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Investigation of Antibacterial Properties during Hardening of Dental Cements

Xiyuan Jiang

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Biology Education Centre and Biology Education Centre and Department of Engineering Sciences,
Uppsala University, Uppsala University

Supervisor: Håkan Engqvist

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Summary

Secondary caries is a major cause of revision and replacements of dental materials. There are several species of bacteria which are involved in the formation of secondary caries, where *Streptococcus mutans* is one of the most frequent. Thus, it is quite practical to evaluate the effect on antibacterial activity of dental cements using *Streptococcus mutans*. In this investigation, six different dental cements were evaluated: RelyX™ Unicem self-adhesive universal resin cement, Ketac™ Cem Aplicap™ glass ionomer luting cement, Harvard zinc phosphate cement, Ceramir® Crown & Bridge, Calcium Aluminate and Glass Ionomer cement. Agar Diffusion Test (ADT) and Resazurin Test were used in this study to evaluate the degree of antibacterial properties of the dental cements.

Compared with the control group, the Calcium Aluminate and Ceramir cements demonstrated strongest antibacterial properties in a long period time with the resazurin test ($P \leq 0.05$), while the Glass Ionomer Cement exhibited no antibacterial properties. Zinc phosphate and RelyX Unicem show little antibacterial properties. In the agar diffusion test, no antibacterial activity was observed for any of the tested cements, and resazurin test may be a more suitable test than agar diffusion test to evaluate antibacterial properties of dental cements.

The antibacterial properties of dental materials may be attributed to the pH during setting and after maturation, and release of ions, such as fluoride ions. During this study, antibacterial effects of different pH and different concentration of fluoride were also investigated. The changing of pH value during and after setting of dental cements and the release of fluoride ions from cements contribute to the antibacterial properties, and these effects are used to try to explain antibacterial properties of each dental material.

1. Introduction

Prosthetic dentistry is the dental specialty including restoration or replacement of teeth, such as crowns, bridges and dentures. Due to the rapidly growing amount of elderly people in the world, prosthetic dentistry is increasing in importance. Thus dental cements are widely used in dental and orthodontic applications to cement materials and obtain a functional unit in the mouth. For ideal dental cements, several properties are desired, such as low solubility, low viscosity, high bond strength to various dental materials, high compressive and tensile strength, anticariogenic activity, etc (1). The present dental cements originate from resins, hybrids and the ceramic classes of materials. One main design difficulty with dental cements is their environment as a bond between two rigid materials or one prosthetic material and dentine. A bond should last for many years under high loads, moist and attacked by bacteria.

1.1. The formation of dental plaque

The bacterial pressure in the oral cavity is high and the risk of plaque formation and subsequent caries is constantly a risk for patients. With dental treatments, secondary caries is a major cause of revision and replacements of dental materials. The environment of the oral cavity is quite complicated, and there are numerous species of micro-organisms that co-exist in the oral cavity, which provides a condition for micro-organisms to survive and proliferate (2). The bacteria in the oral cavity are involved in the development of many oral diseases such as demineralization process of marginal enamel and dentin and secondary caries (3). Secondary caries is one of the most important factors to affect the longevity of dental cements (4). There are several species of bacteria isolated from dental plaque, such as *Lactobacilli*, *Streptococcus mutans*, *Streptococcus sobrinus*, etc (5), which may induce the formation of secondary caries. *Streptococcus mutans* is one of the most frequent bacteria involved in dental caries (4). These cariogenic bacteria could degrade fermentable carbohydrates to acids to demineralise tooth tissue (6). Thus, it is quite practical to evaluate the antibacterial activity of dental cements with these bacteria, and the ability of dental cements to inhibit the formation of secondary caries (4).

In general the starting points for bacterial colonization are irregularities on dental surfaces. These irregular surfaces give high resistance to shear forces and bacteria can proliferate (2). After placement of a dental material on or in a tooth, depending on application there is always a risk of microleakage to occur between material and tooth substance. This is a common clinical phenomenon and happens more or less with all materials (6). For instance, the polymerization shrinkage of dental cements based on resins during hardening may lead to gap formation between the tooth-cement interface, and oral fluids, ions, some molecules and bacteria could penetrate into this gap (6) (7). The gaps between the material and teeth are easy for

oral micro-organisms to colonize and the shortage of mechanical disturbance and oxygen favors the growth of anaerobic *Streptococcus mutans* (8). Then dental plaque is formed, and followed by secondary caries. Thus antibacterial properties may be a very important characteristic of dental cement.

1.2. Dental cements studied in investigation

There are several different cements used in the clinical practice such as resins, glass ionomers, zinc phosphates, ceramics, and hybrids. In this investigation, antibacterial properties of 6 different dental cements has been evaluated; RelyX™ Unicem self-adhesive universal resin cement (manufactured by 3M ESPE), Ketac™ Cem Aplicap™ glass ionomer luting cement (manufactured by 3M ESPE), Harvard zinc phosphate cement (manufactured by HARVARD Dental International GmbH), Ceramir® Crown & Bridge (manufactured by Doxa Dental AB), Calcium Aluminate and Glass Ionomer cement (supplied by Doxa Dental AB), see Table 1.

Table 1. Compositions of all the dental cements used in this investigation

	Powder	Liquid	Type	Supplier	P/L ratio¹
RelyX™ Unicem (RelyX)	Alkaline fillers, silanated fillers, initiator components, pigments	Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, initiator components, stabilizers	Resin cement	3M ESPE	In capsules
Ketac™ Cem Aplicap™ (Ketac)	Glass powder, pigments	Polycarboxylic acid, tartaric acid, water, conservation agents	Glass ionomer	3M ESPE	In capsules
Harvard zinc phosphate cement (Zinc)	Zinc oxide, magnesium oxide	o-phosphoric acid	Zinc phosphate	Harvard Cement	3/2
Ceramir® Crown & Bridge (Ceramir)	Calcium aluminate, strontium fluoride, polyacrylic acid, tartaric acid, strontium alumino fluoride glass	Water, accelerators	BioCeramic	Doxa Dental	3.2/1
Calcium Aluminate (CA)	Calcium Aluminate	Water, accelerators	BioCeramic	Doxa Dental	2.5/1
Glass Ionomer cement (GIC)	Poly acrylic acid, tartaric acid, strontium alumino fluoride glass, strontium fluoride	Water	Glass ionomer	Doxa Dental	3.2/1

¹P/L ratio stands for the ratio of power and liquid when mixing.

1.3. Effects on antibacterial properties of dental cements

The antibacterial properties of dental materials may be attributed to the pH during setting and after maturation and release of ions, such as fluoride ions (6). Fluoride is well known as an anticariogenic agent used in drinking water and a variety of other vehicles, and there are several mechanisms involved in this effect, such as reduction of demineralization, enhancement of remineralization, and inhibition of microbial growth and metabolism (9). Fluoride can influence the metabolism of bacteria in oral cavity in multiple ways (10).

1.4. Popular assays for evaluating antibacterial properties of dental cements

The agar diffusion test (ADT) and direct contact test (DCT) are two popular assays for evaluating the antibacterial properties of dental cements used.

ADT is based on placing dental cement specimens on agar plates, which are seeded with micro-organisms, and then the degree of antibacterial activity of each sample is evaluated by taking gauge of inhibition zone around specimens after incubation (6). However, this assay has several disadvantages, and there are some acknowledged limitations in ADT. This assay just has ability to measure released water-soluble components, so the results of ADT depend on the solubility and diffusion properties of both dental cements and media. For dental cements, ideal ones are expected to have low solubility and less diffusion, so at this time, ADT may not detect the antibacterial activity. The results of ADT are usually semiquantitative. It is also difficult to control the variables during testing, such as the density of bacterial inocula, growth medium, storage conditions of agar plates, etc (6) (11) (12) (13).

To avoid the limitations ADT has, DCT has been developed to quantitatively measure the effect of physical direct and close contact between the tested materials and bacteria. Classic DCT was first described by Weiss et al (14). It is based on the growth of bacteria in 96-well microtiter plates after direct contact between materials and bacteria. The growth of bacteria in each well was measured and recorded at 650nm continuously at 37 degrees every 30 minutes, by using a temperature-controlled spectrophotometer (13). Compared with ADT, DCT is independent of the solubility of antibacterial components from cements and diffusion properties. Also, the DCT simulates the clinical situation, so the results of DCT are more convincing than ADT (11) (12) (13).

In this investigation, resazurin is used in the direct-contact test. Resazurin is a fluorescent blue dye used as an indicator of cell viability. Metabolically active cells

can reduce resazurin to resorufin and dihydroresorufin, from blue color to pink color. Resazurin is nonfluorescent, and resorufin is fluorescent. The degree of fluorescence can be measured colorimetrically or fluorometrically, and represents bacterial cell viability after direct contact, and then the antibacterial properties of each dental materials can be found out. One of the advantages of resazurin is that it is non-toxic to cells and stable in the culture medium. The resazurin test consumes less time, and it is quite convenient to control, compared with the classic DCT (15) (16) (17).

1.5. Aim of the study

The objective of this study is to assess and compare the antibacterial properties of six different dental cements on *S. mutans* during hardening by using the resazurin test and agar diffusion test, and also test the effects of different pH and different concentration of fluoride on the bacterial survival and attempt to explain the results and mechanisms according to the characteristics of each dental cement.

2. Materials and methods

2.1. Specimens preparation

Six types of dental cements were investigated. The brands, types, suppliers and mixing ratio used in this study are shown in Table 1.

RelyX Unicem capsule was inserted in the Aplicap™ Activator (manufactured by 3M ESPE), and the activator lever was pushed completely down and held down for 2-4 seconds. Then the RelyX Unicem capsule was triturated in high speed by CapMix™, manufactured by 3M ESPE, for 15 seconds. The capsule was inserted in the Aplicap Applier after mixing, and the nozzle was opened as far as possible. Then the cement was covered the entire mold evenly, and the surface of the filled mold was placed over by a plastic film. The mold was 1.5 mm deep, and the diameter was 5mm. After exposing to the artificial visible blue light for 60 seconds, the cement was cured. Then the plastic film was removed, and specimens were incubated in the 37°C oven in a dry condition.

The preparation for Ketac™ Cem Aplicap™ was similar to RelyX Unicem at beginning. After mixing by CapMix™ for 10 seconds, the capsule was inserted in the Aplicap Applier, and the cement was spread into the mold. Then the samples were incubated in the 37°C oven in the moist condition for 10 min. After setting, the specimens were removed out of mold, and incubated in the 37°C oven in the moist condition.

The Harvard zinc phosphate cement was mixed in the proportions 3/2 (powder/liquid). A spatula was used to mix the powder and liquid in 90 seconds, to achieve a homogenous consistency. The mixed cement was transferred into mold and incubated in the 37°C oven in the moist condition for 7 min. After setting, the specimens were removed out of mold, and incubated in the 37°C oven in the moist condition.

The P/L ratio of mixing for Ceramir, Calcium Aluminate, and GIC are shown in Table 1. The setting time for all of them was 10 min at 37°C and moist condition. Then all the specimens were incubated in the 37°C oven in the moist condition.

All the specimens were allowed to age in the oven for 10 min, 1 day, and 7 days before testing, and six repeats for the same experiment.

2.2. Bacterial strain and growth conditions

The indicator bacterial strain which was used to determine the growth inhibition activity of the 6 different dental materials was *Streptococcus mutans*, and the original strain was supplied by Department of Engineering sciences, Nanotechnology and Functional Materials group, which was isolated from dental plaque. This strain was activated and grown in the brain-heart infusion broth (BHI) medium for 24 hours at 37°C anaerobically (without shaking) from the plate of frozen-stock cultures.

2.3. Agar diffusion test

For agar diffusion test, 100 µl of bacterial suspension which was activated in the BHI medium from frozen-stock cultures were spread on 1% BHI agar plates evenly, and the plates were dried for 1 hour at room temperature. Then the specimens with different aging time (in sextuple) were inserted in the surface of BHI agar plates previously inoculated with *S. mutans* vertically and were incubated anaerobically for 17 hours at 37°C. After incubation, the agar plates were examined for inhibition of bacterial growth. Under these conditions, the semidiameter of inhibition zone was measured and recorded. Each of the experiment with different materials and different times were carried out in sextuple.

2.4. Resazurin test

For the resazurin test, besides the six different materials with three different aging time (in sextuple) as testing group, six specimens made of Poly (methyl methacrylate) (PMMA) were used as control group, which is the same size as testing specimens, 1.5 mm in deep and 5 mm in diameter. A microtiter plate (96-well, flat-bottom Transparent Polystyrol) was used, and all the specimens, both testing group and control group were put into it, one specimen for one well. The original *S. mutans* suspension of 5 µl which was activated in the BHI medium from frozen-stock cultures were added on the surface of each specimen to make sure directly and closely contact between the testing micro-organism and the testing materials. Then the plate was incubated in 37°C for 1 hr (check dryness every 15 min). MH broth 135 µl and 10×resazurin 15 µl was added to each well, and the plate was incubated in 37°C for 100 min (check color from blue to pink every 15 min). The standard curve was also done at the same time, and the dilution rate were 0.1×, 0.08×, 0.06×, 0.04×, 0.02×, 0.01×, 0.008×, 0.006×, 0.004×, 0.002×, 0.001×. Multimode microplate reader (Infinite® 200 PRO, TECAN) was used to measure the degree of fluorescence for each well.

2.5. Antibacterial effects of pH

For the pH effects test, 10 different pH buffers were used (pH 1, 2, 3, 4, 5, 6, 7, 9, 10, and 11). The components for reference, control group, and test group were shown in the Table 2, and 3 repeats for each sample.

Table 2. The components of reference, control group and test group

	BHI broth	pH buffer	ddH ₂ O	<i>S. mutans</i> ³
Reference ¹	4 ml	—	6 ml	—
Control group ²	3.4 ml	—	6 ml	0.6 ml
Test group	3.4 ml	6 ml	—	0.6 ml

¹Reference was used to calibrate the optical density (OD) of spectrophotometer under 600nm, considered as the baseline;

²Six ml ddH₂O was used in the control group instead of pH buffer, whose pH is around 7, and have no effects on the growth of bacteria;

³The original *S. mutans* suspension was also obtained by activating the strain from frozen-stock culture in the brain-heart infusion broth (BHI) for 24 hours at 37°C anaerobically.

After mixing them with the proportion as Table 3 described, a pH detector was used to measure the pH value for both control group and test group and to check how much the BHI broth medium would influence the pH value of every pH buffer, and this measured pH was named Working pH. All the control group and test group were incubated in the 37°C, and data were recorded in optical density (OD) units measured by spectrophotometer at 600 nm at 0, 2, 4, 6, 8, and 10 hours. Optical density is the measure of the amount of light absorbed by a suspension of bacterial cells with the use of spectrophotometer. The values can be used to measure turbidity, which in turn is used to estimate the number of bacteria in the solution, thereby estimating the outgrowth of bacteria in the tubes.

2.6. Antibacterial effects of fluoride

For the fluoride effects test, different concentration of fluoride was used. Sodium fluoride (NaF) solution was used. BHI broth medium, ddH₂O, and 10⁴ ppm NaF solution were mixed to reach the different concentrations of fluoride, from 0 ppm to 2000 ppm. Then 50 µl *S. mutans* suspension was added to each sample. For each experiment, there were three repeats. The bulk volume was 10 ml.

All samples were incubated at 37°C for 8 hours, and data were recorded in OD units measured by spectrophotometer at 600 nm after 8 hours.

2.7. Statistical analysis

The data from the direct-contact test were processed in SPSS 19 (Statistical Package for the Social Sciences, version 19). SPSS 19 (SPSS Inc) was used to do a univariate analysis of variance for two factors (material and time), seven different materials (including control), and three different times. Multiple comparisons were made using Scheffe's method. Intraindividual differences between the various materials and the various time were tested, where p value < 0.05 indicated statistical significant differences, and with a 95% confidence interval (99% CI).

3. Results

3.1. Antibacterial properties of six dental cements

The results of Agar Diffusion Test are shown in Figure 1, and it showed no antibacterial activity of the test cements for any of the test groups. It had been hoped that there would be a clear inhibition zone around the specimens. However, none of the test cements showed a clear zone.

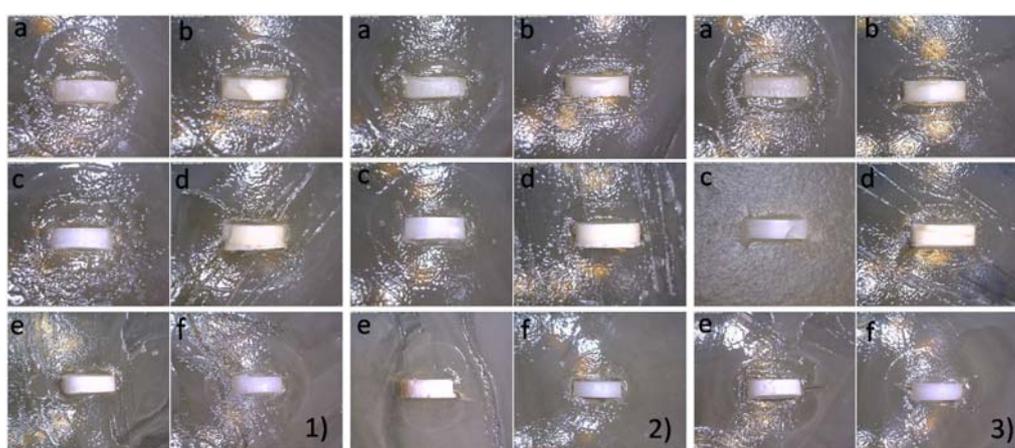


Figure 1. Results of agar diffusion tests of 1) 10 min, 2) 1 day, and 3) 7 days specimens for 6 different materials. (a) Rely X, (b) Ketac, (c) Ceramir, (d) Zinc phosphate, (e) Calcium Aluminate (CA), (f) Glass Ionomer Cement (GIC).

The results of resazurin test are shown in Table 3 and Figures 2 and 3. Usually when resazurin is reduced to resorufin and dihydroresorufin by active bacterial cells, it exhibits fluorescence itself, and this fluorescence can be detected by Multimode Micromode Reader. The strength of fluorescence represents the amount of viable bacterial cells, and it means the higher value of fluorescence, the larger amount of viable bacteria. Table 3 shows the degree of fluorescence for different experiment. After direct contacting between tested dental materials and *S. mutans*, bacterial cells may be killed by dental materials, or may be not killed, or even grow on the surface of materials, so the amount of bacterial cells remaining on the surface of materials after contacting varies. Thus, according to degree of fluorescence of different experiments, the amount of remaining bacteria on the surface can be known, thereby the degree of antibacterial properties of the six dental cements. Lower value represents better bacteria inhibition. The control group (PMMA) showed 8.9 ± 0.3 ($\times 10^3$) of degree of fluorescence. And Figure 2 shows the standard curve of this test,

of which the dilution rate are 0.1x, 0.08x, 0.06x, 0.04x, 0.02x, 0.01x, 0.008x, 0.006x, 0.004x, 0.002x, 0.001x. It represents the relation between concentration of viable bacterial cells and degree of fluorescence is linear ($R^2 = 0.97974$).

Table 3. Degree of fluorescence for resazurin test, six different materials within three different aging times.

	10 min ($\times 10^3$)	1 day ($\times 10^3$)	7 days ($\times 10^3$)
Control		9.0 \pm 0.3 ¹	
RelyX	6.6 \pm 1.0	8.2 \pm 0.2	8.1 \pm 0.4
Ketac	12.9 \pm 0.7	12.0 \pm 1.2	10.3 \pm 0.6
Ceramir	6.2 \pm 1.0	6.7 \pm 0.5	7.7 \pm 0.4
Zinc	7.2 \pm 1.9	9.5 \pm 1.4	9.8 \pm 2.8
CA	3.7 \pm 0.4	3.1 \pm 0.1	2.5 \pm 0.1
GIC	13.3 \pm 0.7	14.4 \pm 0.9	12.9 \pm 1.0

¹All values are the mean of six experiments \pm standard deviation.

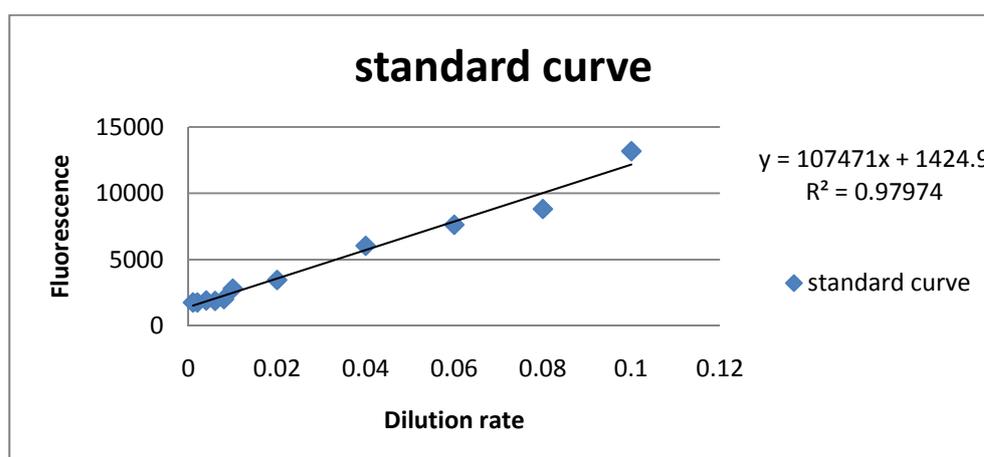


Figure 2. Standard curve for resazurin test. R2 = 0.98.

Figure 3 is obtained by SPSS 19. Each point on these curves was the average of the degree of fluorescence measured in six repeated wells. The strongest antibacterial activities were exhibited by CA, and there was significant difference compared with control samples ($P \leq 0.0001$). Ceramir also showed antibacterial properties. Compared with control samples PMMA, there was significant difference ($P = 0.008$). Ceramir had better antibacterial activities for shorter aging time, compared with CA.

There were two dental cements, RelyX and Zinc Phosphate, which did not show significant antibacterial properties compared with control group. However, the

samples with 10 min aging time have some activities ($P \leq 0.05$). The spread in data for some of the time points were large. One reason for the large spread was the very low volume of *S. mutans* suspension added on the surface of specimens. This in combination with an uneven bacteria concentration in the solution possibly resulted in a large spread.

Ketac and GIC do not show inhibition of bacterial growth, compared with control PMMA and other dental cements.

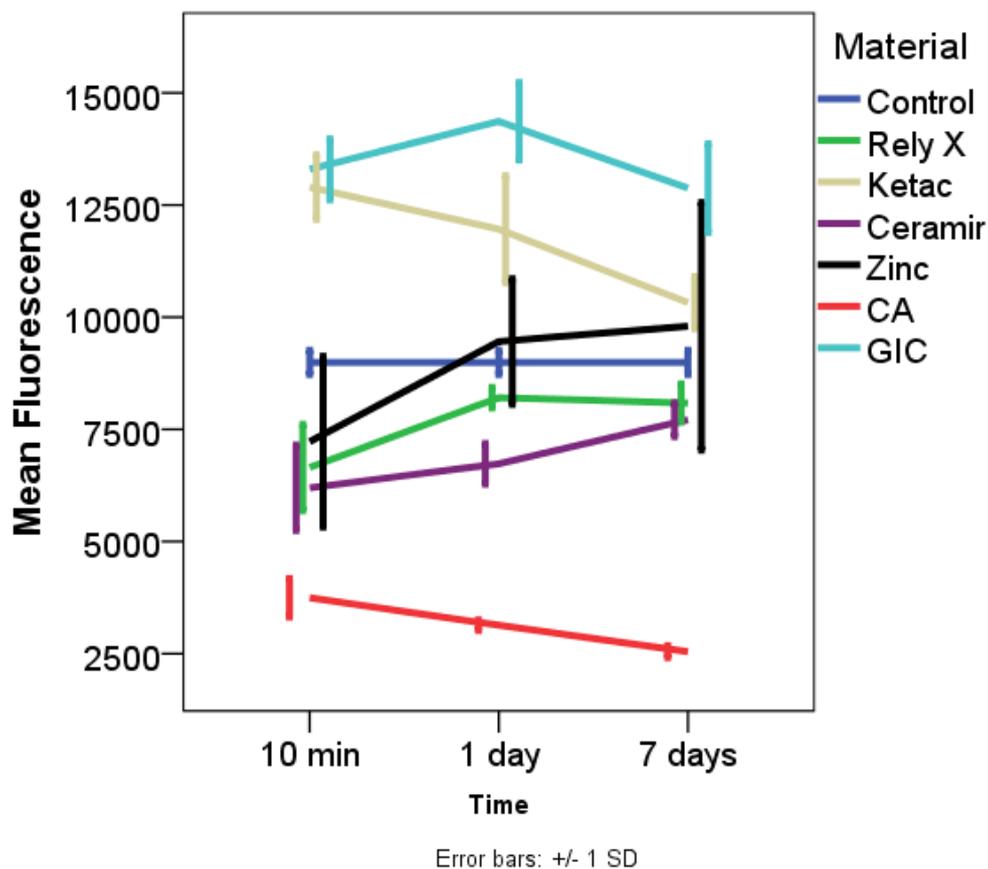


Figure 3. Antibacterial effect of six different dental cements (average of the degree of fluorescence with error bars) compared with control material (PMMA).

3.2. Antibacterial effects of pH

The results of the tests of the antibacterial effects of pH are shown in Table 4 and Figures 4 and 5. The data in Table 4 shows the OD of *S. mutans* in different pH buffer at a wavelength of 600nm for every 2 hours as measured by spectrophotometer. These OD values represent the amount of light that is absorbed by bacterial cells, which is proportional to cell density of suspension cultures. It means that the larger the OD value, the higher concentration of bacterial cells in the liquid. So it is used to estimate the concentration of bacterial cells in the liquid.

In this test, working pH is from pH 1.54 to 9.04. Figure 4 presents the outgrowth of bacteria in different pH conditions. Each point on these curves was the average of the OD measured in three repeated tubes at the same time. In this graph, from pH 1.54 to 6.32, and also above pH 9.04, bacteria did not grow any more during 10 hours. Bacteria have a strong growth from pH 6.99 to pH 8.56, especially at neutral pH, compared with control group. Figure 5 presents the tendency of antibacterial effects from acidic pH to neutral and to alkaline.

Table 4. Optical density (OD) of *S. mutans* in different pH at 600nm for every 2 hours measured by spectrophotometer.

	0 hr	2 hr	4 hr	6 hr	8 hr	10 hr
Control(7.07) ¹	0.08±0.01 ²	0.13±0.01	0.39±0.06	0.75±0.02	0.90±0.09	0.88±0.08
pH1 (1.54)	0.09±0.00	0.09±0.00	0.08±0.00	0.08±0.00	0.07±0.00	0.08±0.00
pH2 (3.45)	0.09±0.01	0.09±0.00	0.09±0.00	0.09±0.00	0.08±0.00	0.09±0.00
pH3 (3.90)	0.09±0.00	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.01	0.08±0.00
pH4 (4.45)	0.08±0.00	0.08±0.00	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00
pH5 (5.24)	0.08±0.00	0.08±0.00	0.07±0.00	0.07±0.00	0.06±0.00	0.07±0.00
pH6 (6.32)	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.00	0.09±0.01
pH7 (6.99)	0.08±0.00	0.12±0.02	0.36±0.05	0.69±0.05	1.04±0.06	1.02±0.04
pH9 (8.05)	0.08±0.00	0.10±0.01	0.17±0.01	0.33±0.05	0.52±0.10	0.69±0.11
pH10(8.56)	0.09±0.01	0.10±0.01	0.14±0.03	0.28±0.07	0.42±0.09	0.50±0.11
pH11(9.04)	0.09±0.01	0.09±0.00	0.09±0.00	0.10±0.00	0.11±0.00	0.12±0.00

¹The value in bracket stands for working pH, which is measured by pH meter after mixing *S. mutans* and pH buffer.

²All values are the mean for three experiments ± standard deviation

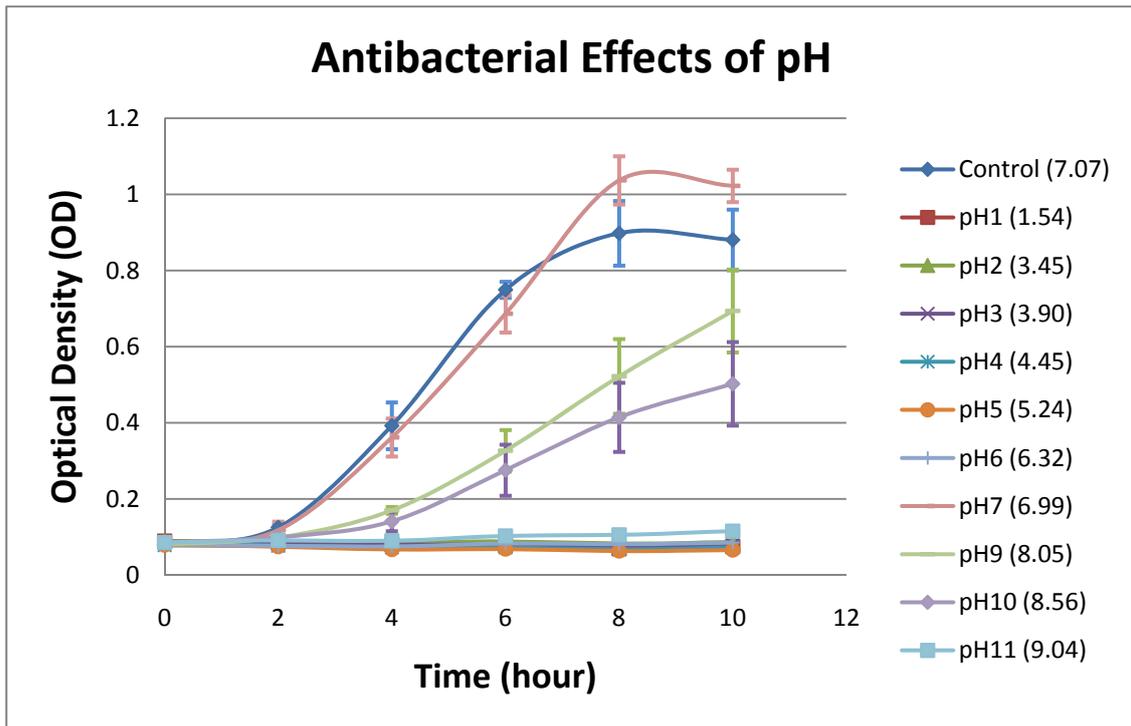


Figure 4. The outgrowth of *S. mutans* in different pH values.

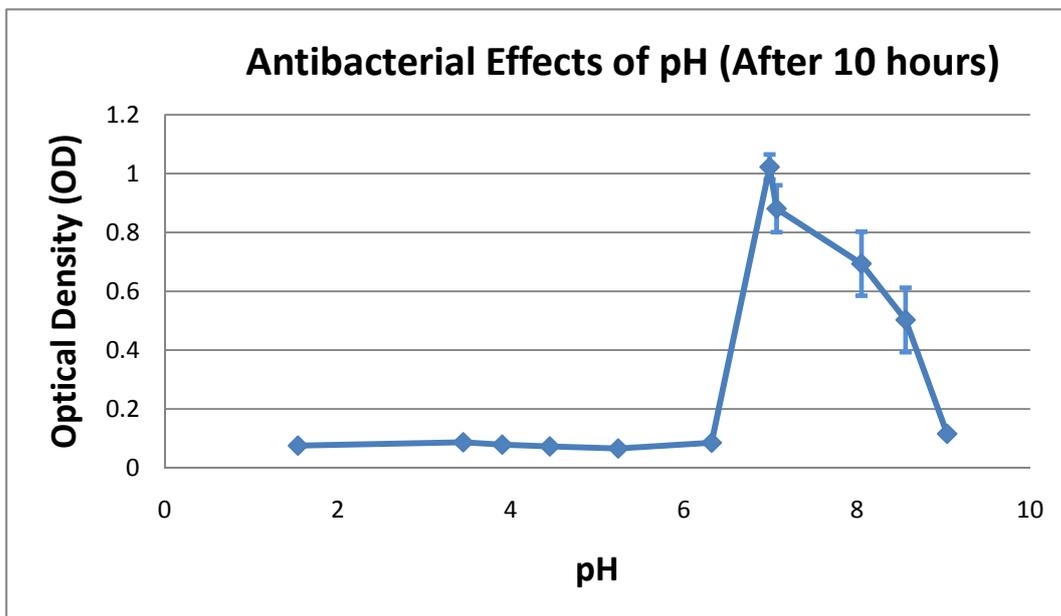


Figure 5. Tendency for antibacterial effects of different pH after 10 hours incubation

3.3. Antibacterial effects of fluoride

The general trend was that the higher concentration of fluoride in the environment, the stronger influence on the growth of *S. mutans*, and the better antibacterial activities, see Table 5 and Figure 6. When the concentration reaches 1400 ppm, the degree of antibacterial activities go to steady. Each point in Figure 6 was the average of the OD measured in three repeated tubes at the same time after incubating eight hours.

Table 5. Optical density (OD) of *S. mutans* in different concentration of fluoride at 600nm measured by spectrophotometer after 8 hours.

Fluoride Concentration (ppm)	Optical Density (OD)
0	0.874±0.022 ¹
200	0.648±0.043
400	0.630±0.025
600	0.544±0.003
800	0.346±0.047
1000	0.386±0.056
1200	0.373±0.025
1400	0,260±0.023
1600	0.231±0.051
1800	0.234±0.008
2000	0.235±0.045

¹All values are the mean of three experiments ± standard deviation

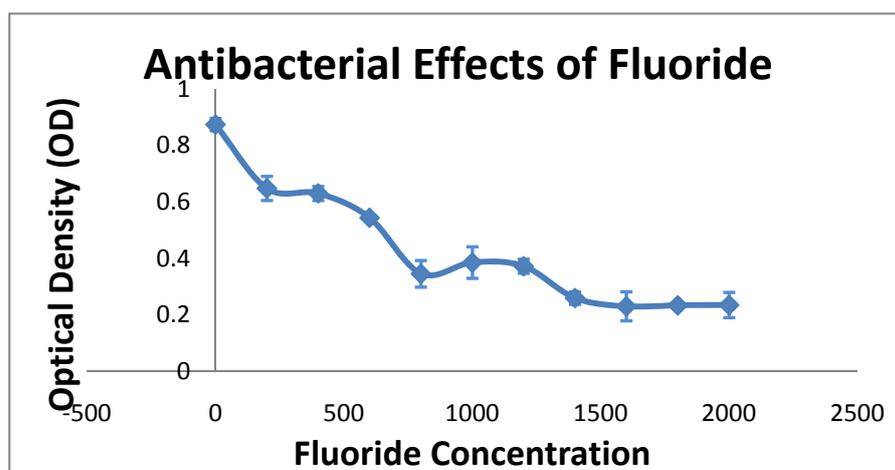


Figure 6. Tendency for antibacterial effects of different fluoride concentration.

4. Discussion

The bacterial pressure in the oral cavity is high and the risk of plaque formation and caries is constantly a risk for patients. So for an ideal dental cement, antibacterial properties are potentially of great importance because secondary caries are the major cause of failure of dental restorations, and this property determines, in many cases the longevity of dental cements.

During this investigation, two assays were used to evaluate the antibacterial activity for dental cements, ADT and resazurin test. The test did not show antibacterial properties for any of the cements. From resazurin test, the strongest antibacterial activity is exhibited by CA and Ceramir. RelyX and Zinc phosphate have not shown obvious antibacterial properties. GIC and Ketac do not show any inhibition of bacterial growth at all ($P \leq 0.05$).

The antibacterial properties of dental cements are considered to be attributed to 1) the release of fluoride ions from dental cements; 2) the pH level of dental cements when setting or after setting (6).

Usually, the pH of freshly mixed dental cements is low, and often rises to a neutral level or even alkaline level during the setting reaction or at the process of maturing (18). According to Figure 5, the growth of *S. mutans* significantly decreases below pH 6.99, and is completely inhibited below pH 6.32. Similarly, *S. mutans* could not grow above pH 9.04. Usually, bacteria can not grow at a pH value below 5, and it is the minimum growth pH for organisms (10). Based on these results, the antibacterial properties of some dental cements investigated in this study might be explained. For example, pure Calcium Aluminate has the strongest antibacterial properties in this investigation. The initial pH after setting is about 5, and then rather fast reaches a pH above 9, and ends at about 11.5. This dental material has a lower initial pH while setting, and the final pH of them is quite alkaline, which also can inhibit the growth of *S. mutans*. So, the strong antibacterial activities of this cement may be due to the trend of pH changing after mixing. However, pH change after setting can not explain all phenomena. Ceramir, which shows obvious antibacterial activities, has an initial pH at 5.4, and ends at 8.5. Ketac Cem and GIC, used in this study, never reach neutral levels but end up at a pH of about 6. From Figure 5, this would mean that they should both display rather high antibacterial activities. However, in the results from resazurin test, Ketac Cem and GIC do not show any inhibition of bacterial growth. Zinc phosphate cement has a lower initial setting pH, and can reach to pH 4.0 in 3 min after setting (18). But in this investigation, Zinc phosphate does not have obvious antibacterial properties. These phenomena indicate that pH is not directly associated with antibacterial properties of dental cements, but fluoride release is associated with the antibacterial properties directly, even when the pH reaches around 7 (19).

Fluoride is widely used as an anticariogenic agent in many dental products, such as toothpaste, and mouthrinse (20). There are various mechanisms involved in this effect, such as the reduction of demineralization, the enhancement of remineralization, and the inhibition of bacterial growth and metabolism (9). In this investigation, only inhibition of bacterial growth and metabolism will be discussed. The result of the antibacterial effect of fluoride indicated that fluoride might influence the growth and metabolism of *S. mutans* directly.

Direct inhibition of enzymes is the major way for fluoride (as F^- or HF) to influence bacterial cells. Examples of such enzymes are the glycolytic enzyme enolase, the proton-extruding ATPase, acid phosphatase, pyrophosphatase, peroxidase and catalase, and all these enzymes are involved in bacterial metabolisms (6) (10). Previous studies have found that usually fluoride can not penetrate through the cell wall and membranes of bacteria in ionized form, F^- , but in the form of HF. HF acts as a transmembrane proton carrier to discharge a ΔpH across the cell membrane. Proton-extruding ATPases, which are located in the membrane are overloaded and disturbed to extrude proton as usual, because due to the penetration of HF, protons just come back into cells (6). Once HF crosses into bacterial cells, it dissociates again into H^+ and F^- . H^+ can lead to intracellular acidification, which can inhibit the activity of glycolytic enzymes, and F^- is released and binds to some specific sites of enzymes or other protein to interfere with their functions (10) (20) (21). Fluoride can also form some complexes with other metals, such as AlF_4^- , and $BeF_3^- \cdot H_2O$, and these complexes can mimic phosphate and have effects on enzymes and regulatory phosphatases (10).

For a dental cement to have an ideal release profile from an antibacterial perspective, it is important to have both a strong initial release of fluoride and a stable and constant release of fluoride at a lower level. If there is a strong initial fluoride release from dental cement after setting, the gap or microgroove between dental cement and tooth which is due to the shrinkage of cements can be concentrated with fluoride, and it is hard for bacteria to grow in this area. It means that the viability of bacteria in this area will be decreased, which can reduce the formation of dental plaque. And the low level constant release of fluoride could maintain the activity of bacteria in the plaque at low level, and could reduce the risk of caries (22).

So the release of fluoride of dental cements leads to the antibacterial properties. There are many types of dental cements in the market including fluoride components, but not all the cements with fluoride components have obvious antibacterial properties. The antibacterial properties of dental cement can not be attributed to the fluoride contents, because not all of the fluoride contents can be released from dental cement. Some of the fluoride content just acts as the filler component which is the bracket for other components, and it is inert with regard to antibacterial activity (13). It is indicated in this investigation that GIC does not show inhibition of bacterial growth. Ketac Cem exhibits low antibacterial properties, and it is also

considered to be due to the low fluoride release of this cement, as mentioned in previous studies (19). GIC has the same level of initial fluoride release as Ketac Cem and thus has the same level of antibacterial properties. From Table 1, strontium fluoride is one of the components of the powder part in GIC. However, strontium fluoride in GIC acts as filler component, and is considered to be inert. So the release of fluoride from these cements is small.

In this investigation, Ceramir shows relatively strong antibacterial properties, compared with GIC. As mentioned before, considering the effects of pH, both of them should have had antibacterial activities (GIC is acidic and Ceramir is first acidic and then basic). Also the fluoride release and the potential release of Calcium and other ions are similar. One possible explanation for the antibacterial properties for Ceramir is the continued reaction with water during hardening, resulting in an alkaline pH for a longer period of time than for the GIC.

RelyX Unicem cement is dual-curing, self-adhesive universal resin cement for permanent cementation. It also releases fluoride ions. However, during the setting process, the initial pH-value after immediately mixing is considerably acidic, below 1. Then the pH-value starts to increase to a neutral level in 24 hours to about 7.5 (23). The final pH of RelyX is just located in the pH scale which could not inhibit the bacterial growth. Thus, at the beginning, the 10 min samples exhibited some antibacterial activity, but this property could not last long. Zinc phosphate cement also does not exhibit significant antibacterial activity, and the lack of antibacterial agents and relatively high pH might result in this poor activity of this cement.

For future investigation, there has to be more focus on the mechanisms and connection to the clinical situation. The release of fluoride from different dental materials at different pH levels will be needed to understand the relation between pH and amount of fluoride release. Also, since the dental cements are intended to be long lasting in their clinical service, it could be interesting that samples are left for longer times in larger volume of bacteria suspensions and samples are withdrawn for analysis at different times.

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