

## Which is More Effective as Intracanal Medicament on *Enterococcus Faecalis*, Chlorhexidine Gel™ or Activ Point™?

Irmaleny<sup>1\*</sup>, Yolanda<sup>1</sup>, Sri Susilawati<sup>2</sup>, Meirina Gartika<sup>3</sup>,  
Pratiwi Yudhia Dewi<sup>4</sup>, Sri Rahmi Fitri<sup>5</sup>, Sarah Shafira<sup>6</sup>

1. Department of Conservative Dentistry, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia.
2. Department of Dental Public Health, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia.
3. Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia.
4. Clinical Dental Student, Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia.
5. Diskes Lantamal II, Padang, Indonesia.
6. Dental Private Practice, Bandung, Indonesia.

### Abstract

The aim of this study was to evaluate the effectiveness of chlorhexidine gel™ compared to chlorhexidine activ point™ as an intracanal medicament against *Enterococcus faecalis* (ATCC 29212) by counting the *E. faecalis* colony count as well as testing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test.

Calculation of the *E. faecalis* colony counts was using the Total Plate Count (TPC) technique while the MIC and MBC test was using two microplates 96 well. The result using the Kruskal Wallis test and then the Mann Whitney U-Test on day 1, 3, 7, and 14.

The decrease occurred more in the gel group on day 1 (1857.50 ± 903.377); 3 (322.50 ± 109.048); 7 (225.00 ± 55.677) and 14 (189.25 ± 117.709) compared to the active point group. The MIC of chlorhexidine gel™ against *E. faecalis* was 0.5%, while chlorhexidine point™ was 1.25%. The MBC of chlorhexidine gel™ for *E. faecalis* is 1%, while chlorhexidine point™ is 2.5%.

There was a significant difference in the decrease of bacterial colonies number. The MIC and MBC of chlorhexidine activ point™ were higher than chlorhexidine gel™. Chlorhexidine gel™ has better antibacterial properties and is more effective as an intra-canal medicament against *E. faecalis* (ATCC 29212).

**Experimental article (J Int Dent Med Res 2023; 16(3): 1091-1097)**

**Keywords:** Chlorhexidine gel™, chlorhexidine activ point™, bacterial colonies counts, minimum inhibitory concentration, minimum bactericidal concentration.

**Received date:** 11 July 2023

**Accept date:** 09 August 2023

### Introduction

Bacteria and their products are the main causes of pulpal and periapical disease. Root canal treatment aims to eliminate microorganisms and their by-products and create an environment that makes bacteria unable to grow in the root canal.<sup>1,2</sup> Failure of endodontic treatment can occur due to inadequate treatment or persistent bacterial infection in the root canal system and periapical.<sup>3</sup> *E. faecalis* is the most common bacterial species found in cases of failed endodontic treatment. *E. faecalis* is one of

the most resistant pathogens of all root canal microflora because of its ability to form biofilms, enter dentinal tubules, survive in low pH, and be resistant to various intracanal drugs.<sup>1,4</sup> Prevalence of *E. faecalis* in cases of endodontic failure ranged from 24-77%.<sup>4,5,6,7</sup>

Successful endodontic treatment is based on crown access to the root canal, adequate root canal shaping and cleaning, irrigation, medicament, and proper root canal filling.<sup>8,9</sup> The use of intracanal medicaments between appointments is recommended to reduce the number of bacteria.<sup>8,9,10</sup> The ideal medicaments should have antibacterial properties, be able to reduce residual microbial biofilm, not irritate the periapical tissue, help regenerate periapical tissue, and be easy to apply and clean.<sup>11,12</sup>

Chlorhexidine is an antimicrobial agent with a broad spectrum because it can fight gram-positive, gram-negative, and facultative aerobic

#### \*Corresponding author:

Irmaleny,  
Department of Conservative Dentistry, Faculty of Dentistry,  
Universitas Padjadjaran, Bandung, Indonesia.  
E-mail: irmaleny@unpad.ac.id

and anaerobic bacteria, especially *E. faecalis*.<sup>13</sup> Chlorhexidine is used as an intracanal medicament because it can enter the dentinal tubules and cell walls of microorganisms.<sup>11</sup> Chlorhexidine can inhibit and kill bacteria by changing the protein structure of the bacterial cell wall membrane by increasing the permeability of the membrane. Increased permeability of the cell wall membrane causes cell wall leakage so that the cytoplasm or fluid inside the bacterial cell comes out of the cell and the bacteria will lyse or die.<sup>14</sup>

Chlorhexidine gel<sup>TM</sup> as a medicament is effective in reducing the growth of *E. faecalis*, but it has the disadvantage of being difficult to reach apically and to clean.<sup>15,16</sup> Chlorhexidine active point can be used as an alternative material so that intra-canal medicaments can reach the apical end and are easy to clean.<sup>17</sup> Chlorhexidine active point<sup>TM</sup> has a higher concentration (5%) with ISO standard size from number 15-40 which makes this material easy to apply.<sup>18</sup>

This study aims to evaluate the effectiveness of chlorhexidine gel<sup>TM</sup> with chlorhexidine activ point<sup>TM</sup> as an intracanal medicament against *E. faecalis* (ATCC 29212) by counting the *E. faecalis* bacteria colonies, as well as the minimum inhibitory concentration test and minimum bactericidal concentration test.

### Materials and methods

*E. faecalis* (ATCC 29212) was obtained from a frozen preparation of the Microbiology Laboratory of School of Pharmacy, Bandung Institute of Technology, Indonesia, cultured on Mueller Hinton Agar (MHA) slants and incubated for 24 hours at 37°C. One loop was taken from the slanting agar and transferred into a tube containing 10 ml of Mueller Hinton Broth (MHB), then incubated for 24 hours at 37°C. Using a spectrophotometer at  $\lambda 625$  nm, the absorbance of the suspension is 0.08 – 0.12 or adjusted to the standard turbidity of 0.1 McFarland. The culture suspension of *E. faecalis* was diluted with a ratio of 1:20 to produce a population of 10<sup>6</sup> CFU/ml.<sup>52</sup>

Counting the number of *E. faecalis* colonies with Total Plate Count (TPC)

Dilution of *E. faecalis* was carried out by adding 1 ml of *E. faecalis* suspension to 9 ml of 0.9% NaCl (10<sup>-1</sup> dilution). Then 1 ml of 10<sup>-1</sup> dilution suspension was added to 9 ml of 0.9%

NaCl (10<sup>-2</sup> dilution), carried out until 10<sup>-6</sup> dilution. Then, 1 ml of the 10<sup>-6</sup> dilution suspension was put into a petri dish and added 20 ml of liquid MHA whose temperature was around 50°C. The suspension was homogenized by shaking the petri dish, and was carried out in triplicate. After solid suspension, put it in an incubator at 37°C for 24 hours. Samples were divided into 8 groups namely A-1 chlorhexidine activ point<sup>TM</sup> (roeko activ point<sup>TM</sup>) #LOT 183148 1 day, A-2 chlorhexidine activ point<sup>TM</sup> 3 days, A-3 chlorhexidine activ point<sup>TM</sup> 7 days and A-4 chlorhexidine activ point<sup>TM</sup> 14 days. Group B-1 chlorhexidine gel<sup>TM</sup> (Gluko-CheX 2% gel<sup>TM</sup>) #LOT 1011 1 day, B-2 chlorhexidine gel<sup>TM</sup> 3 days, B-3 chlorhexidine gel<sup>TM</sup> 7 days and B-4 chlorhexidine gel<sup>TM</sup> 14 days. The number of bacterial colonies was counted using the colony counting method with a colony counter which is expressed in units of CFU/mL.

### Minimum Inhibitory Concentration Test and Minimum Bactericidal Concentration Test

Two 96-well microplates were added with 100  $\mu$ L of MHB media except for well column 12, row 1 to row 3. The well in column 1 only contained MHB media as a media control. Well column 2 contains MHB medium added with 10  $\mu$ L of *E. faecalis* suspension as a bacterial control. Serial dilution of chlorhexidine gel<sup>TM</sup> was carried out by adding 100  $\mu$ L of chlorhexidine gel<sup>TM</sup> into well column 12, then into wells in column 11 which contained 100  $\mu$ L of MHB and then homogenized. The mixture of MHB and chlorhexidine gel<sup>TM</sup> in well column 11 was taken using a micropipette as much as 100  $\mu$ L and then put into well column 10 which already contained 100  $\mu$ L MHB and homogenized. This procedure was carried out until well column 3. Well in column 3 containing a mixture of 200  $\mu$ L of MHB and chlorhexidine gel<sup>TM</sup>, discarded 100  $\mu$ L to equalize the amount of the mixture in the well. This procedure is carried out up to three repetitions.

Well in column 11, rows 4 to 6, 200  $\mu$ L of MHB was added. Dilution of chlorhexidine activ point<sup>TM</sup> in well column 12 rows 4 to 6 was carried out by inserting one chlorhexidine activ point<sup>TM</sup> into well column 12 containing 100  $\mu$ L MHB and then homogenizing. Well, in column 11 containing 200  $\mu$ L MHB, one chlorhexidine activ point<sup>TM</sup> was added and then homogenized. A mixture of chlorhexidine activ point<sup>TM</sup> and MHB in well column 11 was taken as much as 100  $\mu$ L

and then put into well column 10. This treatment was continued until well column 3. Well in column 3 containing a 200 µL mixture of MHB and chlorhexidine activ point™ was removed as much as 100 µL to equalize the amount of the mixture in the well. The procedure is carried out up to three repetitions.

The 2% chlorhexidine solution as the control variable was serially diluted by taking 100 µL of 2% chlorhexidine with a micropipette and putting it into the well in column 12, then 100 µL of 2% chlorhexidine into the well in column 11 which already contained 100 µL MHB and then homogenized. The procedure was carried out until well column 3. Well in column 3 which contained 200 µL of MHB and 2% chlorhexidine, discarded 100 µL to equalize the amount of the mixture in the well. The procedure is carried out up to four repetitions. Well, in column 3 to column 12, 10µL of E. faecalis suspension was given. This treatment was also carried out on the second 96 well microplate. Then incubated at 37 °C for 24 hours.

Microplate 96 well which has been incubated, seen for turbidity. Well on the chlorhexidine activ point™ line, chlorhexidine gel™, and the clearest 2% chlorhexidine solution were used as MIC. One ose was taken from each well and inoculated onto the plate with MHA medium, incubated at 37°C for 24 hours, then observed the growth of the microbial colonies that appeared. Plates with no growth of microbial colonies and concentrations higher than MIC were designated as MBC.

Chlorhexidine Gel™	Concentration	MC	BC	0.003	0.007	0.015	0.031	0.06	0.12	0.25	0.5	1	2
KM	-												
KB	0.001	-											
0.003	0.001	1.000	-										
0.007	0.001	1.000	1.000	-									
0.015	0.001	1.000	1.000	1.000	-								
0.031	0.001	1.000	1.000	1.000	1.000	-							
0.06	0.001	1.000	1.000	1.000	1.000	1.000	-						
0.12	0.001	1.000	1.000	1.000	1.000	1.000	1.000	-					
0.25	0.001	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-				
0.5	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-			
1	1.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-		
2	1.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1.000	-	

**Figure 1.** Results of the Mann Whitney U-Test Comparison of the Minimum Inhibitory Concentration of Chlorhexidine Gel™.

Chlorhexidine Activ Point™	Concentration	MC	BC	0.007	0.015	0.03	0.07	0.15	0.31	0.625	1.25	2.5	5
KM	-												
KB	0.001	-											
0.007	0.001	1.000	-										
0.015	0.001	1.000	1.000	-									
0.03	0.001	1.000	1.000	1.000	-								
0.07	0.001	1.000	1.000	1.000	1.000	-							
0.15	0.001	1.000	1.000	1.000	1.000	1.000	-						
0.31	0.001	1.000	1.000	1.000	1.000	1.000	1.000	-					
0.625	0.001	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-				
1.25	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-			
2.5	1.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-		
5	1.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1.000	-	

**Figure 2.** Results of the Mann Whitney U-Test Comparison of the Minimum Inhibitory Concentration of Chlorhexidine Activ Point™.

Control	Concentration	CM	BC	0.003	0.007	0.015	0.031	0.06	0.12	0.25	0.5	1	2
KM	-												
KB	0.008	-											
0.003	0.008	1.000	-										
0.007	0.008	1.000	1.000	-									
0.015	0.008	1.000	1.000	1.000	-								
0.031	0.008	1.000	1.000	1.000	1.000	-							
0.06	0.008	1.000	1.000	1.000	1.000	1.000	-						
0.12	0.008	0.008	0.008	0.008	0.008	0.008	0.008	-					
0.25	1.000	0.008	0.008	0.008	0.008	0.008	0.008	0.008	-				
0.5	1.000	0.008	0.008	0.008	0.008	0.008	0.008	0.008	1.000	-			
1	1.000	0.008	0.008	0.008	0.008	0.008	0.008	0.008	1.000	1.000	-		

**Figure 3.** Results of the Mann Whitney U-Test Comparison of the Minimum Inhibitory Concentration of Control.

Variable	Groups		P Value
	Chlorhexidine gel™ N=4	Chlorhexidine activ point™ N=4	
<b>Number of Bacterial Colonies Day 1</b>			
Mean ± Std	1857.50±903.377	15125.00±5146.115	0.013**
Median	1840.000	15200.00	
Range (min-max)	980.00-2770.00	10100.00-20000.00	
<b>Number of Bacterial Colonies Day 3</b>			
Mean ± Std	322.50±109.048	1945.00±504.413	0.029**
Median	370.000	2040.000	
Range (min-max)	160.00-390.00	1250.00-2450.00	
<b>Number of Bacterial Colonies Day 7</b>			
Mean ± Std	225.00±55.677	882.50±27.537	0.000**
Median	235.000	885.000	
Range (min-max)	150.00-280.00	850.00-910.00	
<b>Number of Bacterial Colonies Day 14</b>			
Mean ± Std	189.25±117.709	14700.00±3070.287	0.002**
Median	220.000	15000.000	
Range (min-max)	27.00-290.00	1.08E+006-1.80E+006	

**Table 1.** Number of E. faecalis colonies on days 1, 3, 7, and 14 in the groups of chlorhexidine gel™ and chlorhexidine activ point™. \*\*p value < 0.01 : very significant.

**Results**

Total Colonies of E. faecalis  
 The number of E. faecalis colonies can be seen in Table 1.

Gel	Concentration	Clear		Muddy		P-Value	Note
		f	%	f	%		
	2%	6	100%	0	0%	0,000	< - MIC= (0.5% + 0.12%)/2=0.31%
	1%	6	100%	0	0%	0,05	
	0.50%	6	100%	0	0%		
	0.25%	3	50%	3	50%		
	0.12%	3	50%	3	50%		
	0.06%	2	33%	4	67%		
	0.031%	1	17%	5	83%		
	0.015%	1	17%	5	83%		
	0.007%	0	0%	6	100%		
	0.003%	0	0%	6	100%		
	BC	0	0%	6	100%		
	MC	6	100%	0	0%		

**Table 2.** The Kruskal Wallis Test Comparison of the Minimum Inhibitory Concentration of Chlorhexidine Gel™.

Activ Point	Concentration	Clear		Muddy		P-Value	Note
		f	%	f	%		
	5%	6	100%	0	0%	0,000	< - MIC= (0.5%+1%)/2=1,875%
	2,5%	6	100%	0	0%	0,05	
	1,25%	3	50%	3	50%		
	0,625%	0	0%	6	100%		
	0.31%	0	0%	6	100%		
	0.15%	0	0%	6	100%		
	0.07%	0	0%	6	100%		
	0.03%	0	0%	6	100%		
	0.015%	0	0%	6	100%		
	0.007%	0	0%	6	100%		
	BC	0	0%	6	100%		
	MC	6	100%	0	0%		

**Table 3.** The Kruskal Wallis Test Comparison of the Minimum Inhibitory Concentration of Activ Point™.

Control	Concentration	Clear		Muddy		P-Value	Note
		f	%	f	%		
	2%	4	100%	0	0%	0,000	< - MIC= (0.031%+0.25%)/2=0.14%
	1%	4	100%	0	0%	0,05	
	0.50%	4	100%	0	0%		
	0.25%	4	100%	0	0%		
	0.12%	2	50%	2	50%		
	0.06%	2	50%	2	50%		
	0.031%	2	50%	2	50%		
	0.015%	0	0%	4	100%		
	0.007%	0	0%	4	100%		
	0.003%	0	0%	4	100%		
	BC	0	0%	4	100%		
	MC	4	100%	0	0%		

**Table 4.** The Kruskal Wallis Test Comparison of the Minimum Inhibitory Concentration of Control. BC: Bacterial Control; CM: Media Control.

**Minimum Inhibitory Concentration Test**

The minimum inhibitory concentration of chlorhexidine gel™, active point™, and controls can be seen in Table 2, Table 3, Table 4 and Figure 1, Figure 2, Figure 3

The results of the Kruskal-Walis obtained a p-value of 0.000 <0.05 which indicated that

there was a very significant difference in all treatment concentrations on the inhibition of bacteria. Further test results showed that concentrations of 0.003%, 0.007%, 0.015%, and 0.031% were significantly different from concentrations of 1% and 2% (p-value <0.05). The minimum inhibitory concentration of chlorhexidine gel™ was 0.31%.

Table 2,3, and 4 showed a significant difference in all treatment concentrations on the results of bacterial inhibition on chlorhexidine activ point™ with a p-value of 0.000 <0.05. Further tests obtained a concentration of 0.007%; 0.015%; 0.03%; 0.07%; 0.15%; 0.31%; 0.625% differed significantly with concentrations of 2.5% and 5% (p-value <0.05). The minimum inhibitory concentration of chlorhexidine point™ was 1.875%.

The results of the Mann Whitney U-Test on chlorhexidine gel™, chlorhexidine active point™, and 2% chlorhexidine solution are shown in Figure 1, Figure 2, and Figure 3. Chlorhexidine gel at a concentration of 0.003%; 0.007%; 0.015%; 0.031%; 0.06%; 0.12%; 0.25%; 0.5% and well as control bacteria have different anti-bacterial power with a concentration of 1%; 2% and media control with a p-value of 0.001 <0.05, which means that the difference is statistically significant. All concentrations of chlorhexidine activ point™ tested with the Mann Whitney U-Test showed a difference in the anti-bacterial effect on the bacterial control, concentration 0.007%; 0.015%; 0.03%; 0.07%; 0.15%; 0.31%; 0.62%; 1.25% with a concentration of 2.5%; 5% and media control with a p-value of 0.001. Statistical tests of the concentration of 2% chlorhexidine solution showed that there was a difference in the effectiveness of the anti-bacterial against E. faecalis in the control bacteria, with concentrations of 0.003%; 0.007%; 0.015%; 0.031%; 0.06%; 0.12% with a concentration of 0.25%; 0.5%; 1%; 2% and media control with a p-value of 0.001 <0.05.

**Discussion**

E. faecalis was used in this study because it is a microorganism that can survive in the presence or absence of oxygen, has the ability to enter the dentinal tubules, and causes endodontic treatment failure.<sup>7,9-22</sup> There was a significant difference in the decrease of E. faecalis colonies number on days 1, 3, 7 and 14

in both groups. The chlorhexidine gel™ group experienced a greater decrease because it is biocompatible and water soluble, and contains natrosol compounds which cause chlorhexidine to have longer contact with the dentinal tubules and root canal walls so that the anti-bacterial effect lasts a long time.<sup>22</sup> However, the used of chlorhexidine as intracanal medicament will decrease the amount of endotoxins levels during root canal treatment.<sup>23</sup>

The contact of chlorhexidine with the bacterial wall of *E. faecalis* changed the surface structure of the bacteria which caused a loss of osmotic balance, thereby increasing the permeability of the bacterial cell wall. Chlorhexidine molecules penetrate into the bacteria and damage the cytoplasmic membrane, so the bacteria become lysed and the number of *E. faecalis* decreased.<sup>24-26</sup> The decrease in the number of *E. faecalis* colonies in the chlorhexidine gel group occurred on days 1, 3, and 7. The biggest decrease occurred on the 7th day, while the smallest was on the 14th day. These results are in line with the research by Gomes BPPA et. al that 2% chlorhexidine gel was able to inhibit the growth of *E. faecalis* for up to 15 days.<sup>27</sup>

The number of *E. faecalis* chlorhexidine activ point™ colonies decreased on days 1, 3 and 7. The decrease was smaller than that of chlorhexidine gel™, because its ability to release chlorhexidine ions was not as good as gel. According to Shaaran M, the number of *E. faecalis* chlorhexidine activ point™ colonies decreased until day 7,<sup>12</sup> in line with this study. On the 14th day, there was an increase in the number of colonies, this was due to the absence of the ability to release chlorhexidine ions, so that the mechanism of action of chlorhexidine on the cell walls of *E. faecalis* bacteria was reduced which caused the remaining growth of *E. faecalis* bacteria to occur.

Chlorhexidine activ point™ is used as an intracanal medicament because it can reduce the number of microorganisms in root canals.<sup>12</sup> The effectiveness of chlorhexidine activ point™ is different from chlorhexidine gel™ in reducing *E. faecalis* colony numbers, but it is easy to apply and remove from the root canal. According to Stojanovic.N et.al, chlorhexidine gel™ or activ point™ can reduce the number of bacteria and inhibit bacterial colonization of dentine. Chlorhexidine gel™ is more effective than

chlorhexidine activ point™. Chlorhexidine activ point™ is recommended for persistent periapical inflammation or as an agent for apexification.<sup>28</sup>

The results of the MIC and MBC tests in this study showed that chlorhexidine gel™ could inhibit growth and kill *E. faecalis* at concentrations of 0.5% and 1%. In line with research conducted by Babickaite et al, that 1% chlorhexidine gel had anti-bacterial activity against *Staphylococcus aureus*, *E. faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.<sup>29</sup>

This study results showed that chlorhexidine activ point™ at a concentration of 2.5% could kill *E. faecalis*, while at a concentration of 1.25% could inhibit the growth of *E. faecalis*. Sinha et al. said that the MIC of the chlorhexidine solution was 0.0078% and the MBC was 0.0625%. This difference was influenced by 5µL *E. faecalis* suspension in the 96 well microplate<sup>30</sup>, whereas in this study it was 10µL. Thus, a higher concentration of chlorhexidine solution is needed to inhibit and kill *E. faecalis*.<sup>30,31</sup>

The 2% chlorhexidine solution as a control in this study had a MIC of 0.12%, in line with the research conducted by Karpinski and Szkaradkiewicz that 0.12% chlorhexidine could inhibit bacterial growth.<sup>32</sup> 0.25% MBC was produced in the test using 2% chlorhexidine solution. In contrast to the results of Kohli et al's study, the MIC was lower by 0.03% and the MIC was higher by 1%<sup>33</sup> in a 2% chlorhexidine solution. This difference may be influenced by the number of samples using a micropipette and the homogeneity between the solution and the anti-bacterial sample, so there is a risk of confusion in preparing various anti-bacterial concentrations.

The results showed that the MIC and MBC chlorhexidine activ point™ were higher than chlorhexidine gel™ because the point preparations were rigid which made it difficult for chlorhexidine ions to be released. Besides that, the natrosol content is biocompatible and water-soluble, causing Chlorhexidine ion gel™ to have longer contact with bacteria.<sup>12,34</sup> A 2% chlorhexidine solution has the lowest MIC and MBC because chlorhexidine ions are released more quickly in liquid preparations, so a lower concentration can inhibit growth and kill *E. faecalis*.<sup>29</sup> Chlorhexidine gel™ and activ point™ are used as intra-canal medicaments because

they can eliminate bacteria and their by-products within 24 hours in vitro.<sup>35</sup>

MIC and MBC of chlorhexidine gel™ smaller than chlorhexidine activ point™, but larger than 2% chlorhexidine solution. At low concentrations, chlorhexidine will bind to the bacterial cell wall membrane which can affect the osmotic pressure balance of the bacterial cell and stop the bacterial replication process. However, at high concentrations, the positive molecules of CHX will bind to the negative molecules on the bacterial cell wall membrane and changing the osmotic pressure of the bacterial cytoplasm and resulting in cell lysis.<sup>36,37</sup>

Chlorhexidine activ point™ is effectively used in endodontic treatment in cases of the open apex and the presence of periapical inflammation because it is easy to insert or remove from the root canal and the length of action can be estimated. The use of 2% chlorhexidine solution as an intra-canal medicament has the risk of being able to pass the apical foramen of the tooth so that it cannot come into contact with the root canal wall. This causes the remaining bacteria attached to the root canal walls not to disappear and the chlorhexidine solution that exits apically can cause inflammation.<sup>38,39</sup>

## Conclusions

There was a significant difference in the average number of bacterial colonies on days 1, 3, 7, and 14 in the chlorhexidine gel™ and activ point™ groups, with a decrease in the number of bacterial colonies more in the chlorhexidine gel. The minimum inhibitory concentration of chlorhexidine gel™ against *E. faecalis* was 0.5%, while chlorhexidine activ point™ was 1.25%. The minimum bactericidal concentration of chlorhexidine gel™ for *E. faecalis* is 1%, while chlorhexidine activ point™ is 2.5%. Chlorhexidine gel has better antibacterial properties and is more effective as an intra-canal medicament against *E. faecalis* (ATCC 29212).

## Acknowledgements

This Study was Funded by The Competence Research of Universitas Padjadjaran Lecturers 2019

## Declaration of Interest

The authors report no conflict of interest.

## References

1. Singh H, Kur M, Dhillon J, Saini M, Kaushal A. A Comparative Evaluation of Antibacterial Efficacy of 'Calcium Hydroxide Plus Points', 'Active Points' and 'Combi Point' Against *Enterococcus Faecalis* in Endodontic Therapy: an in Vitro Analysis. *J Dental Health, Oral Disorders & Therapy*. 2015; 2(5):1-5
2. Naik B, Shetty S, Yeli M. Antimicrobial Activity of Gutta Percha Point Containing Root Canal Medications Against *E. faecalis* and *Candida Albicans* in Simulated Root Canal – an in Vitro Study. *J Endodontology*. 2013; 25(2):1-10
3. Murvindran V, James DR. Antibiotics as an Intracanal Medicament in Endodontics. *J Pharmaceutical Sciences and Research*. 2014; 6(9): 297- 301
4. Singh H, Kapoor P. A Comparative Evaluation of Antibacterial Efficacy of 'Active Point' and 'Combi Point' as Intra – Canal Medicaments Against *Enterococcus Faecalis*: an Ex Vivo Study. *J OHDM* 2014; 13(2): 1-7
5. Kusgoz A, Ozcan E, Arslan I, Inci M. Antibacterial Activity of Calcium Hydroxide Combined With Triple Antibiotic Pasta Against *Enterococcus Faecalis*; an in Vitro Study. *J Cumhuriyet Dent*. 2013; 16(1): 25-30.
6. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus Faecalis*: its Root Treatment Failure and Current Concepts in Treatment. *Journal of Endodontics*. 2006; 31: 93- 98
7. Bhardwaj BB. Role of *Enterococcus Faecalis* in Failure of Endodontic Treatment. *Int. J. Curr. Microbial. App. Sci* 2013; 2(8); 272–277
8. Gonçalves LS, Rodrigues RCV, Andrade Junior CV, Soares RG, Vettore MV. The effect of sodium hypochlorite and chlorhexidine as irrigant solutions for root canal disinfection: A systematic review of clinical trials. *J Endod*. 2016; 42(4): 531.
9. Wang Z, Shen Y, Haapasalo M. Dentin extends the antibacterial effect of endodontic sealers against *Enterococcus faecalis* biofilms. *J Endod*. 2014; 40(4): 505–8.
10. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part I. In vitro studies. *Restor Dent Endod*. 2014 Nov; 39(4): 241–52.
11. Samanth SA, Varghese SS. The most effective concentration of chlorhexidine as a mouthwash- systematic review. *J Pharm Sci Res*. 2017; 9(2): 233–6.
12. Sharaan M. An evaluation of antimicrobial effect of calcium hydroxide gutta percha points and chlorhexidine points in the treatment of infected root canals (In Vivo Study). *Gulf Med J*. 2014; 3(in vivo. guttapercha containing caoh and chx): 211–21.
13. Tandan M, Hegde MN, Hegde P. Effect of four different intracanal medicaments on the apical seal of the root canal system: A dye extraction study. *Indian J Dent Res*. 2014; 25(5): 607–12
14. Fiorillo L. Chlorhexidine gel use in the oral district: A systematic review. *Gels*. 2019; 5(2): 1–16.
15. Vasudeva A, Sinha DJ, Tyagi SP, Singh NN, Garg P, Upadhyay D. Disinfection of dentinal tubules with 2% Chlorhexidine gel, Calcium hydroxide and herbal intracanal medicaments against *Enterococcus faecalis*: An in-vitro study. *Singapore Dent J*. 2017; 38: 39–44.
16. Shrigondekar V, Pawar J, Gulve M, Samuel R, Kolhe S, Aher G. Comparative Evaluation of Antimicrobial Efficacy of Sodium Hypochlorite, Chlorhexidine, Neem and Green Tea Against *E. Faecalis* - an in Vitro Study. *Int J Adv Res*. 2019; 7(4): 449–53.
17. Stojanovic N, Kronic J, Mladenovic I, Stojanovic Z, Apostolska S, Zivkovic S. Influence of different forms of calcium hydroxide and chlorhexidine intracanal medicaments on the outcome of endodontic treatment of teeth with chronic apical periodontitis. *Srp Arh Celok Lek*. 2017; 146(3–4): 143–8.

18. Jhamb A, Masamatti V kumarS, Tiwari S, Nair D, Agarwal J, Chaurasia V. In vitro evaluation of antimicrobial activity of different Gutta-percha points and calcium hydroxide pastes. *J Int Soc Prev Community Dent* [Internet]. 2014 May; 4(2): 92. Available from: <http://www.jispcd.org/text.asp?2014/4/2/92/137648>
19. Mozayeni MA, Haeri A, Dianat O, Jafari AR. Antimicrobial Effects of Four Intracanal Medicaments on Enterococcus Faecalis: An in Vitro Study. *J Iranian Endodontic*. 2014; 9 (3): 195-198.
20. Rodrigues.C, V Oporto.G. Clinical Implications of Enterococcus Faecalis Microbial Contamination in Root Canals of Devitalized Teeth. Literature Review. *Revista Odontologia Mexicana*, July-September 2015; 1(19): 1-6
21. Suchitra.U, Kundabala.M. Enterococcus Faecalis: An Endodontic Pathogen. Department of Conservative Dentistry, Manipal College of Dental Science, Mangalore. 2006; 18(11): 1-12
22. Gomes.BPFA, Vinna. ME, Zaia.AA. Chlorhexidine in Endodontics. *J Brazilian Dental* 2013; 21(2): 89-102.
23. Boreak, N. Effect of Bio Mechanical Preparation on Endotoxin Levels in Patients with Chronic Apical Periodontitis. *Journal of International Dental and Medical Research*. 2021; 15(1):158-164. Available from : <http://www.iidmr.com>
24. Mohamed MA. The Effect of Experimental 2 % Chlorhexidine Gel Compared to Calcium Hydroxide Paste as Intracanal Medicaments on Fracture Resistance and Microhardness of Root Canal Dentin. A Thesis Submitted to the Faculty of Oral and Dental Medicine, Cairo University, in Partial Fulfillment of Requirement for Master Degree in Endodontics. 2006; 1(1): 13-65
25. Parveen. N, Anjum.NA, Lal.V. Ahmed. B. Effectiveness of 2% Chlorhexidine Gel in Reducing Intracanal Bacterial Count. *J Pakistan Oral & Dental* .2014; 43(2): 2-6
26. Tandan. M, Hegde. MN, Hegde. P. Effect Four Different Intracanal Medicaments on the Apical Seal of the Root Canal System: a Dye Extraction Study. *J Indian Dental Research* , 2014: 25(5): 1-8
27. Gomes Bp, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L, et al. Effectiveness of 2% Chlorhexidine Gel and Calcium Hydroxide Against Enterococcus Faecalis in Bovine Root Dentine in Vitro. *Int Endod J*. 2003; 36: 267-75.
28. Stojanovic N, Kronic J, Mladenovic.at al. Influence Different froms of Calcium Hydroxide and Chlorhexidine Intracanal Medicaments Outcome of Endodontic Treatment of Teeth with Chronic Apical Periodontitis. Original Article Departement of Restorative Dentistry Endodontic. 2017; 1(1): 9. Available from: <http://doi.org/10.2298/SARH170221139s>
29. Babickaite, Ramanauskienė, Grigonis, Vaskienė, Daunoras, Klimiene, et al. DETERMINATION OF ANTIMICROBIAL ACTIVITY OF CHLORHEXIDINE GEL. *Acta Pol Pharm*. 2016; 73(6): 1623.
30. Joy Sinha D, D S Nandha K, Jaiswal N, Vasudeva A, Prabha Tyagi S, Pratap Singh U. Antibacterial Effect of Azadirachta indica (Neem) or Curcuma longa (Turmeric) against Enterococcus faecalis Compared with That of 5% Sodium Hypochlorite or 2% Chlorhexidine in vitro. *Bull Tokyo Dent Coll*. 2017; 58(2): 103–9.
31. Li J, Xie S, Ahmed S, Wang F, Gu Y, Zhang C, et al. Antimicrobial activity and resistance: Influencing factors. *Front Pharmacol*. 2017; 8(JUN): 1–11.
32. Karpiński TM, Szkaradkiewicz AK. Chlorhexidine - Pharmacobiological activity and application. *Eur Rev Med Pharmacol Sci*. 2015; 19(7): 1321–6.
33. Kohli D, Hugar SM, Bhat KG, Shah PP, Mundada M V, Chandrashekar M Badakar. Comparative evaluation of the antimicrobial susceptibility and cytotoxicity of husk extract of Cocos nucifera and chlorhexidine as irrigating solutions against Enterococcus Faecalis, Prevotella Intermedia and Porphyromonas Gingivalis – An in-vitro study. *J Indian Soc Pedod Prev Dent*. 2018; 36(2): 142–50.
34. Bhandari S, T S A, Patil CR. An in Vitro Evaluation of Antimicrobial Efficacy of 2% Chlorhexidine Gel, Propolis and Calcium Hydroxide Against Enterococcus faecalis in Human Root Dentin. *J Clin Diagn Res*. 2014/11/20. 2014 Nov; 8(11): ZC60–3.
35. Abbaszadegan A, Gholami A, Ghahramani Y, Ghareghan R, Ghareghan M, Kazemi A, et al. Antimicrobial and cytotoxic activity of cuminum cuminum as an intracanal medicament compared to chlorhexidine gel. *Iran Endod J*. 2016; 11(1): 44–50.
36. Amoian B, Omidbakhsh M, Khafri S. The clinical evaluation of Vi-one chlorhexidine mouthwash on plaque-induced gingivitis: A double-blind randomized clinical trial. *Electron Physician*. 2017; 9(9): 5223–8.
37. Vishnu Prasanna SG, Lakshmanan R. Characteristics, Uses and Side effects of Chlorhexidine-A Review. *IOSR J Dent Med Sci e-ISSN* [Internet]. 2016; 15(6): 57–9. Available from: [www.iosrjournals.org](http://www.iosrjournals.org)
38. Kumar A, Tamanna S, Iftekhhar H. Intracanal medicaments - Their Use in Modern Endodontics: A Narrative review. *J Oral Res Rev*. 2019; 11: 89–90.
39. Gandhi K, Maganti RS, Kaur H, Vinod KS, Verma P. Formulation and evaluation of Sol-Gel drug delivery system for intracanal pH sensitive controlled delivery of chlorhexidine. *J Clin Diagnostic Res*. 2017; 11(4): ZC68–72.