Research Article

Repurposing Napabucasin as an Antimicrobial Agent against Oral Streptococcal Biofilms

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Objectives. Disruption of microbial biofilms is an effective way to control dental caries. Drug resistance and side effects of the existing antimicrobials necessitate the development of novel antibacterial agents. The current study was aimed at investigating the antibacterial activities of the repurposed natural compound napabucasin against oral streptococci. Methods. The minimum inhibitory concentration, minimum bactericidal concentration, minimum biofilm inhibition concentration, and minimum biofilm reduction concentration of Streptococcus mutans, Streptococcus gordonii, and Streptococcus sanguinis were examined by a microdilution method. Cytotoxicity of napabucasin against human oral keratinocytes, human gingival epithelia, and macrophage RAW264.7 was evaluated by CCK8 assays. The dead/live bacterium and exopolysaccharide in the napabucasin-treated multispecies biofilms were evaluated by confocal laser scanning microscopy. Microbial composition within the napabucasin-treated biofilms was further visualized by fluorescent in situ hybridization and qPCR. And the cariogenicity of napabucasin-treated biofilms was evaluated by transverse microradiography. Results. Napabucasin exhibited good antimicrobial activity against oral streptococcal planktonic cultures and biofilms but with lessened cytotoxicity as compared to chlorhexidine. Napabucasin reduced the cariogenic S. mutans and increased the proportion of the commensal S. gordonii in the multispecies biofilms. More importantly, napabucasin significantly reduced the demineralization capability of biofilms on tooth enamels. Conclusion. Napabucasin shows lessened cytotoxicity and comparable antimicrobial effects to chlorhexidine. Repurposing napabucasin may represent a promising adjuvant for the management of dental caries.

1. Introduction

Dental caries is one of the most prevalent diseases incurring large expenditures worldwide [1, 2]. It is a slowly progressive chronic disease initiated by oral biofilms and associated with multiple risk factors [3]. Cariogenic bacteria such as Streptococcus mutans dynamically compete with commensal bacteria including Streptococcus sanguinis and Streptococcus gordonii within the oral biofilm. Given the disequilibrium of the microbial ecology, the microbial metabolism of carbohydrates can lead to continuous decline of pH at the biofilm and tooth hard tissue interface, consequently causing demineralization of tooth hard tissue, and dental caries gradually occurs [3–7].

Mechanical plaque control is the mainstay for the control of oral biofilms and dental caries, but it heavily relies on individuals’ compliance. To supplement mechanical plaque control, mouth rinses with antiplaque properties are well recommended [8]. Chlorhexidine (CHX) is one of the most common antimicrobial agents used as mouth rinse [9–11]. However, longtime usage of CHX could cause drug resistance and side effects such as taste confusions or tooth staining
Therefore, alternative antimicrobial agents with comparable effectiveness but lessened side effects are needed for the better control of the oral biofilm [12].

Drug repurposing has garnered increasing attention as an alternative strategy to identify new antimicrobial agents for its efficiency in reducing time, cost, and risks associated with the development of novel antibiotics [13, 14]. In an effort to repurpose existing drugs as antibacterial agents, we have recently screened from a library of bioactive molecules against *Streptococcus mutans* and identified the natural compound napabucasin (NAP) (Figure 1(a)), namely, 2-acetylfuro-1,4-naphthoquinone. 2-Acetylfuro-1,4-naphthoquinone is one of the chemical constituents first isolated from *Newbouldia laevis* [15]. *N. laevis* is widely used in the African folk medicine and has been reported to reduce dental caries and other diseases [16]. Previous studies reported the antibacterial activity of 2-acetylfuro-1,4-naphthoquinone against Escherichia coli, *Streptococcus faecalis*, and *Staphylococcus aureus* [15, 17]. A recent study also showed its antimycobacterial activity for the treatment of tuberculosis [18]. In addition, the NAP is in phase III clinical trials for the treatment of cancers (i.e., gastric cancer, pancreatic cancer, and colorectal cancer) [19–23]. However, there is no data to support its activity against oral pathogens.

The purpose of this study is to investigate the antimicrobial activity of NAP against oral streptococci.

## 2. Materials and Methods

### 2.1. Test Bacteria and Chemicals

*Streptococcus mutans* UA159, *Streptococcus gordonii* DL1, and *Streptococcus sanguinis* ATCC 10556 were kindly provided by the State Key Laboratory of Oral Diseases (Sichuan University, Chengdu, China). *S. mutans*, *S. gordonii*, and *S. sanguinis* were routinely grown at 37°C under aerobic condition (5% CO₂) in brain heart infusion broth (BHI; Difco, Sparks, MD). Inoculum for the experiment was adjusted to 1 × 10⁶ CFU/mL for *S. mutans*, *S. gordonii*, and *S. sanguinis* based on the OD₆₀₀ₙₙ versus CFU/mL graph of each bacterium and further 1 : 100 diluted in the growth culture. When needed, medium was supplemented with 1% sucrose (designated BHIS).

Napabucasin was purchased from Bide Pharmatech Ltd. and prepared in DMSO at a stock concentration of 100 mg/mL.

### 2.2. Susceptibility Tests

#### 2.2.1. Bacterial Susceptibility Test

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of NAP against *S. mutans*, *S. gordonii*, and *S. sanguinis* were determined by a microdilution method in BHI, as described previously [24, 25]. The concentrations of NAP ranged from 0.97 to 1000 μg/mL (twofold dilutions). BHI broth containing equivalent DMSO (1% to 0.001%, v/v) was used as a solvent control and ran simultaneously to control for the possible growth inhibition caused by the added DMSO. CHX was used as a positive control, cell control (test bacteria and BHI broth) was used as a negative control, and BHI broth was used as a blank control.

#### 2.2.2. Biofilm Susceptibility Test

The minimum biofilm inhibition concentration (MBC) was used to evaluate the effect of NAP on biofilm formation [26]. *S. mutans*, *S. gordonii*, or *S. sanguinis* (1 × 10⁷ CFU/mL, 10 μL/well) were grown in BHI with twofold serial dilution of NAP (200 μL/well) ranging from 0.12 to 125 μg/mL at 37°C for 24 h. A parallel study was also performed with BHIS as a negative control. Then, the supernatants from the wells were decanted, and the adherent biofilm was washed three times with PBS to remove the planktonic cells. Fixed with methanol for 15 min and air-dried at room temperature, the biofilm was stained with 0.1% (wt/vol) crystal violet (Sigma) for 5 min, rinsed with deionized water until blank control wells were colorless, and added 200 μL of 95% ethanol to each crystal violet-stained well. Subsequently, the plate was rocked 30 min at room temperature, and the absorbance at 595 nm was recorded. The percentage of inhibition was calculated using the equation: (A₄₅₀ of negative control – A₄₅₀ of the test group)/A₄₅₀ of negative control × 100%. The MBC was defined as the lowest agent concentration that showed 90% or more inhibition of biofilm formation.

The effect of NAP on the 1-day-developed biofilm was examined by the minimum biofilm reduction concentration (MBRC). *S. mutans*, *S. gordonii*, or *S. sanguinis* (1 × 10⁷ CFU/mL, 200 μL/well) in BHIS was added to a 96-well polystyrene tissue culture plate. After anaerobic incubation at 37°C for 24 h, the supernatants were removed and washed with PBS three times without disrupting the integrity of biofilms. Fresh BHIS containing NAP ranging from 0.12 to 125 μg/mL were added to each well and incubated at 37°C for 24 h. The negative control was biofilms in BHIS without NAP. The biofilm was fixed, air-dried, stained, and quantified as described above. The MBRC was defined as the lowest agent concentration that showed 90% or more reduction of the biofilm.

#### 2.3. In Vitro Cytotoxicity/Viability Assay

Cell viability was evaluated by using the Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) assay as described by Park et al. [27]. Test cells were human oral keratinocytes (HOK), human gingival epithelia (HGE), and macrophage RAW264.7 (RAW264.7). Cells were plated in 96-well plates at 10,000 cells/well in minimum essential medium with the Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic. The cells were grown in an incubated environment with 5% CO₂ at 37°C for 24 h. A parallel study was also performed with BHIS as a negative control. Then, the cells were washed with PBS twice and were added with the fresh medium (200 μL/well). Each well was added with 10 μL of CCK-8, and after incubation in the CO₂ incubator for 1 h to 1.5 h, absorbance was measured at the wavelength of 450 nm. The cell viability was calculated according to the following formula: (% = (A₄₅₀ of test group – A₄₅₀ of blank control)/(A₄₅₀ of negative control – A₄₅₀ of blank control) × 100%.

#### 2.4. Multispecies Biofilm Imaging

The multispecies biofilms were cultivated in accordance with a previous study [30].
Overnight cultures of *S. mutans*, *S. gordonii*, and *S. sanguinis* were simultaneously inoculated (inoculum ratio = 1:1:1). The chemotaxis chamber μ-Slide, which has extremely low values of birefringence and autofluorescence, was used for bacterial culture and confocal microscopy [31]. Bacterial suspensions (1 × 10^5 CFU/mL for each strain) were mixed in 300 μL BHI containing 1% sucrose (BHIS) in the μ-Slide (8 wells, 80826, Ibidi) at 37°C for 24 h. Then, biofilms were exposed to PBS, 62.5 μg/mL NAP, and 0.2% CHX for 3 days (5 min, three times per day). This short-term repeated treatment was to simulate the daily exposure to the mouth rinses [32].

For dead/live imaging, biofilms were stained with fluorescent LIVE/DEAD BacLight Bacterial Viability stain (Molecular Probes, Invitrogen) according to the manufacturer’s instructions. The labeled biofilms were imaged with a DMIRE2 confocal laser scanning microscope (Leica, Wetzlar, Germany) equipped with a 60x oil immersion objective lens.

For extracellular polysaccharide (EPS) staining, the bacterial cell and the EPS were stained with SYTO 9 (Molecular Probes) and Alexa Fluor 647-labeled dextran conjugate (Molecular Probes) as described previously [33]. The biofilms were captured with a Leica DMIRE2 confocal laser scanning microscope as in live/dead imaging.

For fluorescent in situ hybridization imaging, biofilms were fixed in 4% paraformaldehyde overnight and investigated by species-specific probes [34]. The multispecies biofilms were imaged with a confocal laser scanning microscope (FV1000, Olympus, Tokyo, Japan). All three-dimensional reconstructions of the biofilms were performed with Imaris 7.0.0 (Bitplane, Zürich, Switzerland). The quantification of the dead/live and EPS/bacteria
2.5. DNA Isolation and Real-Time PCR. Total DNA of biofilms were isolated and purified using a TIANamp Bacteria DNA kit (TIANGEN, Beijing, China). The purity and concentration of DNA were detected with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The extracts were stored at -20°C until use. TaqMan real-time polymerase chain reaction (Life Technologies, Carlsbad, CA, USA) was used to quantify the absolute number of *S. mutans*, *S. gordonii*, and *S. sanguinis* as described by the manufacturer (Takara, Dalian, China).

2.6. Transverse Microradiography. Human teeth free of white spots, cracks, and other defects that had been extracted for periodontal or impacted reasons were collected under a protocol approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University (WCHSIRB-D-2018-107). Crowns were separated from roots and cut into four sections measuring 5 mm × 5 mm × 2 mm by using a diamond-coated saw (Struers Minitom; Struers, Copenhagen, Denmark) under continuous water cooling. The enamel slabs were embedded in polymethylmethacrylate and painted with two layers of acid-resistant nail varnish, leaving a 4 mm × 4 mm window of the exposed enamel surface. Then, these surfaces were polished progressively with waterproof silicon carbide abrasive papers (800–4000 grit; Struers, Copenhagen, Denmark) and were ultrasonically cleaned in a deionized water for 5 min to remove the residual abrasives. All slabs were sterilized with ethylene oxide [35]. The 30 specimens were randomly divided into 3 groups: PBS, 62.5 μg/mL NAP, and 0.2% CHX. Enamel slabs obtained from the same tooth were evenly distributed to each test group.

Overnight cultures of *S. mutans*, *S. gordonii*, and *S. sanguinis* (1 × 10^7 CFU/mL) were simultaneously inoculated on enamel slabs in a 24-well cell culture plate with BHIS at 37°C. Slabs with biofilms were then exposed to PBS, 62.5 μg/mL NAP, and 0.2% CHX for 5 days (5 min, three times per day). The 5-day demineralization duration was in accordance with previous studies [36, 37]. All specimens were then washed in PBS and refreshed with BHIS after every exposure. After 5 days of treatment, specimens were taken out and rinsed with PBS to remove the biofilms. Then, specimens were cut again and polished with a hand plane—parallel from both sides with waterproof silicon carbide abrasive papers (800–4000 grit; Struers, Copenhagen, Denmark) to thickness ranging around 150 nm [38]. X-ray films of experimental lesions were acquired with an X-ray generator (Softex, Japan) equipped with a microradiography camera and then were further examined using a Zeiss AXIO Imager A2 microscope (Carl Zeiss, Germany). Quantitative data was acquired by a calibrated analysis system TMR2006 (Inspektor Research Systems BV, Netherlands) [39]. Data are obtained as the mean of 10 separate samples.

2.7. Statistical Analysis. All experiments were repeated at least three times independently. One-way analyses of variance (ANOVAs) and the Student-Newman-Keuls test were used to compare differences. Differences were considered significant when *P* < 0.05. Statistical analyses were performed with the SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA).

### Table 1: MICs, MBCs, MBICs, and MBRCs of NAP and CHX against *S. mutans*, *S. gordonii*, and *S. sanguinis* strains.

| Strain       | Planktonic cells (μg/mL) | Biofilm | Planktonic | Biofilm |
|--------------|--------------------------|---------|------------|---------|
|              | MIC NAP | MIC CHX | MBC NAP | MBC CHX |
| *S. mutans*  | 3.91 | 1.95 | 15.63 | 7.81 | 1.95 | 0.98 | 62.50 | 62.50 |
| *S. gordonii* | 0.49 | 3.91 | 0.98 | 7.81 | 0.49 | 3.91 | 15.63 | 15.63 |
| *S. sanguinis* | 0.49 | 0.49 | 3.91 | 1.95 | 1.95 | 3.91 | 3.91 |

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MBIC: minimum biofilm inhibition concentrations; MBRC: minimum biofilm reduction concentrations.

3. Results

3.1. NAP Exhibits Good Antimicrobial Activity against Oral Streptococci. NAP inhibited the planktonic growth of *S. mutans*, *S. gordonii*, and *S. sanguinis* with minimum inhibitory concentration (MIC) ranging from 0.49 μg/mL to 3.91 μg/mL and minimum bactericidal concentration (MBC) ranging from 0.98 μg/mL to 15.63 μg/mL. Besides, NAP inhibited the biofilms of *S. mutans*, *S. gordonii*, and *S. sanguinis* with minimum biofilm inhibitory concentration (MBIC) ranging from 0.49 μg/mL to 1.95 μg/mL and minimum biofilm reduction concentration (MBRC) ranging from 3.91 μg/mL to 62.5 μg/mL (Table 1). CHX, as a positive control, inhibited the planktonic growth and biofilms of *S. mutans*, *S. gordonii*, and *S. sanguinis* with MIC ranging from 0.49 μg/mL to 1.95 μg/mL, MBC ranging from 3.91 μg/mL to 7.81 μg/mL, MBIC ranging from 0.98 μg/mL to 3.91 μg/mL, and MBRC ranging from 3.91 μg/mL to 62.5 μg/mL (Table 1).

3.2. NAP Shows Lessened Cytotoxicity against Human Oral Cells Relative to Chlorhexidine. The cytotoxicity of NAP against human oral keratinocytes (HOK), human gingival epithelia (HGE), and macrophage RAW264.7 (RAW264.7) was evaluated by measuring the cell viability after drug exposure duration of 5 min. NAP showed lessened cytotoxicity against HOK, HGE, and RAW264.7 compared with CHX. More importantly, the IC50 of NAP on HOK, HGE, and RAW264.7 (IC50 > 62.5 μg/mL) were higher than its minimal biofilm reduction concentrations against an oral streptococcal biofilm (MBRC ranging from 3.91 μg/mL to 62.5 μg/mL), suggesting that NAP is safe for use as an antimicrobial agent at the exposure duration (Figures 1(b)–1(d)).

3.3. NAP Inhibits the Development of Multispecies Biofilms. The antimicrobial effects of NAP were further evaluated with multispecies biofilms. Both NAP and CHX treatment disrupted the structural integrity of multispecies biofilms significantly (Figure 2(a)). NAP treatment further reduced
the bacteria within the oral streptococcal biofilms as compared to CHX (Figure 2(b)). In addition, the biofilms treated with NAP showed an equivalent dead/live cell ratio as well as an EPS/bacteria ratio as compared to the CHX-treated ones (Figures 2(c)–2(e)). Fluorescent in situ hybridization imaging and qPCR were conducted to evaluate the effect of NAP on multispecies biofilm composition. As shown in Figures 3(a) and 3(b), both NAP and CHX treatments significantly reduced the total amount of streptococci. NAP inhibited S. mutans and S. sanguinis but increased the proportion of the commensal S. gordonii within the multispecies biofilms.

3.4. NAP Reduces the Demineralization Capability of Streptococcal Biofilms on Enamel. We further evaluated the inhibitory effects of NAP on the cariogenicity of oral biofilms by quantifying biofilm-induced demineralization on human enamel slabs. As shown by the transverse microradiography data, the depth of a biofilm-induced lesion and the mineral loss of enamel were significantly reduced when treated with CHX and NAP compared to the negative control. NAP and CHX showed a comparable inhibitory effect on the biofilm-induced demineralization (Figure 4).

4. Discussion

As a biofilm-associated chronic disease, effective biofilm control is critical for dental caries management. Antimicrobial agents, as supplements to insufficient mechanical removal, have been used to control oral biofilms for years. Clinical trials have shown that long-time usage of antimicrobial mouth rinses can significantly reduce S. mutans in saliva [40, 41] and reduce the incidence of caries among people at high caries risk [42, 43]. However, CHX had only a superficial bactericidal effect on dental plaque and exhibited noticeable cytotoxicity as dose increases [44, 45]. Long-term repeated exposure to CHX could induce drug resistance in oral microbes such as S. gordonii, Enterococcus faecalis, Fusobacterium nucleatum, and Porphyromonas gingivalis [46, 47]. In addition, CHX can cause tooth or tongue staining and
taste confusions in patients [48]. The adversary effects of CHX necessitate the development of novel agents to control oral biofilms. Here, we demonstrated that the natural compound NAP possessed comparable antimicrobial activity with CHX against oral streptococcal biofilms but with relatively lower cytotoxicity, representing a promising novel

**Figure 3:** Effects of NAP on the composition shift of multispecies biofilms. (a) Representative fluorescent in situ hybridization (FISH) images of multispecies biofilms treated with NAP, CHX, and phosphate-buffered saline (PBS). *S. mutans* (*S. m.*, green), *S. gordonii* (*S. g.*, blue), and *S. sanguinis* (*S. s.*, red) were labeled with species-specific FISH probes. Images were captured with a fluorescence microscope at 60x magnification. (b) The ratio of *S. mutans*, *S. gordonii*, and *S. sanguinis* in multispecies biofilms quantified by qPCR. *P < 0.05.

**Figure 4:** The antidemineralization effect of NAP against multispecies biofilms. (a) Representative transverse microradiography (TMR) images of human enamel discs exposed to 5-day biofilm-induced experimental demineralization. The high-density regions represent the sound enamel tissues, while the low-density shadows indicate the caries-like lesions. (b) Lesion depth and (c) mineral loss were calculated. Data are presented as mean ± SD. *P < 0.05.
agent in the control of dental caries. NAP is also an anticancer drug that is in phase III clinical trials for cancer treatment. Drug repurposing is an effective drug development strategy [49]. The repurposed use of the anticancer drug toremifene showed good antimicrobial activity against oral pathogens P. gingivalis and S. mutans by damaging the bacterial membrane [50]. The antiasthma drug zafirlukast also showed potent antimicrobial activity against P. gingivalis and S. mutans [51]. The repurposed use of NAP in the current study also showed potent antimicrobial activity against oral streptococci in either planktonic culture or biofilm.

Oral biofilms are microbes embedded within an EPS matrix that functions as a “glue” to form a cohesive and adherent ecosystem. EPS is well recognized as a critical cariogenic factor of a streptococcal biofilm [5, 33, 52–54]. Disruption of EPS can disperse the biofilm and increase the sensitivity of bacteria to antibiotics [54, 55]. Previous studies verified that proanthocyanidins (PACs) in cranberry could reduce the amount of EPS, break down microarchitecture of the cariogenic biofilm, and reduce the incidence of smooth-surface caries in rats [56, 57]. The current study found that NAP could significantly inhibit EPS production in the biofilm and thus disrupted the integrity of the streptococcal biofilm, further supporting its potential use as a plaque control measure that could supplement the management of dental caries.

The cariogenicity of the oral biofilm is closely associated with microbial interactions between cariogenic S. mutans and commensal streptococci such as S. sanguinis and S. gordonii. Disequilibrium within the plaque biofilms is the initiating event that mediates the transition from health to disease [58]. Changes in host diet such as excessive carbohydrate consumption promote accumulation of acid-producing organisms that trigger ecological alteration towards cariogenic microbiota [5]. The most typical acid-producing organism, S. mutans, can drive the dysbiosis of the oral microbial ecology and ultimately lead to the occurrence of dental caries [59, 60]. Inhibiting S. mutans with increasing relative abundance of commensal streptococci is believed to be “ecologically safe” for the control of oral biofilms [6, 24, 53, 61]. Our previous study showed that the combinatorial use of arginine and NaF could inhibit S. mutans but enrich the commensal S. sanguinis in the multispecies biofilms, representing an ecological approach to the management of dental caries [6]. In this study, multispecies biofilms consisting of S. mutans, S. gordonii, and S. sanguinis were established and we found that NAP could suppress cariogenic S. mutans but increase the proportion of S. gordonii within the multispecies biofilms. More importantly, NAP treatment could significantly halt the biofilm-induced demineralization of tooth enamel, suggesting that NAP could be a good candidate for daily use mouth rinse.

Biocompatibility is a critical factor for daily use mouth rinse. Based on the clinical data reported in the previous phase I and II study, patients with advanced malignancies received napabucasin orally at a dose of 240 mg twice a day. Adverse events were generally mild and predominantly included diarrhea, abdominal pain, nausea, and fatigue [22, 62]. The current study further evaluated the cytotoxicity of NAP on human oral cells such as HOK, HGE, and macrophage RAW264.7. Lower cytotoxicity of NAP against all these cells was observed as compared to that of CHX. These data further support repurposing NAP as an antimicrobial compound that can be tropically used for the control of oral biofilms.

5. Conclusion

In summary, this study for the first time demonstrated that NAP exhibited good antibacterial capability against oral streptococcal biofilms with lower cytotoxicity. NAP can disperse the multispecies biofilms and reduce the biofilm-induced demineralization of tooth hard tissue with decreasing relative abundance of S. mutans in the biofilms. Further in vivo studies are still needed to translate this promising repurposed natural compound to the management of dental caries.

Data Availability

All data used during the study are available in the article and can be solicited from the corresponding author.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors’ Contributions

LYF, XX, and ZXD conceived and designed the experiment. KXY, YT, ZZC, and PX performed the experiments. KXY, JY, and LCG performed the statistical analysis. KXY wrote the first draft of the manuscript. YT, LYF, and XX help revise the manuscript. All authors read and approved the submitted version. Xinyi Kuang and Tao Yang contributed equally to this work.

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References

[1] N. J. Kassebaum, E. Bernabé, M. Dahiya, B. Bhandari, C. J. L. Murray, and W. Marcenes, “Global burden of untreated caries: a systematic review and metaregression,” Journal of Dental Research, vol. 94, no. 5, pp. 650–658, 2015.
[2] B. Söder, M. Yakob, J. H. Meurman, L. C. Andersson, and P. Ö. Söder, “The association of dental plaque with cancer mortality in Sweden. A longitudinal study,” BMJ Open, vol. 2, no. 3, article e001083, 2012.
[3] R. H. Selwitz, A. I. Ismail, and N. B. Pitts, “Dental caries,” The Lancet, vol. 369, no. 9555, pp. 51–59, 2007.
[4] P. D. Marsh, “Microbial ecology of dental plaque and its significance in health and disease,” Advances in Dental Research, vol. 8, no. 2, pp. 263–271, 2016.
[5] X. Xu, F. Chen, Z. Huang et al., “Meeting report: a close look at oral biofilms and microbiomes,” International Journal of Oral Science, vol. 10, no. 3, p. 28, 2018.

[6] X. Zheng, X. Cheng, L. Wang et al., “Combinatorial effects of arginine and fluoride on oral bacteria,” Journal of Dental Research, vol. 94, no. 2, pp. 344–353, 2014.

[7] X. Zheng, K. Zhang, X. Zhou et al., “Involvement of gshab in the interspecies competition within oral biofilm,” Journal of Dental Research, vol. 92, no. 9, pp. 819–824, 2013.

[8] J. C. Gunsonnley, “Clinical efficacy of antimicrobial mouth rinses,” Journal of Dentistry, vol. 38, Supplement 1, pp. S6–10, 2010.

[9] Y. Wang, J. Li, W. Sun, H. Li, R. D. Cannon, and L. Mei, “Effect of non-fluoride agents on the prevention of dental caries in primary dentition: a systematic review,” PLoS One, vol. 12, no. 8, article e0182221, 2017.

[10] S. Jenkins, M. Addy, and R. G. Newcombe, “A comparison of cetylpyridinium chloride, triclosan and chlorhexidine mouth rinse formulations for effects on plaque regrowth,” Journal of Clinical Periodontology, vol. 21, no. 6, pp. 441–444, 1994.

[11] C. G. Jones, “Chlorhexidine- is it still the gold standard?,” Periodontology 2000, vol. 15, no. 1, pp. 55–62, 1997.

[12] X. Kuang, V. Chen, and X. Xu, “Novel approaches to the control of oral microbial biofilms,” Biomed Research International, vol. 2018, Article ID 6498932, 13 pages, 2018.

[13] C. R. Chong and D. J. Sullivan Jr., “New uses for old drugs,” Nature, vol. 448, no. 7154, pp. 645–646, 2007.

[14] T. T. Ashburn and K. B. Thor, “Drug repositioning: identifying and developing new uses for existing drugs,” Nature Reviews. Drug Discovery, vol. 3, no. 8, pp. 673–683, 2004.

[15] V. Kuete, K. O. Eyong, G. N. Folefoc et al., “Antimicrobial activity of the methanolic extract and of the chemical constituents isolated from Newbouldia laevis,” Pharmazie, vol. 62, no. 7, pp. 552–556, 2007.

[16] H. M. Burkill, “The useful plants of west tropical Africa,” Royal Botanic Gardens, vol. 1, 1985.

[17] V. Kuete, S. Alibert-Franco, K. O. Eyong et al., “Antibacterial activity of some natural products against bacteria expressing a multidrug-resistant phenotype,” International Journal of Antimicrobial Agents, vol. 37, no. 2, pp. 156–161, 2011.

[18] C. Li, Y. Tang, Z. Sang et al., “Discovery of napabucasin derivatives for the treatment of tuberculosis,” MedChemComm, vol. 10, no. 9, pp. 1635–1640, 2019.

[19] K. O. Eyong, P. S. Kumar, V. Kuete, G. N. Folefoc, E. A. Nkengfack, and S. Baskaran, “Semisynthesis and antitumoral activity of 2-acetylfuranonaphthoquinone and other naphthoquinone derivatives from lapachol,” Bioorganic & Medicinal Chemistry Letters, vol. 18, no. 20, pp. 5387–5390, 2008.

[20] S. Fiorito, F. Epifano, C. Bruyere, V. Mathieu, R. Kiss, and S. Genovese, “Growth inhibitory activity for cancer cell lines of lapachol and its natural and semi-synthetic derivatives,” Bioorganic & Medicinal Chemistry Letters, vol. 24, no. 2, pp. 454–457, 2014.

[21] S. Bannwitz, D. Krane, S. Vortherms et al., “Synthesis and structure-activity relationships of lapacho analogues. 2. Modification of the basic naphtho[2,3-b]furan-4,9-dione, redox activation, and suppression of human keratinocyte hyperproliferation by 8-hydroxynaphtho[2,3-b]thiophene-4,9-diones,” Journal of Medicinal Chemistry, vol. 57, no. 14, pp. 6226–6239, 2014.

[22] J. M. Hubbard and A. Grothey, “Napabucasin: an update on the first-in-class cancer stemness inhibitor,” Drugs, vol. 77, no. 10, pp. 1091–1103, 2017.

[23] M. B. Sonbol, D. H. Ahn, D. Goldstein et al., “Canstem111p trial: a phase iii study of napabucasin plus nab-paclitaxel with gemcitabine,” Future Oncology, vol. 15, no. 12, pp. 1295–1302, 2019.

[24] X. Xu, X. D. Zhou, and C. D. Wu, “The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of Streptococcus mutans,” Antimicrobial Agents and Chemotherapy, vol. 55, no. 3, pp. 1229–1236, 2011.

[25] H. Koo, P. L. Rosalen, J. A. Curry et al., “Effect of a new variety of Apis mellifera propolis on Mutans streptococci,” Current Microbiology, vol. 41, no. 3, pp. 192–196, 2000.

[26] G. X. Wei, A. N. Campagna, and L. A. Bobek, “Effect of muc7 peptides on the growth of bacteria and on streptococcus mutans biofilm,” The Journal of Antimicrobial Chemotherapy, vol. 57, no. 6, pp. 1100–1109, 2006.

[27] J. B. Park, G. Lee, B. G. Yun, C. H. Kim, and Y. Ko, “Comparative effects of chlorhexidine and essential oils containing mouth rinse on stem cells cultured on a titanium surface,” Molecular Medicine Reports, vol. 9, no. 4, pp. 1249–1254, 2015.

[28] L. Schmidt, V. Zyla, K. Jung et al., “Cytotoxic effects of octenidine mouth rinse on human fibroblasts and epithelial cells - an in vitro study,” Drug and Chemical Toxicology, vol. 39, no. 3, pp. 322–330, 2016.

[29] J. Schmidt, V. Zyla, K. Jung et al., “Effects of octenidine mouth rinse on apoptosis and necrosis of human fibroblasts and epithelial cells - an in vitro study,” Drug and Chemical Toxicology, vol. 41, no. 2, pp. 182–187, 2018.

[30] K. Zhang, S. Wang, X. Zhou et al., “Effect of antibacterial dental adhesive on multispecies biofilms formation,” Journal of Dental Research, vol. 94, no. 4, pp. 622–629, 2015.

[31] P. Zengel, A. Nguyen-Hoang, C. Schüldhammer, R. Zantl, V. Kähl, and E. Horn, “M-slide chemotaxis: a new chamber for long-term chemotaxis studies,” BMC Cell Biology, vol. 12, no. 1, pp. 21, 2011.

[32] M. W. Mustafa, S. Unghaiboon, N. Phadoongsombut, K. Pangsumboon, S. Chelae, and S. Mahattanadul, “Effectiveness of an alcohol-free chitosan-curcuminoid mouthwash compared with chlorhexidine mouthwash in denture stomatitis treatment: a randomized trial,” Journal of Alternative and Complementary Medicine, vol. 25, no. 5, pp. 552–558, 2019.

[33] J. Xiao, M. I. Klein, M. L. Falsetta et al., “The exopolysaccharide matrix modulates the interaction between 3d architecture and virulence of a mixed-species oral biofilm,” PLoS Pathogens, vol. 8, no. 4, article e1002623, 2012.

[34] W. Jiang, Y. Wang, J. Luo et al., “Effects of antimicrobial peptide gh12 on the cariogenic properties and composition of a cariogenic multispecies biofilm,” Applied and Environmental Microbiology, vol. 84, no. 24, 2018.

[35] R. Z. Thomas, J. L. Ruben, J. T. ten Bosch, and M. C. N. J. M. Huysmans, “Effect of ethylene oxide sterilization on enamel and dentin demineralization in vitro,” Journal of Dentistry, vol. 35, no. 7, pp. 547–551, 2007.

[36] S. L. Eversole, K. Saunders-Burkhardt, and R. V. Faller, “Erosion prevention potential of an over-the-counter stabilized snf2 dentifrice compared to 5000 ppm f prescription-strength products,” The Journal of Clinical Dentistry, vol. 26, no. 2, pp. 44–49, 2015.
[37] D. M. S. Dos Santos, J. G. Pires, A. B. Silva, P. M. A. Salomão, M. A. R. Buzalaf, and A. C. Magalhães, “Protective effect of 4% titanium tetrafluoride varnish on dentin demineralization using a microcosm biofilm model,” *Caries Research*, vol. 53, no. 5, pp. 576–583, 2019.

[38] Q. Han, B. Li, X. Zhou et al., “Anti-caries effects of dental adhesives containing quaternary ammonium methacrylates with different chain lengths,” *Materials*, vol. 10, no. 6, p. 643, 2017.

[39] X. Zheng, J. He, L. Wang et al., “Ecological effect of arginine on oral microbiota,” *Scientific Reports*, vol. 7, no. 1, article 7206, 2017.

[40] P. Agarwal and L. Nagesh, “Comparative evaluation of efficacy of 0.2% chlorhexidine, listerine and tuli extract mouth rinses on salivary streptococcus mutants count of high school children—rct,” *Contemporary Clinical Trials*, vol. 32, no. 6, pp. 802–808, 2011.

[41] R. Jayaprakash, A. Sharma, and J. Moses, “Comparative evaluation of the efficacy of different concentrations of chlorhexidine mouth rinses in reducing the mutants streptococci in saliva: an in vivo study,” *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, vol. 28, no. 3, pp. 162–166, 2010.

[42] H. M. van Rijkom, G. J. Truin, and M. A. van’t Hof, “A meta-analysis of clinical studies on the caries-inhibiting effect of chlorhexidine treatment,” *Journal of Dental Research*, vol. 75, no. 2, pp. 790–795, 2016.

[43] B. W. Chaffee, J. Cheng, and J. D. B. Featherstone, “Non-operative anti-caries agents and dental caries increment among adults at high caries risk: a retrospective cohort study,” *BMC Oral Health*, vol. 15, no. 1, p. 111, 2015.

[44] E. Zaura-Arite, J. van Marle, and J. M. ten Cate, “Confocal microscopy study of undisturbed and chlorhexidine-treated dental biofilm,” *Journal of Dental Research*, vol. 80, no. 5, pp. 452–458, 2014.

[45] Y. C. Li, Y. H. Kuan, S. S. Lee, F. M. Huang, and Y. C. Chang, “Cytoxicity and genotoxicity of chlorhexidine on macrophages in vitro,” *Environmental Toxicology*, vol. 29, no. 4, pp. 1127–1138, 2014.

[46] S. Wang, H. Wang, B. Ren et al., “Do quaternary ammonium monomers induce drug resistance in cariogenic, endodontic and periodontal bacterial species?,” *Dental Materials*, vol. 33, no. 10, pp. 1127–1138, 2017.

[47] H. Kitagawa, N. Izutani, R. Kitagawa, H. Maezono, M. Yamaguchi, and S. Imaizato, “Evolution of resistance to cationic biocides in streptococcus mutants and enterococcus faecalis,” *Journal of Dentistry*, vol. 47, pp. 18–22, 2016.

[48] D. Shungin, S. Haworth, K. Divaris et al., “Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data,” *Nature Communications*, vol. 10, no. 1, article 2773, 2019.

[49] S. Saputo, R. C. Faustoferri, and R. G. Quivey Jr., “A drug repositioning approach reveals that streptococcus mutants is susceptible to a diverse range of established antimicrobials and nonantibiotics,” *Antimicrobial Agents and Chemotherapy*, vol. 62, no. 1, article e01674, 2018.

[50] E. Gerits, V. Defraine, K. Vandamme et al., “Repurposing tamoxifen for treatment of oral bacterial infections,” *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 3, 2017.

[51] E. Gerits, I. van der Massen, K. Vandamme et al., “In vitro activity of the antiasthmatic drug zafirlukast against the oral pathogens Porphyromonas gingivalis and Streptococcus mutans,” *FEMS Microbiology Letters*, vol. 364, 2017.

[52] R. J. Lamont, H. Koo, and G. Hajishengallis, “The oral microbiota: dynamic communities and host interactions,” *Nature Reviews. Microbiology*, vol. 16, no. 12, pp. 745–759, 2018.

[53] W. H. Bowen, R. A. Burne, H. Wu, and H. Koo, “Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments,” *Trends in Microbiology*, vol. 26, no. 3, pp. 229–242, 2018.

[54] H. Koo, M. L. Falsetta, and M. I. Klein, “The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm,” *Journal of Dental Research*, vol. 92, no. 12, pp. 1065–1073, 2013.

[55] H. Koo, R. N. Allan, R. P. Howlin, P. Stoodley, and L. Hall-Stoodley, “Targeting microbial biofilms: current and prospective therapeutic strategies,” *Nature Reviews. Microbiology*, vol. 15, no. 12, pp. 740–755, 2017.

[56] D. Kim, G. Hwang, Y. Liu et al., “Cranberry flavonoids modulate cariogenic properties of mixed-species biofilm through exopolysaccharides-matrix disruption,” *PLoS One*, vol. 10, no. 12, article e0145844, 2015.

[57] H. Koo, S. Duarte, R. M. Murata et al., “Influence of cranberry proanthocyanidins on formation of biofilms by streptococcus mutants on saliva-coated apatite surface and on dental caries development in vivo,” *Caries Research*, vol. 44, no. 2, pp. 116–126, 2010.

[58] L. Samaranayake and V. H. Matsubara, “Normal oral flora and the oral ecosystem,” *Dental Clinics of North America*, vol. 61, no. 2, pp. 199–215, 2017.

[59] J. M. Tanzer, J. Livingston, and A. M. Thompson, “The microbiology of primary dental caries in humans,” *Journal of Dental Education*, vol. 65, no. 10, pp. 1028–1037, 2001.

[60] L. Guo, J. S. McLean, Y. Yang et al., “Precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 112, no. 24, pp. 7569–7574, 2015.

[61] Y. Ge, P. W. Caufield, G. S. Fisch, and Y. Li, “Streptococcus mutans and streptococcus sanguinis colonization correlated with caries experience in children,” *Caries Research*, vol. 42, no. 6, pp. 444–448, 2008.

[62] R. Sugarman, R. Patel, S. Sharma, D. Plenker, D. Tuveson, and M. W. Saif, “Pharmacokinetics and pharmacodynamics of new drugs for pancreatic cancer,” *Expert Opinion on Drug Metabolism & Toxicology*, vol. 15, no. 7, pp. 541–552, 2019.