Supplementary Information

A Facile and Universal Strategy to Endow Implant Materials Antibacterial Ability via Alkalinity Disturbing Bacterial Respiration

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1. EXPERIMENTAL SECTION

1.1. Materials Preparation

Polyetheretherketone (PEEK) with a thickness of 1 mm was incised into the square plate (10 mm*10 mm), and then polished and ultrasonically cleaned. The obtained sample was denoted as PEEK. Besides, 0.50 g of ZrO_2 powder (400 nm in diameter) was made into a disk (6 mm in diameter) via isostatic cool pressing and then calcined for 6 hours at 1300 °C. Afterwards, the obtained ZrO_2 disk was polished, and then ultrasonically cleaned. The obtained sample was denoted as ZrO_2. Next, PEEK and ZrO_2 samples were placed in the vacuum chamber, and the chamber pressure was controlled below 4.0×10^{-3} Pa. Then pure Ar gas was introduced into the chamber to regulate the working pressure to 0.5 Pa, and a radio frequency magnetron sputtering was applied to deposit MgO film on PEEK and ZrO_2 samples by using pure MgO target (purity:＞99.99%). The sputtering parameters (power and time) were 300W for 40 min, and the obtained samples were denoted as M-PEEK and M-ZrO_2, respectively.

1.2. Samples Characterization

The morphologies of the surfaces of polished Ti, PEEK, M-PEEK, ZrO_2, and M-ZrO_2 samples and the morphology of cross-section of MgO film on M3 were observed by scanning electron microscopy (SEM, Magellan 400, FEI, USA), and the element content and distribution were detected via the equipped energy spectrum system (EDS). Carbon films were coated on the surfaces of ZrO_2/M-ZrO_2 before conducting EDS analysis.

1.3. Mg ion release

The samples were immersed in 10 mL of normal saline at 37 °C without stirring for 1, 4, 7, and 14 days. The extract solution was collected at every time point. The amount of Mg ion release was detected via inductively coupled plasma atomic emission spectroscopy (Varian Liberty 150, USA).

1.4. Antibacterial Ability Assessment

The procedures of this part are similar to those described in the main body, and the only difference is the volume of bacteria solution inoculated on samples. The surface area of the ZrO_2 disk is smaller than that of PEEK or Ti plate, therefore 20 μL bacterial suspensions were introduced on ZrO_2/M-ZrO_2 samples (60 μL for Ti and PEEK).

1.5. Cytotoxicity Assessment

The MC3T3-E1 cells with 5.0×10^4 cell/mL were seeded on various samples. Cell proliferation on samples at 1, 4, and 7 days were estimated via alamarBlue assay. The morphologies of cells cultured on samples for 4 days were observed by SEM, and the cell live/dead staining experiment was conducted at 4 days. These procedures are the same as those described in the main body, except for that of live/dead staining cells on PEEK/M-PEEK. To eliminate the impact of strong fluorescence produced by PEEK, the cells were firstly detached from PEEK/M-PEEK sample surfaces by using trypsin and then were collected by centrifugation. After that, the cells were suspended and stained by using a 100 μL PBS mix solution containing propidium iodide (PI, 5 μM) and calcein-AM (2 μM).

1.6. Statistical Analysis

Statistical analysis was conducted by GraphPad Prism statistical software package. One/two-way analysis of variance was applied to assess the statistical significance of the difference (p), and $p < 0.05$ was regarded as statistically significant.

2. RESULTS AND DISCUSSION

2.4. Surface Structure and Chemistry

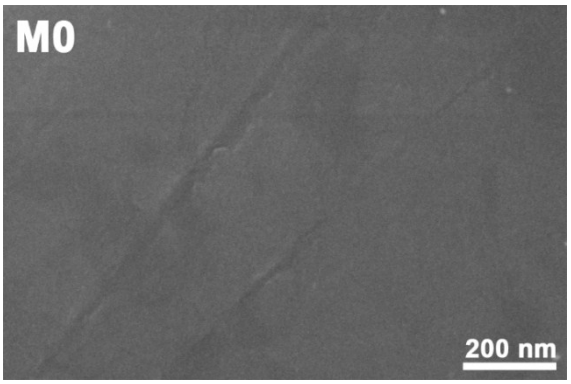

Figure S1. SEM morphologies of polished Ti (d).

Figure S1 shows the SEM morphology of polished titanium (M0). The surface of M1 showed almost flat topography, and only some scratches caused by sanding can be observed.

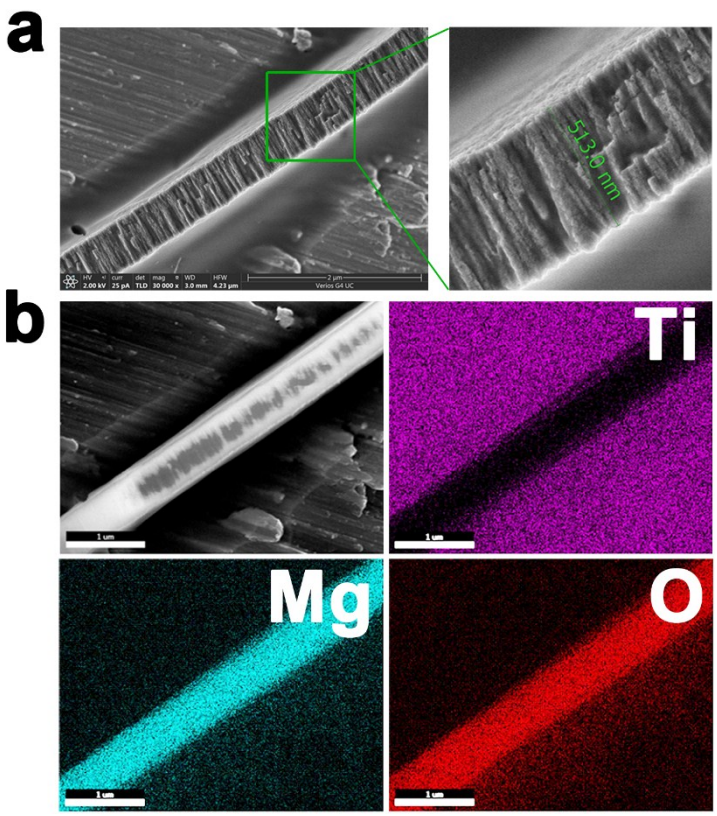

Figure S2. SEM morphologies of the cross-section of MgO film on M3 (a); and the cross-sectional view and elemental mapping images (b).

The SEM morphologies of the section of MgO film on M3 are shown in Figure S2a. The thickness of the MgO film is 513.0 nm. Besides, it can be found that the film consists of plenty of columnar crystals, which was probably resulted from the small bulk/surface diffusion caused by the low temperature of Ti substrates and the high melting point of MgO. The corresponding EDS mapping images of MgO film on M3 were displayed in Figure S2b, confirming the successful fabrication of MgO film on titanium.

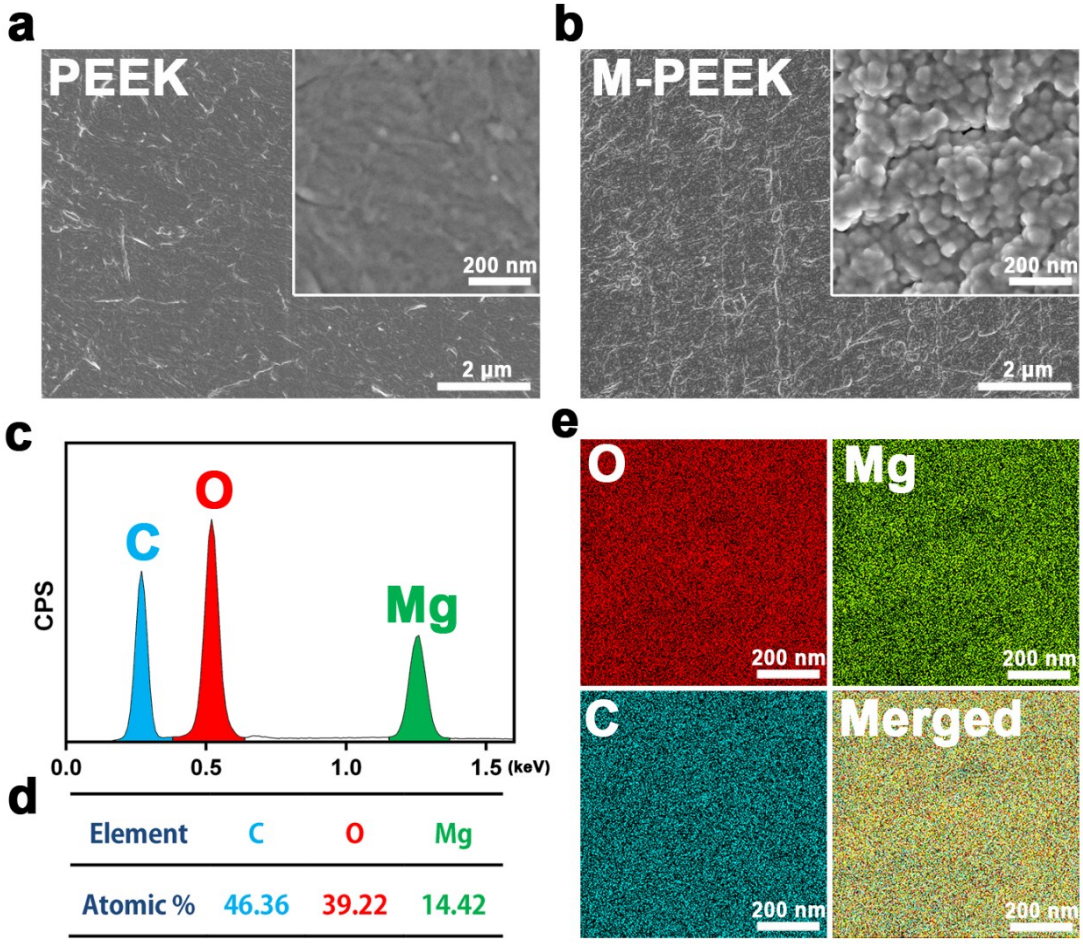

Figure S3. SEM morphologies of PEEK (a) and M-PEEK (b) at low and high magnification; EDS spectra of M-PEEK (c); surface elemental content of M-PEEK (d); and EDS mapping images of the high magnification area of M-PEEK(e).

As shown in Figure S3a and b, the surface topography of M-PEEK is rougher than that of PEEK. Moreover, some nanoparticles were observed on M-PEEK, and it seems like that these MgO nanoparticles fused together with PEEK substrate, which

was probably resulted from the melting of PEEK surface, which was caused by the continuous heating of high energy particles during the sputtering process. The results of EDS spectra (Figure S3c) and surface elemental contents (Figure S3d) clearly display the presence of Mg, O, and C elements on the surface of M-PEEK. EDS mapping images (Figure S3e) show that Mg, O, and C elements evenly distributed on the surface of M-PEEK, indicating the successful fabrication of MgO film on PEEK.

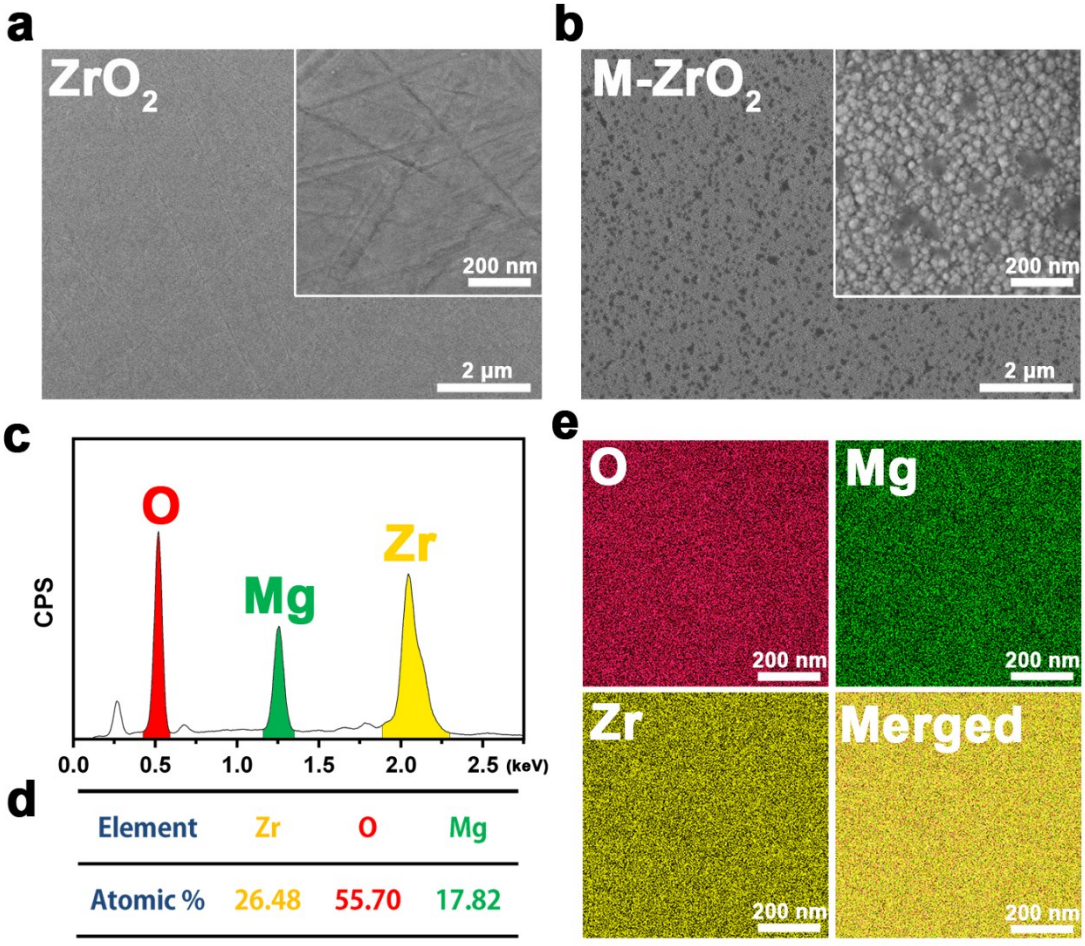

Figure S4. SEM morphologies of ZrO_2 (a) and M-ZrO_2 (b) at low and high magnification; EDS spectra of M-ZrO_2 (c); surface elemental content of M- ZrO_2 (d); and EDS mapping images of the high magnification area of M-ZrO_2 (e).

As shown in Figure S4a and b, the surface topography of ZrO_2 is almost flat topography except for some scratches caused by sanding. After sputtering MgO, plenty of nanoparticles appeared on the surface of ZrO_2. The results of EDS spectra

(Figure S4c) and surface elemental contents (Figure S4d) confirm the presence of Mg, O, and C elements on the surface of M-ZrO_2. EDS mapping images (Figure S4e) show that Mg, O, and Zr elements evenly distributed on the surface of M-ZrO2, indicating the successful fabrication of MgO film on ZrO_2.

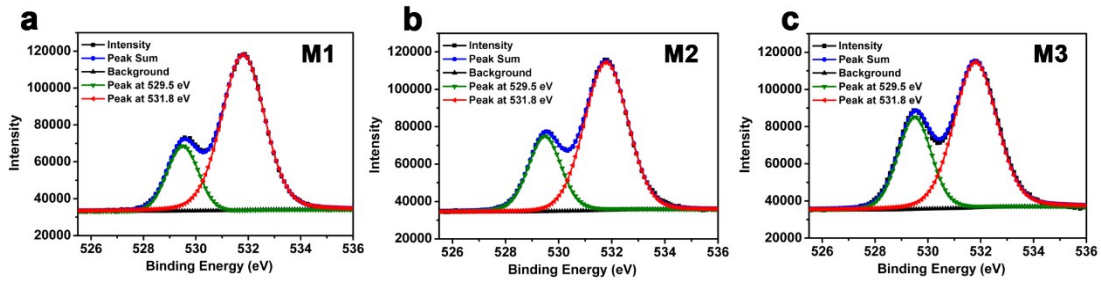

Figure S5. O 1s high resolution XPS spectra of M1 (a), M2 (b), and M3 (c) sample surfaces.

Figure S5 presents the O1s peak of the surfaces of M1, M2, and M3, which can be divided into two peaks at 529.5 eV and 531.8 eV, assigned to O of MgO lattice, and O of hydroxyl, respectively. The shapes of two peaks on M1, M2 and M3 are similar, but the intensities of the peak at 529.5 eV on M 1, M2 and M3 are obviously different. The corresponding trend is as follows: M1 < M2 < M3, which is probably ascribed to the different thicknesses of MgO films on M1, M2, and M3. The thicker film would lead a stronger signal of O of MgO lattice, but the signal of O of hydroxyl had little relation with the thickness of the MgO film.

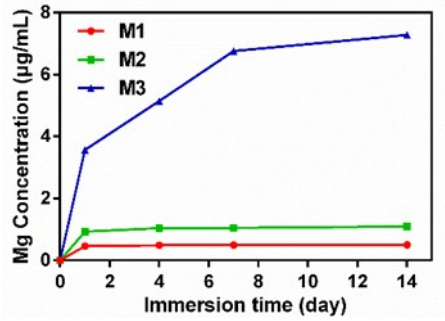

Figure S6. Cumulative Mg ion release amounts in saline solution after immersing M1, M2 and M3 samples for various days.

Figure S6 presents the result of cumulative Mg ions released from M1, M2, and M3 samples. After immersion for 1 day, negligible amount of Mg ions were detected from M1 and M2 groups, indicating that the MgO films on M1 and M2 dissolved completely within 1 day. However, M3 sample still released magnesium ion after

immersion for 7 days, indicating that MgO film on M3 can maintain at least 7 days.

2.5. Antibacterial Ability

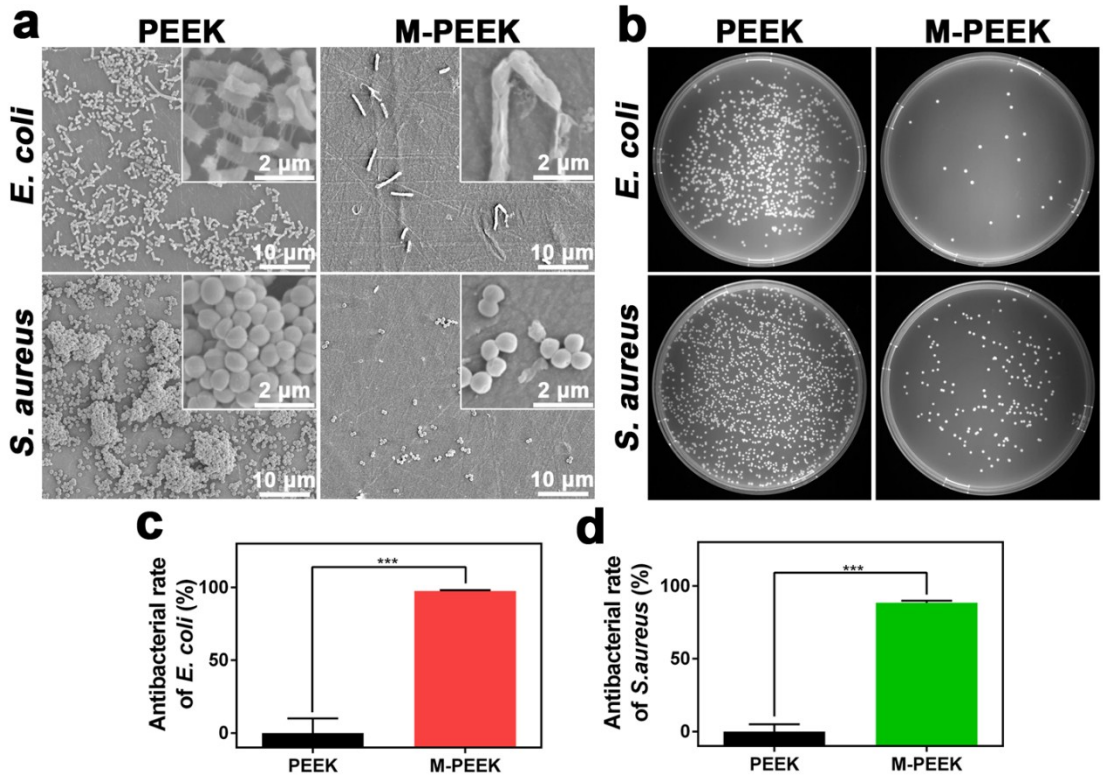

Figure S7. SEM morphologies of *E. coli* and *S. aureus* cultured on PEEK and M-PEEK at low and high magnification (a); photographs of re-cultivated *E. coli* and *S. aureus* colonies on agar (b); and the quantitative results of antibacterial rates of *E. coli* (c) and *S. aureus* (d). *** $p < 0.001$.

Figure S7a shows the SEM morphologies of *E. coli* and *S. aureus* on the PEEK and M-PEEK surfaces after cultured for 12 h. A large amount of *E. coli* and *S. aureus* adhered on PEEK sample, exhibiting a typical baculiform and spherical morphology with intact membrane, indicating good growth of *E. coli* and *S. aureus* on unmodified PEEK. For M-PEEK, the amount of *E. coli* and *S. aureus* significantly declined, and some *E. coli* and *S. aureus* with broken morphology were observed, indicating good antibacterial ability of M-PEEK. The results of bacterial colony are shown in Figure S7b. The amounts of *E. coli* and *S. aureus* colony of M-PEEK are significantly less than that of PEEK groups, and the corresponding antibacterial rates are presented in Figure S7c and d. The antibacterial rates of M-PEEK against *E. coli* and *S. aureus* are 97.50 ± 0.29% and 88.44 ± 0.71%, respectively, further verifying the good antibacterial ability of the PEEK modified by MgO magnetron sputtering.

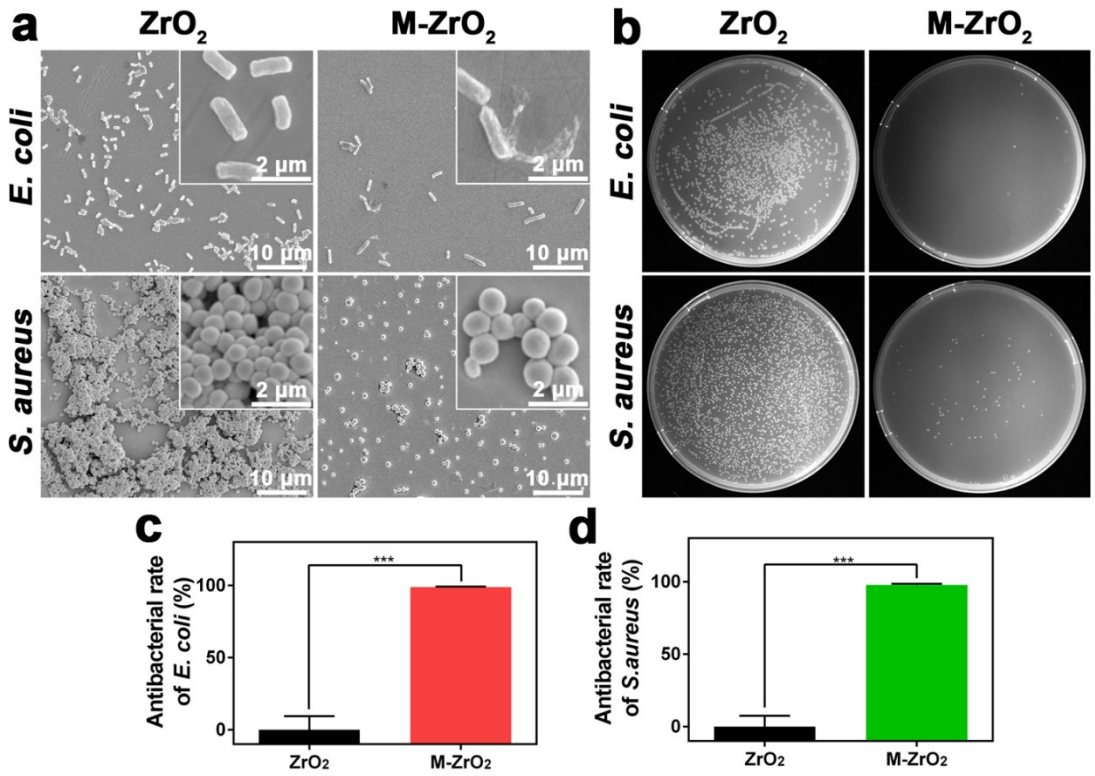

Figure S8. SEM morphologies of *E. coli* and *S. aureus* cultured on ZrO_2 and M-ZrO_2 samples at low and high magnification (a); photographs of re-cultivated *E. coli* and *S. aureus* colonies on agar (b); and the quantitative results of antibacterial rates of *E. coli* (c) and *S. aureus* (d). *** $p < 0.001$.

Figure S8a shows the SEM morphologies of *E. coli* and *S. aureus* on the ZrO_2 and M- ZrO_2 surfaces after cultured for 12 h. Similar to PEEK, plenty of *E. coli* and *S. aureus* adhered on ZrO_2 sample, exhibiting a typical baculiform and spherical morphology with intact membrane, indicating good growth of *E. coli* and *S. aureus* on unmodified ZrO_2. For M-ZrO_2, the amount of both *E. coli* and *S. aureus* significantly declined, and some *E. coli* and *S. aureus* with broken morphology were observed, indicating good antibacterial ability of M-ZrO_2. The results of the bacterial colony are shown in Figure S8b. The amounts of *E. coli* and *S. aureus* colony of M- ZrO_2 are significantly less than that of ZrO_2 groups, and the corresponding antibacterial rates are presented in Figure S8c and d. The antibacterial rates of M- ZrO_2 against *E. coli* and *S. aureus* are 98.80 ± 0.20% and 97.70 ± 0.50%, respectively, further verifying the good antibacterial ability of the ZrO_2 modified by MgO magnetron sputtering.

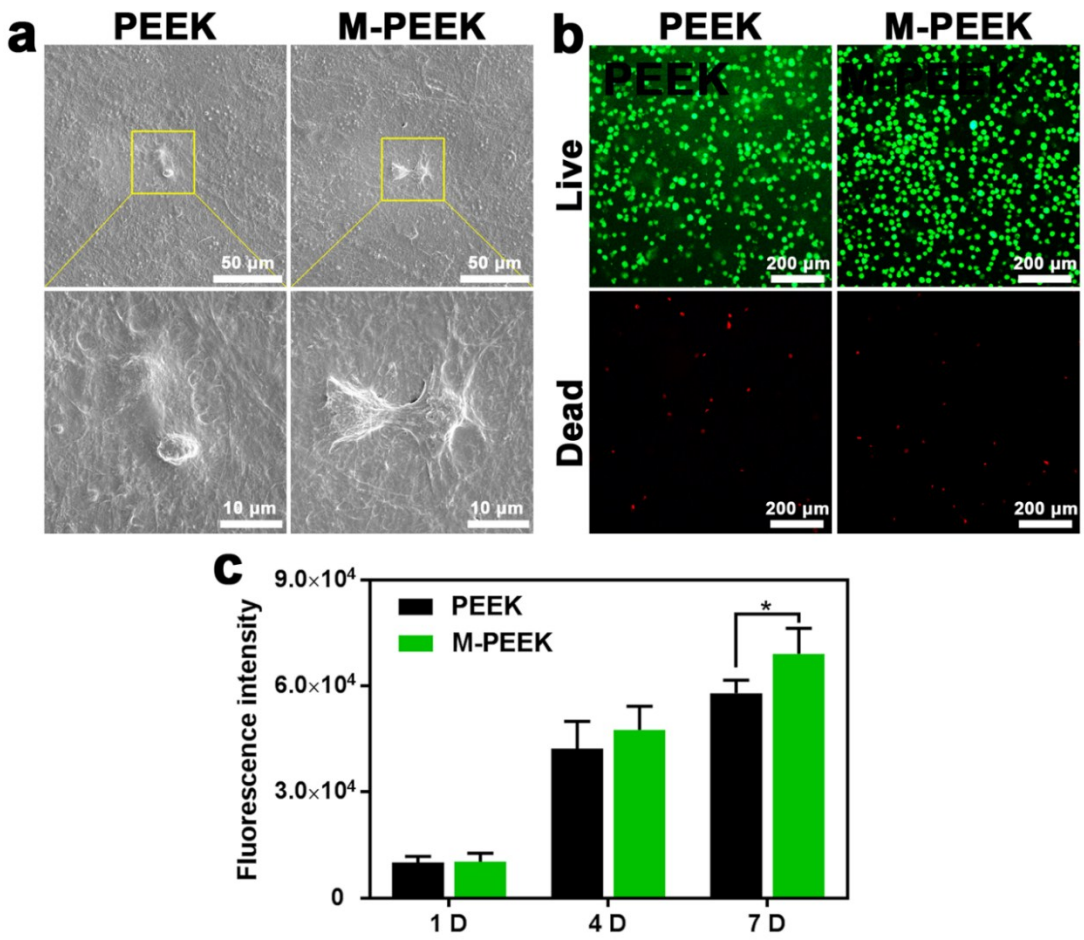

Figure S9. SEM morphologies of MC3T3-E1 cells cultured on PEEK and M-PEEK for 4 days at low and high magnification (a); fluoroscopy images of live/dead (green/red) staining of MC3T3-E1 cells cultured on PEEK and M-PEEK for 4 days (b); fluorescent intensity of alamarBlue reduced by MC3T3-E1 cells cultured on PEEK and M-PEEK for 1, 4 and 7 days, * $p < 0.05$.

The SEM morphologies of MC3T3-E1 cells cultured on PEEK and M-PEEK for 4 days are shown in Figure S9a. The surfaces of PEEK and M-PEEK were completely covered by cells. Besides, plenty of live cells were observed from PEEK and M-PEEK groups, and only a small amount of dead cells can be found. Moreover, there exists no significant difference about the amounts of both live and dead cells of PEEK and M-PEEK groups, indicating the nontoxicity of MgO modified PEEK. The results of the quantitative proliferation rate of cells (Figure S9c) show no obvious difference between PEEK and M-PEEK groups at 1 and 4 days, and the proliferation rate of cells on M-PEEK was slightly larger than that on PEEK at 7 days, further confirming the good cytocompatibility of MgO modified PEEK.

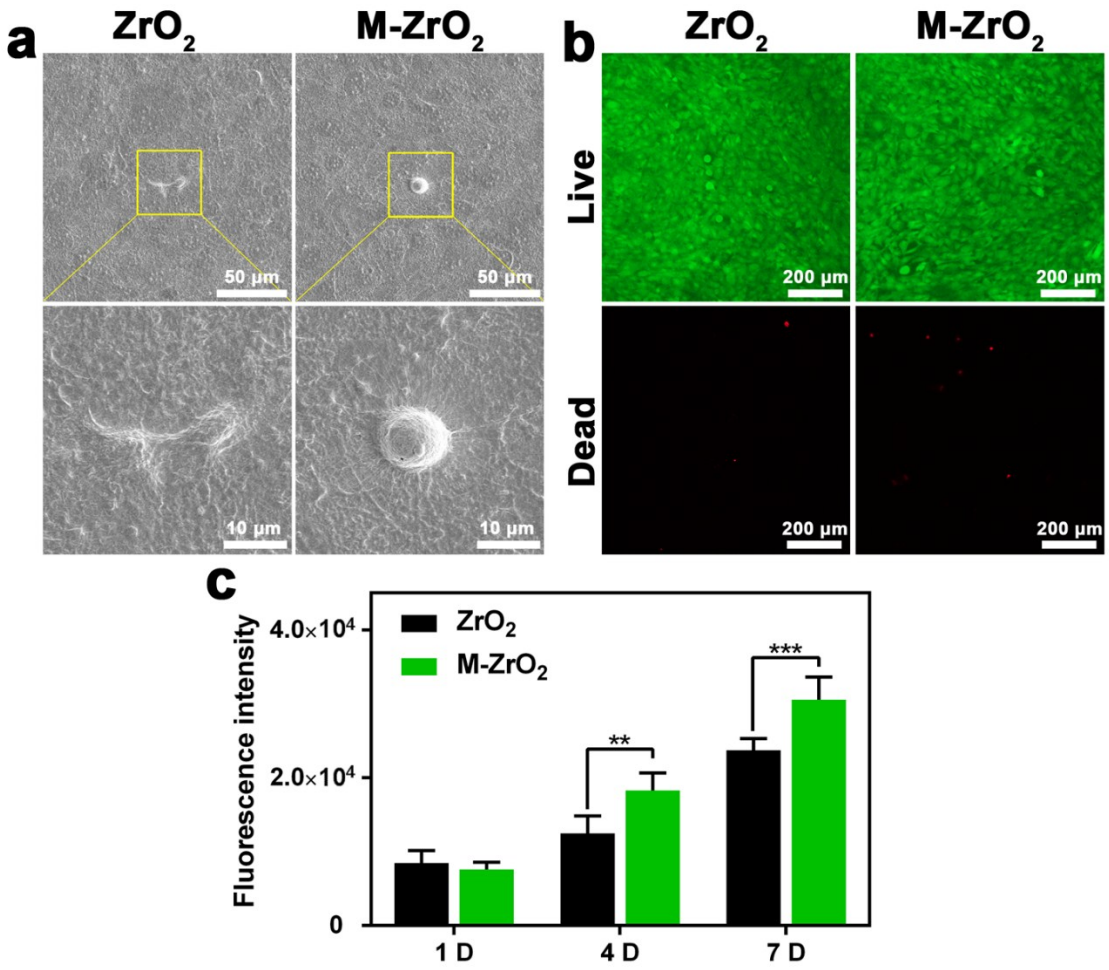

Figure S10. SEM morphologies of MC3T3-E1 cells cultured on ZrO_2 and M-ZrO_2 for 4 days at low and high magnification (a); fluoroscopy images of live/dead (green/red) staining of MC3T3-E1 cells cultured on ZrO_2 and M-ZrO_2 for 4 days (b); fluorescent intensity of alamarBlue reduced by MC3T3-E1 cells cultured on ZrO_2 and M-ZrO_2 for 1, 4 and 7 days, ** $p < 0.01$, *** $p < 0.001$.

The SEM morphologies of MC3T3-E1 cells cultured on ZrO_2 and M-ZrO_2 for 4 days are shown in Figure S10a. Similar to PEEK and M-PEEK, cells completely covered the surface of ZrO_2 and M-ZrO_2, and a very large number of live cells and a negligible amount of dead cells were observed on ZrO_2 and M-ZrO_2 (Figure S10b), indicating the nontoxicity of MgO modified ZrO_2. In addition, the proliferation rates of cells on M-ZrO_2 at 4 and 7 days were bigger than that of ZrO_2 (Figure S10c), confirming the excellent cytocompatibility of MgO modified ZrO_2.