Synthetic antigen-binding fragments (Fabs) against *S. mutans* and *S. sobrinus* inhibit caries formation

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*Streptococcus mutans* and *Streptococcus sobrinus* are the main causative agents of human dental caries. Current strategies for treating caries are costly and do not completely eradicate them completely. Passive immunization using nonhuman antibodies against Streptococcal surface antigens has shown success in human trials, however they often invoke immune reactions. We used phage display to generate human antigen-binding fragments (Fabs) against *S. mutans* and *S. sobrinus*. These Fabs were readily expressed in *E. coli* and bound to the surface *S. mutans* and *S. sobrinus*. Fabs inhibited sucrose-induced *S. mutans* and *S. sobrinus* biofilm formation in vitro and a combination of *S. mutans* and *S. sobrinus* Fabs prevented dental caries formation in a rat caries model. These results demonstrated that *S. mutans* and *S. sobrinus* Fabs could be used in passive immunization strategies to prevent dental caries. In the future, this strategy may be applied towards a caries therapy, whereby Fabs are topically applied to the tooth surface.

Dental caries is one of the most common global chronic diseases caused by the formation of biofilms on the tooth surface¹. Gram-positive acidogenic and aciduric bacterial species, most often mutants streptococci in humans, have been associated with caries initiation and progression². In addition to mutants streptococci (*Streptococcus mutans* and *Streptococcus sobrinus*), other microbial species have been isolated from caries including: salivarius group streptococci (*S. salivarius, S. vestibularis*) and *S. parasanguinis* as well as lactobacilli (*L. gasseri, L. johnsonii, L. casei, L. paracasei*) and Veillonella species (*V. atypica, V. dispar, V. parvula*)²-⁴. Modern molecular and microbial culture techniques have revealed that a range of bacteria can contribute to the caries process at different stages².

Dental caries are a complex biofilm-mediated disease that occurs when acidogenic/aciduric bacteria obtain a selective advantage over other members of the oral flora, disrupting the balance of the plaque biofilm and initiating the caries⁶. Mutans streptococci and lactobacilli can dominate advanced stages of caries formation where increased biofilm acidification results in the biofilm becoming less microbially diverse⁶.

The process of caries formation is modulated by complex interactions between acid-producing bacteria and host factors⁸-¹¹. Streptococcal surface fibrillar adhesins (e.g. antigen I/II) control attachment to the surface of the tooth by adhering to salivary agglutinin. Cell-wall-associated glucosyltransferases (GTFs) produce adhesive glucans from sucrose, which cell wall-associated glucan-binding proteins (GBP) of *S. mutans* bind, resulting in glucan-mediated aggregation¹². Water insoluble glucans produced by *S. mutans* GTFs are the most important for building the biofilm structure¹³. Other isoforms of GTFs and glucan produced by various oral streptococci contribute to biofilm formation in a synergistic manner¹⁴. *S. mutans* metabolism and acid production also contribute...
to the pathogenesis of dental caries. S. mutans can metabolize a wide variety of carbohydrates, leading to the production of lactic acid. The acid diffuses into tooth enamel and dissolves the mineral underneath the tooth surface. If the mineral dissolution is not reversed, then early lesions result in caries. New caries preventive therapies may be developed by inhibiting biofilm formation through suppression of mutants streptococci.

Despite efforts in promoting oral hygiene and fluoridation, approximately 35% of the global population suffers from tooth decay and cavities in permanent teeth. Current strategies for treating caries are limited to removal of the diseased part of the tooth and placing a suitable restoration, which is costly and does not eradicate caries on other teeth. As a result, dentistry is beginning to move from the surgical model for treating tooth decay (placing restorations) to identification of early carious lesions and preventing or treating them with non-surgical methods.

Strategies for caries prevention, such as brushing, professional cleaning, antimicrobial peptides, sugar substitutes, and chemoprophylactic agents such as classical antibiotics, chlorhexidine, and triclosan are effective against dental biofilm, but their retention in the oral cavity is poor. Passive immunization by applying mucosal vaccinations such as S. mutans and S. sobrinus antigens GTFs, antigen I/II, GBP, and virulence-associated immunomodulatory extracellular proteins (VIP) at inductive sites leads to increases in IgA secretion and can be effective in preventing caries formation. Many other vaccine immunogens such as synthetic peptides, DNA-based active vaccines, and mucosal adjuvants have been successful in animal models. The murine monoclonal antibody Guy’s 13, which specifically recognizes the S. mutans and S. sobrinus, has been used to prevent caries formation in non-human primates and in human clinical trials. A humanized scFv based on Guy’s 13 antibody is capable of aggregating S. mutans in vitro. To date, no vaccines have been brought to market, mainly due to the difficulty in inducing and maintaining protective antibody in oral fluids.

There has been a growing interest in using antibody fragments to treat caries. Advantages of using antibody fragments are their low production costs and enhanced tissue penetration. We used phage display to generate Fabs against S. mutans and S. sobrinus. By selecting the Fab-phage library against live bacteria, we were able to generate Fabs against antigens presented in their native context in an unbiased manner. Using this strategy, we obtained Fabs that bound to S. mutans and S. sobrinus, blocked biofilm formation in vitro, and prevented dental caries formation in rat caries model.

Results and Discussion

Selection of antigen-binding fragments (Fabs) against S. mutans and S. sobrinus. To generate synthetic human Fabs against S. mutans and S. sobrinus, we conducted five rounds of phage display selection using a synthetic Fab phage library against S. mutans and S. sobrinus (Fig. 1a). We observed an increase in phage retention on the bacteria with sequential rounds of selection up to the fifth round (Fig. 1b). During the fourth and fifth rounds of selection, we included subtractive binding steps. For the S. mutans and S. sobrinus selections, we removed members of the Fab-phage library that bound E. coli and S. mutans and S. sobrinus, respectively. During selection rounds with subtractive panning, we observed a modest drop in the number of phage retained on S. mutans or S. sobrinus (Fig. 1b). Subsequent rounds of selection resulted in increased phage retention for subtracted phage pools (Fig. 1b). At the end of fifth round, we sequenced 20 phage clones from each selection.

For the S. mutans selection, sequences present at the highest frequencies were Fabs SM-1, SM-10, and SM-12 (Fig. 1c) and for the S. sobrinus selection Fabs SS-2 and SS-14 were most frequent sequences (Fig. 1c). The highest frequency Fabs in both selections, SM-1 and SS-2, had identical sequences. We also performed a competitive subtracted phage pools (Fig. 1b). At the end of fifth round, we sequenced 20 phage clones from each selection.

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To test the binding selectivity of S. mutans and S. sobrinus Fabs, we used ELISA, immunofluorescence, and flow cytometry. We performed the ELISA by adding a fixed concentration of Fabs to S. mutans, S. sobrinus, E. coli, and bovine serum albumin (BSA) immobilized on ELISA plates. Fabs interacted with both S. mutans and S. sobrinus and in most cases Fabs bound slightly better to S. mutans relative to S. sobrinus (Fig. 2a). SM-C-1 had the highest ELISA signals for both S. mutans and S. sobrinus (Fig. 2a). SM-12 Fab bound weaker to both S. mutans and S. sobrinus, and SM-C-4 Fab bound non-specifically to S. mutans and S. sobrinus, E. coli, and BSA (Fig. 2a). Except for SM-C-1 and SM-12, Fabs generally bound better to the bacteria that they were selected against, but also showed cross reactivity to S. sobrinus, indicating that antigens targeted by the Fabs were shared between S. mutans and S. sobrinus.

To visualize Fab binding to S. mutans and S. sobrinus, we performed immunofluorescence with Fabs SM-10, SM-12, SMC-1, SS-2, and SS-C-1. We coated 10^6 CFU/mL of bacterial cell suspension on ELISA plates and added 5μg purified Fabs. Consistent with ELISA results, Fabs bound to both S. mutans and S. sobrinus (Fig. 2b).

To confirm that Fabs bound to single cell suspensions of S. mutans and S. sobrinus, we disrupted filaments of S. mutans and S. sobrinus cells by sonication. We added 1μM Fabs to 10^6 CFU/tube of suspended bacterial and analyzed binding using flow cytometry with an anti-FLAG R-Phycocerythin-conjugated secondary antibody. Consistent with ELISA and immunofluorescence results, we observed that SM-10, SM-12, SS-2, and SS-C1 Fabs
bound to both *S. mutans* and *S. sobrinus* with Fabs binding better to the bacteria that they were selected against (Fig. 3a,b). These results were generally consistent with binding results observed in the ELISA. Although the flow cytometry results indicated that the binding of SM-12 to *S. mutans* was tighter than SS-2 and SS-C-1, it was the least strongest interaction in the ELISA assay. A nonspecific Fab against human cell surface receptor EphB6 was used as a negative control for Fab binding and it did not bind to *S. mutans* and *S. sobrinus*. To check the specificity of these Fabs against *S. mutans* and *S. sobrinus*, a gram-negative *E. coli* strain was used as a negative control for bacteria binding and none of the Fabs interacted with *E. coli* (Fig. 3c).

In general Fabs bound better to the bacteria that they were selected against, although the *S. mutans* Fabs still bound to *S. sobrinus* and vice versa, indicating antigens that Fabs targeted were shared between *S. mutans* and *S. sobrinus*. *S. mutans* Fabs SM-1 and SM-C-1 were identical to *S. sobrinus* Fabs SS-2 and SS-C-10, respectively, confirming that these Fabs recognized common antigens expressed on both *S. mutans* and *S. sobrinus*. The cross reactivity of Fabs was not unexpected as it has previously been shown that a murine monoclonal antibody against SAI/II protein of *S. mutans* recognizes the SAI/II protein of both *S. mutans* and *S. sobrinus*. Further, a monoclonal antibody against antigen B of *S. downei* is specific for both *S. sobrinus* and *S. downei*.

**Figure 1.** Phage display selection of Fabs against *S. mutans* and *S. sobrinus*. (a) Phage display selection scheme for isolating Fabs against *S. mutans* and *S. sobrinus*. Five rounds of phage display were performed against *S. mutans* or *S. sobrinus*. During the fourth and fifth rounds of selection, subtractive panning steps were included. For the *S. sobrinus* and *S. mutans* selections, members of the Fab-phage library were removed that bound *E. coli* and *S. mutans* and *S. sobrinus*, respectively. (b) The frequency of Fab-phage eluted during different rounds of selection against *S. mutans* and *S. sobrinus*. (c) Frequency of the top CDRL3 and CDRH3 sequences of Fabs against *S. mutans* and *S. sobrinus*. 
**S. mutans and S. sobrinus Fabs reduced in vitro biofilm formation.** To assess the effect of *S. mutans* and *S. sobrinus* Fabs on biofilm formation, we used an in vitro biofilm formation assay that measures biofilm formation on a polystyrene plate surface. We added Fabs (3µM) to cell suspensions of *S. mutans* or *S. sobrinus* in BHI media with 1% sucrose in a 96 well plate and incubated at 37°C for 20 hours. *S. mutans* or *S. sobrinus* cultured in BHI with 1% sucrose were used as positive controls for biofilm formation. *S. mutans* or *S. sobrinus* cultured in BHI without any sucrose were used as negative controls. Fab SM-10 showed the best blocking of *S. mutans* biofilm formation, followed by Fab SM-12 (Fig. 4a). *S. sobrinus* did not show any blocking effects on *S. mutans* biofilm formation, followed by Fab SS-C-1 (Fig. 4a). None of the Fabs interfered with the normal growth of *S. mutans* and *S. sobrinus*. SM-10, which blocked *S. mutans* biofilm the most, was also the tightest binder to *S. mutans*. Fabs SS-2 and SS-C-1 showed the lowest *S. mutans* biofilm inhibition, which was consistent with their weak binding properties to *S. mutans*. SS-2, which showed the highest *S. sobrinus* biofilm inhibition, was the strongest binder to *S. sobrinus*. In general, we found that Fabs with strong binding properties showed high biofilm inhibition. Previous reports have also shown that polyclonal and monoclonal antibodies to Streptococcal surface antigens inhibit adherence of *S. mutans* cells to tooth surfaces35–37 and prevent caries formation in non-human primates and in human clinical trials38.

**S. mutans and S. sobrinus Fabs prevent dental caries formation in rat caries model.** For the rat caries model, we used a combination of *S. mutans* and *S. sobrinus* Fabs that showed the greatest biofilm formation blocking effects in vitro (SM-10 and SS-2). Three groups of 20-day old (day 1 after weaning) rats were used for this study. All experimental groups (groups 1, 2 and 3) were infected with *S. mutans* and *S. sobrinus* at day 1 of the experiment and then two times per week. All groups were also given the cariogenic diet for a total of 4 weeks. For the treatment, we used 50µg of Fabs and delivered Fabs twice a week in the oral cavity for the duration of the experiment (total of 4 weeks). Group 1 did not receive Fabs (Fig. 5a, b). Group 2 only received 2 weeks of Fab treatment (Fig. 5a, c, d). Group 3 received 4 weeks of Fab treatment (Fig. 5a, e). At the end of the 4th week, we sacrificed rats and caries were detected by micro-CT. Compared to control group, we observed a significant higher inhibition of caries formation in rats treated with Fabs. Caries were found in four rats out of five in the control group (Fig. 5b), where we observed a total of five caries per sixty molars (Fig. 5c). In contrast, caries were detected only in 1 rat out of five for groups 2 and 3 (Fig. 5b), where we observed one caries per sixty molars (Fig. 5c). There was no difference between 2 weeks and 4 weeks of Fab treatment (local application), suggesting that Fabs show efficacy in this model as early as 2 weeks following the initial application. This experiment was repeated two times with similar results showing an overall 5-fold reduction in total number of dental caries for
rats treated with Fabs. This in vivo rat model study indicated that combined application of Fabs SM-10 and SS-2 significantly reduced caries formation despite rats receiving a cariogenic diet.

In conclusion, we isolated Fabs that cross-reacted with gram-positive S. mutans and S. sobrinus although at different levels and not with the gram-negative E. coli, suggesting that they recognize a shared epitope between the gram-positive bacteria. Future work will aim at defining Fab epitopes to determine whether they are specific for mutans streptococci. Epitopes shared by a range of cariogenic bacteria may be show better efficacy in clinical trials, especially for caries when mutans streptococci are present at low levels.

We showed that synthetic human Fabs could be generated against the cell surface of oral pathogenic bacteria and that oral application of Fabs reduced dental caries in a rat caries model. This strategy allows Fab production in large quantities in microbial culture systems, which would be more suitable to scale up for the routine control of dental caries. Secretory IgEs are generally more preferable for oral applications as they are most abundant in saliva, resistant to proteolysis, and have higher retention time in oral cavity compare to IgGs or Fabs. Since synthetic Fabs are made using recombinant DNA technology, our system allows Fabs to be engineered easily to
make human secretory IgE or IgG. This would prevent adverse immune responses seen with nonhuman antibodies. Passive immunization using synthetic Fabs is much safer and easier to administer locally compared with approaches using active vaccination. In the future, this strategy might lead towards an inexpensive and convenient general treatment for dental caries.

Methods

**Bacterial strains and culture conditions.** *S. mutans* (ATCC700610) and *S. sobrinus* (ATCC27351) were cultured on brain heart infusion (BHI) agar (Difco) for 24–48 h at 37 °C and 5% CO₂. Single colonies were isolated and used to inoculate 3 mL of BHI and cultured overnight at 37 °C with 5% CO₂. For phage display, 10 mL of BHI was inoculated with 0.5 mL of an overnight culture and grown at 37 °C with 5% CO₂ to an OD₆₀₀ of 1.0. For biofilm-induced competitive panning, 10 mL of BHI with 1% sucrose was inoculated with overnight cultures of *S. mutans* and *S. sobrinus* and grown in BHI with 1% sucrose for 20 hrs at 37 °C in a 5% CO₂ aerobic atmosphere.

**Selection protocol.** The selection protocol is outlined in Fig. 1a. Target antigens for panning were live *Streptococcus mutans* and *Streptococcus sobrinus* cells. The subtraction panning was performed using *E. coli* (DH10B) and *S. mutans* (in case of *S. sobrinus*) or *S. sobrinus* (in case of *S. mutans*). For the selection, 10 mL of BHI was inoculated with overnight cultures of *S. mutans* or *S. sobrinus* and cultured until they reached an OD₆₀₀ of 1. Bacteria were pelleted by centrifugation and used for selections. For sucrose-induced biofilm competitive selections, 10 mL of BHI supplemented with 1% sucrose was inoculated with overnight cultures of *S. mutans* or *S. sobrinus* and cultured until they reached an OD₆₀₀ of 1. Bacteria were pelleted by centrifugation and used for selections.
S. sobrinus and cultured at 37 °C with 5% CO₂ for 4 hours. Bacteria were centrifuged and cell pellets were collected for selections. Approximately 10⁷ bacterial cells were washed twice with phosphate-buffered saline (PBS), re-suspended in 1 ml of PBS containing 1% BSA, and incubated in a 1.5 ml micro-centrifuge tube on a rotator for 1 hr at room temperature. This and subsequent incubations were carried out using micro-centrifuge tubes that had been blocked with 1% BSA in PBS for 1 hr at room temperature. After 1 hr, 10¹⁰ phage particles containing antibody fragments (Fab) were added to the tube containing 10⁶ cells in 500 μl PBS-1% BSA and rotated for an additional 1 hr at room temperature to allow phages to bind to the bacteria. After incubation, selection tubes were washed eight times with PBS containing 0.05% Tween-20 (PBT) to remove unbound phages. During final wash, re-suspended bacterial cells were moved to a fresh tube that was pre-blocked with 1% BSA in PBS. To elute bound phages, the cell pellet was re-suspended in 500 μl of 100 mM HCl and rotated at room temperature for 5 min. The tube was centrifuged for 2 min at 10,000 RPM and the supernatant containing eluted phages was transferred to a new micro-centrifuge tube and neutralized with 1 M Tris–HCl pH 8.0. Eluted phages were used to infect NEBρE coli cells (NEB). Phage were either amplified for use in the next round of selection or plated on LB media containing carbenicillin and kanamycin to isolate E. coli harboring individual phagemids. Subtractive panning was performed at rounds 4 and 5. During subtractive panning, phage pools amplified from round three were pre-blocked with E. coli and then the unbound phage pool was further incubated with S. mutans (in case of S. sobrinus) or S. sobrinus (in case of S. mutans). During sucrose-induced biofilm competitive selections, suspended bacteria were first incubated with FabS that were isolated from non-biofilm selections and then phage particles were added to the bacteria.

**DNA sequencing.** At the end of the fifth round of selection, phage infected NEBρE coli cells were plated on LB media containing antibiotic carbenicillin and kanamycin to isolate single colonies. Colony PCR was performed to confirm the correct size of the Fab. For Sanger sequencing, phagemids from twenty E. coli colonies after fifth round of panning were isolated. Phagemids were sequenced on 3500 Genetic Analyzer (Thermo Fisher Scientific) using TGS163 and TGS164 primers (Table S1).

**Fab Cloning, Expression, and Purification.** Selected Fabs were PCR-amplified using TGS157 and TGS160 primers (Table S1) from phagemids. The PCR-amplified Fab amplicon was cloned into SacI- and XhoI-digested pCW-LIC Fab expression vector (Addgene) using Gibson assembly. Each Fab contained the light chain signaling sequence, light chain (VL-CL), FLAG tag, heavy chain signaling sequence, heavy chain (VH-CH1), GGS linker, and C-terminal His, tag in the order listed. Fab expression plasmids were transformed into BL21 (DE3) competent E. coli cells (NEB) and plated on 2YT media containing carbenicillin. Single E. coli colonies were cultured in overnight expression Instant TB media (Novagen) for 20–24 hrs. Cells were pelleted and suspended in Protein L Binding buffer (Sodium Phosphate 20 mM, 0.15 M NaCl, pH 8.0). Cells were disrupted at 35 Kpsi using the cell disruptor (Constant System LTD. USA). Cell debris was removed by centrifuging at 12,000 RPM for 20 min. Supernatant was collected and filtered through a 0.45-micron membrane filter (Minisart, Sartorius stedim). Fabs were purified on a GE Healthcare AKTA FPLC system using a HiTrap Protein L column (GE Healthcare). Fabs were eluted from the Protein L column using IgG elution buffer (Fisher Scientific) and neutralized with neutralization buffer (1 M Tris–HCl pH 9.0). Purified Fabs were dialyzed overnight in PBS and concentrated using 10 K MWCO filter (Millipore). Fabs were filter sterilized and stored at −20 °C. Fabs were quantified using the Bicinchonic acid assay (Pierce™ BCA Protein Assay Kit, Thermo Scientific) following manufacturer’s instructions.

**Enzyme linked immunosorbent assay (ELISA).** To test the binding of phage display generated Fabs, 96-well Maxisorp ELISA plates (Nunc) were coated with bacterial cell suspension containing 10⁵ cells per well in PBS for 1 hr at 37 °C followed by overnight at 4 °C. Plates were washed three times with PBS and blocked with 2% milk powder in PBS for 2 hrs at room temperature. Purified Fabs (5 μg per well) were incubated with cells for 2 hrs at room temperature. After incubation with Fabs, the plate was washed three times with PBS containing 0.01% Tween 20 followed by an additional three washes with PBS. Cell bound Fabs were detected using anti-His horseradish peroxidase-conjugate (Amersham Biosciences, Piscataway, NJ, USA). ELISAs were developed with 3,3′,5,5′-tetramethylbenzidine (TMB) (Sigma). Reactions were stopped by the addition of H₂SO₄ after 20 min and readings taken at OD₄₅₀ using plate reader (Spectra Max, Molecular Devices).

**Immunofluorescence.** Bacterial cell suspensions at a concentration of 10⁶ CFU/ml were coated on 96-well plate and blocked with 2% milk powder in PBS for 1 hr at room temperature. Purified Fabs (5 μg per well) were incubated with bacteria cells for 1 hr at room temperature. Following three washes with 1× PBS, FITC conjugated Anti-FLAG antibody was added at 1:5000 dilution to each well and incubated for 1 hr at room temperature. Bacteria were washed three times in PBS followed by three additional washes with PBS. Fluorescence images were taken using fluorescence microscope (EVOS, Advance Microscopy Group).

**Flow cytometry.** Bacteria growing on BHI were centrifuged and washed once with PBS. To break filaments of S. mutans and S. sobrinus, bacteria cells were re-suspended in 1 ml of ice-cold PBS and sonicated using a microtip probe sonicator for 20 secs at 10% power with a setting of a 0.5 second impulse, followed by a 0.5 sec break. For flow cytometry analyses, bacterial cell suspensions at a concentration of 10⁶ CFU/tube were incubated with different Fabs (1 μM) at room temperature for 60 min. Bacteria cells were washed three times with PBS, pH 7.4. Anti-FLAG® R-Phycoerythrin Conjugate (ProZyme) was added at 1:3000 dilution to each tube and incubated for 30 min at room temperature. Bacteria cells were washed three times in PBST followed by three additional washes with PBS and re-suspended in 500 μL of PBS. Bacteria cell fluorescence was monitored using a flow cytometer (MACS Quant, Miltenyi Biotec) with 561 nm excitation and 586–541 nm emission wavelengths. Flow cytometry data were analyzed using Flowjo (Tree Star).
**In vitro biofilm assay.** The effect of different Fabs on *S. mutans* and *S. sobrinus* biofilm formation were examined using the microdilution method\(^1\). Briefly, overnight culture of *S. mutans* and *S. sobrinus* were prepared to a final concentration of 10⁶ CFU/mL and grown in BHI for 24 hrs at 37°C in a 5% CO₂ aerobic atmosphere with the addition of 1% sucrose for biofilm development. Different Fabs were added to the culture at 0 hour at a final concentration of 3μM. After 24 hours, before biofilm quantification, the culture growth was measured at OD₅₉₀nm using a plate reader. The cell suspension was carefully removed and each well was washed three times with 1× PBS. The remaining attached cells were fixed with 96% ethanol for 15 min and then plates were dried. Wells were stained with 120μL of 0.1% crystal violet (Fisher) for 20 minutes. The crystal violet stain was aspirated and wells were washed three times with 150 μL of PBS. Wells were air-dried and retained crystal violet was solubilized with 150μL of 30% (v/v) glacial acetic acid (Fisher). The plate was incubated at room temperature for 20 min with shaking. The biofilm formation was quantified by measuring the OD₆₀₀nm using a plate reader.

**In vivo rat caries model.** The animal experiment protocol was reviewed and approved by the Ethical Committee on Animal Research at the University of Michigan. Fifteen days old Sprague-Dawley rats were purchased from Charles River (Wilmington, MA). Rats then were screened for infection with *S. mutans* and *S. sobrinus*. Fifteen infection-free rats were given *S. mutans* and *S. sobrinus* (kindly proved by Dr. Christopher Fenno, School of Dentistry, University of Michigan) twice a week for 4 weeks starting at day 21 after birth. All rats were fed high cariogenic diet 2000 (Envigo, Madison, WI) and 5% sucrose water ad libitum\(^2\). Rats were randomly divided into three groups. Each group had 5 rats. All groups received the bacteria mixture applied directly on their teeth with a brush twice a week for total 4 weeks. Rats in the first group were treated with a mixture of Fabs (SM-10 and SS-2) applied directly on teeth surface using a brush starting at day 1 of the experiment. The second group was treated with the same Fabs mixture twice a week starting from the 3rd week for a total of two weeks; the last group was the vehicle control group; rats in this group were treated with PBS twice a week for 4 weeks. Upon completion of the 4 weeks the rats were sacrificed under anesthesia with a ketamine/xylazine mixture and mandibles and maxilla collected, fixed with 70% ETOH for 24 hours and analyzed by micro-CT.

**Micro-CT.** The dissected samples were scanned with micro-computed tomography (SkyScan 1272 Micro-CT at 60 kV, with 6.0 μm pixel size). The scanned images were reconstructed with Nrecon (Bruker, Manning Park, MA) and DataViewer (Bruker).

**Availability of data and materials.** The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author Contributions
C.R.G. and P.P. conceived the idea. M.K.A. performed phage display, generated the antibodies, purified, and validated the antibodies. K.A. performed ELISA, immunofluorescence, flow cytometry and in vitro biofilm experiments. L.Z., R.W. and S.P. designed and/or performed in vivo rat study. M.K.A., C.R.G., and P.P. interpreted experimental observations, designed figures, and wrote the manuscript. All authors read and approved the final manuscript.

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