Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Construction of an Antibacterial Defect-low Hybrid Layer by a Facile PEI Electrostatic Assembly Promotes Dentin Bonding

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Key words: dentin bonding durability, electrostatic assemble, antibacterial, defect-low hybrid layer, hydrogel-like bonding interface.

Materials and Methods

1. The microtensile bond strength of wet bonding in Spectrum Bond system.

Demineralized dentin surface was pretreated with 1% PEI smeared for 20s, 1% PEI smeared for 80s and 10% PEI smeared for 20s, respectively. The conditional dentin surface was coated with Spectrum Bond adhesive, then 3-mm-thick resin was piled (4 teeth er group). Samples were subjected to a tensile force at rate of 1 mm/min until failure.

2. The antibacterial rate of different concentration PEI aqueous solution.

A monoclonal colony of *Escherichia coli* (*E. coli*, ATCC25922) was selected to 10mL Luria-Bertani (LB, QDRS Biotec, CHN) liquid medium and cultured at 37℃ in an atmosphere of 5% carbon dioxide until it reached the exponential phase for bacterial growth. The concentration of the bacterial solution was diluted to 1.0×10^6 CFU/mL with LB liquid medium for subsequent use.

900 μL of the above bacterial suspension was seeded into 100 μL of LB containing different concentration of PEI aqueous solution. LB liquid medium was used as a positive control, and LB without PEI was used as a negative control. After being statically incubated with PEI for 8 h, 100 μL of bacterial suspension from each sample was pipetted out and measure the OD value (600 nm) by a MD-M5 microplate reader. Three replicates were used for all groups. The antibacterial ratio (%) was calculated as follows:

$$Antibacterial\ ratio\ (\%) = (1 - \frac{A_s - A_n}{A_p - A_n}) * 100\%$$

Where A_s, A_n and A_p are the absorbance of samples, negative control and positive

control.

3. Surface tension test

The surface tension was measured by a video-based cintact angle measing device (OCA 20, Dataphsice, Germany). The sodium polyacrylate (Aladdin, Shanghai, CHN) aqueous solution was mixed with PEI in a ratio of 1:1, which the final concentration was 0.1 wt%.

4. The broad-spectrum antibacterial rate of different concentration PEI aqueous solution.

A monoclonal colony of *Escherichia coli* (*E. coli*, ATCC25922) was selected to 10mL Luria-Bertani (LB, QDRS Biotec, CHN) liquid medium and cultured at 37℃ in an atmosphere of 5% carbon dioxide until it reached the exponential phase for bacterial growth. The concentration of the bacterial solution was diluted to 1.0×10^6 CFU/mL with LB liquid medium for subsequent use. Similar treatments were performed for *Staphylococcus aureus* (*S. aureus*, 25923). The calculation of antibacterial rate was mentioned in 3.

5. Broad-spectrum antibacterial activity of PEI by CLSM.

The culture of bacteria has been mentioned above. 3 mm × 3 mm dentin disk (n=3 for each group) were placed in a 48-well plate after ultraviolet sterilization for 1 hour. 1.0×10^6 CFU/mL of *E. coli* or *S. aureus* was added into each well and incubated at 37℃ for 12 h. Then samples were stained using a LIVE/DEAD bacterial viability and counting kit (Invitrogen, USA) and observed by CLSM (Leica dmi8, Germany).

Figure S1. The microtensile bond strength of wet bonding in Spectrum Bond system.

Figure S2. The antibacterial rate of different concentration PEI aqueous solution.

Figure S3. The surface tension of sodium polyacrylate solution with/without PEI.

Figure S4. The broad-spectrum antibacterial rate of different concentration PEI aqueous solution.

Figure S5. Broad-spectrum antibacterial activity of PEI by CLSM.

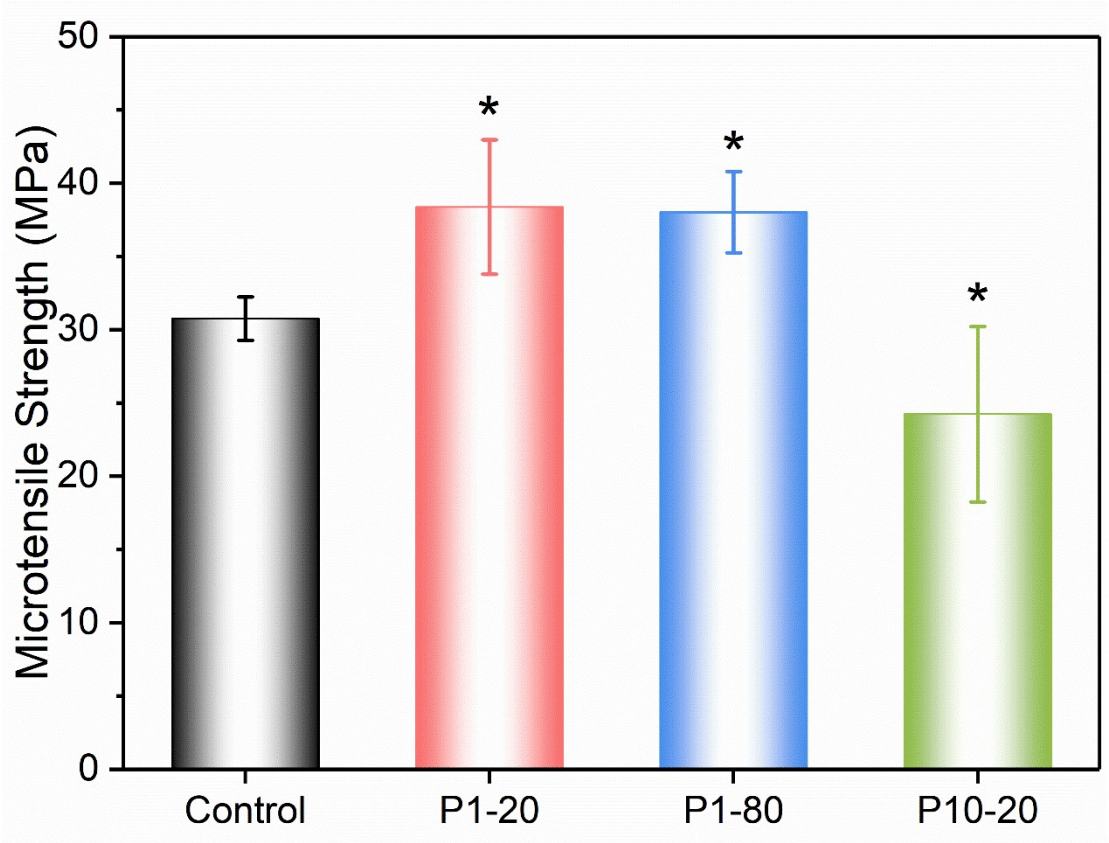

Figure S1. The microtensile bond strength of wet bonding in Spectrum Bond system.

The four groups mean control, applying 1% PEI aqueous solution for 20s, 1% PEI aqueous

solution for 80s and 10% PEI aqueous solution for 20s, respectively. * $p < 0.05$ vs. Control group.

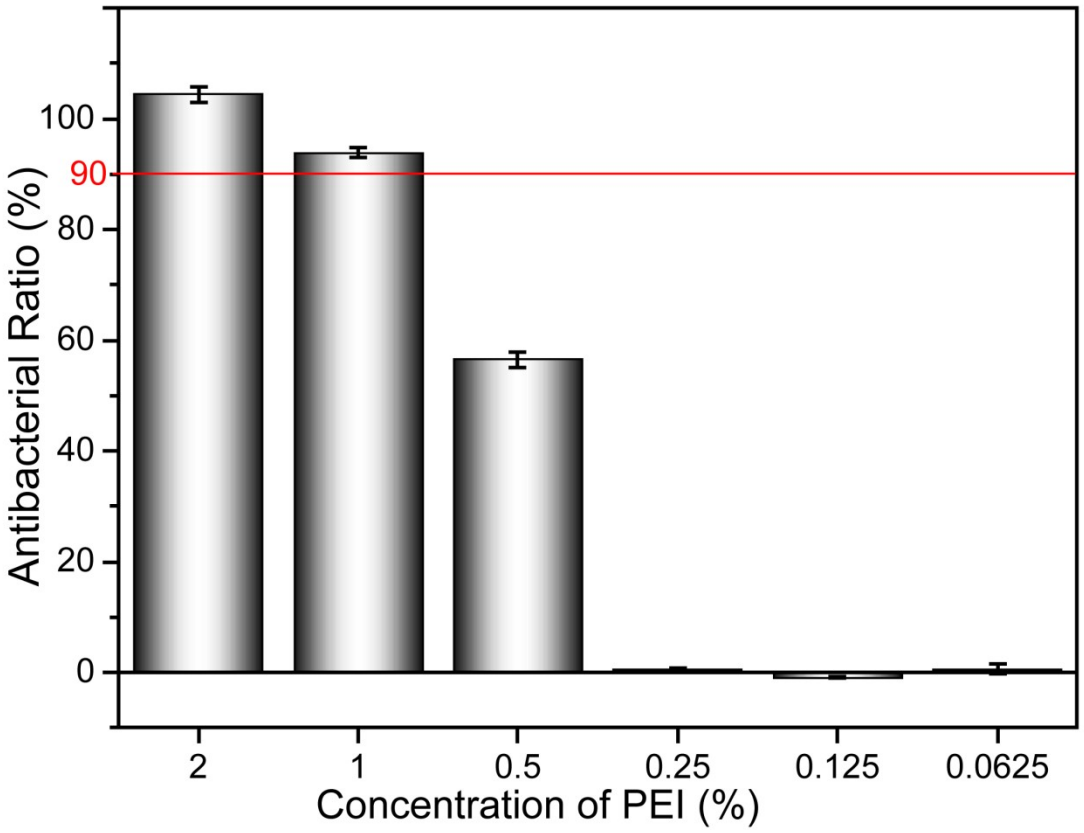

Figure S2. The antibacterial rate of different concentration PEI aqueous solution. There is obvious antibacterial effect when PEI concentration is more than 1%.

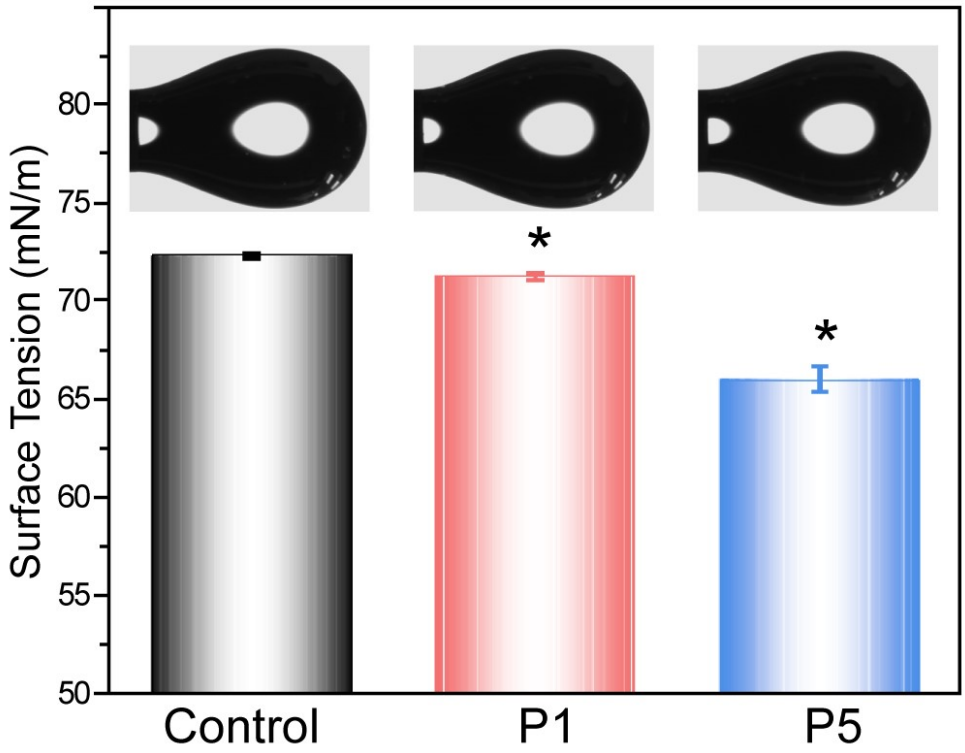

Figure S3. The surface tension of sodium polyacrylate solution with/without PEI. * p < 0.05 vs. Control group.

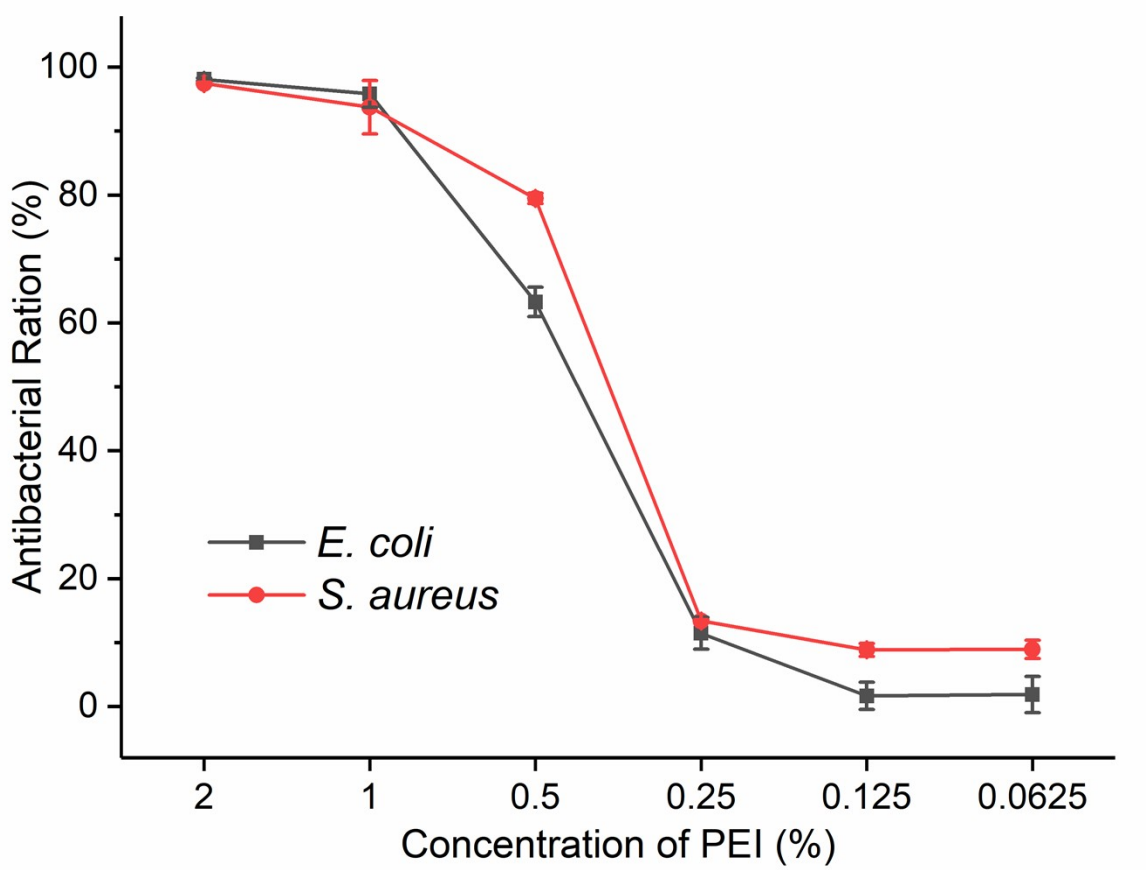

Figure S4. The broad-spectrum antibacterial rate of different concentration PEI aqueous solution. There is obvious antibacterial effect when PEI concentration is more than 1%.

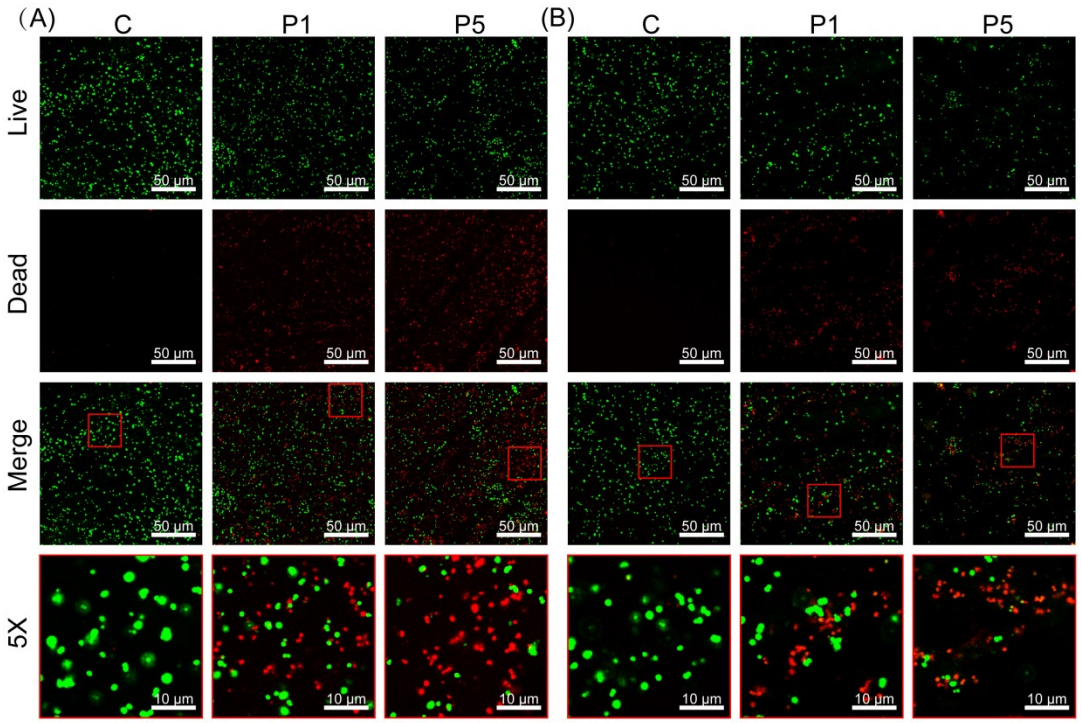

Figure S5. Broad spectrum antibacterial activity of PEI. (A) The destructive effect of dentin matrix with conditioning of *E. coli* (n=3). (B) Inhibitory effect of dentin matrix with conditioning of *S. aureus* (n=3).