Article

Discovery Medicine 2025; 37(199): 1520–1533
https://doi.org/10.24976/Discov.Med.202537199.132

Enhancing Antibacterial Properties of Flowable Dental Composites With Chlorhexidine: A Balance Between Efficacy and Bonding Strength – An *In Vitro* Study

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Published: 20 August 2025

Background: The incorporation of chlorhexidine (CHX) into dental composite resins (DCR) offers antibacterial benefits but may affect their mechanical and esthetic properties. This *in vitro* study aimed to evaluate the impact of different CHX concentrations (0.12%–3%) on polymerization, hardness, bond strength, shade, and antibacterial activity.

Methods: A flowable composite resin was enriched with CHX at various concentrations. The degree of conversion (DC%) was measured using Fourier-transform infrared spectroscopy. Hardness was assessed with a Shore-D durometer, and shear bond strength (SBS) was tested on human dentin surfaces. Shade variations were evaluated using spectrophotometry. Antibacterial efficacy was determined by measuring inhibition halos against *Streptococcus mutans* (*S. mutans*), *Lactobacillus fermentum* and *Lactobacillus paracasei*. Scanning electron microscopy (SEM) analyzed CHX distribution in the resin matrix.

Results: CHX up to 1.5% did not significantly alter DC%, but the concentration of 3% impaired polymerization ($p = 0.049$ vs. control). Both hardness and SBS decreased significantly compared to the control group from concentrations as low as 0.2% for hardness and 0.12% for SBS ($p < 0.001$ in both cases). Notably, SBS values fell below 5 MPa at a concentration of 1% CHX. Shade modifications exceeded the perceptibility threshold, with CHX-containing composites appearing lighter. SEM images revealed inhomogeneous CHX distribution and microgaps in the resin matrix. Antibacterial tests demonstrated a dose-dependent inhibition of bacterial growth, with significant effects at 0.5%–1% CHX.

Conclusion: While CHX incorporation enhances antibacterial properties, concentrations above 0.5% compromise mechanical properties and esthetics, limiting clinical applicability. Future research should explore strategies to optimize CHX release and maintain mechanical integrity, such as hybrid antibacterial agents or controlled-release systems, to balance antibacterial efficacy with structural stability.

Keywords: dental composite resin; chlorhexidine; antibacterial properties; shear bond strength; dental materials; tooth decay

Introduction

For almost 20 years, dental composite resins (DCR) have become the "gold standard" in conservative dentistry [1]. They are polymer materials used for over 60 years for direct and indirect tooth restoration [2]. These materials are constituted of an organic and an inorganic phase that are linked together by a coupling agent [3,4]. The need to improve their mechanical strength and dimensional stability has led over time to several modifications in their composition [4]. The shape, size, nature and percentage of inorganic fillers can be changed by the addition, for example, of nano-fillers which give the composite resins higher wear resistance [5]. The nature of the organic fraction of the matrix can also be modified as it includes the components of the resin phase and its diluents, a priming system, pigments and polymerization stabilizers [4]. The most frequently found monomers in DCR are bisphenol A-glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), and urethane dimethacrylate (UDMA), which will assemble together during the polymerization process (photopolymerization in daily practice) [6]. The conversion rate (or degree of conversion (DC%)) corresponds to the proportion of monomers assembled into polymers and is directly linked to the mechanical properties of the composite resins. *In vitro*, it can be measured by Fourier-Transform Infrared Spectroscopy (FTIR) [7]. A notable disadvantage of this polymerization process is its shrinkage. Indeed, when as-

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sembling monomers into polymers, covalent bonds bring the particles together and reduce the volume of the material [8,9]. This has a direct consequence on the quality of the restoration, and in particular on its lifespan, as this reduction in volume promotes the formation of micro-hiatus, the degradation of the tooth/composite seal and the occurrence of secondary caries [10].

Thus, even if dental composite resins are aesthetic and conservative for dental tissues, reasons why they have gradually replaced amalgams, they still present many worrying clinical failures, which may come from dimensional variation or instability in a wet environment [11,12]. At the end, they present a much lower lifespan than amalgams [1,13]. In fact, the micro-hiatuses are easily colonized by bacterial plaque containing cariogenic bacteria, such as *Streptococcus mutans* (*S. mutans*) [14] and secondary caries can occur through acid production and demineralization of hard tissues [15,16]. In fact, dental caries is a multifactorial disease, strongly influenced by dietary habits and oral microbiota, especially *Streptococcus mutans*. This cariogenic pathogen is a key target in the development of preventive strategies due to its critical role in biofilm formation and acid production [17]. Recent studies have highlighted the potential of probiotics, such as *Lactobacillus paracasei* (*L. paracasei*), in modulating oral microbiota and inhibiting cariogenic biofilms [18]. Notably, the *L. paracasei* ET-22 strain has been shown to reduce *S. mutans* biofilm formation and extracellular polysaccharide production *in vitro*, as well as prevent dental caries *in vivo* through its ability to regulate the microbial structure of dental plaque [18]. These findings underscore the relevance of selecting *L. paracasei* and as a model for protective oral bacteria and *S. mutans* as a representative cariogenic bacterium for antibacterial evaluations.

When a new decay develops around an existing restoration, it necessitates additional dental intervention, which invariably leads to further loss of healthy tooth structure, compromising the long-term integrity of the tooth [19]. However, limiting polymerization shrinkage does not seem sufficient to reduce the risk of secondary caries, the bacterial factor being the main responsible [20].

The addition of antibacterial compounds in DCR appears to be an interesting approach in order to control the development of secondary caries [21,22]. The destruction of bacteria can be managed by direct contact or by the release of antibacterial compounds. The first mechanism creates a rupture of the bacterial cell membrane through the electrostatic attraction between the resin and the bacteria. The main agent used is a quaternary ammonium compound (QAC), which is incorporated into the resinous matrix, allowing a long-lasting bactericidal and bacteriostatic effect [15]. Thus, the resin would be able to release antibacterial charges into the oral environment, therefore helping to kill bacteria through oxidative stress, growth inhibition, or suppression of genetic material synthesis [23].

Chlorhexidine, which is widely known and used in dentistry in the prevention and treatment of bacterial infections [24], is recognized as a first-line treatment in the fight against plaque and gingivitis [25], provided it is always combined with good oral hygiene habits [26]. By disrupting bacterial walls [20], chlorhexidine effectively inhibits bacteria, such as *Streptococcus mutans*, *Porphyromonas gingivalis* or *Staphylococcus aureus* [24]. This would therefore be interesting in the fight against secondary caries.

Thus, the aim of this study was to develop and characterize a dental composite resin with antibacterial activity brought by chlorhexidine. The null hypothesis for this work is that the incorporation of chlorhexidine particles into a composite resin before light-curing does not change its conversion rate, nor its mechanical strength. The shade and the antibacterial activity of the newly designed resins are also evaluated in this study.

Materials and Methods

Samples Conception

Chlorhexidine powder (ThermoFisher Scientific, Waltham, MA, USA) was added in various mass concentrations (0.12%, 0.2%, 1%, 1.5%, 2% and 3%) by mechanical stirring to an existing flowable composite resin (GrandioSO Light Flow, A3 Shade, VOCO, Cuxhaven, Germany) composed of (in descending order according to content) barium aluminium borosilicate glass, silicon dioxide, 2-hydroxyethyl dimethacrylate (HEDMA), Bis-GMA, tricyclodecane dimethanol dimethacrylate (TCDDMA), fumed silica, TEGDMA, ethoxylated bisphenol A dimethacrylate (Bis-EMA) initiators, stabilisers and pigments. More precisely, 1 g pellets were prepared by slow mixing, for example, for the 1% concentration, 0.01 g of chlorhexidine with 0.99 g of resin. The mixture was prepared with the uncured resin and the photopolymerization process was initiated immediately after the mechanical stirring, to avoid any sedimentation, with a light emitting diode lamp during 20 s at a power of 1200 mW/cm^2 (D-Light Pro, GC Europe, Leuven, Belgium). Control samples were made of the flowable DCR alone.

Degree of Conversion (DC%)

The degree of conversion of the composite resin was analyzed one day after the light-curing procedure to take into account the post-polymerization that could have occurred after the experiments. According to our previously published protocols [27], 0.005 g of each sample was isolated, manually crushed and mixed with 0.4 g of potassium bromide (≥99%, Sigma-Aldrich, St. Louis, MO, USA) to design pellets under a press. The degree of conversion among the specimens was calculated using FTIR (SpectrumOne, Perkin Elmer, Waltham, MA, USA) configured via an absorbance mode for measurements between wavelengths of 400 cm^{-1} and 4000 cm^{-1} (n = 4 for each group of

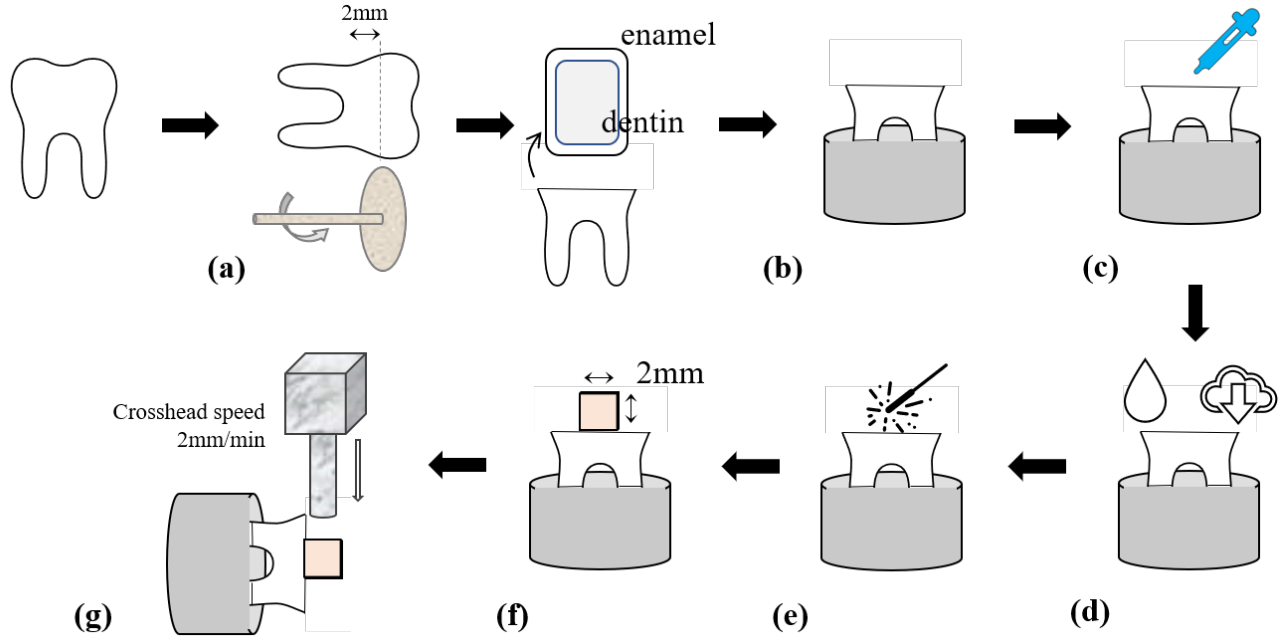

Fig. 1. Protocol applied for shear bond strength testing. (a) The teeth were cut to expose dentin surfaces and (b) then included in hard plaster cylinders. (c) Dentin etching was performed, followed by (d) water cleaning and slight drying. (e) The adhesive was applied and light-cured, (f) as well as the 2 mm side piece of dental composite resin. Finally, (g) the shear bond test was performed at a crosshead speed of 2 mm/min. The figure was created using Microsoft PowerPoint (Microsoft 365, Microsoft Corporation, Redmond, WA, USA).

composite resin). Background spectra were launched prior to running samples through an empty mold. The pellets were then placed inside the FTIR chamber and cured spectra were collected. The conversion degree was calculated as the change in the ratios between the intensities of the absorbance peaks corresponding to the aliphatic (1638 cm^{-1}) and aromatic (1608 cm^{-1}) C=C bonds, which was always in relation to the unpolymerized material. Precisely, the following equation was applied to calculate the DC% [28]:

$$DC\% = \left(1 - \frac{\left(\frac{1638cm^{-1}}{1608cm^{-1}}\right)\ \text{cured}}{\left(\frac{1638cm^{-1}}{1608cm^{-1}}\right)\ \text{uncured}}\right) \times 100$$

Please note that the polymerization being very complicated for the 3%-chlorhexidine material. Thus, this condition was not used beyond the DC% measurement experiments.

Hardness

The hardness of the samples was assessed using a Shore-D durometer (TEKCOPLUS Ltd., Hong Kong, China). One day after polymerization of the pellets (1 cm-diameter, 2 mm-thickness), a single trained operator (AP) carried out indentation measurements in triplicate per sample (n = 5). As Shore-D hardness is relative between samples evaluated under the same conditions, all measurements were carried out during the same operating time.

Shear Bond Strength

Sixty healthy human molars, extracted for orthodontic, periodontal or wisdom tooth reasons, have been collected in Toulouse Hospital (Service d'Odontologie, Hôpitaux de Toulouse, Toulouse, France) in strict compliance with the rules for collecting surgical waste as laid down and recorded by our hospital. Indeed, in accordance with international regulations since the Declaration of Helsinki, this tissue bank has been registered since 2022 by the department of Research and Innovation of the French Ministry of Higher Education, Research and Innovation (Bioethics Unit) under the acronym DENTABOUCHE (n°DC-2022-5010) in order to respect the traceability of surgical waste and the non-opposition of patients to entrust it to a tissue bank. Before the beginning of the study, the teeth were conserved in a 1% chloramine solution in order to decontaminate them as well as maintain their hydration and their integrity [29]. The teeth used were stored for a maximum of one month after collection. To be suitable for use in the study, the teeth had to present a healthy crown and root—i.e., those who presented a decay, a restoration of any type, a crack or a fracture were excluded.

The whole protocol applied for shear bond strength (SBS) testing is presented in Fig. 1. Precisely, the crowns of the teeth were sectioned perpendicularly to their longitudinal axis, 2 mm above the cement-enamel junction, with a low-speed diamond disc under irrigation (IsoMet 2000, Buehler, Leinfelden-Echterdingen, Germany). This cut al-

lowed for the exposure of a healthy surface of dentin on which the bonding procedure could be performed. The roots of each tooth were then included in hard plaster cylinders to prepare for the future shear bond strength tests.

For all the teeth, the dentin was etched for 15 s with 35%-orthophosphoric acid (Vococid, VOCO, Cuxhaven, Germany), rinsed and slightly dried. A universal adhesive used on a 2-step etch-and-rinse mode was then applied on the dentin (FuturaBond U, VOCO, Cuxhaven, Germany) and photopolymerized for 10 s at a power of 1200 mW/cm^2 (D-Light Pro, GC Europe, Leuven, Belgium).

The 60 prepared teeth were randomly assigned to one of the six groups (n = 10), characterized individually by a different composite resin bonded on the surface: conventional flowable light-curing biomaterial alone (control) or chlorhexidine-enriched DCR at mass concentrations of 0.12, 0.2, 1, 1.5 or 2%. Each cylinder was positioned into a specific support on which was fixed a plastic mold centrally perforated and placed in contact with dentin to receive a calibrated resin pin. The flowable composite resin was thus injected in the mold and light-cured for 20 s at a power of 1200 mW/cm^2 (D-Light Pro, GC Europe, Leuven, Belgium). After mold removal, the samples could be placed horizontally on the platform of a universal testing machine (UltraTester Bond Strength Testing Machine, Ultradent, South Jordan, UT, USA) whose indented tip was positioned in contact with the resin pad. The SBS test was carried out at a speed of 2 mm/min until the restoration was separated from the tooth and the resistance value was registered (in MPa).

Shade Evaluation

To evaluate the shade of each resin, the colorimetric parameters of the pellets prepared for hardness evaluation were evaluated in the CIELab colorimetric space, before hardness measurements, with an electronic spectrophotometer (EFI ES-1000 UVcut i1 Eye-One Pro Spectrophotometer, X-Rite Europe, Regensdorf, Switzerland). The device gave a L*a*b* score that contained information concerning luminosity (L*), positioning on the green-red axis (a*) and positioning on the blue-yellow axis (b*). A mean value for each of the three values was determined in each group, then a final ΔE score was calculated using the following formula:

$$\Delta E = \sqrt{(L_c^* - L_m^*)^2 + (a_c^* - a_m^*)^2 + (b_c^* - b_m^*)^2}$$

(where "c" indicates the values obtained in the control group and "m" the values obtained in the mixed materials (resin + chlorhexidine) groups).

Scanning Electron Microscopy

Composite resin samples with or without chlorhexidine were sputter-coated with platinum (Leica EM MED020, Leica Microsystems GmbH, Wetzlar, Germany) and examined by scanning electron microscopy (FEI Quanta FEG 250, ThermoFisher Scientific, Waltham, MA, USA) to determine the incorporation of chlorhexidine into the resin. Representative scanning electron microscopy (SEM) images of each study group were captured at magnifications of 50, 200, 1000 and 3000.

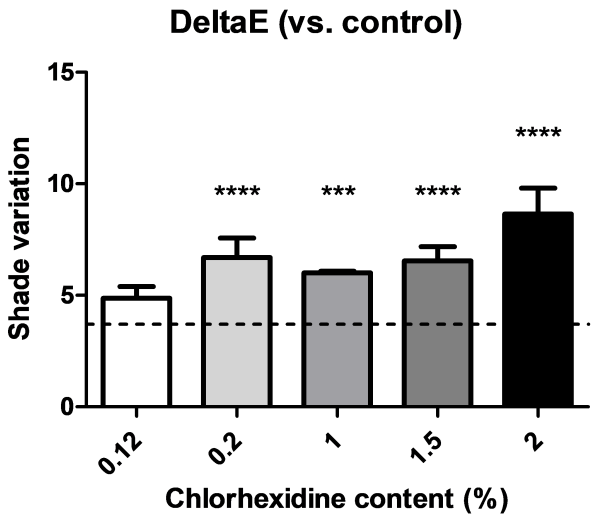

Fig. 2. Diagram showing shade differences according to chlorhexidine concentrations in flowable composite resins (n = 5). The unmodified biomaterial is not shown, since it is used as a reference for calculating the ΔE score. The dotted line indicates the threshold of 3.7 for visual perception of a shade difference and the statistical analyses refer to the difference with control (*** is for $p < 0.001$ and **** is for $p < 0.0001$).

Antibacterial Properties

The three oral bacteria (*Streptococcus mutans*, *Lactobacillus fermentum* (*L. fermentum*) and *Lactobacillus paracasei*) were chosen to carry out the antibacterial tests and obtained from the Leibniz Institute DSMZ microbial collection (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH). *S. mutans* was selected due to its well-documented role as a cariogenic pathogen, known for its acidogenicity and biofilm formation, which are critical factors in the development of dental caries [30]. *L. paracasei*, although generally considered a beneficial bacterium, was included because of its ability to persist in biofilms and its potential involvement in oral dysbiosis under certain conditions, making it a relevant model for testing antibacterial properties [31]. *L. fermentum* is considered one of the dominant Lactobacillus species in carious lesions, contributing to the progression of dental caries [32].

Tests were carried out after obtaining results on the materials' mechanical properties. Thus, for *Streptococ-*

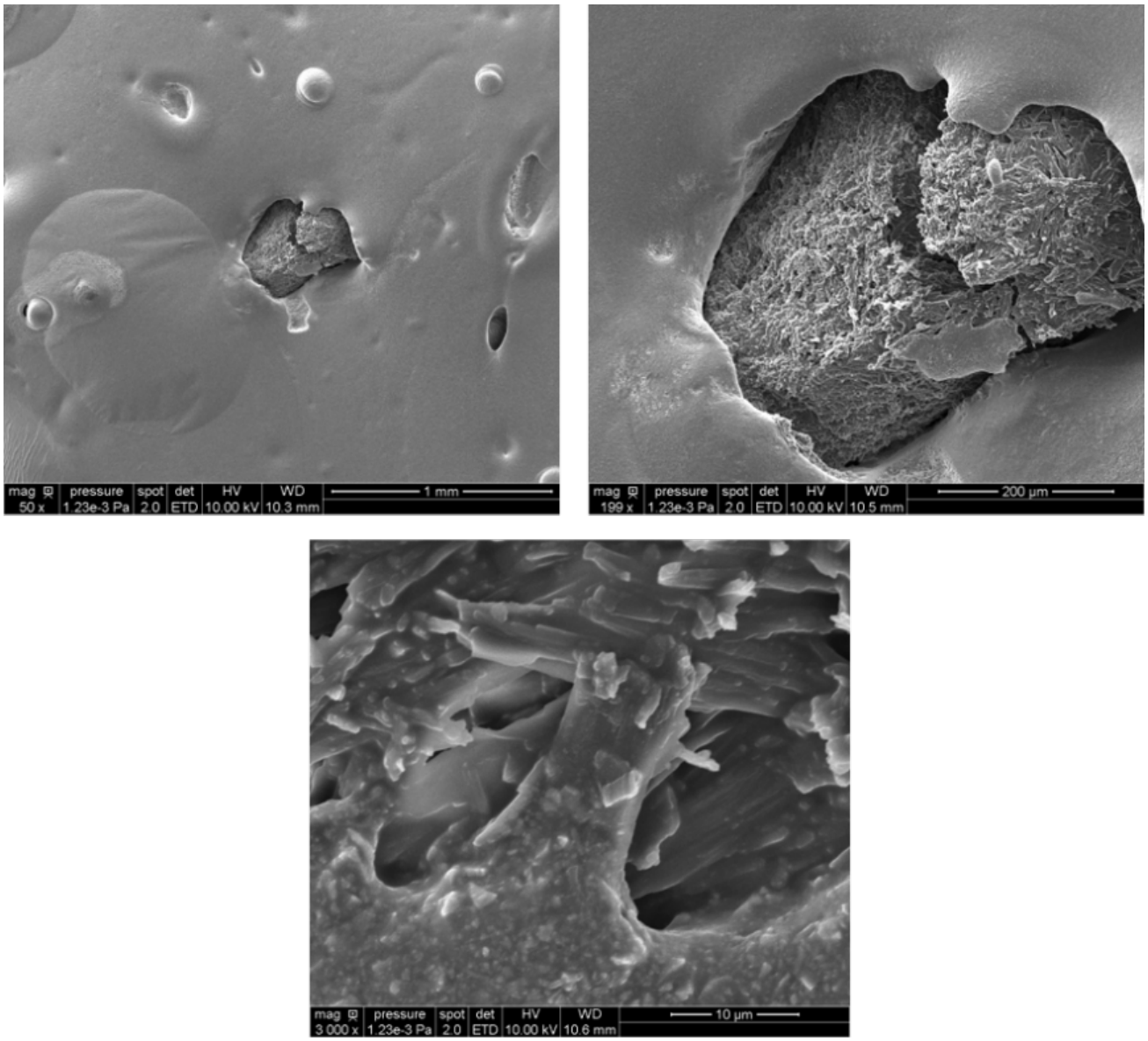

Fig. 3. Scanning electron microscopy (SEM) images of a 1% chlorhexidine sample.

cus mutans and *Lactobacillus fermentum*, only three composite resin conditions were evaluated: 0%, 0.2% and 0.5% chlorhexidine. More concentrations were chosen for *Lactobacillus paracasei* (0%, 0.12%, 0.2%, 1% and 2% chlorhexidine) due to its different implication in cariogenic biofilm. After inoculation of the petri dish, composite resin disks of approximately 6 mm diameter and 50 mg were placed in the center of the dish, with different concentrations of chlorhexidine. The size of the growth inhibition halos was measured after 5 days with the Mesurim 2 software (Institut Français de l'Education, Lyon, France). To validate the antibacterial activity, a control culture using a dental composite resin without chlorhexidine was performed to evaluate if the biomaterial itself does not exhibit intrinsic antibacterial properties.

Statistical Analysis

Results for DC%, hardness, shear bond strength, shade evaluation and antibacterial halos all consisted of quantitative variables. They were thus presented as mean $\pm$ standard deviation. After verification of data normal distribution and variance equality (respectively with Shapiro-Wilk and Levene's robust tests), we chose to compare the groups (of chlorhexidine content) with non-parametric Kruskal-Wallis tests and Mann-Whitney tests for pair analyses. All the tests were launched on Stata v.13.0 (StataCorp, College Station, TX, USA) and Prism 5 (GraphPad, San Diego, CA, USA). The level of significance was set at a p-value < 0.05.

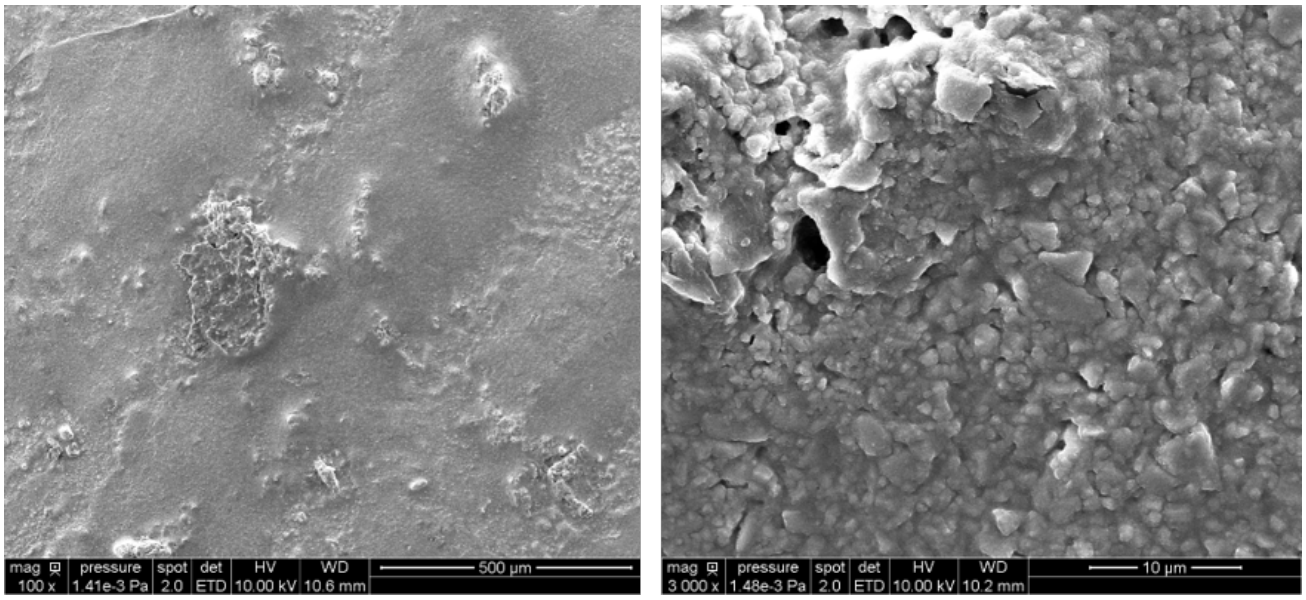

Fig. 4. SEM images of a 0.2% chlorhexidine sample.

Table 1. Conversion rate of chlorhexidine-enriched dental composite resins (n = 4).

Chlorhexidine content	Control (0%)	0.12%	0.2%	1%	1.5%	2%	3%	*p*
DC (%)	33.88 ± 4.4 ^{a}	29.99 ± 9.05	30.28 ± 10.07	39.24 ± 7.19	35.4 ± 12.39 ^{b,c}	22.42 ± 8.43 ^{b}	20.76 ± 9.8 ^{a,c}	0.11

DC%, degree of conversion. Similar letters indicate a statistically significant difference between the two groups concerned.

Results

Degree of Conversion

The degree of conversion of chlorhexidine-enriched composite resins did not vary from the control for concentrations up to and including 2% (Table 1). Above this level, a significant reduction in conversion rate was observed ($p = 0.0493$ versus control). The very poor polymerization of certain samples containing 3% chlorhexidine led us not to use this condition for the other tests undertaken in the course of this work.

Hardness

Consistently, the results of hardness followed the same trend as those of DC% (Table 2). Here, only the 0.12% chlorhexidine concentration conferred a hardness similar to that of the unmodified control resin ($p = 0.59$). Beyond that, the reduction in hardness was significant for all the modified materials.

Shear Bond Strength

The addition of chlorhexidine reduced in all conditions the ability of resins to be bonded on dentin surfaces (Table 3). From a concentration of 0.2% of chlorhexidine, shear bond strength felt below 10 MPa ($p < 0.0001$ versus control) and even dropped below the symbolic value of 5 MPa for concentrations of 1% or more.

Shade Evaluation

The addition of chlorhexidine modified the shade of composite resins (Fig. 2). For all groups, the change in color parameters was above the visual detection limit of 3.7 [33,34]. Biomaterials appeared lighter after the addition of chlorhexidine, which will necessitate the design of a specific shade guide for these DCR, different from the chlorhexidine-free starting material, in the event of further development.

Scanning Electron Microscopy

Scanning electron microscopy images showed inhomogeneous incorporation of chlorhexidine particles into the resin (Figs. 3,4). The observations highlighted a close promiscuity between resin and chlorhexidine, with nevertheless the presence of a small space of a few microns between the two structures (Fig. 3). Apart from these small gaps which could represent preferred fracture sites during mechanical testing, the external surface of the resin did not appear to be altered.

Antibacterial Properties

The measure of inhibition halos after 5 days of incubation revealed a dose-dependent antibacterial effect, logically linked to chlorhexidine concentrations. The three tested bacteria, *L. paracasei*, *L. fermentum* and *S. mutans*, exhibited distinct sensitivities, reflecting their differential

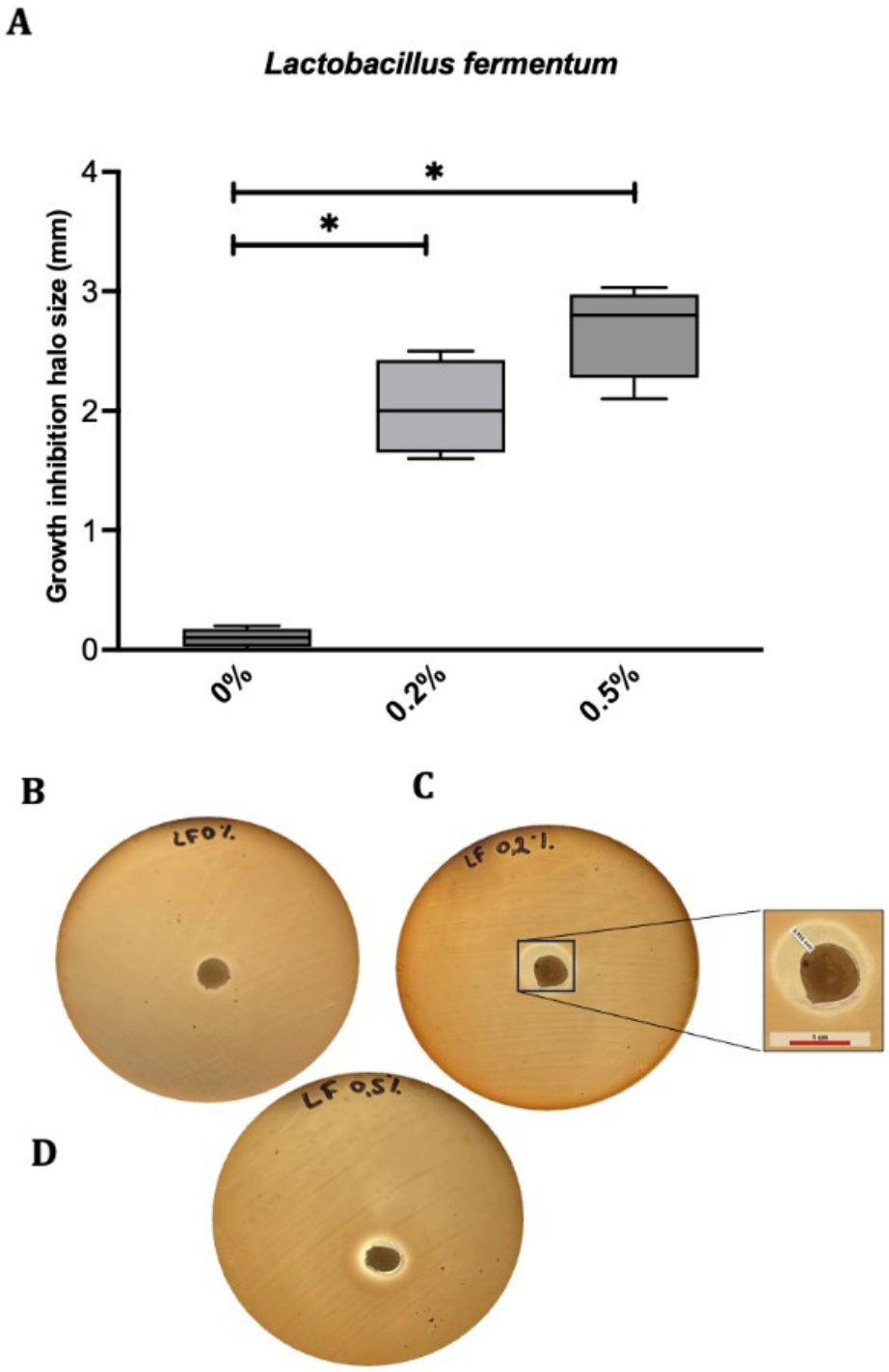

Fig. 5. Antibacterial effects on *Lactobacillus fermentum*. (A) Diagram showing the sizes of the inhibition halos of the different composite resins on *Lactobacillus fermentum*, at 0%, 0.2% and 0.5% chlorhexidine (n = 4). A * symbol indicates a statistically significant difference between groups ($p < 0.05$). (B–D) Photographs of *Lactobacillus fermentum* petri dishes after 5 days of incubation in the presence of composite resin with or without chlorhexidine, at different concentrations: 0% (B), 0.2% (C), 0.5% (D).

biological responses to the antibacterial agent. For *L. fermentum* (Fig. 5), chlorhexidine inhibited bacterial growth in comparison with the chlorhexidine (CHX)-free material ($p = 0.029$ and 0.028, respectively, for 0.2 and 0.5% conditions).

For *L. paracasei* (Fig. 6), chlorhexidine concentrations of 0.12% and 0.2% produced comparable inhibition halos, suggesting limited antibacterial efficacy at these low doses. A significant increase was observed at 1% ($p = 0.0294$), indicating heightened sensitivity to this concentra-

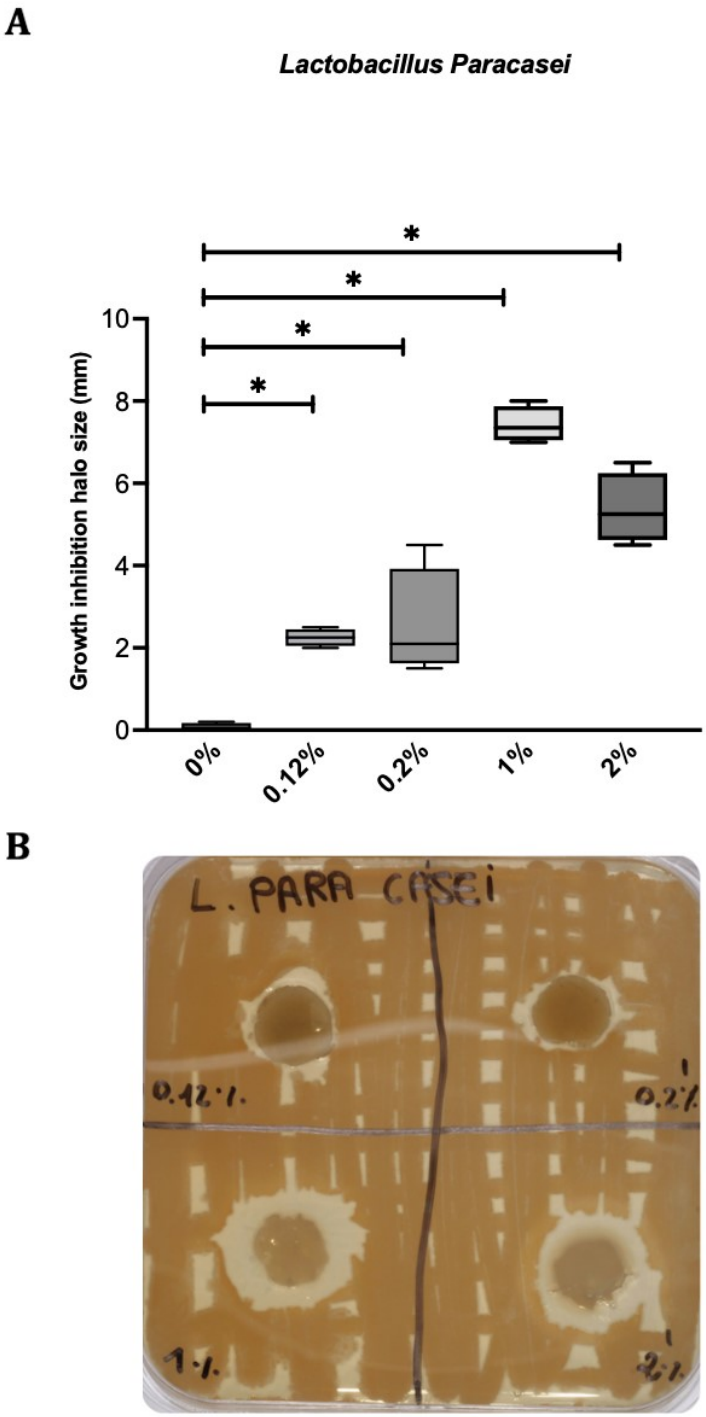

Fig. 6. Antibacterial effects on *Lactobacillus paracasei*. (A) Diagram showing the sizes of the inhibition halos of the different composite resins on *Lactobacillus paracasei*, at 0%, 0.12%, 0.2%, 1% and 2% chlorhexidine (n = 4). The * symbols indicate a statistically significant difference between groups ($p < 0.05$). (B) Photographs of *L. paracasei* petri dishes after 5 days of incubation in the presence of composite resin with or without chlorhexidine, at different concentrations, 0.12%, 0.2%, 1% and 2% chlorhexidine.

tion. Moreover, at lower concentrations of chlorhexidine, the inhibition zone diameters exhibited higher variability among replicates, possibly due to threshold effects near the minimum inhibitory concentration.

A significant inhibition halo size was observed for *S. mutans* (Fig. 7) at 0.2% and 0.5% CHX concentration compared to 0% CHX ($p < 0.05$).

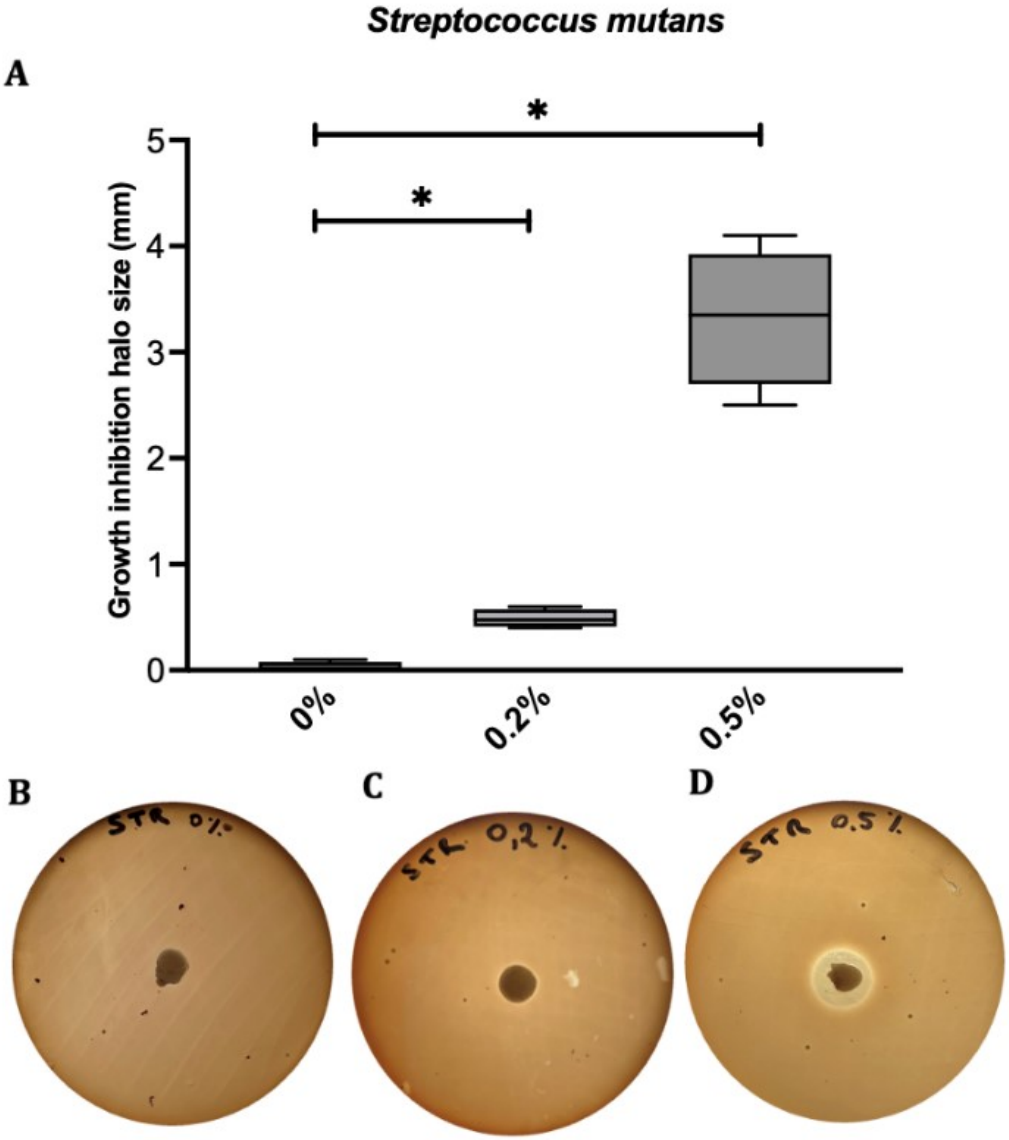

Fig. 7. Antibacterial effects on *Streptococcus mutans*. (A) Diagram showing the sizes of the inhibition halos of the different composite resins on *Streptococcus mutans*, at 0%, 0.2% and 0.5% chlorhexidine (n = 4). A * symbol indicates a statistically significant difference between groups ($p < 0.05$). (B–D) Photographs of *Streptococcus mutans* petri dishes after 5 days of incubation in the presence of composite resin with or without chlorhexidine, at different concentrations 0% (B), 0.2% (C), 0.5% (D).

Our results confirm the dose-dependent antibacterial efficacy of chlorhexidine in composite resins. While lower concentrations of chlorhexidine (0.12%–0.2%) showed limited inhibition, a significant effect was observed at 0.5% and higher. *L. paracasei* responded at 0.12% with stronger inhibition at 1%, whereas *L. fermentum* was only tested at 0.2% and showed measurable inhibition at this concentration.

Discussion

The integration of antibacterial agents into dental composite resins aims to enhance their protective effects against secondary caries. This study investigated the effects of different chlorhexidine concentrations on the polymerization, mechanical properties, and antibacterial activity of a flowable composite resin. Its findings highlighted the potential of chlorhexidine-enriched resins for antibacterial applications, warranting further studies on their clinical relevance and mechanical stability.

Table 2. Hardness values for flowable dental composite resins, obtained with Shore-D durometer (n = 5).

Chlorhexidine content	Control (0%)	0.12%	0.2%	1%	1.5%	2%	*p*
Shore-D durometer	88.83 ± 1.04 ^{a,b,c,d}	88.30 ± 0.45 ^{e,f,g,h}	83.90 ± 0.96 ^{a,e,i,j,k}	80.90 ± 0.42 ^{b,f,i,l,m}	78.80 ± 0.76 ^{c,g,j,l}	74.3 ± 0.57 ^{d,h,k,m}	0.0001

Similar letters indicate a statistically significant difference between the two groups concerned.

Table 3. Resistance to shear bond stress (n = 10).

Chlorhexidine content	Control (0%)	0.12%	0.2%	1%	1.5%	2%	*p*
Shear bond strength (MPa)	22.1 ± 2.52 ^{a,b,c,d,e}	16.75 ± 2.75 ^{a,f,g,h,i}	8.2 ± 0.95 ^{b,f,j,k,l}	0.82 ± 0.32 ^{c,g,j}	1.78 ± 1.9 ^{d,h,k}	0.44 ± 0.17 ^{e,i,l}	<0.0001

Similar letters indicate a statistically significant difference between the two groups concerned.

The degree of conversion remained stable for chlorhexidine concentrations up to 2%, with a significant decline beyond this threshold. The poor polymerization observed at 3% chlorhexidine led us to exclude this concentration from further testing. These findings align with previous studies showing that excessive incorporation of antibacterial agents can disrupt polymer network formation, reducing material stability [35]. Similarly, hardness values followed the same trend as DC%, with significant reductions at concentrations above 0.12%. Shear bond strength was also negatively affected by chlorhexidine incorporation, dropping below 10 MPa from 0.2% and reaching critically low levels (<5 MPa) at 1% and higher. These results are consistent with previous research indicating that antimicrobial additives can interfere with resin-dentin bonding, potentially compromising long-term restoration durability. In terms of esthetics, the addition of chlorhexidine modified the shade of composite resins, with color variations exceeding the perceptibility threshold. Biomaterials appeared lighter, which suggests that future developments should include a dedicated shade guide to accommodate these differences. This is particularly interesting in our case, with the DCR we chose to use, because the resin became whiter and not brown, as previous studies have already reported in the past [16]. Furthermore, scanning electron microscopy revealed an inhomogeneous incorporation of chlorhexidine particles into the resin. The encapsulation of chlorhexidine within the polymer matrix without tight bonds suggests the potential formation of microgaps, which could serve as preferred fracture sites. However, no significant surface alteration of the resin was observed, indicating that these structural changes might not critically affect the overall integrity of the material.

The antibacterial tests demonstrated a dose-dependent inhibition of bacterial growth, confirming the efficacy of chlorhexidine as an antiseptic. However, the sensitivity varied among the tested bacteria. *Lactobacillus fermentum* was inhibited by 0.2%, although variability in inhibition halos was observed at this concentration. A more consistent effect was achieved at 0.5%. *Lactobacillus paracasei* exhibited limited inhibition at 0.12% and 0.2%, with a significant increase at 1%, suggesting heightened sensitivity at higher concentrations. *Streptococcus mutans* showed minimal inhibition at 0.2%, with a substantial increase at concentrations above 0.5%.

This result may appear contradictory to the probiotic role often attributed to *Lactobacillus paracasei*. However, recent evidence suggests that the behavior of *L. paracasei* is strain-specific and context-dependent. Several strains, such as *L. paracasei* ET-22 and *L. paracasei* L9, have demonstrated protective effects against dental caries by inhibiting *S. mutans* biofilm formation and modulating oral microbiota *in vivo* [18,31]. Conversely, other studies have reported that *L. paracasei* and related Lactobacillus species are frequently isolated from deep dentinal lesions and may contribute to caries progression due to their acidogenic and aciduric properties [32,36]. Therefore, the inhibition of *L. paracasei* is not inherently harmful and may even be beneficial in dysbiotic or high-risk carious environments. Our study supports this nuanced interpretation, emphasizing the need to consider strain-level differences and ecological context when evaluating the antibacterial properties of dental materials.

These results highlighted the necessity of optimizing chlorhexidine concentration to achieve effective antibacterial action without compromising mechanical properties. Lower concentrations (≤0.2%) may be insufficient to control cariogenic bacteria such as *S. mutans*, whereas higher concentrations (>1%) pose risks of mechanical degradation. The 1% concentration was not tested for *L. fermentum* and *S. mutans* due to preliminary data suggesting limited additional efficacy beyond 0.5%, combined with potential concerns about compromising mechanical properties. Testing this concentration could have provided additional insights

The choice of antibacterial agents plays a crucial role in determining the balance between antibacterial efficiency and mechanical performance. Various alternative strategies have been explored, including quaternary ammonium compounds (QACs) [37], antimicrobial peptides [38], and

nanoparticles [20]. QAC-based resins have shown effectiveness against *S. mutans* but rely on direct contact, limiting their antibacterial diffusion. These compounds kill bacteria only upon direct contact with the resin surface, leaving surrounding bacteria unaffected, which reduces their overall efficiency. Antimicrobial peptides exhibit promising bactericidal properties, but their degradation by oral enzymes limits their durability. Nanoparticles such as silver, zinc oxide, or magnesium oxide have demonstrated strong antibacterial activity with minimal impact on mechanical properties at low concentrations [23,39]. Silver nanoparticles, also proposed in dentistry for antibacterial biomaterials used in implantology and periodontics [40], offer broad-spectrum antibacterial effects against Gram-positive and Gram-negative bacteria, fungi, and viruses [41]. However, concerns about biocompatibility remain, as silver nanoparticles can cross biological barriers and negatively impact mitochondrial function [42,43]. The size of these particles plays a crucial role in biofilm disruption and cytotoxicity, as smaller particles tend to be more toxic [44]. Fluoride nanoparticles incorporated into bioactive glasses have also demonstrated antibacterial properties by releasing alkaline species and promoting the formation of a mineralized layer over time [45].

Chlorhexidine represents a well-balanced alternative, offering broad-spectrum antibacterial activity with relatively low toxicity at controlled doses. However, its rapid release and elution in saliva may reduce long-term efficacy [24]. Further tests need to be carried out over the long term. The characterization of the biomaterial in our article focused on its properties immediately after its design, but an ageing study with observation of the evolution of its porosity and mechanical properties could constitute interesting avenues. In adhesive dentistry, chlorhexidine has been tested in different stages of the bonding protocol, such as its incorporation into the etching or adhesive system, showing promising *in vitro* results [46]. However, clinical studies have not yet confirmed that chlorhexidine improves the prognosis of bonded restorations [47]. Furthermore, previous *in vitro* experiments have shown that chlorhexidine, when combined with active calcium phosphate, can exhibit remineralizing effects by promoting hydroxyapatite precipitation [48,49]. Exploring these properties within composite resins could offer a dual benefit by limiting secondary caries while reinforcing the mineral structure of dentin or enamel.

One of the challenges associated with the incorporation of antibacterial agents into DCR is the potential degradation of mechanical properties over time. The resins may undergo mechanical modifications when stored in an aqueous environment, leading to a loss of strength and stability [50]. This is particularly relevant given the long-term exposure of dental materials to oral fluids. Additionally, although the incorporation of nanoparticles has shown promising antibacterial effects, their non-uniform distribution within the resin matrix could adversely affect flexural strength. However, some authors suggest that low concentrations of silver, zinc oxide, or magnesium oxide nanoparticles may not only preserve mechanical properties but even enhance them under certain conditions [51,52]. While our findings support the potential of chlorhexidine-enriched composites, further studies are required to address several limitations, for example, the durability of antibacterial effects over extended periods. Future research should investigate slow-release formulations to enhance sustained antibacterial action. Strategies such as bioactive fillers [53], surface modifications, or hybrid antibacterial agents (e.g., chlorhexidine combined with QACs or nanoparticles) could help maintain mechanical properties while preserving antibacterial activity [23,39,42,54]. Furthermore, the question of biocompatibility remains a crucial consideration. While chlorhexidine has been widely used in dentistry with an established safety profile, its long-term effects when incorporated into restorative materials require further investigation [13,24]. Similarly, alternative antibacterial agents such as silver nanoparticles, while effective, require careful assessment of potential cytotoxic effects, particularly given their ability to penetrate biological tissues.

Overall, this study highlights the complex interplay between antibacterial efficacy and mechanical integrity in composite resins. Chlorhexidine at moderate concentrations (0.5%–1%) appears to offer a reasonable compromise, but further optimization is needed to enhance its long-term benefits in restorative dentistry. Future research should focus on improving the stability of these formulations, exploring alternative antibacterial strategies, and validating their clinical effectiveness to develop next-generation antibacterial composites capable of improving restoration longevity and patient outcomes. Furthermore, while this study did not evaluate the specific local release concentration of chlorhexidine under high-flow conditions mimicking the oral environment, future research could employ methods such as applying a gradient of chlorhexidine solutions to filter paper disks matching the material sections to better determine the release kinetics and effective antibacterial levels of these mixed materials with varying incorporation percentages. The limited solubility of chlorhexidine in water raises the concern that contact with oral moisture could lead to larger pores within or at the edges of the material, potentially reducing mechanical strength and increasing the risk of microleakage; while our protocol ensured thorough mixing and limited the chlorhexidine concentration to help maintain structural integrity, future work will include porosity assessment and microleakage analysis to address these concerns in more detail.

Conclusion

This study highlighted the challenge of balancing antibacterial efficacy and mechanical integrity in CHX-

enriched dental composites. While concentrations above 0.5% effectively inhibit cariogenic bacteria, they compromise polymerization, hardness, and bond strength. Additionally, the inhomogeneous distribution of CHX particles observed in SEM analysis suggests potential weak points in the resin matrix. The esthetic impact, with visible color changes, also raises concerns for clinical applications. To optimize these materials for clinical use, future research should explore slow-release formulations, hybrid antibacterial agents, and reinforcement strategies to maintain structural stability while providing sustained antibacterial effects. Moreover, long-term *in vivo* studies are needed to assess the durability, biocompatibility, and overall clinical performance of CHX-enriched composite resins.

Availability of Data and Materials

Data are available upon reasonable request addressed to the corresponding author.

Author Contributions

AP, AM, PL and DY contributed to data acquisition. MM and CM contributed to the analysis and interpretation of data. TC and VBB contributed to the conception and design of the study and the manuscript draft. All authors have read and agreed to the final version of the manuscript. All authors have been involved in the drafting and critical revision of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted thanks to the authorization of the French Ministry of Research, Cellule de Bioéthique (DC-2022-5012), for DENTABOUCHE tissue collection and use for scientific purposes. All procedures involving human samples were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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