Supporting Information

Protein-Mediated Anti-Adhesion Surface against Oral Bacteria

Xi Liu [a,b], Liying Peng[c], Jingxin Meng*[a], Zhongpeng Zhu[a,b], Bing Han*[c], and Shutao Wang[a,b]

[a] CAS Key Laboratory of Bio-inspired Materials and Interfacial Science, CAS Center for Excellence in Nanoscience, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100190, P. R. China.

[b] University of Chinese Academy of Sciences, Beijing, 100049, P. R. China

[c] Department of Orthodontics, Peking University School and Hospital of Stomatology, National Engineering Laboratory for Digital and Material Technology of Stomatology, and Beijing Key Laboratory of Digital Stomatology, Peking University, Beijing, 100081, P. R. China

E-mail: mengjx628@mail.ipc.ac.cn and kqbinghan@bjmu.edu.cn

1. Material and methods

Arch wires (AWs) were purchased from Plasdent Corp., USA. Brackets made from stainless steel, ceramic and resin were bought from Xin Ya Co., China. Brain heart infusion, brain heart infusion agar, tryptone, yeast extract and agar were obtained from Oxoid Co., England. 1 M Tris-HCls with the pH from 4 to 7 were bought from Beijing Applygen Technologies Inc, China. Acetone (>99.5%, AR) and alcohol (≥99.8%, GR) were from Beijing Chemical Co., China. Bovine serum albumin (BSA) and fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) were bought from Sigma-Aldrich Co, USA. 1H, 1H, 2H, 2H-Perfluorodecyltrimethoxysilane (FAS) were bought from J&K Chemical, China. Silica (SiO_2) quartz crystal microbalance (QCM) sensors were purchased from Q-sense, Sweden. HyClone® Phosphate buffered saline (PBS) was purchased from Thermo Scientific, USA. Deionized water (>1.82 MΩ cm, Milli-Q system) was used. All the chemicals were used directly without further modification.

2. Apparatus and characterization

Scanning electron microscope (SEM) images were recorded using an S-4800 instrument (HITACHI, Japan). UV-VIS-NIR absorbance measurement was realized by UV-2600 (SHIMADZU, Japan). Static contact angles were measured on a Dataphysics OCA20 contact-angle system (FILDERSTADT, Germany). Fluorescence images and corresponding intensities of Bare-AW, FAS-AW and FITC-BSA-AW could be observed with a fluorescence microscope (Nikon Ti-E, Japan). All QCM measurements were carried out at 25 °C by using Q-Sense E1 system (Q-Sense Sweden). Prior to binding assays between BSA and sensors (i.e., FAS-SiO_2), the QCM chamber was washed with deionized water and ethyl alcohol, then dried with N_2 flow. Then, Tris-

HCl solution was injected into the chamber at a flow rate of 100 μL min^{-1}. After obtaining the base line, the 10 mg/ml BSA in 1 M Tris-HCl solution was injected into the chamber (100 μL min^{-1}). When the absorption curves became stable, Tris-HCl solution was injected to acquire desorption curves of BSA at different pH value. All QCM curves were recorded by Q-Sense software and analyzed by Qtools. Surface Zeta potentials under different pH value were obtained from SurPASS 3 of Anton Paar. Atomic force microscope (AFM) images with the area of 1 μm × 1 μm were tested by employing Olympus LEXT Nano Search Microscope (OLS4500) with dynamic mode.

3. Bacteria culture

Streptococcus mutans (*S. mutans*, UA159) was used as the model bacteria because it was the dominating bacteria leading to dental decay.[1, 2] For *S. mutans*, a single bead was taken from its freezing solution in -80 °C and incubated for 48 h on heart infusion agar (OXOID, England) at 37 °C in an atmosphere enriched with 5% CO_2. Then, one clone was isolated from the agar and incubated with 1 ml of nutrient broth (OXOID, England) overnight at 37 °C in an atmosphere enriched with 5% CO_2. After reaching the required growth phase, *S. mutans* were harvested by centrifugation at 4500 g for 10 min at room temperature and washed with PBS three times. Subsequently, they were suspended in PBS at the final concentration 1 × 10^8 colony-forming units (CFU)/mL.

4. Fabrication of fixed appliances modified by BSA

BSA was successfully modified on fixed appliances (e.g., arch wires, AWs) by taking advantages of hydrophobic interaction[3] between BSA and FAS modified AWs (FAS-AWs). To remove surface contamination, AWs were firstly ultrasonicated in acetone,

ethanol, and deionized water for 30, 30 and 30 min, respectively. Then, AWs were treated with FAS in a decompression environment at 80 °C overnight, fabricating FAS modified AWs (FAS-AWs). Later, FAS-AWs were immersed in BSA solution with different concentrations (from 0.5 to 20 mg/ml) overnight at room temperature. Finally, these materials were adequately washed with deionized water for three times and dried with N_2 flow, obtaining BSA modified AWs (BSA-AWs).

5. Anti-adhesion performance of fixed appliances

To test the anti-adhesion performance, Bare-AWs and BSA-AWs were immersed in bacteria suspensions (*S. mutans*, 10^8 CFU/mL) for 10, 30, 150 and 300 min at 37 °C. After that, these materials were gently washed and fixed with 2.5% glutaraldehyde (GA) in PBS for 1 h at room temperature. After washing the samples with PBS three times, the samples were gradiently dehydrated through a series of alcohol concentrations (30%, 50%, 70%, 85%, 95% and 100%), each concentration keeping 15 min. Finally, the adhesion of the *S. mutans* was examined through a SEM and fluorescent microscope.

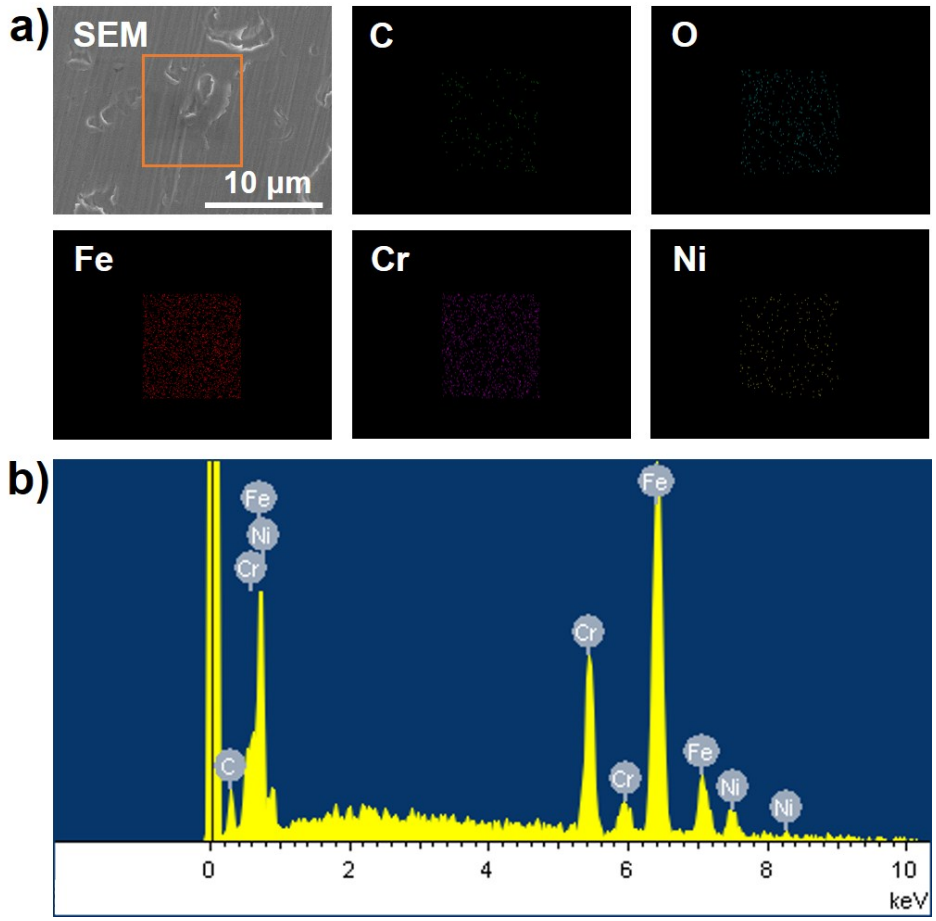

Fig. S1. The surface morphology and chemical composition of Bare-AW. a) SEM image of Bare-AW and corresponding EDS mappings in selected area including C, O, Fe, Cr and Ni elements. b) The EDS spectrum of Bare-AW. The scale bars are 10 μm.

Table S1. The atomic ratio of different elements in Bare-AW.

	C	O	Fe	Cr	Ni
Atomic Ratio(%)	9.95	6.96	23.77	2.6	56.72

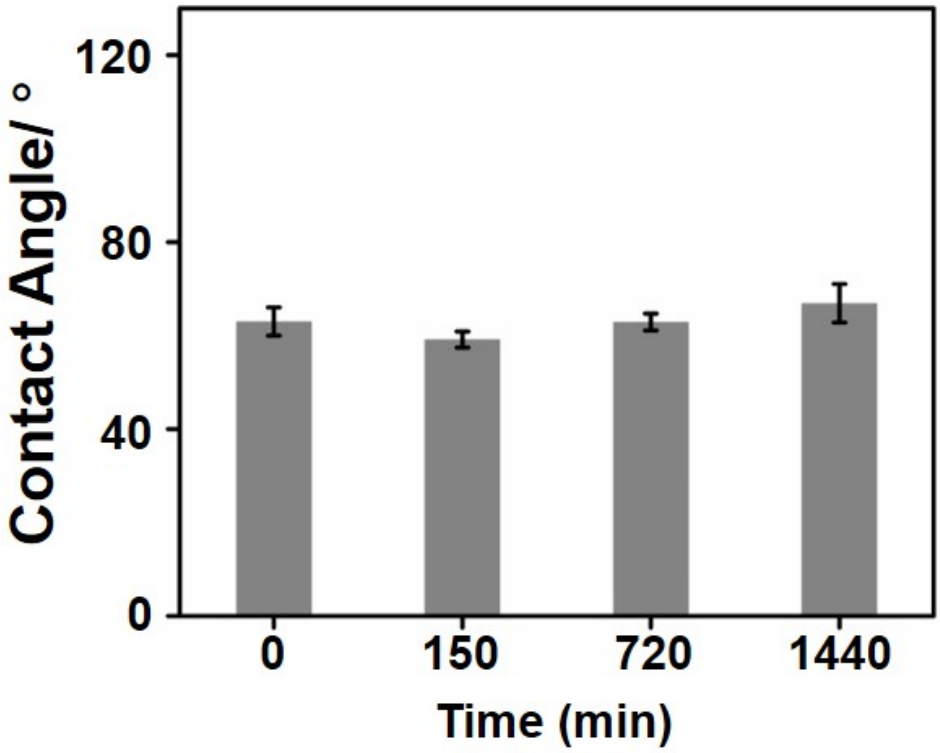

Fig. S2. The measurements of contact angles of BSA-modified surfaces after immersing in buffer solution for different time. The error bar represents the standard deviation from three repeats.

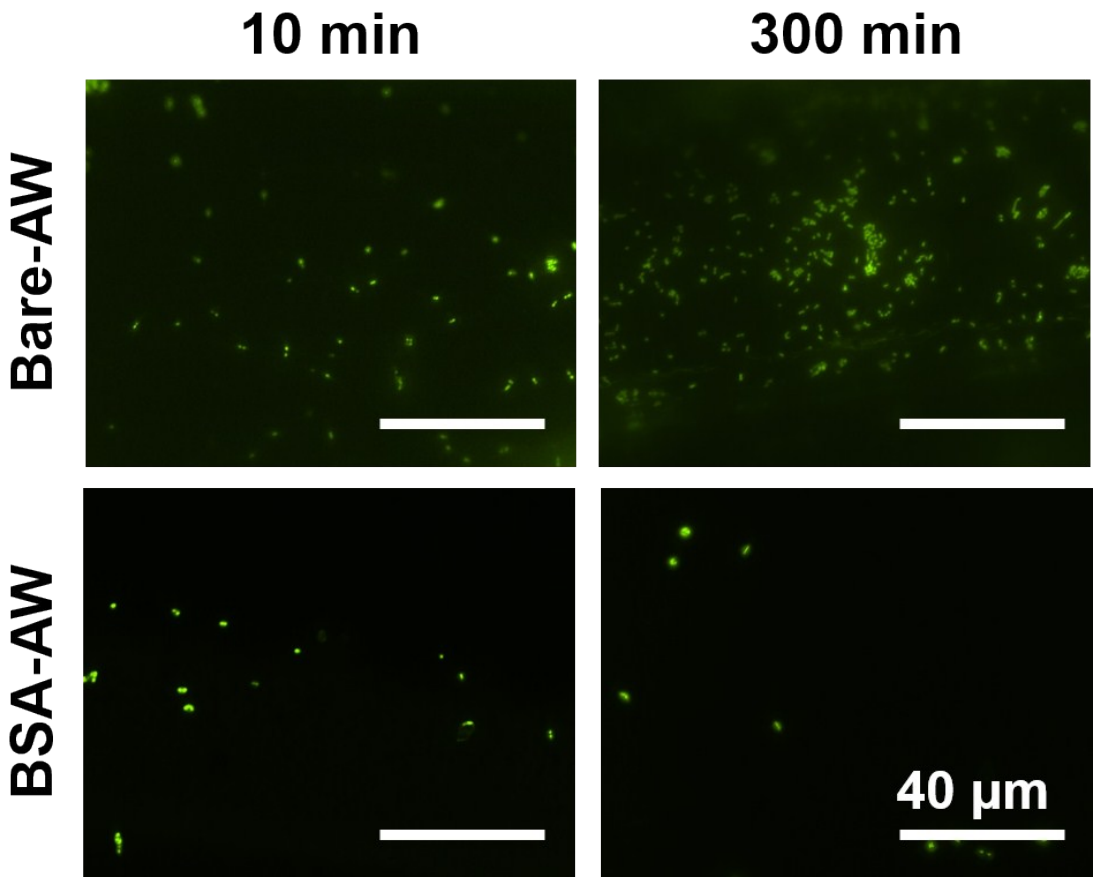

Fig. S3. The fluorescence images of Bare-AW and BSA-AW after incubating in oral bacteria suspensions with 10 and 300 min, respectively. The scale bars are 40 μm.

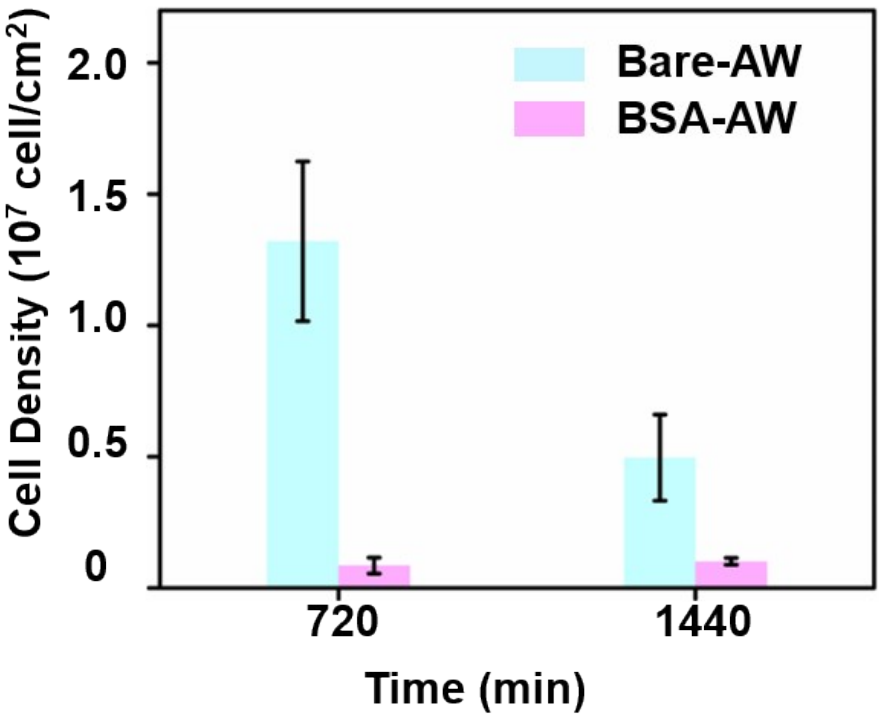

Fig. S4. The quantitative evaluations of bacteria adhesion performance on Bare-AW and BSA-AW at 720 and 1440 min. The error bar represents the standard deviation from three repeats.

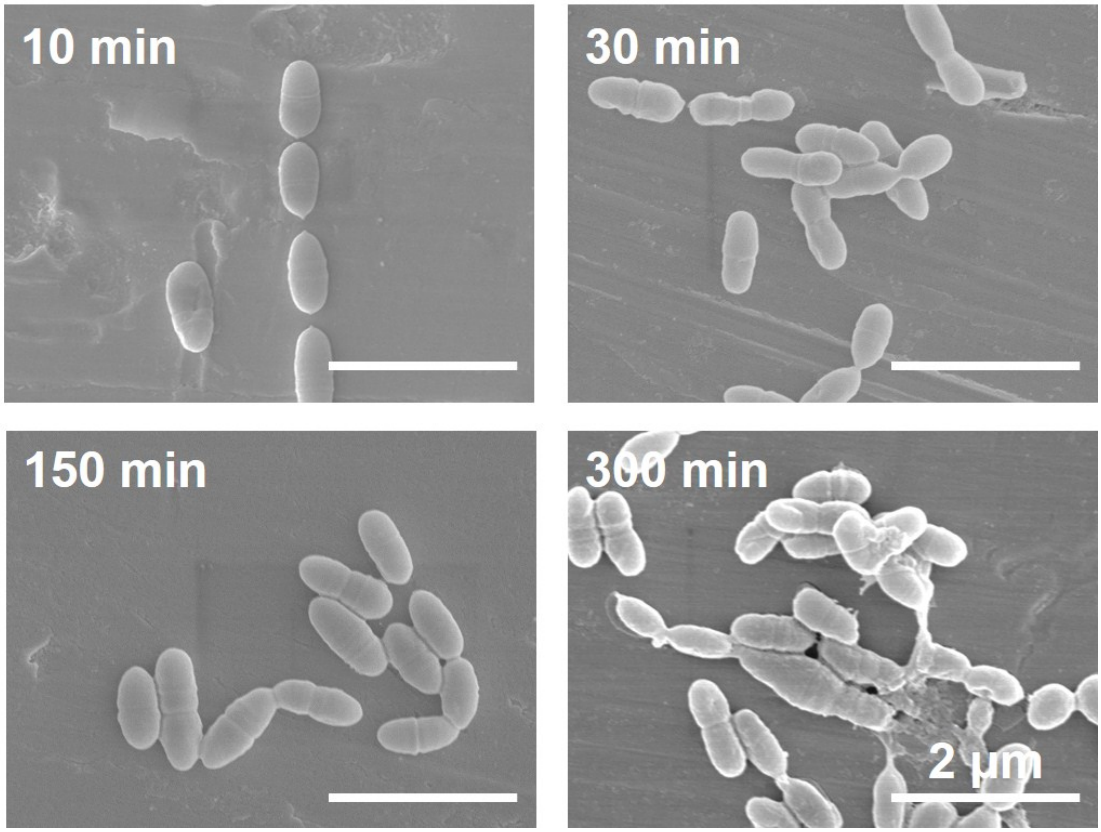

Fig. S5. The SEM images of BSA-AW after incubating in oral bacteria suspensions with time from 10 to 300 min. The scale bars are 2 μm.

References

1. F. Lundström and B. Krasse, *Eur. J. Orthod.*, 1987, **9**, 109.

2. C. Chambers, S. Stewart, B. Su, J. Sandy and A. Ireland, *Br. Dent. J.*, 2013, **215**, 505.

3. H. L. Liu, Y. Y. Li, K. Sun, J. B. Fan, P. C. Zhang, J. X. Meng, S. T. Wang and L. Jiang, *J. Am. Chem. Soc.*, 2013, **135**, 7603.