Assessment of Bacterial Accumulation on Newly Designed Directly Printed Aligner With a Chamber Containing Fluoride Release: An In Vitro Study

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ABSTRACT

Introduction: Clear aligner therapy creates a confined intraoral environment that may promote bacterial biofilm accumulation and enamel demineralization. Directly 3D-printed aligners offer greater design flexibility than thermoformed appliances, allowing the incorporation of internal structures such as a fluoride-releasing chamber, a feature not previously evaluated as an integrated, rechargeable component of the aligner itself.

Aim: To evaluate fluoride release from a newly designed, directly printed clear aligner containing an internal fluoride-releasing chamber, and to assess its antibacterial effect against Streptococcus mutans and Lactobacillus acidophilus.

Materials and Methods: Twenty clear aligners were fabricated by direct 3D printing and divided into a fluoride group (internal chamber filled with 1.23% sodium fluoride gel) and a non-fluoride control group, each subdivided by thickness (0.75 mm and 1.0 mm; n = 5/subgroup). Fluoride release into artificial saliva was measured over 28 days using a calibrated ion-selective electrode. Antibacterial effect was assessed after a 24-hour dual-species biofilm challenge using colony-forming unit (CFU) counts, crystal violet biomass quantification, and scanning electron microscopy (SEM).

Results: Fluoride release peaked on day one (25.20 ppm for 0.75 mm; 18.41 ppm for 1.0 mm) then declined significantly, remaining detectable for approximately 19 days. Cumulative release did not differ significantly between thicknesses (35.13 ± 4.80 vs 27.96 ± 1.22 ppm·day; p = 0.059). Fluoride-releasing aligners showed significantly lower CFU counts, lower crystal violet absorbance, and near-complete absence of bacterial growth on SEM compared with controls.

Conclusion: A directly printed aligner with an internal, manually rechargeable fluoride chamber provides sustained antibacterial activity against cariogenic bacteria, with a single loading maintaining release for approximately 19 days, comparing favorably with previously reported fluoride-coated aligners.

Keywords: Clear aligner; 3D printing; Fluoride release; Streptococcus mutans; Lactobacillus acidophilus; Antibacterial effect; Biofilm.

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Introduction:

Clear aligner therapy (CAT) has become one of the most important advances in contemporary orthodontics, driven by patient demand for an esthetic, removable alternative to conventional fixed appliances[1]. Despite its clinical advantages, clear aligners create a confined environment between the appliance and the tooth surface that may promote bacterial adhesion and biofilm maturation, and aligner wear has been associated with increased colonization by *Streptococcus mutans* and *Lactobacillus* species[2]. Fluoride remains the cornerstone of caries prevention because of its ability to inhibit bacterial metabolism and enhance enamel remineralization[3], but conventional fluoride delivery through toothpastes and rinses depends heavily on patient compliance.

Bioactive modifications of clear aligners, including fluoride-releasing coatings, have demonstrated antibacterial and remineralizing potential in laboratory studies[4], and dual-function, pH-responsive coatings combining antibacterial and remineralizing activity have also been described[5]. However, such surface-adsorbed coatings are inherently prone to wear and are limited by the fluoride content of a single application, and their in vitro release performance is known to be highly sensitive to specimen- and protocol-related variables[6]. Directly 3D-printed aligners offer substantially greater design flexibility than thermoformed appliances, since printing does not constrain the appliance to a uniform, pre-manufactured thickness[1,7], raising the possibility of embedding a discrete, reloadable internal reservoir rather than a fixed surface coating, an approach that has not previously been evaluated for clear aligners.

Cariogenic biofilm formation on both thermoformed and directly printed aligner materials is well documented, with the extent of accumulation influenced by material composition, surface characteristics, and exposure time[8,9,10]. *Streptococcus mutans* acts as a primary, acidogenic colonizer, while aciduric organisms such as *Lactobacillus* species contribute to the later stages of the cariogenic process[11,12,13]. Sodium fluoride has been shown to reduce acid production and glucan synthesis by *S. mutans* biofilms through interference with bacterial carbohydrate metabolism[14,15]. Given the susceptibility of aligner-wearing patients to bacterial accumulation and the limitations of existing fluoride-releasing coatings, the present study aimed to evaluate fluoride release from a newly designed, directly printed clear aligner containing an internal, rechargeable fluoride-releasing chamber, and to assess the effect of this release on the accumulation of *S. mutans* and *L. acidophilus* on the aligner surface.

Materials and methods

This in vitro experimental study evaluated a newly designed, directly three-dimensionally (3D) printed clear aligner incorporating an internal chamber for sustained fluoride release. Twenty aligners were fabricated using

an LCD-based 3D printer (Anycubic Photon Mono M5s, 12K resolution) and a biocompatible photocurable resin (Tera Harz TA28, Graphy Inc., Seoul, South Korea), with a standardized layer thickness of 50 µm and a 35° build orientation to minimize support contact and layer-line artifacts[1,7]. An internal chamber (6.436 × 2.763 × 3.535 mm) was incorporated into the aligner shell using Direct Aligner Design (DAD) software, positioned to house a fluoride-releasing gel.

Specimens were divided into a fluoride group (Group F; chamber filled with 1.23% sodium fluoride gel, loaded via an insulin syringe) and a non-fluoride control group (Group N), each further subdivided by aligner thickness (0.75 mm and 1.0 mm; five specimens per subgroup, twenty aligners in total). For the antibacterial evaluation, a dual-species biofilm model combining *Streptococcus mutans* (ATCC 25175) and *Lactobacillus acidophilus* (DSM 20079) was used to simulate a cariogenic challenge on the aligner surface[11,12,13]. Standardized inocula were combined in a 1:1 ratio and incubated with aligner segments in Brain Heart Infusion broth supplemented with 1% sucrose for 24 hours at 37°C under microaerophilic conditions. Antibacterial activity was subsequently assessed using colony-forming unit (CFU) counts on chromogenic agar, crystal violet biomass quantification (absorbance at 595 nm), and scanning electron microscopy (SEM), following the general principle that culture-based counts and structural assessments provide complementary information on biofilm status[16].

Fluoride release into artificial saliva was measured over a 28-day period using a potentiometric ion-selective electrode (Metrohm 905 Titrando system with a fluoride ISE, model 6.0502.150), following calibration against standards spanning 0.019–190 ppm mixed with a Total Ionic Strength Adjustment Buffer (TISAB), an approach selected because fluoride output measured in vitro is highly sensitive to specimen surface area, immersion volume, and measurement frequency, which were therefore standardized across all specimens and time points[6]. Data were analyzed using descriptive statistics, the exact Mann–Whitney U test with Holm correction for between-group comparisons at each time point, the Friedman repeated-measures test for temporal change within each group, and comparison of the area under the concentration–time curve from day 1 to day 20 (AUC1–20) between thickness groups, with significance set at $p < 0.05$.

Results :

Fluoride release followed a pronounced early-burst pattern in both thickness groups, with the highest mean concentrations recorded on day one (25.20 ± 6.44 ppm for the 0.75 mm group and 18.41 ± 2.53 ppm for the 1.0 mm group), followed by a rapid, statistically significant decline (Friedman test, $p < 0.001$ for both groups) over the subsequent two weeks (Table 1, Figure 1). Fluoride remained detectable for approximately 19 days before falling below the electrode detection limit. Cumulative fluoride release (AUC1–20) was numerically higher in the 0.75 mm group (35.13 ± 4.80 ppm·day) than in the 1.0 mm group (27.96 ± 1.22 ppm·day), but this difference did not reach statistical significance (exact Mann–Whitney U = 20.0, $p = 0.059$), and no individual day-to-day comparison remained significant after Holm correction.

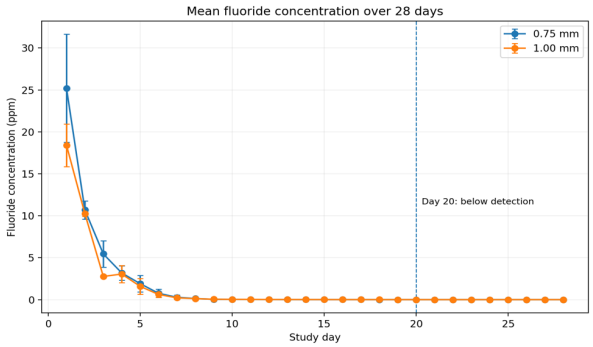

Figure 1: *Mean fluoride concentration (± SD) over 28 days in the 0.75-mm and 1.0-mm thickness groups.*

Table 1: Fluoride concentration (ppm, mean ± SD) by study day and thickness group

Study day	0.75 mm	1.0 mm
Day 1	25.20 ± 6.44	18.41 ± 2.53
Day 3	5.45 ± 1.58	2.76 ± 0.11
Day 7	0.28 ± 0.26	0.22 ± 0.20
Day 14	0.01 ± 0.00	0.01 ± 0.00
Day 19	0.0015 ± 0.0002	0.0020 ± 0.0004
Day 20	0.0000	0.0000
AUC1–20 (ppm·day)	35.13 ± 4.80	27.96 ± 1.22 ($p = 0.059$)

The fluoride-releasing aligners produced a marked and consistent antibacterial effect across all three assays compared with the non-fluoride control (Table 2). Mean CFU counts were approximately 4×10^8 CFU in the control group, compared with 8×10^7 CFU in the 0.75 mm fluoride group and 1.5×10^8 CFU in the 1.0 mm fluoride group (Figure 2). Crystal violet absorbance (OD_{570}) was similarly reduced, from 0.80 in the control group to 0.28 and 0.65 in the 0.75 mm and 1.0 mm fluoride groups, respectively. SEM examination confirmed these findings: the control group showed abundant *Streptococcus mutans* and *Lactobacillus* coverage (Figure 3a), whereas the fluoride groups showed a marked reduction, with near-complete absence of bacterial growth in the 0.75 mm fluoride group (Figure 3b).

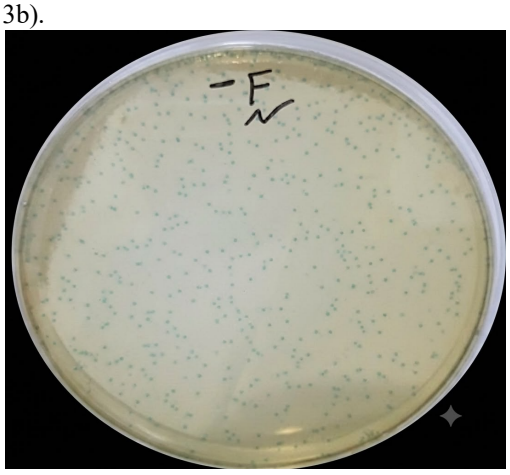

Figure 2: *Colony forming units on a Group N (no-fluoride) agar plate.*

Table 2: Antibacterial outcomes by group (Mean values)

Parameter	Control (Group N)	Fluoride 0.75 mm	Fluoride 1.0 mm
Mean CFU count	~4×10^8	~8×10^7	~1.5×10^8
Crystal violet OD570	0.80	0.28	0.65

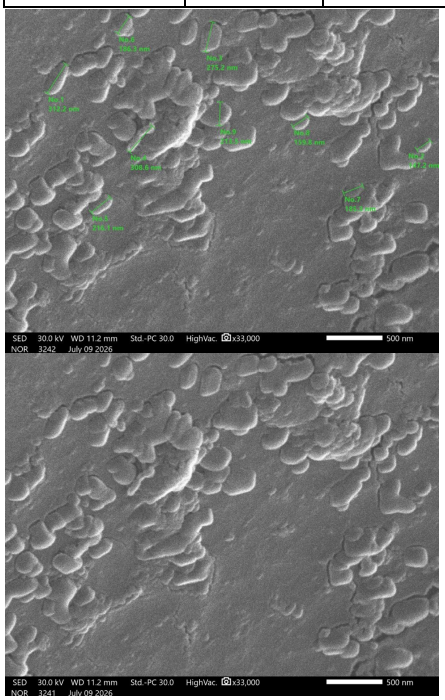

Figure 3: *SEM images of the control group (a, left) and the 0.75 mm fluoride-releasing group (b, right) after 24 hours, showing abundant bacterial coverage in the control versus complete absence of bacterial growth in the fluoride group.*

Discussion :

This study demonstrates that a directly printed clear aligner with an internal fluoride-releasing chamber can provide sustained antibacterial activity against cariogenic bacteria. The early-burst release pattern observed here is consistent with previous reports on fluoride-releasing dental materials: Bayrak et al.[17] described a comparable profile for fissure sealants, and Yan et al.[4] reported a similar pattern for a fluoride-coated clear aligner, with the greatest output recorded during the initial days of immersion. Notably, a single loading of the present chamber sustained detectable fluoride release for approximately 19 days, a duration longer than the 14-day continuous release window reported for the fluoride-coated aligner of Yan et al.[4], suggesting that housing the fluoride source within a discrete internal reservoir can support a longer single-application release period than a thin surface-adsorbed coating, despite the coating's additional passive ion-exchange recharge capacity.

The antibacterial findings are mechanistically consistent with the established action of fluoride on cariogenic bacteria, which interferes with bacterial carbohydrate metabolism and reduces glucan synthesis by *Streptococcus mutans*[14,15]. The marked reduction observed in both *S. mutans* and the more aciduric *Lactobacillus acidophilus*[12,13] suggests that the concentrations released, at least during the early period, were sufficient to suppress both the primary colonizer and the secondary aciduric organism, although this should be interpreted with the caveat that only a single, static 24-hour biofilm challenge was evaluated rather than a dynamic, repeated-exposure model.

An important practical feature of the present design is that, because the gel is housed within an accessible chamber rather than a permanently bonded coating, it can be manually recharged with fresh fluoride gel whenever clinically indicated, a renewability advantage not directly shared with previously reported fluoride-coated[4] or nanoparticle-coated[18,19,20] aligner systems, which rely on passive ion-exchange or a fixed, depletable surface reservoir. No statistically significant difference in cumulative release was found between the two thicknesses tested, which may reflect the modest sample size (five specimens per subgroup) rather than a true absence of a thickness-dependent diffusion effect, since fluoride-release measurements are known to be sensitive to specimen-related factors[6]. Limitations of the present in vitro model, including the use of artificial rather than natural, pellicle-conditioned saliva[21] and the absence of direct enamel remineralization outcome measures[22,23], should be addressed in future research before clinical extrapolation.

Conclusion:

The present study demonstrated that a directly 3D-printed clear aligner incorporating an internal, rechargeable fluoride-releasing chamber provides sustained fluoride release and a significant antibacterial effect against *Streptococcus mutans* and *Lactobacillus acidophilus*. A single gel loading maintained detectable fluoride release for approximately 19 days, comparing favorably with previously reported fluoride-coated aligner systems, while the accessible chamber design allows the protective effect to be renewed through manual recharging. Collectively, these findings support the feasibility of directly printed aligners with internal fluoride chambers as a multifunctional orthodontic appliance capable of combining tooth movement with sustained antibacterial protection.

Clinical Recommendations:

1. Directly printed aligners with internal fluoride-releasing chambers may be considered as an adjunctive antibacterial strategy for orthodontic patients at elevated risk of white spot lesion development, pending further clinical validation.
2. Clear clinical protocols and validated intervals for manually recharging the fluoride chamber should be established before this practice is recommended to patients.
3. Given the greater fluoride release and antibacterial effect observed with the thinner (0.75 mm) aligners, thickness may be an additional design consideration when planning fluoride-releasing appliances.
4. The mechanical properties and orthodontic force delivery of chamber-modified aligners should be verified clinically to confirm that the internal chamber does not compromise treatment efficiency.

Research Recommendations:

1. In Vitro Nature of the Study. Laboratory conditions cannot fully replicate the complex oral environment, including salivary flow, pH fluctuations, and daily aligner insertion and removal.
2. Single Loading Protocol Evaluated. Only a single gel loading was tested; the durability and antibacterial efficacy of the chamber across multiple manual recharge cycles remain to be evaluated.
3. Static Biofilm Model. A single 24-hour static biofilm challenge was used; dynamic, repeated-exposure models would more closely simulate intraoral conditions.
4. Dual-Species Biofilm Only. The polymicrobial complexity of the oral cavity was not fully represented by the two-species model used.
5. No Direct Remineralization or Mechanical Assessment. Enamel remineralization outcomes and the mechanical/force-delivery properties of the modified aligner were not directly evaluated.
6. Small Sample Size per Subgroup. Larger samples are needed to more definitively establish or exclude a thickness-dependent effect on fluoride release.

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