Supporting Information

Effect of Local Alkaline Microenvironment on the Behaviors of Bacteria and Osteogenic Cells

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EXPERIMENTAL SECTION

Bacteria counting

After incubation for 24 h, the bacteria adhered on sample, the sample and the liquid on it were transferred to a test tube with 3 mL sterile normal saline together. Then, the tube was violently shaken for 60 s via vortex oscillator to separate bacteria from sample surface. After that, 100 μL bacterial suspension was inoculated on Luria-Bertani or Nutrient Broth No 2 agar culture mediums, and then re-cultured at 37 °C for 18 h. The bacterial colonies were counted according to the National Standard of China GB/T 4789.2 protocol and the antibacterial rate = (C-T)/C × 100%, where C and T were the average count of bacteria in control group (Ti) and test groups (LDH, LDH-250 and LDH-500), respectively. Each bacteria suspension was re-cultured on three agar culture mediums and the results were expressed as means ± SD (standard deviation).

Effect of pH on bacteria

To study the effect of pH on cell viability and Reactive Oxygen Species (ROS) level of bacteria, the pH of saline solution was adjusted from 7.0 to 12.0 ± 0.05 (at 1 intervals) by using NaOH solution. Afterward, a drop of bacterial suspension (60 μL with 10^7 cfu/ml) diluted ten-fold by these saline solutions with different pH was dropped onto the carry sheet glass and incubated 24 h at 37 °C (three replicates). Cell viability and ROS level of bacteria were evaluated by using alamarBlue assay and ROS Assay Kit, respectively. The detailed steps are described in the main body.

Effect of pH on cell proliferation

To study the effect of pH on the proliferation of rBMSCs and MC3T3-E1 cells, the pH of full α-MEM medium was adjusted from 7.5 to 9.0 ± 0.05 (at 0.5 intervals) by using NaOH solution, and the full α-MEM medium with pH = 7.4 ± 0.05 was the control group. The rBMSCs and MC3T3-E1 cells with a density of 0.5 × 10^4 cell/mL were seeded on a 96-well plate. After culturing for 1 day at 37 °C, the mediums were changed by fresh mediums with pH = 7.4, 7.5, 8.0, 8.5 and 9.0 ± 0.05 in succession, and the cells were cultured for another 1 and 4 days (three replicates). At the end of period, the cells

were washed with PBS two times followed by incubation for 2 h with 0.5 mL of medium mixed with 10% (vol) alamarBlue. Afterward, the fluorescence intensity of reduced alamarBlue in the medium was examined with an enzyme labeling instrument at 560 nm extinction wavelength and 590 nm emission wavelength, respectively.

RESULTS AND DISCUSSION

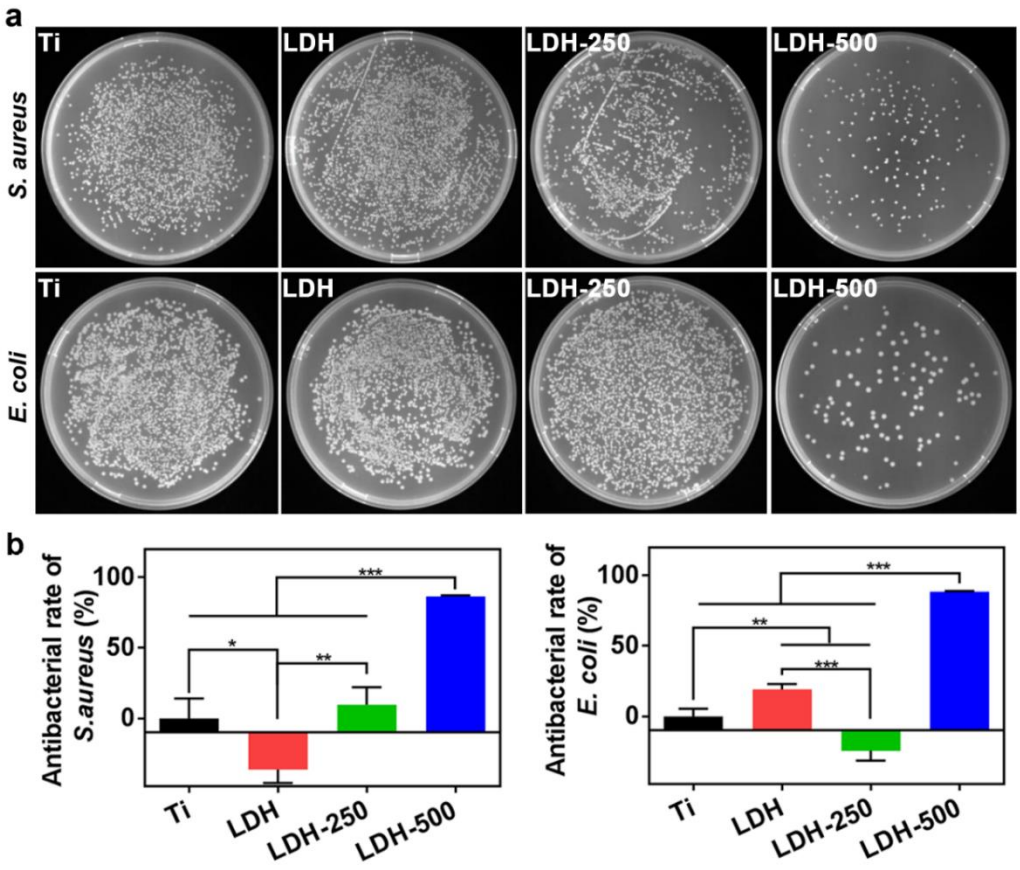

Figure S1. Photographs of re-cultivated *S. aureus* and *E. coli* colonies on agar (a) and the quantitative analysis of antibacterial rates of *S. aureus* and *E. coli* (b). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S1 show the results of bacteria counting of *S. aureus* and *E. coli*. The colony amounts of both *S. aureus* and *E. coli* on LDH-500 sample are significantly less than that on other samples. The corresponding antibacterial rates of LDH-500 sample against *S. aureus* and *E. coli* are 86.27% and 88.44%, respectively. Nevertheless, the LDH and

LDH-250 samples have shown little antibacterial abilities, and even promoted the growth of bacteria. As mentioned above, the viability and number of bacteria on LDH and LDH-250 surfaces declined, indicating that the growth of bacteria was suppressed. However, once the restraining bacteria are in a nutrient-rich environment, they would recover and grow rapidly, which result in this indistinguishable colony counts.

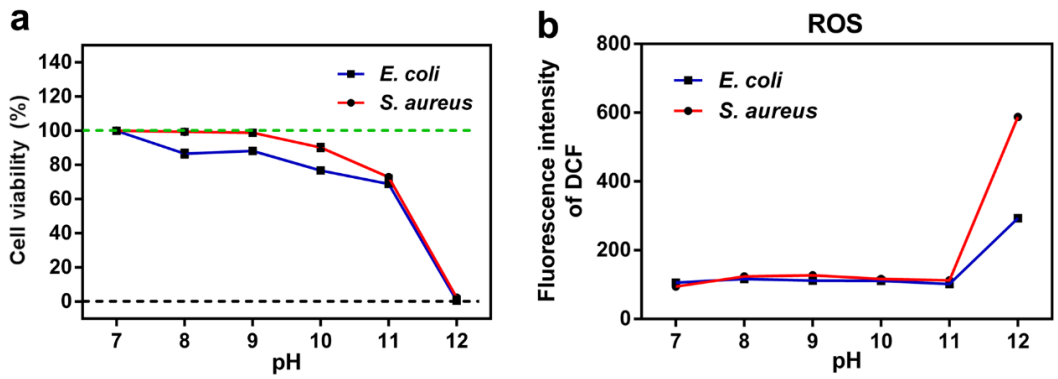

Figure S2. Cell viability (a) and intracellular ROS levels (b) of *S. aureus* and *E. coli* in medium with different pH value cultured for 24 h.

The cell viability and intracellular ROS level of bacteria cultured with various alkaline mediums are shown in Figure S2. When the pH value is less than 9.0, the cell viability and intracellular ROS level of both *E. coli* and *S. aureus* have no significant change. With the increase of pH from 9.0 to 11.0, the cell viabilities of *E. coli* and *S. aureus* declined to 72.87% ± 0.38 and 68.85% ± 0.51, respectively. However, the intracellular ROS level of *E. coli* and *S. aureus* almost remained constant. The results indicate that alkaline medium with pH from 9.0 to 11.0 could inhibit bacterial cell viability but induce no oxidative stress. What's more, as the pH up to 12.0, the cell viability of *E. coli* and *S. aureus* sharply declined to 0.64% and 2.36%, respectively, indicating that alkaline medium with pH more than 11.0 could effectively kill bacteria. Meanwhile, the intracellular ROS level of *E. coli* and *S. aureus* cultured in medium with pH 12 increased to about 3 and 6 times compared with other groups, indicating that stronger alkalinity would induce bacteria producing more ROS.

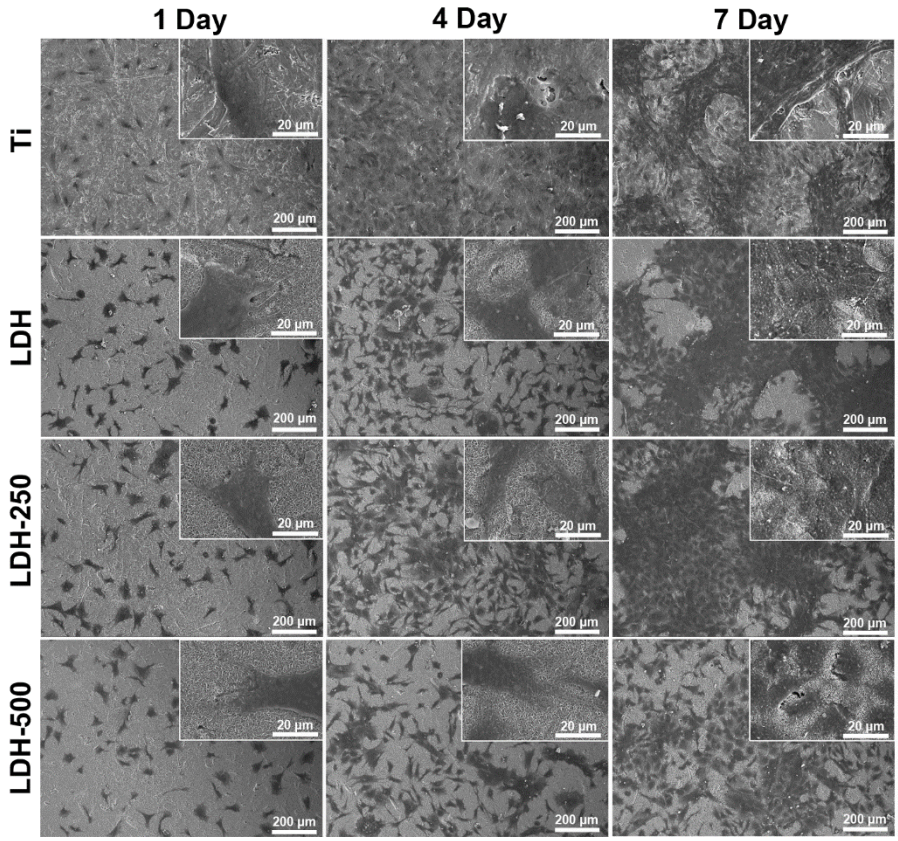

Figure S3. SEM morphologies of rBMSCs cells cultured on sample surfaces for 1, 4 and 7 days at low and high magnification.

The corresponding SEM morphologies of rBMSCs cells cultured on the sample surfaces for 1, 4 and 7 days are shown in Figure S3. When cultured for 1 day, there is no significant difference among all samples. Prolonged to 4 and 7 days, the surface of Ti was completely covered by cells. However, cells barely covered partial area of LDH, LDH-250 and LDH-500 sample surfaces, and the covering area of cells on LDH-500 sample are smaller than that on LDH and LDH-250 samples. The results indicate that the proliferation of rBMSCs cells cultured on the modified Ti surfaces is inferior to that on Ti surface, and LDH-500 sample shows worst cell proliferation among all groups.

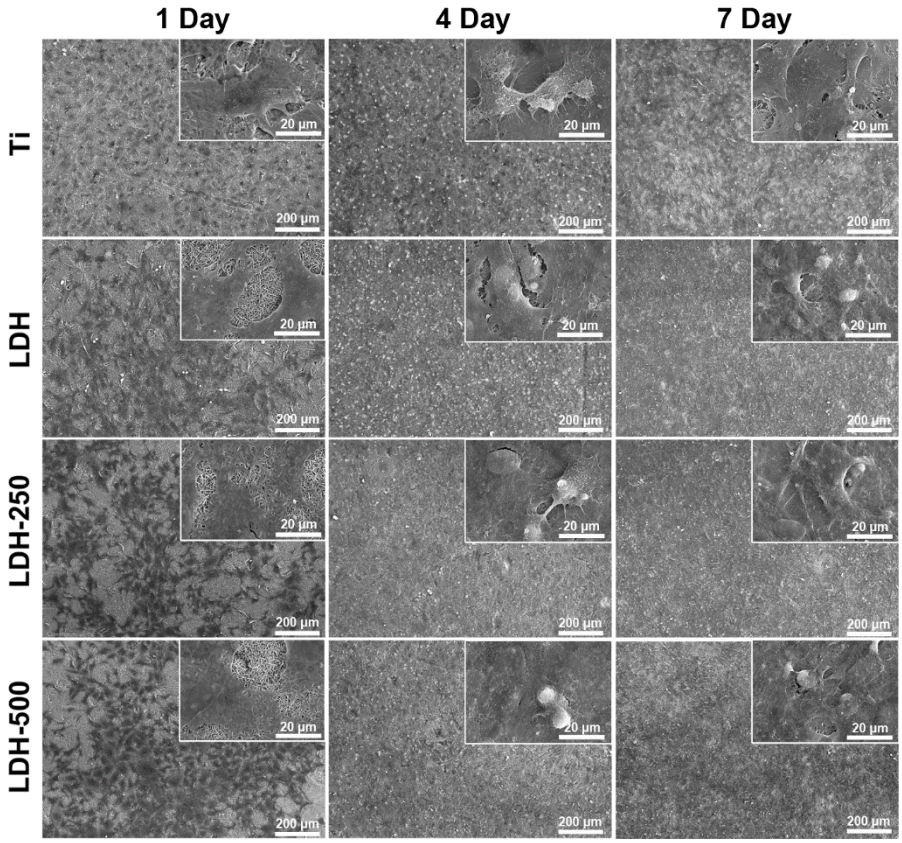

Figure S4. SEM morphologies of MC3T3-E1 cells cultured on sample surfaces for 1, 4 and 7 days at low and high magnification.

For MC3T3-E1 cells, when cultured for 1 day, the Ti surface was completely covered by cells, and most area of modified Ti samples were covered by cells as well. With time prolonging to 4 and 7 days, one or more layers of cells can be found on all sample surfaces, and there is no significant difference among all sample surfaces.

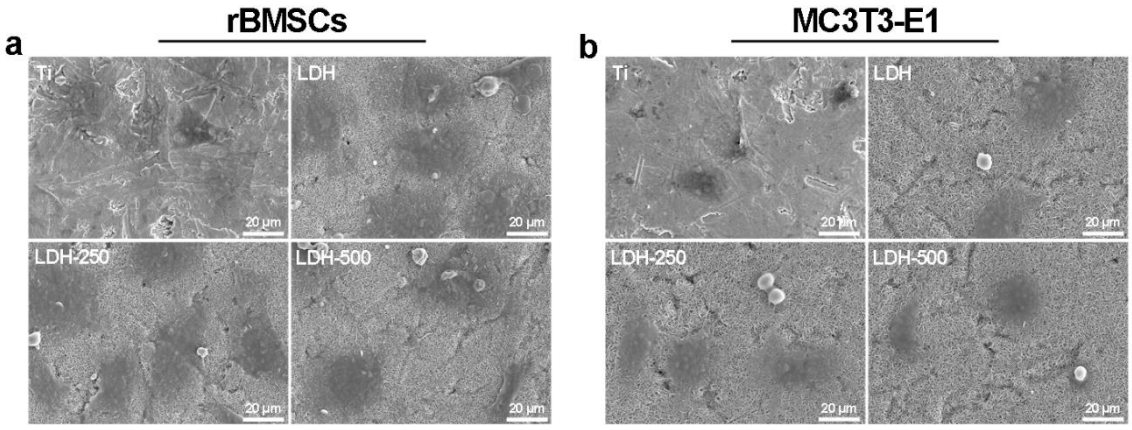

Figure S5. SEM morphology of rBMSCs (a) and MC3T3-E1 (b) cells cultured on various sample surfaces for 4 h.

The SEM morphologies of rBMSCs and MC3T3-E1 cells cultured on all samples for 4 h were observed. As shown in Figure S5, almost all rBMSCs and MC3T3-E1 cells completely spread on the surface of Ti. However, there still exist some round cells on the surfaces of LDH, LDH-250 and LDH-500 samples. The results show that initial adhesion and spreading of rBMSCs and MC3T3-E1 cells were inhibited on the LDH, LDH-250 and LDH-500 sample surfaces.

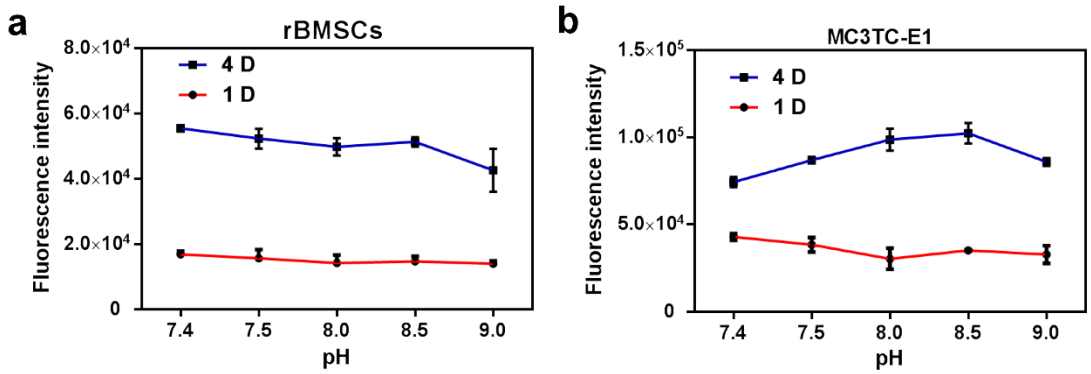

Figure S6. Effects of pH on the proliferation of rBMSCs (a) and MC3T3-E1 (b) cells analyzed by fluorescent intensity of reduced alamarBlue.

As shown in Figure S6, the pH of medium has a distinct effect on the cells proliferation. At the first day, both of rBMSCs and MC3T3-E1 cells cultured with various alkaline medium shows slight difference. However, as time prolonged to 4 days, the proliferation of rBMSCs cells was reduced with the increase of pH, especially for pH 9.0 ($p < 0.001$, vs the control group pH 7.4), but the other groups showed no significant difference. Moreover, the proliferation rate of MC3T3-E1 cells presents the Gaussian distribution with increase of pH. The maximum proliferation rate occurred at pH 8.5, which has significant difference to the control group pH 7.4 ($p < 0.001$). The results indicate that the alkaline medium could promote the proliferation of MC3T3-E1 cells when pH < 9.0 and inhibit the proliferation of rBMSCs cells when pH > 8.5.