Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Oral biofilm elimination by combining iron-based nanozymes and hydrogen peroxide-producing bacteria

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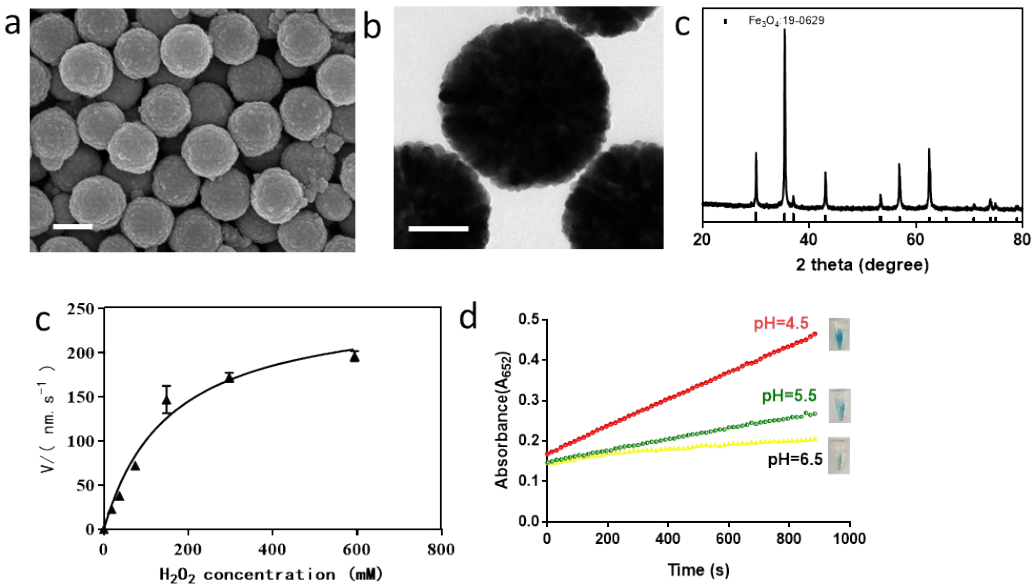

Figure S1. IONzymes characterization and catalytic assay. (a) The scanning electron microscope (SEM) image of IONzymes Scale bar: 200 nm. (b) The transmission electron microscope (TEM) image of IONzymes. The scale bars: 100 nm. (c) XRD characterization of IONzymes in the phases of Fe_3O_4. (c) Michaelis-Menten kinetics of peroxidase-like activity of IONzymes (with varied [H_2O_2]). (d) Peroxidase-like activity of IONzymes at different pH as determined by a colorimetric assay using 3,3',5,5 ' -tetramethylbenzidine (TMB). The catalytic reaction of TMB (which serves as a peroxidase substrate) in the presence of H_2O_2 produces a blue color (insert).

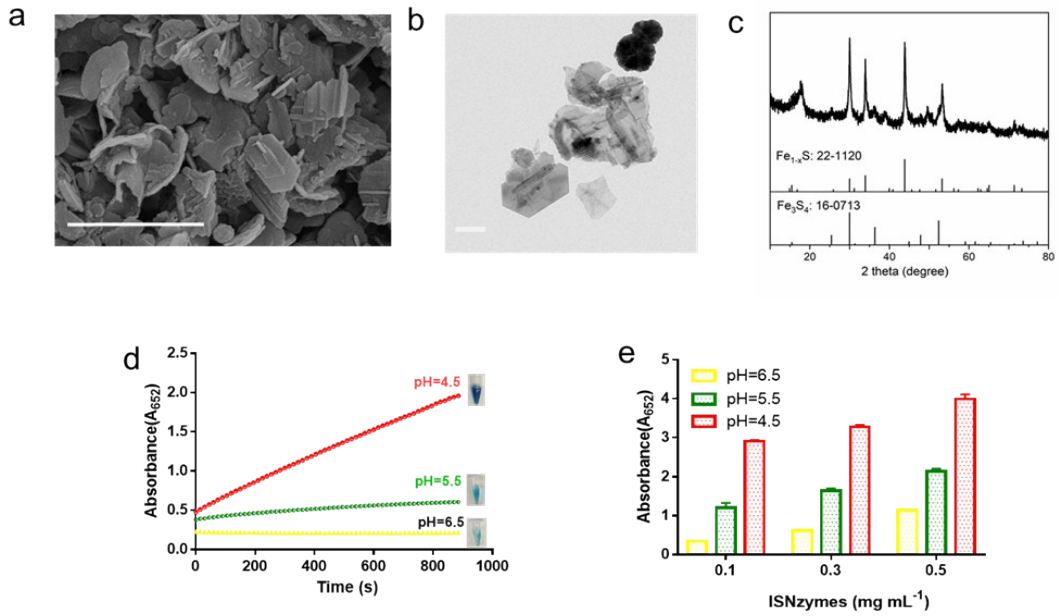

Figure S2. ISNzymes characterization and catalytic activity assay. (a) SEM image of ISNzymes with sheet-like hexagonal nanostructure. Scale bar: 1μm. (b) TEM)image of ISNzymes. The scale bars: 100 nm. (c) XRD characterization of ISNzymes indicated the two phases of $Fe_{1-x}S$ and Fe_3S_4. (d) Peroxidase-like activity of ISNzymes at different pH as determined by a colorimetric assay using 3,3’ ,5,5 ’ -tetramethylbenzidine (TMB). The catalytic reaction of TMB (which serves as a peroxidase substrate) in the presence of H_2O_2 produces a blue color (insert). (e) Peroxidase-like activity of ISNzymes at different concentrations at pH 4.5, pH 5.5 and pH 6.5.

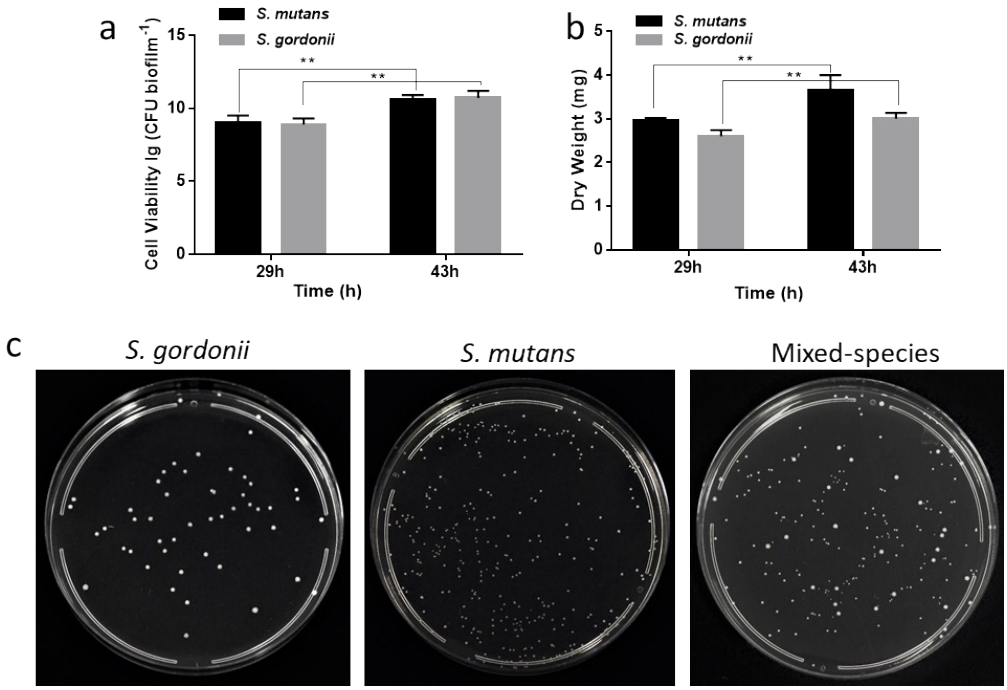

Figure S3 Biofilm assays for *S. mutans* or *S. gordonii* single-species biofilm. (a) Bacterial viability of *S. mutans* or *S. gordonii* in its single-species biofilm. (b) Dry weight *S. mutans* or *S. gordonii* in its single-species biofilm. Data are shown as the mean ± s.d. (c) Colonies of *S. gordornii* and *S. mutans* formed on agar plate. Statistical significance was assessed using an unpaired Student ’s two-sided t -test compared to the control group. **p < 0.01. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate, and representative images are shown.

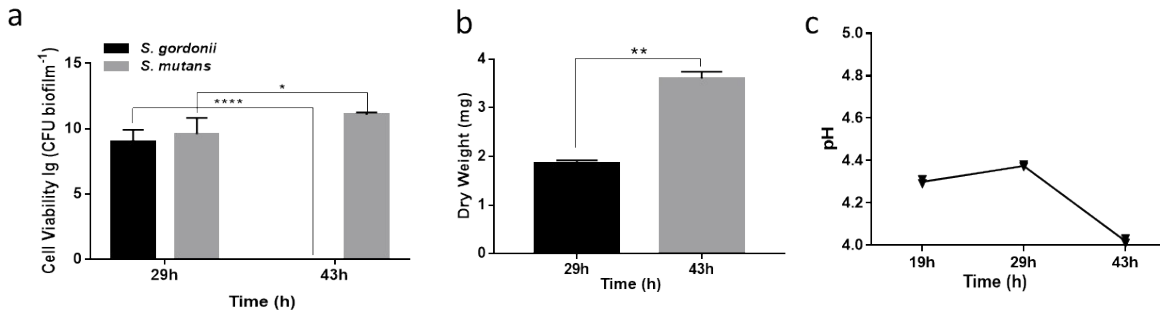

Figure S4. The mixed-species oral biofilms without supplemental *S. gordonii*. (a) Bacterial viability of mixed-species biofilm without supplemental *S. gordonii*. At first, the mixed-species oral biofilms component with *S. mutans* and *S. gordonii*. Starting from 0h, the sHA discs was inoculated with *S. mutans* UA159 and *S. gordonii* DL1 at 10^4 CFU mL^{-1}. *S. gordonii* was not supplied during the period of experiment. (b) The dry weight of mixed-species biofilm without supplemental *S. gordonii*. (c) the pH change of mixed-species biofilm without supplemental *S. gordonii*. Data are shown as the mean ± s.d. Statistical significance was assessed using an unpaired Student 's two-sided t -test compared to the control group. **p < 0.01, **** p < 0.0001. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate, and representative images are shown

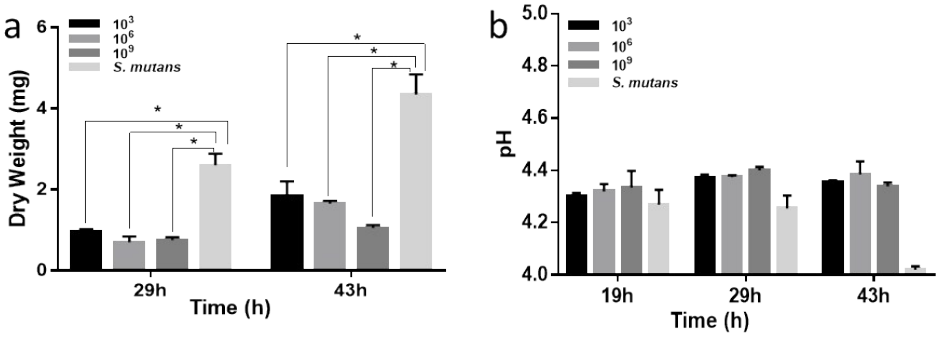

Figure S5. Mixed-species biofilm with supplemental *S. gordonii*. (a) The dry weight of mixed-species biofilm with supplemental *S. gordonii* at different concentration. (b) pH in the mixed-species biofilm with supplemental *S. gordonii* at different concentration. Data are shown as the mean ± s.d. Statistical significance was assessed using an unpaired Student ’s two-sided t -test compared to the control group. *$p < 0.05$. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate, and representative images are shown.

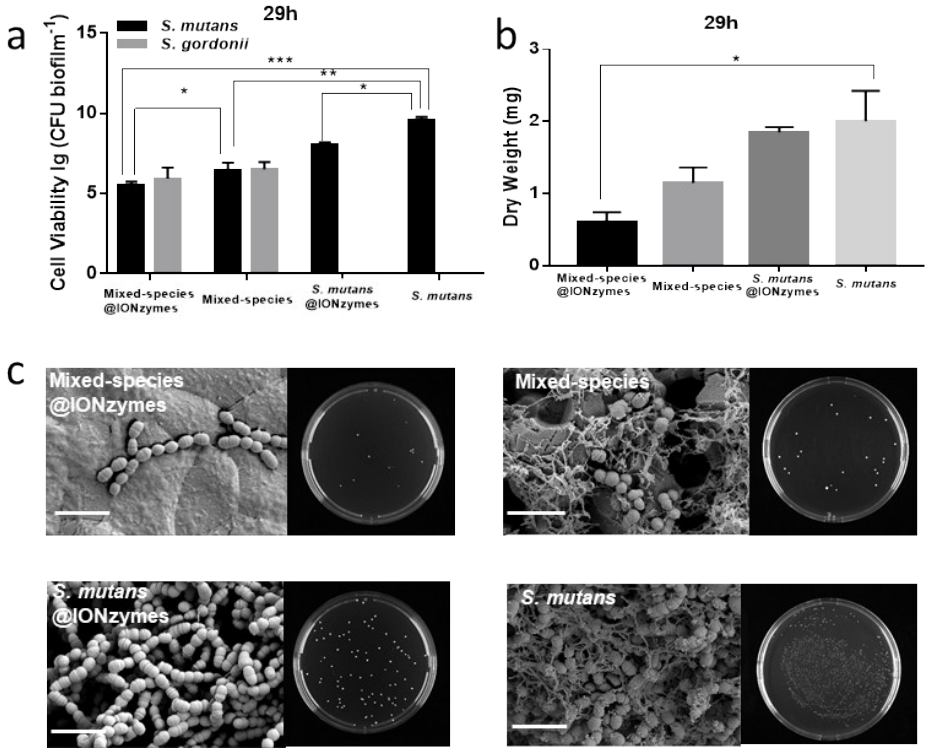

Figure S6. IONzymes enhanced biofilm elimination in 29 h mixed-species biofilm. (a) Total viable cells of *S. mutans* and *S. gordonii* in 29 h mixed-species biofilm treated by IONzymes. (b) biomass (dry weight) of 29 h mixed-species biofilm treated by IONzymes. (b) in each experimental group at the end of experimental period (29h). The growth of bacteria on the UFTYE culture plates and SEM images of 29h-biofilms (c) The representative images of SEM characterization and bacterial killing (culture plates) for 29 h mixed-species biofilm established on sHA discs. Data are shown as the mean ± s.d. Statistical significance was assessed using an unpaired Student ’s two-sided t -test compared to the control group. *p < 0.05, **p < 0.01, ***p < 0.001. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate, and representative images are shown.

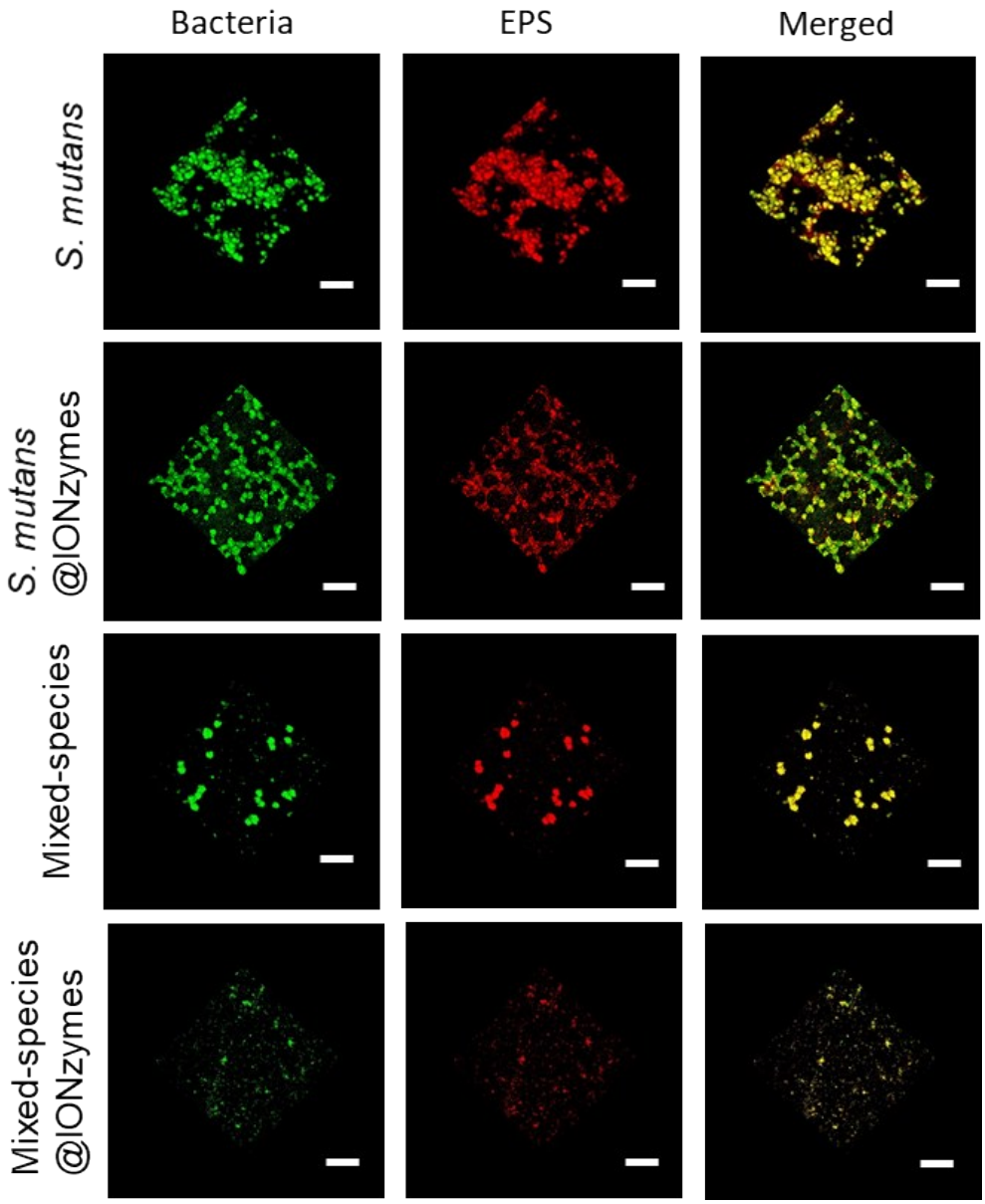

Figure S7. 3D images of mixed-species biofilm (43h) on sHA discs with IONzymes. *S. mutans* and *S. gordonii* cells are stained with SYTO 9 (in green) and EPS were labelled with Alexa Fluor 647 (in red). Scale bars: 200 µm.

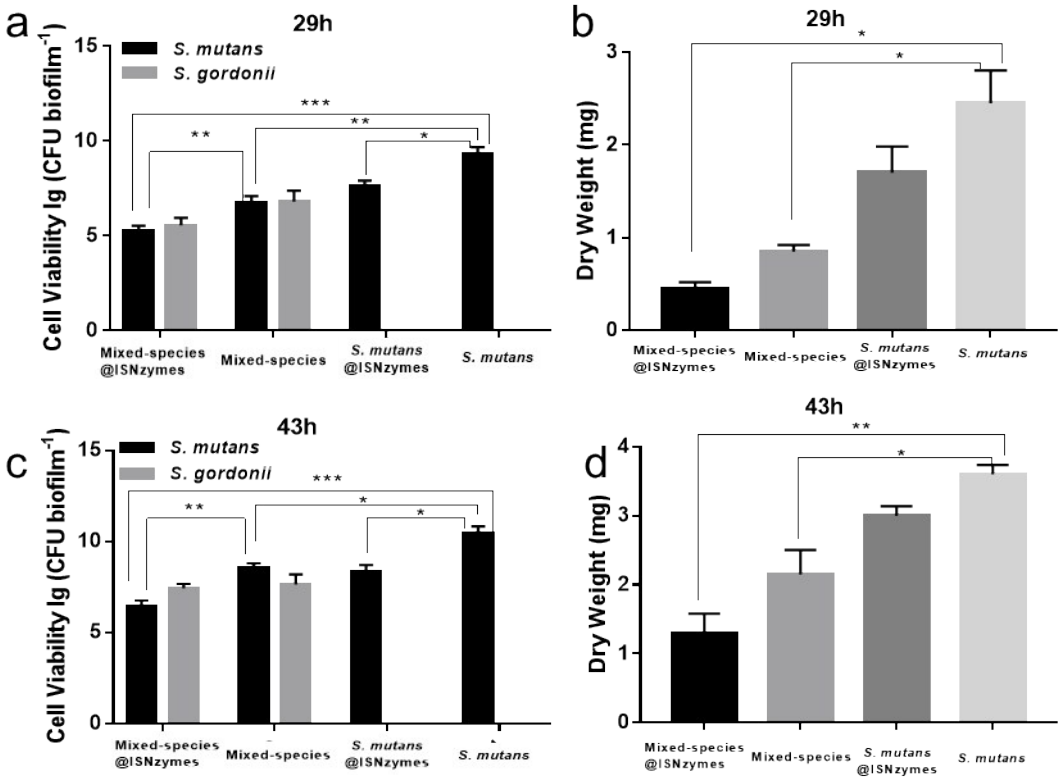

Figure S8. ISNzymes enhanced biofilm elimination of mixed-species biofilms formed on sHA discs. (a) Total viable cells of *S. mutans* and *S. gordonii* in 29 h mixed-species biofilm treated by ISNzymes. (b) biomass (dry weight) of 29 h mixed-species biofilm treated by ISNzymes.(c)Total viable cells of *S. mutans* and *S. gordonii* in 43 h mixed-species biofilm treated by ISNzymes. (d) biomass (dry weight) of 43 h mixed-species biofilm treated by ISNzymes. All biofilms were formed on sHA discs. Data are shown as the mean ± s.d. Statistical significance was assessed using an unpaired Student ’s two-sided t -test compared to the control group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate, and representative images are shown.

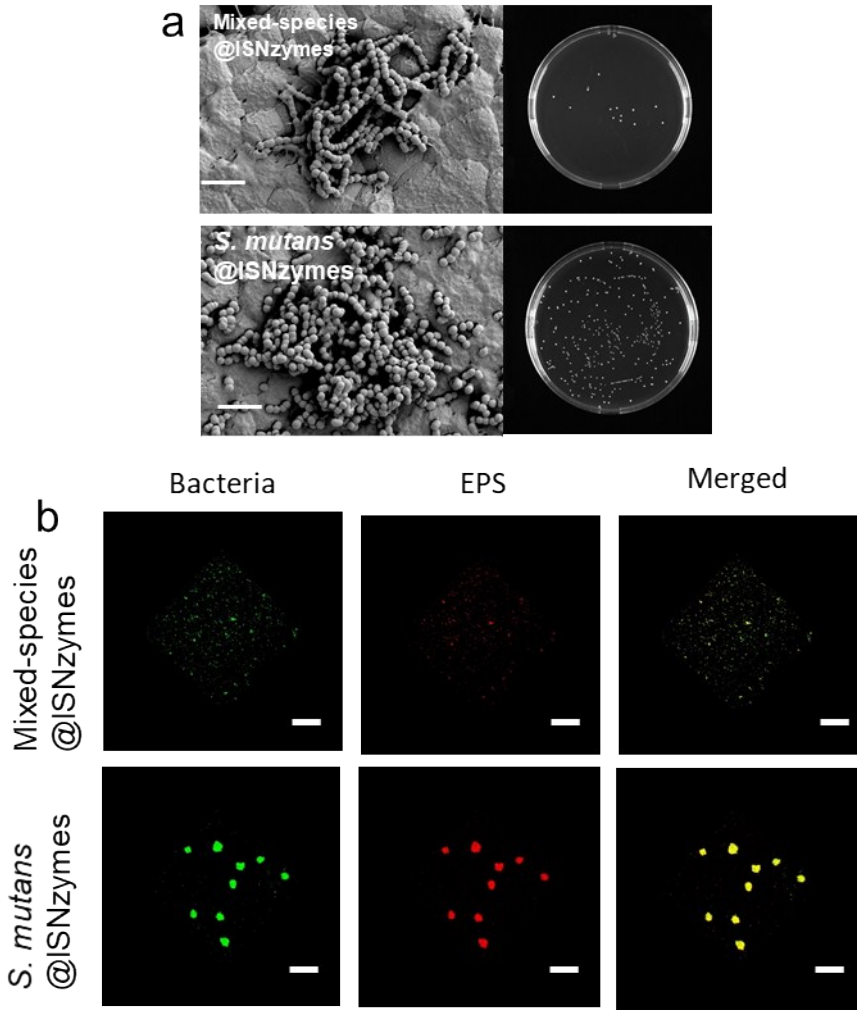

Figure S9. SEM and 3D images for ISNzymes eliminating 43 h mixed-species biofilms on sHA discs. (a) SEM images of 43 h mixed-species biofilm or *S. mutans* biofilm treated with ISNzymes. The scale bars of SEM images are 3 μm. (b) 3D images of SEM images of 43 h mixed-species biofilm or *S. mutans* biofilm treated with ISNzymes. *S. mutans* and *S. gordonii* bacteria were stained with SYTO 9 (in green) and EPS was labelled with Alexa Fluor 647 (in red). Scale bars: 200 μm.

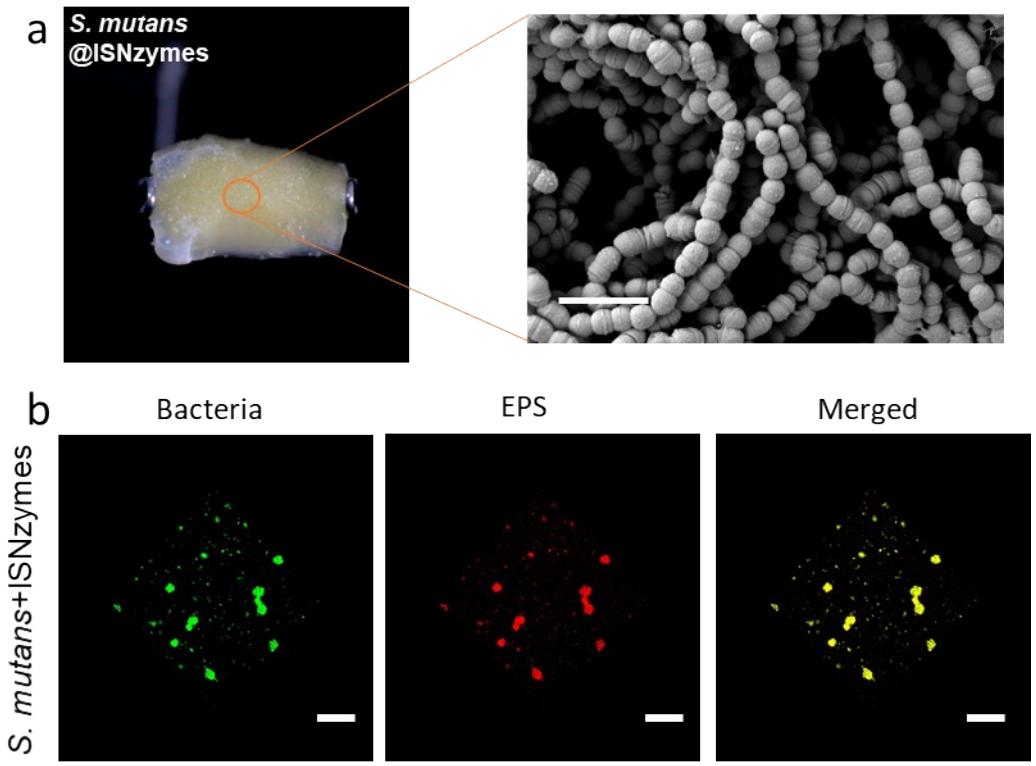

Figure S10. ISNzymes efficiently eliminated *S. mutans* biofilm formed on dentin surface. (a) Photo of dentin surface and SEM image *of S. mutans* biofilm treated by ISNzymes on dentin surface. (b) 3D images of *S. mutans* biofilm treated by ISNzymes on dentin surface. Bacterial cells were stained with SYTO 9 (in green) and EPS were labeled with Alexa Fluor 647 (in red).

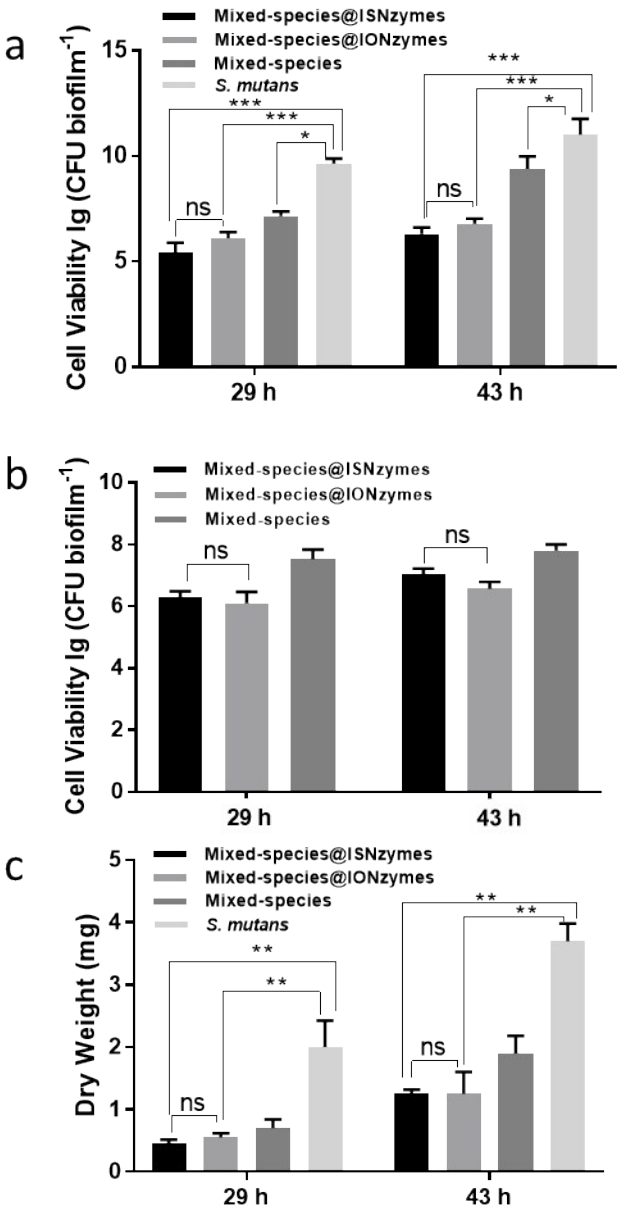

Figure S11. Comparison of ISNzymes and IONzymes in eliminating mixed-species biofilm formed on dentin surface. (a) Total viable cells of *S. mutans* in mixed-species biofilm treated with or without nanozymes. (b) Total viable cells of *S. gordonii* in mixed-species biofilm treated with or without nanozymes. (c) The biomass (dry weight) of mixed-species biofilm treated with nanozymes. Data are shown as the mean ± s.d. Statistical significance was assessed using an unpaired Student ’s two-sided t -test. ns means no significant different, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate, and representative images are shown.

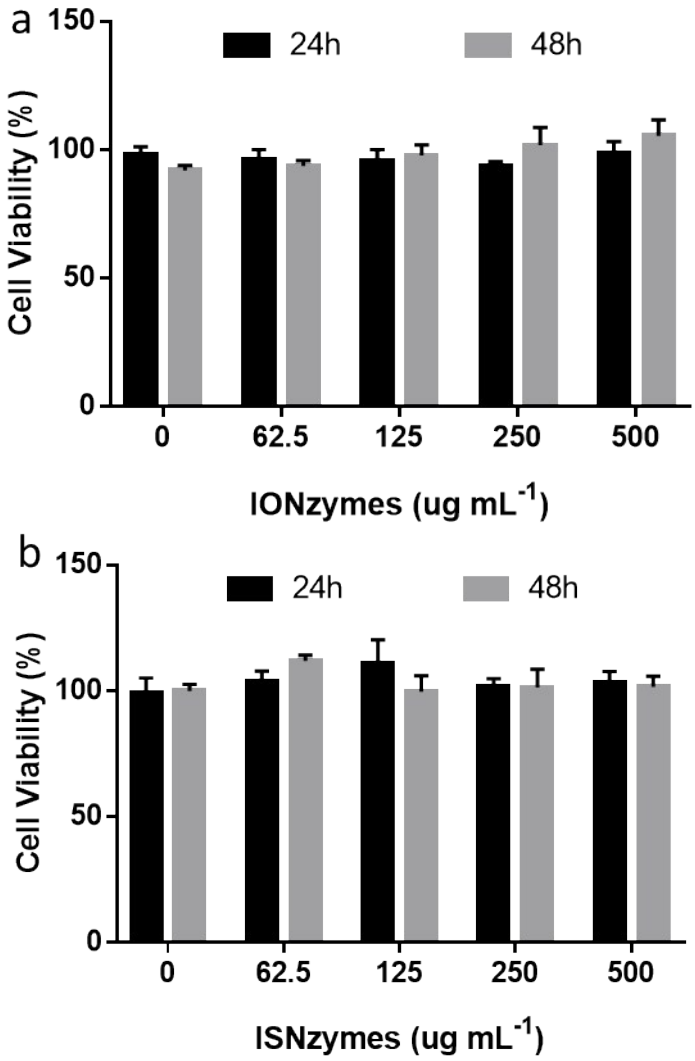

Figure S12. Cytotoxicity assay of nanozymes on HOK cells. Cell viability of HOK cell treated with IONzymes (a) and ISNzymes (b). Data are shown as the mean ±s.d. Mean values and error bars were defined as mean and s.d., respectively. All experiments were performed in triplicate.