Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Local co-delivery and release of antimicrobial peptide and RGD using the porous TiO_2

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Antimicrobial assay

Bacterial culture

Staphylococcus Aureus (*S. aureus*, strain ATCC 29213) and *Escherichia Coli* (*E. coli*, strain ATCC 15224) were purchased from VWR International, LLC. Single colony of the bacteria was inoculated in 5 ml of LB media overnight at 37 ºC with shaking (150 rpm). After that, 1 ml of the bacterial suspension was inoculated in 50 ml of fresh LB media, respectively, which was incubated for 5 h with shaking (250 rpm) at 37 ºC to achieve mid-log phase growth.

Bacterial culture on the film

The bacteria were diluted to PBS with the concentration of 1.0×10^7 cfu/ml. Prior to seeding, the films were placed into a 24-well culture plate. Then 350 μl of bacterial suspension (1.0×10^7 cfu/ml in PBS) was added onto each film to fully cover the surface of it.

Antimicrobial activity of the film

After cultured the bacteria on different films for 0.5 h at 37 ºC, 490 μl of PBS was added into each well, and the samples were washed roughly by using the pipettes. Then we transferred both the bacterial suspension and the samples into a tube, and treated it with ultrasonic for 3 min to ensure the bacteria on the samples could be dispersed into the PBS. After that, the bacterial suspension was transferred to a new tube immediately, and was diluted to 10^0, 10^1 and 10^2 times. Then 10μl of each bacterial suspension was taken to evaluate the viability of bacteria by using agar plates. The films were air-dried, and treated with the same amounts of bacteria for another 30 min after which bacteria suspension was smeared on the agar plates as above for three times.

Live/dead assay of bacteria

After cultured the bacteria on different films at 37 ºC, the ***Inorg*** and ***Inorg-AMP*** films were rinsed gently with PBS for three times and added with 20 μl 0.5% propidium iodide solution as well as 40 μl 0.05% Fluorescein diacetate in distilled water for 5 min at room temperature. After that, the films were washed with distilled water for three times, dried in air and observed with Eclipsc Ti-U (Nikon, Japan).

Cell assay

The influence of the functionalized films on cell viability was tested with the rat bone mesenchymal stem cells (*rBMSCs*) as follows.

Cell culture and seeding

rBMSCs were cultured in High glucose Dulbecco's modified Eagle's medium (H-DMEM) (Hyclone, Logan, Utah) containing 10% fetal bovine serum (FBS) and in a 5% CO_2 atmosphere at 37 ºC. Medium was replaced every third day. The adherent cells were allowed to reach about 80% confluence. Cells were passaged in culture and passage 3-5 (P3-P5) cells were used for the experiments.

All the nanotube films used for cell test were sterilized with 75% ethanol for 2 h before treatment. After sterilization, all the preparation processes were in aseptic condition to integrate the HHC36 peptide. The films were placed individually into the 24-well plates, and the *rBMSCs* were added directly onto each film (30000 cells in 1 mL of H-DMEM containing 10% FBS). They were cultured for indicated times before the test.

CCK-8 assay

After being cultured for 24 h, the biocompatibility of the materials was evaluated with CCK-8 assay. Briefly, at indicated time points, the films were transferred to a new 24-well plate and washed three times with PBS. Then 400 μl of complete medium containing 40 μl of CCK-8 solution was added to each disk. After incubation for 2 h at 37ºC in dark, 150 μl of the CCK-8 solution was transferred to a new 96-well plate and the optical density (OD) value of the solution was measured with an ELISA plate reader (Varioskan Flash 3001, Thermo, Finland) at 450 nm wavelength.

FITC stain assay

After being cultured for 4 and 24 h, the samples were washed lightly with PBS for 2 times, and fixed with neutral methanal (4 vol% in PBS) at room temperature for 30 min. Then 350 μl of FITC solution was added onto the samples, and the system was incubated at room temperature for 30 min. After that, the samples were got out, washed with PBS for 2 times, and observed with Eclipsc Ti-U (Nikon, Japan).

Statistics

The release of the peptide, antimicrobial assay and cell assay were repeated at least three times and the results were expressed as means ± standard deviations. Statistical significance was calculated by the SPSS 17.0 statistical software with the t-tests method.

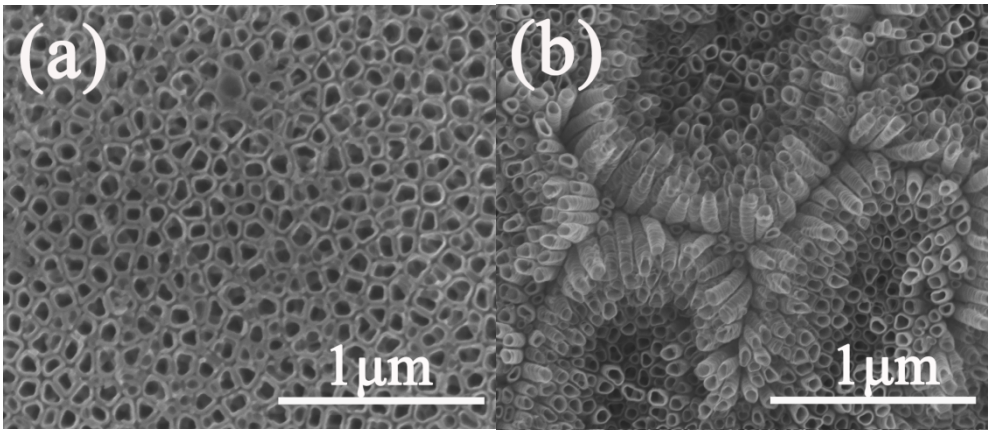

Fig. 1S SEM images of ***Inorg*** (a) and ***Org*** (b) films.

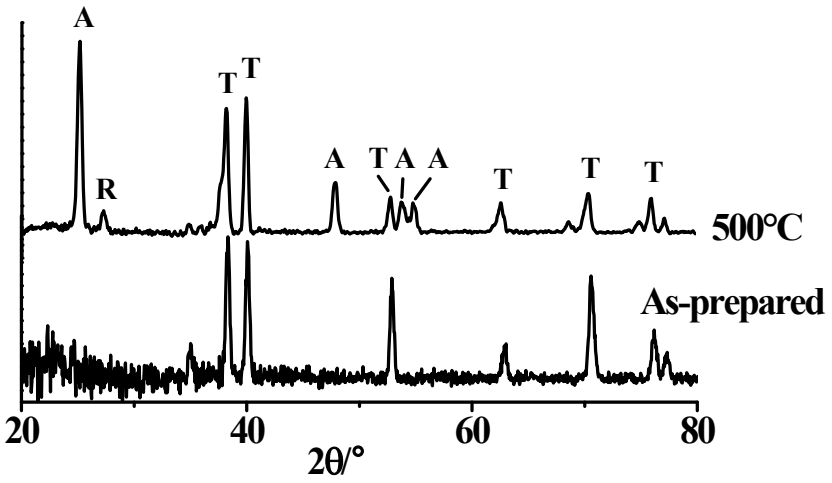

Fig. 2S XRD pattern of the porous TiO_2 film formed in organic electrolyte and before or after being annealed.

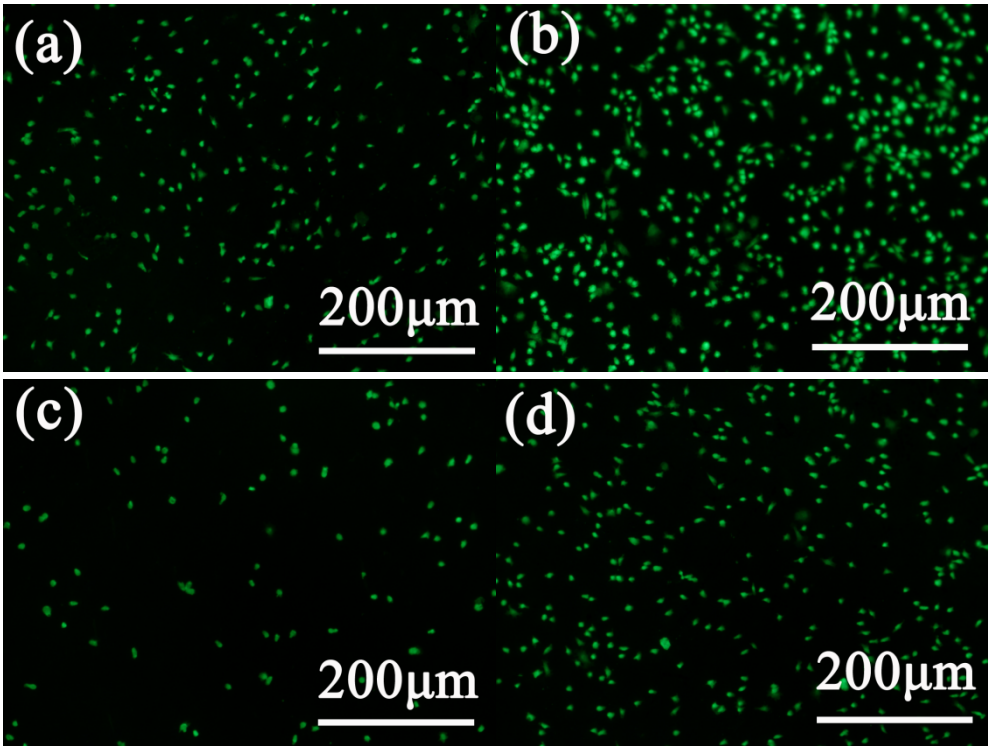

Fig. 3S The fluorescent images of the *rBMSCs* cultured on ***Org*** (a), ***Org-RGD*** (b), ***Org-AMP*** (c) and ***Org-AMP-2RGD*** (d) for 4 h.

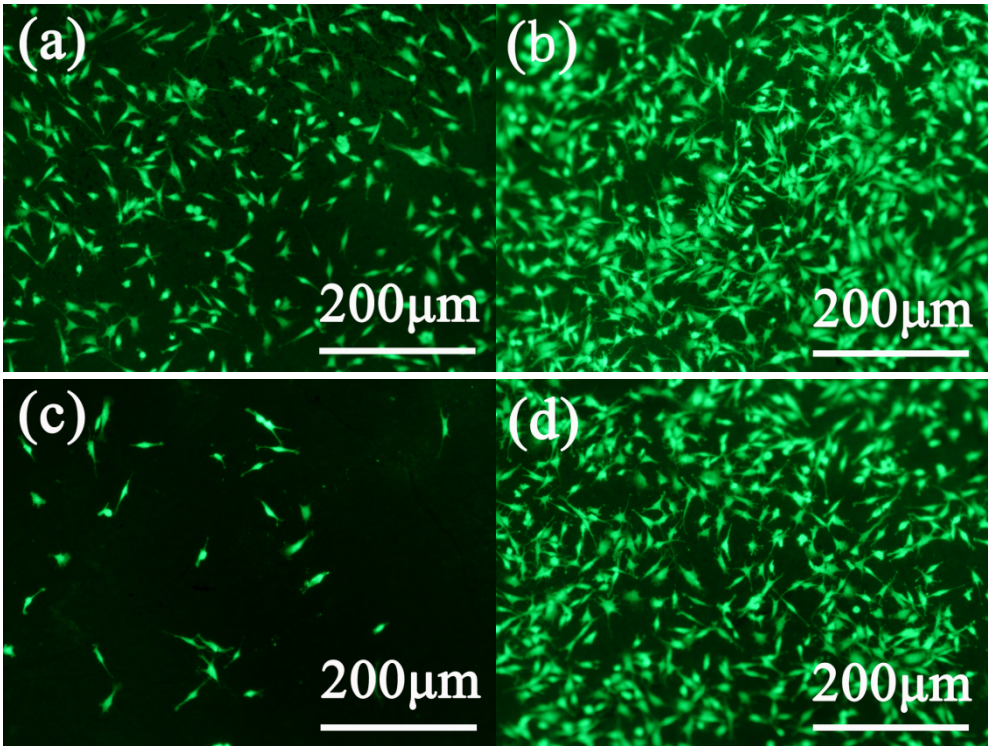

Fig. 4S The fluorescent images of the *rBMSCs* cultured on ***Org*** (a), ***Org-RGD*** (b), ***Org-AMP*** (c) and ***Org-AMP-2RGD*** (d) for 24 h.

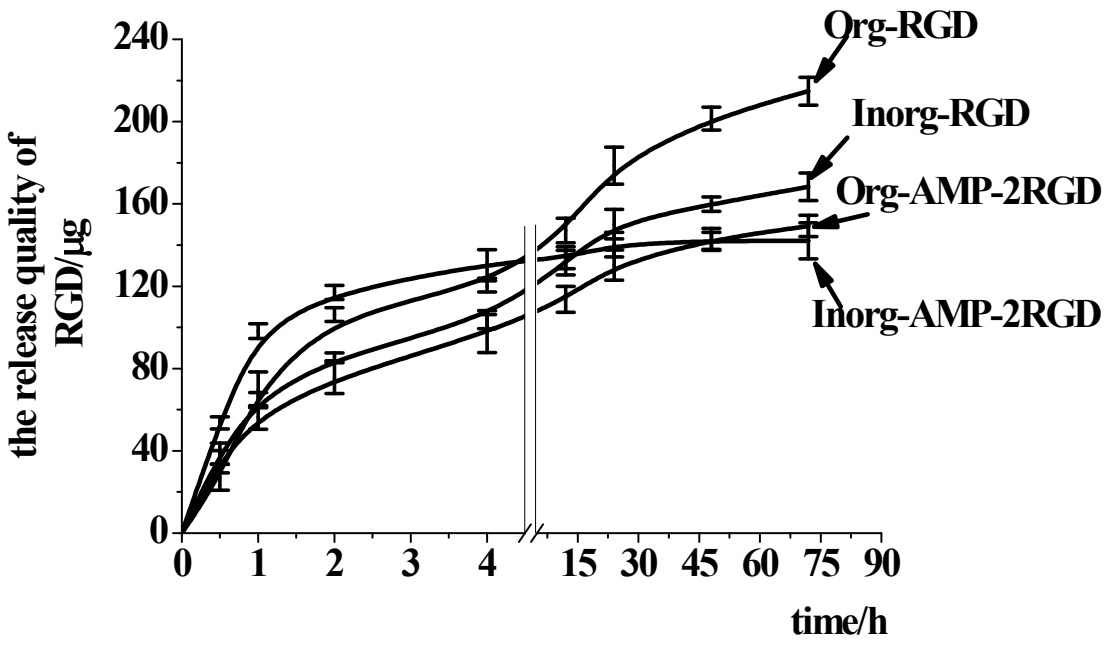

Fig. 5S In vitro release of RGD from the indicated films (n=4).

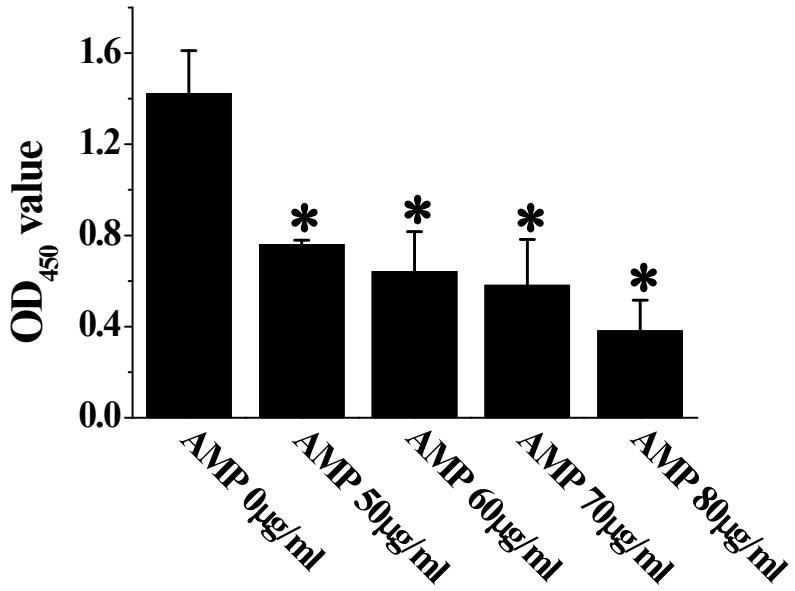

Fig. 6S Cell viability of *rBMSCs* in H-DMEM mediums containing AMP at various concentrations for 24 h (n=4). * denotes significant differences ($p < 0.001$) compared with "AMP 0μg/ml" group.

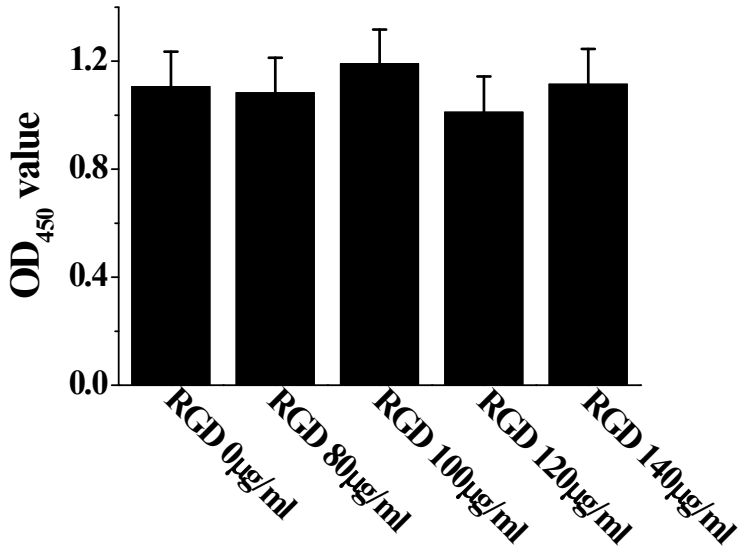

Fig. 7S Cell viability of *rBMSCs* in H-DMEM mediums containing RGD at various concentrations for 24 h (n=4).

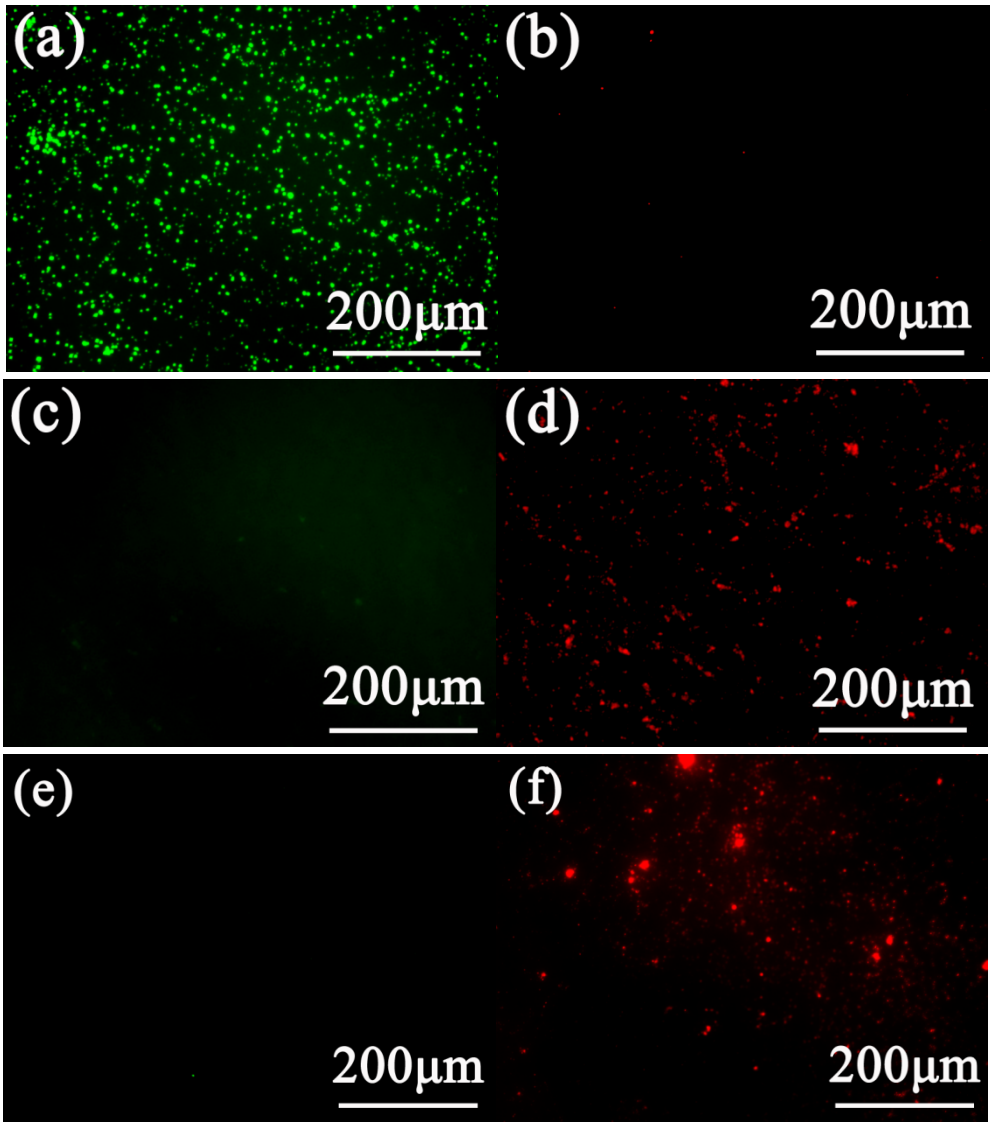

Fig. 8S The fluorescent images of *E. coli* on ***Inorg*** ((a) and (b)) ,***Inorg-AMP*** ((c) and (d)) or ***Inorg-AMP-2RGD***((e) and (f)) films. The (a), (c) and (e) images were got under FITC channel, and the (b), (d) and (f) images were got under TRITC channel. The red bacteria were dead, while the green bacteria were alive.