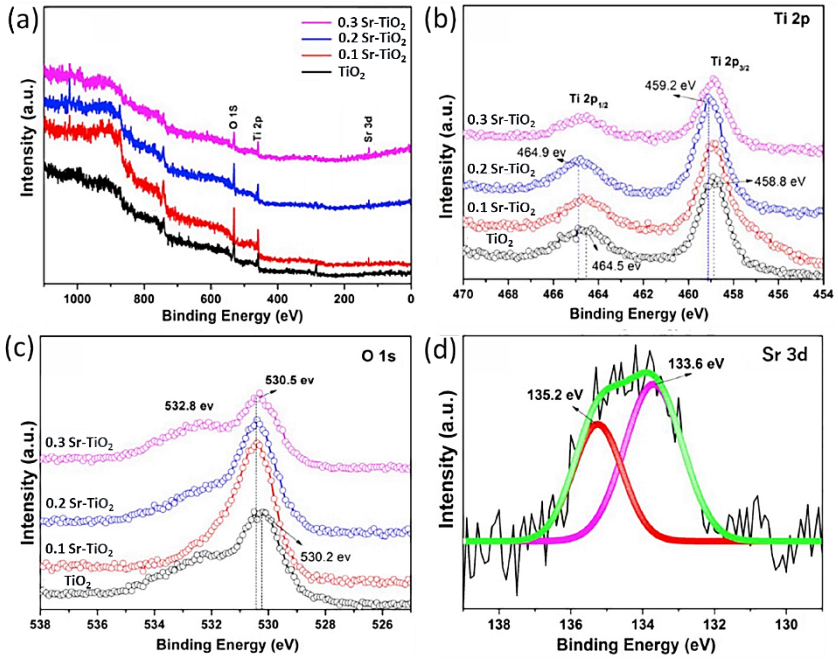

Figure S2 XPS profiles of TiO_2 and different Sr-TiO_2 products. (a) Survey spectrum, (b) Ti 2p, (c) O1 s, and (d) Sr 3d.

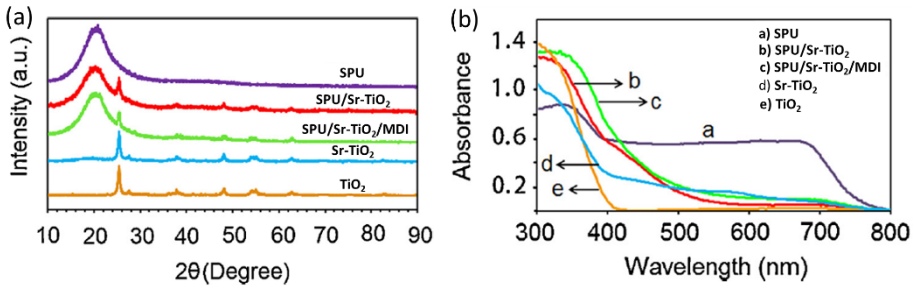

Figure S3 (a) XRD profiles of TiO_2, Sr-TiO_2, SPU/Sr-TiO_2/MDI, SPU/Sr-TiO_2, and SPU samples and their UV-vis DR spectra (b).

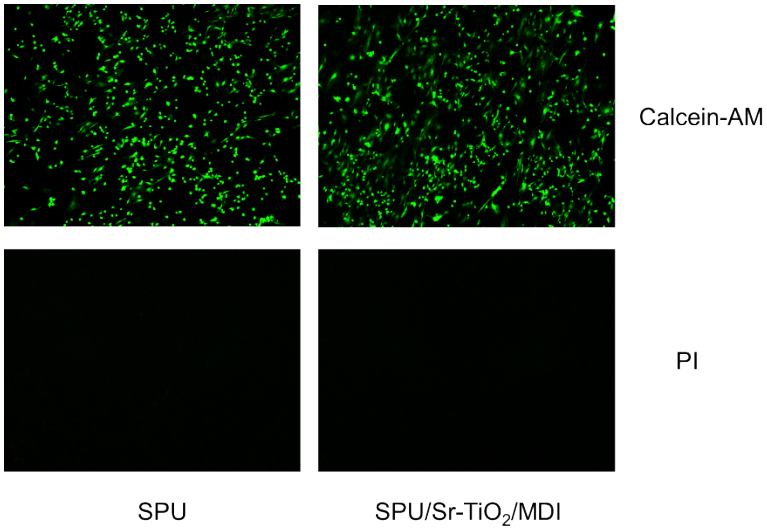

Figure S4 Staining of living/ dead rBMSCs on the surface of different materials.

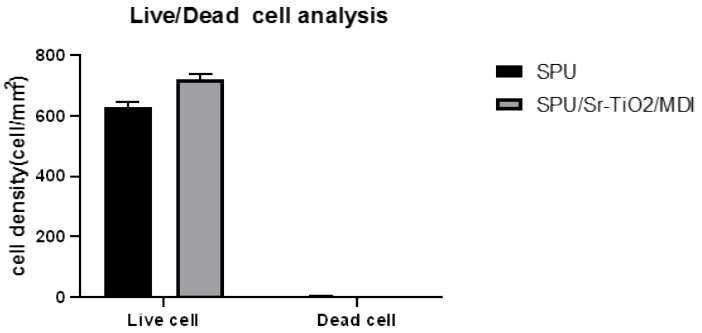

Figure S5 Cell density for the live and dead cell by applying different materials.

Table S1. Comparison of SPU/ Sr-TiO_2/ MDI with different materials in the field.

Material	Common point	Merit	Drawback
SPU/ Sr-TiO_2/ MDI	Promote wound healing, ability to fill the defect tissue, and fine biocompatibility	Anti infection, suitable for periodontitis extraction of affected teeth; Injectability, strong plasticity and convenient operation. Suitable for post extraction site preservation of irregular extraction socket; Hard to cause secondary injury of bone repair site; Stable degradation performance.	The long-term effect needs to be observed.
Collagen sponge		Anti infection, rapid hemostasis, stable blood clot, preventing blood clot from falling off and foreign bodies from falling into the tooth extraction socket, provide a stable environment for blood clot mechanized osteogenesis, suitable for those with multiple bleeding and poor coagulation function.	In the process of absorption, there will be slight inflammatory reaction and low mechanical properties.
Calcium sulfate hemihydrate		Reduce exudation, protect wounds and prevent bleeding; Suitable for patients with oral anticoagulants.	Excessive absorption rate in vivo; Degradation products are easy to lead to local exudation reaction of incision.
BIOSs bone meal		Strong effect on promoting osteogenesis and a large amount of new bone formation, which is conducive to the recovery of patients' wounds and	Immune inflammatory reaction might happens; Inconvenient to operate, requires a special bone powder conveyor for filling.

		surrounding tissues; Suitable for patients with many bone defects.	
Hydroxyapatite artificial bone powder		The effect of promoting osteogenesis is not as good as BIOSs bone powder; Suitable for patients with small-scale bone defects	Low mechanical performance, slow degradation speed and inconvenient operation, Requires a special bone powder conveyor for filling
CGF hydroxyapatite ceramics		Shorten the time of bone healing and improve the quality of bone tissue. Suitable for those with poor bone tissue conditions	High cost and complicated fabrication.