Uptake and release of chlorhexidine by bovine pulp and dentin specimens and their subsequent acquisition of antibacterial properties

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Bovine pulp and dentin specimens were treated with either a 0.02 or 1.00 percent solution of chlorhexidine for either 20 or 40 minutes. Culture determination of the acquisition of antibacterial properties by the treated specimens immediately and 1 week after the treatment was evaluated using the test organism *Streptococcus faecalis*. It was concluded that chlorhexidine is a potent antibacterial agent under the test conditions and that its use as an endodontic irrigating solution should be further evaluated.

The endodontic "triad" of success involves biomechanical preparation, microbial control, and complete obturation of the root canal space. Frostell[1] reported a significantly higher failure rate in teeth infected at the appointment when the root filling is performed. The importance of microbial control to the over-all success of endodontic therapy is supported by other investigators.[2–8] However, there is considerable controversy over the value of the culture test in predicting success. Seltzer and associates[9] showed that the bacteriologic status of the root canal, as determined by cultures, did not influence repair of the periapical tissues. Their findings are supported by other investigators.[10–12] On the other hand, Oliet and Sorin[7] reported a greater degree of healing when teeth were filled under negative culture conditions, and their work is also supported by others.[2–8]

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Although opinions differ on culturing, it can be agreed that contamination and infection in general play a role in diminishing the over-all success rates in endodontics and that the number of potentially pathogenic bacteria must be reduced substantially to promote healing.

Since the dentist is attempting to eliminate most, if not all, contamination and infection from the root canal system prior to filling, it would seem advantageous to have a more reliable method of ensuring immediate and continued bacterial control. This might be accomplished with an antiseptic irrigant, used in conjunction with filing procedures, which could render the dentin walls, pulp fragments, and any remaining organic debris free of viable bacteria. A prolonged, residual antibacterial effect transferred to these tissues would help reduce or eliminate bacterial activity throughout the course of treatment. With this accomplished, a higher percentage of clinically predictable success might be achieved.

The purpose of this investigation was to determine whether chlorhexidine is adsorbed, and then released from, bovine pulp and dentin specimens and whether these specimens acquire antibacterial properties as a consequence of exposure to chlorhexidine.

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Table I. Chlorhexidine uptake and release by bovine pulp specimens

Group*	Mean specimen weight (mg.)	Chlorhexidine uptake (mean μg. chlorhexidine/mg. pulp tissue ± SE)	Chlorhexidine release (mean μg. chlorhexidine/mg. pulp tissue ± SE)	
			After 1 hour	After 20 hours§
A	71.4	26.4 ± 2.5	1.6 ± 0.2	1.6 ± 0.2
B	72.3	26.5 ± 2.2	2.2 ± 0.3	1.7 ± 0.2
C	72.2	15.8 ± 1.5†	1.7 ± 0.2	1.4 ± 0.2
D	64.2	29.5 ± 1.6	3.0 ± 0.2‡	1.7 ± 0.2

*See text for description of groups.
†Significantly lower than the other groups ($p < 0.005$).
‡Significantly higher than the other groups ($p < 0.05$).
§All groups statistically similar.

METHODS AND MATERIALS

Uptake of chlorhexidine

Forty specimens of dental pulp tissue obtained from the crowns and roots of freshly extracted bovine teeth were weighed. Forty blocks of bovine dentin, about 5.5 × 4.5× 1.5 mm., were sanded smooth under a water spray to remove any predentin, cementum, or attached organic debris. The length, width, and thickness of each block were measured with micrometer calipers to determine the surface area of each block. The weight and surface area data were used to express the uptake and release of chlorhexidine per milligram of pulp tissue or per square millimeter of dentin surface, respectively.

The pulp and dentin specimens were placed in screw-cap tubes and randomly divided into four equal groups. Five milliliters of 0.2 percent chlorhexidine* was added to the tubes of Groups A and C. One milliliter of 1 percent chlorhexidine was added to the tubes of Groups B and D. Two additional tubes with chlorhexidine solution but wihout pulp or dentin specimens served as controls. After a treatment period of 20 minutes (Groups A and B) or 40 minutes (Groups C and D), the solution from each tube was transferred to a 10 ml. flask. The tubes were then washed with 5 ml. deionized water and the washing solution was transferred to the flasks. Enough deionized water was added to each flask to adjust the final volume to 10 ml. One milliliter of the solution in each flask was then transferred to screw-cap tubes, and 5 ml. of deionized water and 0.5 ml. of 0.25 percent NaOCL solution was added. The tubes were shaken and allowed to stand for 30 minutes. They were then shaken again and the chlorhexidine concentration was immediately evaluated colorimetrically in a Klett-Summerson photoelectric colorimeter,* previously zeroed with a water blank, using filter No. 42.

*Hibitane Imperial Chemical Industries, Limited, Macclesfield, Chesire, England.

Release of chlorhexidine

To assess chlorhexidine release from the treated pulp and dentin specimens, 5 ml. of deionized water was added to the tubes containing the original specimens and to the control tubes without specimens. The specimens were allowed to stand in the water for 1 hour or 20 hours. One milliliter of each test solution was transferred to screw-cap tubes, and the amount of chlorhexidine released was analyzed as described above.

Acquisition of antibacterial properties by the chlorhexidine-treated specimens

Fifty pulp specimens and fifty dentin specimens were sterilized with ethylene oxide. Following appropriate aeration, the specimens were randomly divided into five equal groups. Group A and C specimens were treated with 0.2 percent chlorhexidine solution for 20 and 40 minutes, respectively. Group B and D specimens were treated with 1 percent chlorhemidine solution for similar periods. Positive control specimens were treated with sterile saline solution for 30 minutes. Five pulp and five dentin specimens from each group were then transferred to sterile Petri dishes and incubated at 37° C. for 1 week. The remaining pulp and dentin specimens were transferred to sterile Petri dishes and inoculated with a 24-hour culture of *Streptococcus faecalis* grown in trypticase-soy broth containing 0.25 percent dextrose. Each inoculum culture was standardized to exhibit an absorbance of 0.50 at 520 nm. in a Coleman spectrophotometer.† The inoculated specimens were incubated at 37° C. for 1 hour to facilitate

*Klett Mfg. Co., New York, N. Y.
†Coleman Junior II-A Linear Absorbance Spectrophotometer, Model 620, Coleman Industries, Maywood, Ill.

Table II. Chlorhexidine uptake and release by bovine dentin specimens

Group*	Mean specimen weight (mg.)	Chlorhexidine uptake (mean μg. chlorhexidine/mm.² dentin specimen ± SE)	Chlorhexidine release (mean μg. chlorhexidine/mm.² dentin specimen ± SE)	
			After 1 hour	After 20 hours§
A	82.34	6.4 ± 1.2	1.6 ± 0.1‡	1.7 ± 0.1
B	83.38	12.7 ± 1.3†	2.0 ± 0.1	1.7 ± 0.1
C	80.82	9.3 ± 1.4	1.7 ± 0.3	1.9 ± 0.1
D	80.04	6.2 ± 1.8	1.8 ± 0.1	1.8 ± 0.1

*See text for group descriptions.
†Significantly higher than groups A and D ($p < 0.01$).
‡Significantly lower than group B ($p < 0.025$).
§All groups statistically similar.

Table III. Antibacterial activity of specimens inoculated with *streptococcus faecalis* immediately after treatment

Group	Specimen treatment solution	Treatment time (minutes)	Mean Klett reading ± SE*	
			Pulp	Dentin
A	0.2 percent Chlorhexidine	20	160 ± 1.0	175 ± 8.1
B	1.0 percent Chlorhexidine	20	162 ± 0.7	164 ± 1.7
C	0.2 percent Chlorhexidine	40	160 ± 0.8	170 ± 3.6
D	1.0 percent Chlorhexidine	40	178 ± 6.3	164 ± 0.3
E	Sterile saline	30	402 ± 1.3	371 ± 3.2
Positive control				
Negative control†			158 ± 0.6	158 ± 0.6

*All groups and negative controls were significantly different from the positive control ($p < 0.001$).
†Sterile growth medium without specimens.

implantation of the organisms onto the specimens. The specimens were then transferred to screw-cap tubes containing 7 ml. of the culture medium and incubated at 37° C. for 24 hours. The degree of bacterial growth was determined by measuring the turbidity of the cultures in the Klett-Summerson colorimeter. The base turbidity of sterile culture medium was used as the negative control.

At the end of 1 week the Petri dishes containing the other half of the originally treated pulp and dentin specimens were removed from the incubator and assayed for the acquisition of antibacterial properties as outlined above. The *t* test was used for statistical analysis of the data.

RESULTS

Uptake and release of chlorhexidine with pulp specimens. As Table I shows, the mean chlorhexidine uptake for the four groups was 24.6 μg./mg. pulp tissue. The mean 1-hour and 20-hour chlorhexidine release was 2.1 and 1.6 μg./mg. pulp tissue, respectively. The highest uptake and release, 29.5, 3.0, and 1.7μg./mg. respectively, were from specimens in Group D, treated with 1.0 percent chlorhexidine solutions for 40 minutes.

Uptake and release of chlorhexidine with dentin specimens. The mean chlorhexidine uptake for the four groups was 8.7 μg./mm.² dentin surface (Table II). The mean 1-hour and 20-hour chlorhexidine release was 1.8 μg./mm.² dentin surface.

Acquisition of antibacterial properties by the specimens immediately after treatment. As indicated in Table III, the average Klett reading for pulp specimens was 165, compared to the negative control sterile medium reading of 158. All groups and negative controls were significantly lower than the positive control. For dentin specimens the average Klett reading was 168. All groups and negative control values were significantly lower than the positive control.

Acquisition of antibacterial properties by the specimens 1 week after completing the treatment. For the pulp specimens the average Klett reading was 168, and for the dentin specimens it was 160 (Table IV). In the case of both pulp and dentin specimens all groups and negative controls had significantly lower Klett values than the positive control.

The findings of this study indicate that chlorhexidine has excellent potential as an intracanal antibacterial agent. The concentrations of chlorhexidine chosen for this study (0.2 and 1.0 percent) were based on past

Table IV. Antibacterial activity of specimens inoculated with *Streptococcus faecalis* 1 week after treatment

Group	*Specimen treatment solution*	*Treatment time (minutes)*	*Mean Klett reading ± SE**	
			Pulp	*Dentin*
A	0.2% Chlorhexidine	20	158 ± 0.6	156 ± 0.7
B	1.0% Chlorhexidine	20	175 ± 2.0	164 ± 1.3
C	0.2% Chlorhexidine	40	167 ± 2.2	158 ± 0.8
D	1.0% Chlorhexidine	40	172 ± 1.8	161 ± 1.5
E	Sterile saline	30	402 ± 1.3	371 ± 3.2
Positive control				
Negative control†			158 ± 0.6	158 ± 0.6

*All groups and negative controls were significantly different from the positive control ($p < 0.001$).
†Sterile growth medium without specimens.

investigations of the effect of chlorhexidine on dental plaque formation. Loe and Rindom Schiott[14] determined that two daily rinses with 0.2 percent solution of chlorhexidine gluconate effectively prevented plaque formation but that two rinses with a 0.1 percent concentration were not effective. In the present study the 1.0 percent concentration was tested to determine if higher concentrations would result in increased antibacterial properties. The specimens were exposed to the chlorhexidine solutions for periods of 20 and 40 minutes to represent an estimated clinical treatment period for cleansing and shaping the canals of single and multirooted teeth, respectively.

The results of the uptake and release of chlorhexidine by pulp and dentin specimens supported the findings of other investigators.[13, 15, 16] More variation was noticed in the uptake and release of chlorhexidine among the pulp specimens than the dentin specimens, possibly because of the wide range of weights in the pulp specimens. The surface area available for interaction with chlorhexidine does not increase linearly with an increase in weight. For example, a pulp specimen weighing 100 mg. will not necessarily have twice the surface area of a specimen weighing 50 mg. Therefore a heavier pulp specimen would be expected to exhibit a correspondingly lower chlorhexidine uptake when it is expressed as micrograms chlorhexidine per milligram tissue. Although the mean specimen weights for groups A, B, and C were almost identical, individual weights varied from 31.4 to 130.5 mg. The lower mean specimen weight for group D may partially explain the higher uptake and release values it exhibited.

With the dentin specimens an effort was made to standardize their surface area by grinding them to approximately equal sizes. The closeness of the individual and mean specimen area values of the different groups eliminated the variable of dissimilar surface area and may have accounted for the greater stability of uptake and release values for the dentin treatment groups. The dentin specimens were sanded smooth before treatment so that any predentin, cementum, or organic debris would be removed. This may account for the lower uptake values recorded for the dentin specimens as compared to the pulp specimens, since the latter were more organic and proteinaceous in nature.

Rolla and associates[15] investigated the interaction of chlorhexidine with hydroxyapatite, the tooth surface, and salivary proteins. The results indicated that chlorhexidine gluconate adsorbs to hydroxyapatite, tooth surface, and salivary mucins and that the adsorbed chlorhexidine is released when the concentration in the environment is low. The authors stated that the interaction of chlorhexidine with acidic proteins may be of greater significance than its affinity for hydroxyapatite.

For an evaluation of the acquisition of antibacterial properties by the chlorhexidine-treated specimens, *Streptococcoccus faecalis* was chosen as the test organism because it is a common isolate from infected root canals.[17] The organism is easy to culture and grows rapidly to full turbidity.

No consistent differences were noted between the treatment groups to indicate that one concentration of chlorhexidine or treatment period was superior to the other in conferring antibacterial properties on treated specimens. Essentially identical antibacterial properties were expressed by pulp and dentin specimens when analyzed immediately after treatment. Therefore it appeared that the treated pulp and dentin specimens acquired similar antibacterial activity. When analyzed 1 week after completing the treatments, the differences between the treatment groups were not of a magnitude or consistency to indicate that one was superior to another in conferring antibacterial properties. Evidently, neither the antibacterial properties of chlorhexidine on the specimen surfaces nor the release of chlorhexidine into solution from the specimen surfaces was diminished due to incubation and storage for 1 week.

It is important at this point to consider the data from a microbiologic and a clinical viewpoint. The mean Klett reading for the negative control (sterile medium)

was 158. The values obtained for the pulp and dentin specimens, both immediately and after 1 week of storage, ranged from 160 to 168. The slight differences between negative control and experimental readings were probably due to the presence of debris from the pulp tissue itself. This means that in all likelihood no bacterial growth occurred in the tubes where the treated specimens were incubated. In other words, treatment with chlorhexidine prevented these specimens from becoming infected when they were placed in contact with *Streptococcus faecalis*. Confirmation of this point was provided by Klett readings obtained after incubating the experimental cultures for 48 to 72 hours. The Klett reading remained essentially the same.

This investigation takes on further meaning when clinical implications are considered from an endodontic viewpoint. Given the following assumptions based on rough estimates, certain speculations can be made. Assume the length of an average root canal to be 16 mm. and the width at midlevel to be 1 mm. The volume of the root canal, representing the maximum volume of intracanal fluid (equivalent to culture medium), would be approximately 13 $mm.^3$. The surface area of the dentin walls of the root canal, representing the area of dentin available to interact with chlorhexidine, would be approximately 52 $mm.^2$. The volume of the culture medium in the experimental test tubes was 7 ml. or 7,000 $mm.^3$. The average area of the dentin specimens was 82 $mm.^2$. These assumptions can be analyzed mathematically by comparing the concentration of chlorhexidine released by the 82 $mm.^2$ of the dentin specimens in 7,000 $mm.^3$ of medium vs. the amount released from 52 $mm.^2$ of root canal surface in a maximum 13 $mm.^3$ of medium. A simple computation demonstrates that the concentration of chlorhexidine in the root canal fluid can be expected to be at least 340 times as great as in the experimental tubes, and much more if one considers that it would be extremely unlikely that all the volume of the canal would be occupied by fluid. The antibacterial properties would be increased even more when the amount of chlorhexidine released by pulp remnants is added to that released by dentin.

CONCLUSIONS

1. Chlorhexidine was taken up by and released from bovine pulp and dentin.

2. As a consequence of their exposure to chlorhexidine, the pulp and dentin specimens acquired antibacterial properties which completely inhibit the growth of *Streptococcus faecalis*.

3. The acquisition of antibacterial properties by the treated pulp and dentin specimens 1 week after completing the treatments was unchanged when compared to immediate evaluation after treatment.

4. The treated pulp and dentin specimens acquired equal antibacterial properties.

5. No treatment method tested was superior to another in conferring antibacterial properties to the specimens.

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