

Figure S1. FE-SEM analysis of the eDNA nanofibers. *S. mutans* UA159 was grown in biofilm medium with glucose as the supplemental carbohydrate source on hydroxylapatite discs. After 24 hours, the biofilms were treated for one hour with deoxyribonuclease (DNase I, 2 mg/ml), ribonuclease H (RNase H, 2 U/ml), dextranase (0.1 mg/ml), proteinase K (2.5 mg/ml) and formic acid (88%), respectively, with controls receiving heat-inactivated enzyme or reaction buffer only instead. Relative to the untreated and controls (not shown), the nanofibrous matrices were completely destroyed by DNase I and significantly reduced by dextranase and formic acid, while little effect was seen with RNase H and Proteinase K.

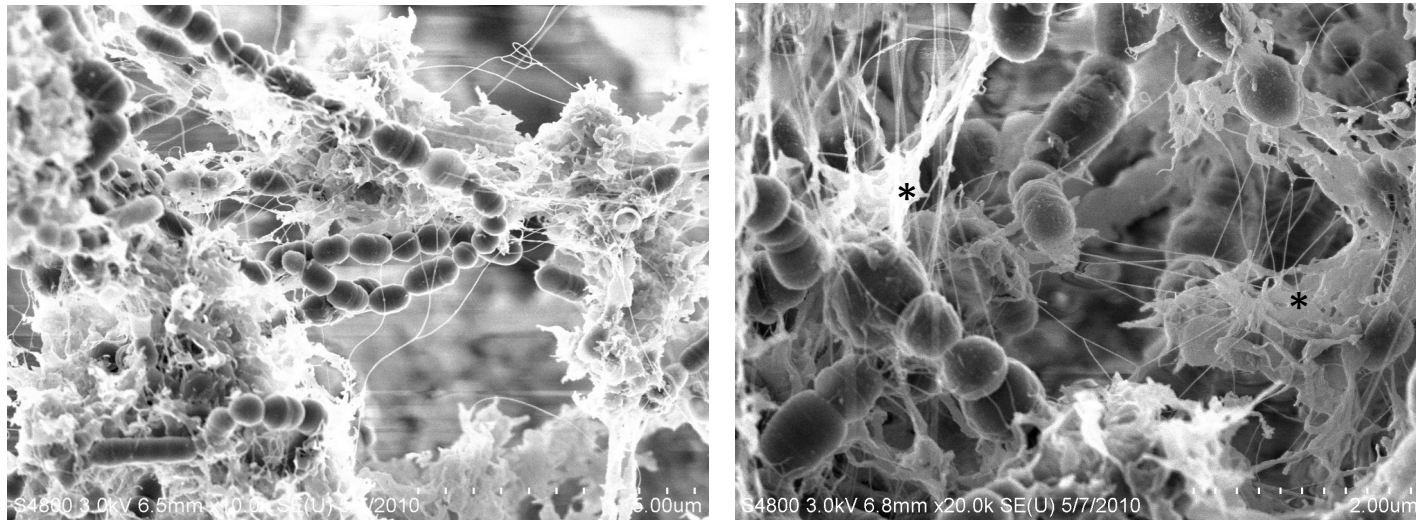


Figure S2. FE-SEM analysis of *S. mutans* biofilms. *S. mutans* UA159 was grown in biofilm medium with glucose (18 mM) and sucrose (2 mM) as the supplemental carbohydrate sources on hydroxylapatite discs. Images show 24 hour biofilms at a magnification of 10K (left) and 20K (right), respectively, with asterisks highlighting interaction of nanofibrous eDNA and wool-like glucans.

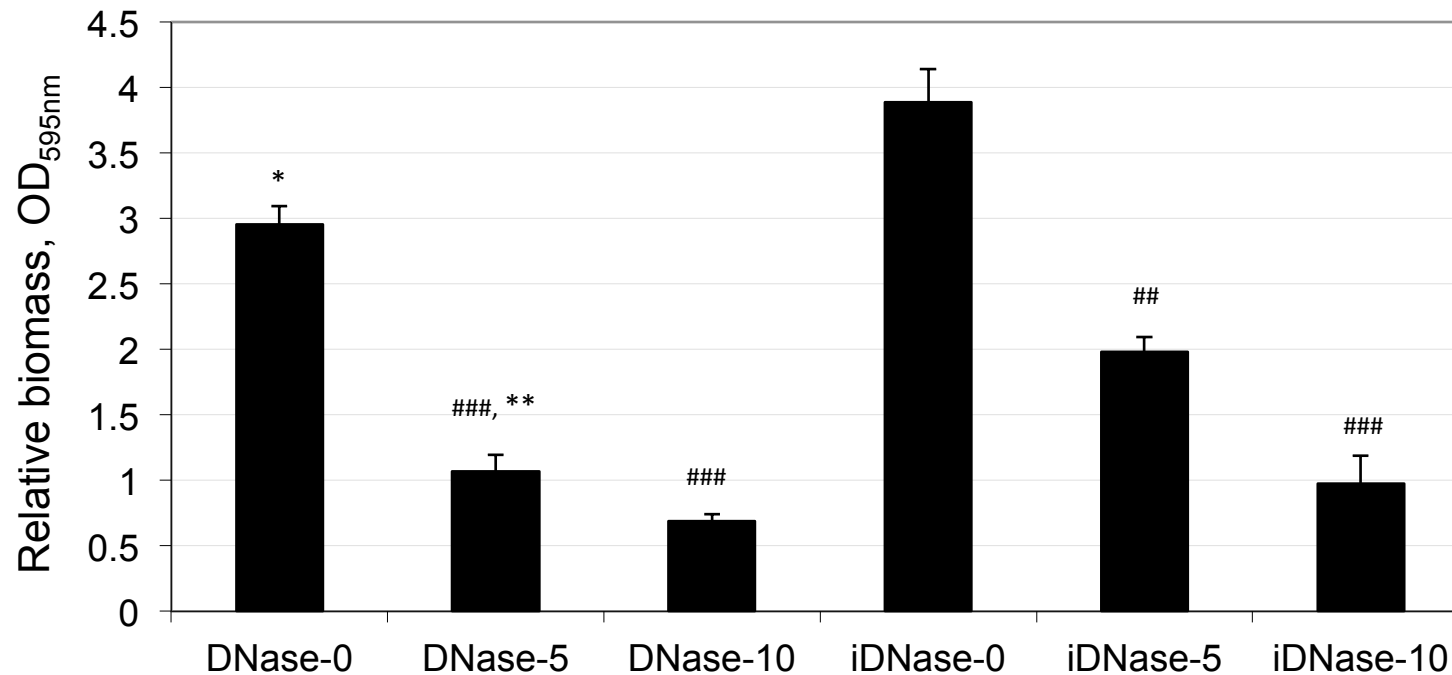


Figure S3. Effect of DNase I on the mechanical stability of *S. mutans* biofilms. 24 hour biofilms grown in FMC GS on 24 well plates were treated with DNase I (DNase) for 30 minutes, and following sonication for 5 (-5) and 10 (-10) seconds, the residual biomasses were stained with crystal violet and measured using a spectrophotometer at 595 nm. Controls were processed similarly but received heat inactivated enzyme (iDNase) instead. Symbols ## and ### indicate statistic difference at  $P < 0.01$  and  $0.001$ , respectively when compared to the ones without sonication (-0) within the same group. Symbols \* and \*\* represent statisitic differences between groups (DNase I treated vs control) at the specified time points at the level of  $P < 0.05$  and  $0.01$ , respectively.

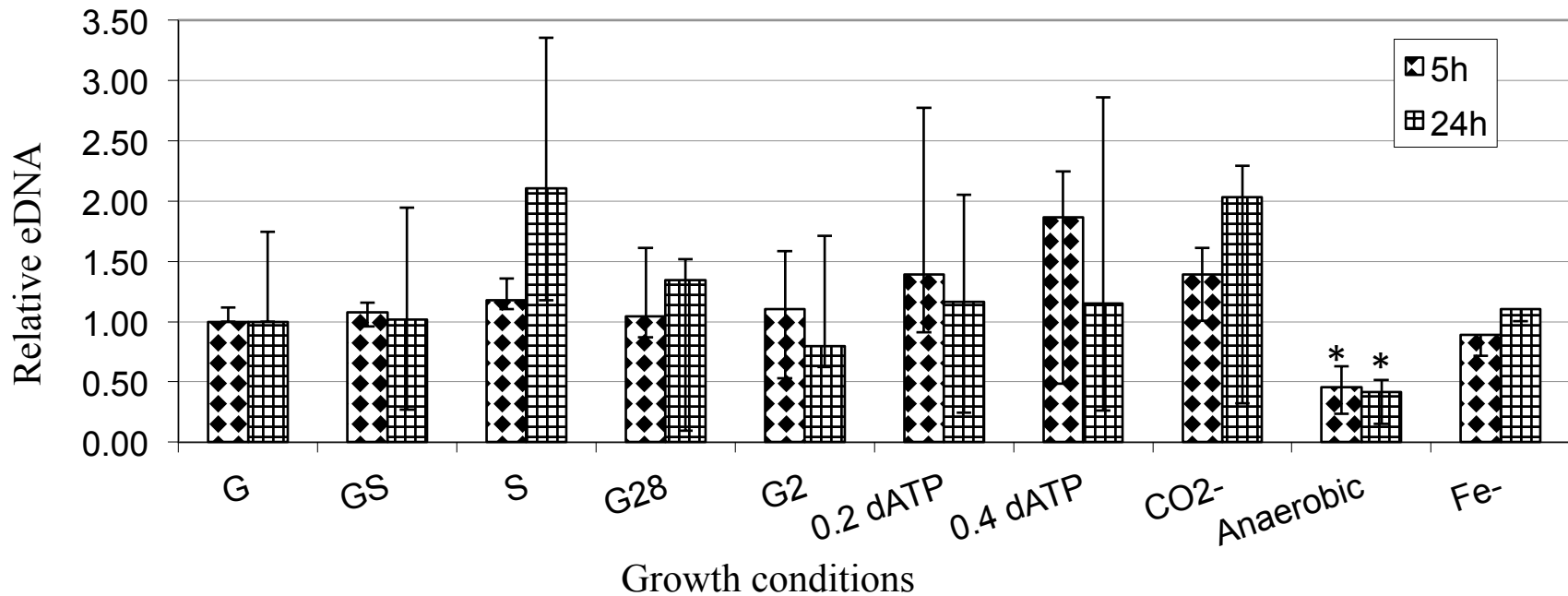


Figure S4. Analysis of eDNA production during growth under different conditions. *S. mutans* UA159 was grown in aerobic chamber with 5% CO<sub>2</sub> in chemically defined medium FMC with glucose (G, 20 mM), sucrose (S, 10 mM) or glucose (18 mM) plus sucrose (2 mM) (GS) for 5 (5h) and 24 (24h) hours. Under certain conditions glucose was provided at 2 and 28 mM, representing glucose limiting and glucose excess, respectively; and dATP was supplemented at 0.2 and 0.4 mM, respectively. CO<sub>2</sub>- and Anaerobic represent growth under aerobic condition without provision of 5% CO<sub>2</sub> and in anaerobic box, respectively. All data were normalized using the results of cultures grown in FMCG as 1. Data presented here represent the average ( $\pm$ standard deviation) of more than 3 independent experiments with an \* representing statistic difference at  $P < 0.05$ , when compared to cultures grown in FMCG with 5% CO<sub>2</sub>.

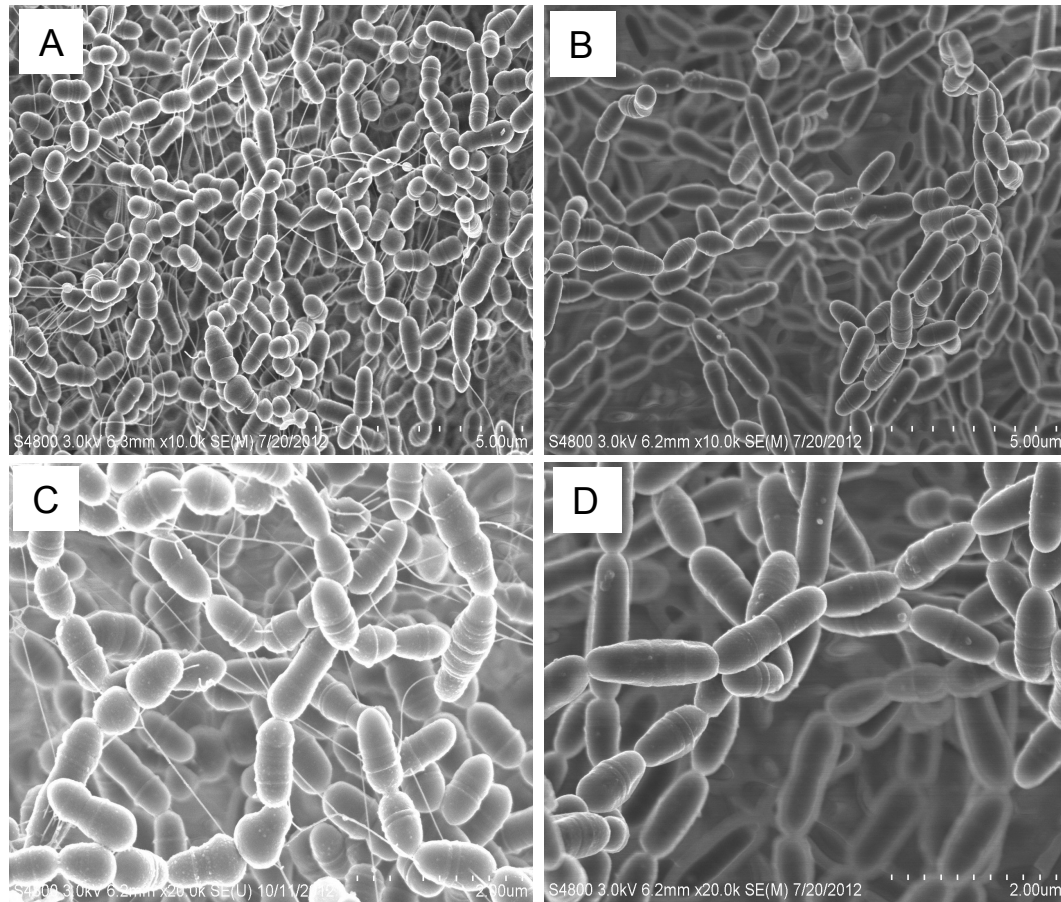


Figure S5. Iron-limitation on *S. mutans* biofilm formation. *S. mutans* UA159 was grown on hydroxylapatitic discs in BM-glucose with (A and C) and without (B and D) inclusion of ferric chloride, and analyzed using FE-SEM. Images show 24 hour biofilms featuring elongated cells when compared to growth under regular BM-glucose. Images A&B were taken at magnification of 10k and C&D at 20k, respectively.