Effects of Temperature and Hypochlorite Concentration on the Rate of Collagen Dissolution

Diana Dumitriu, DMD, MSc,* and Tanase Dobre, PbD†

Abstract

Introduction: The aim of this research was to quantify the effects of different temperature on the rate of collagen dissolution in different concentrations of sodium hypochlorite solution. Methods: A customized laboratory apparatus was used to immerse 23 samples of reticulated collagen matrices and 20 samples of non-reticulated collagen matrices in a continuously agitated solution of 1%–5% (m/v) sodium hypochlorite at 20°C–35°C. The time needed for complete dissolution of each sample was used to generate mathematical equations that described the effects of temperature and hypochlorite concentration on the dissolution of collagen. Results: Both temperature and concentration of sodium hypochlorite were positively correlated with the rate of collagen dissolution. The same speed of dissolution was obtained for 5% sodium hypochlorite at 20°C, 4% solution at 20.8°C, 3% solution at 23.5°C, 2% solution at 26.9°C, and 1% solution at 36°C. Conclusions: Treatment protocols that involve sodium hypochlorite, which is the preferred root canal irrigant, should consider the important effects caused by variations in the use of different irrigant temperatures and concentrations. Further investigation is required to establish the optimal concentration and temperature of sodium hypochlorite solution for root canal irrigation. (J Endod 2015;41:903–906)

Key Words
Collagen, root canal irrigant, sodium hypochlorite, speed of dissolution, tissue dissolution

The aim of root canal treatment is to obtain germ-free and compact-filled canals to prevent diseases such as apical periodontitis. To reach this goal, the soft organic tissue content of the canal must be removed. This is achieved by irrigation of the parts of the canal that cannot be reached by mechanical means. The tissue-dissolving ability of an irrigant is important because it enables the removal of pulp and pulp remnants from the root canal.

Sodium hypochlorite solution has long been advocated as the preferred irrigant for root canal irrigation owing to its tissue-dissolving and antimicrobial activities. To date, there has been no suggestion in the literature about which temperature and concentration of sodium hypochlorite solution are optimal for root canal irrigation. Many authors have demonstrated that sodium hypochlorite dissolves pulp tissue. Others have shown that increasing the concentration or temperature of the solution appears to enhance the solvent effect on pulp tissue. The pH of the solution does not influence the tissue-dissolving ability of the sodium hypochlorite solution. Immersing organic tissue samples in pots filled with sodium hypochlorite solution and a large specimen contact surface increase the ability of sodium hypochlorite to dissolve organic tissue.

Collagen is abundant in the dental pulp and in the vicinity of the canal wall. Because collagen is one of the most compact proteins found within soft tissues, its use in experimental settings provides a worst-case scenario on which to test various parameters. Accordingly, collagen has been used as a substitute for dental pulp in studies to investigate the dissolution effect of sodium hypochlorite. Dissolution of collagen samples appears to occur in two major stages as follows:

1. The surface stage, which involves fragmentation of collagen in fibrils, followed by their liberation into the liquid phase.
2. The liquid phase, which involves hydrolysis of the fibrils into amino acids and their neutralization by conversion to salts and chloramines.

All of the studies that have investigated the variables that affect the dissolution of collagen by sodium hypochlorite have investigated the effects of each factor in isolation. Few of the aforementioned studies have used agitated sodium hypochlorite solution to represent the solution flow in the root canal. The relationship between temperature and sodium hypochlorite concentration that affects the rate of pulp dissolution has yet to be established. The aim of the present study was to obtain a mathematical equation that described the dependence of the rate of tissue dissolution on the temperature and concentration of agitated sodium hypochlorite solution. Knowledge of the correlation between these factors will enable us to choose the optimal correlation between temperature and concentration of sodium hypochlorite solution that will maximize the rate of collagen dissolution.

Materials and Methods

Reticulated collagen matrix (RCG) and non-reticulated collagen matrix (NRCG) samples were obtained from Bucharest Leather Research Institute (Bucharest, Romania). The RCG samples had 25 ± 3 μm fiber diameter, and the NRCG samples had 23 ± 4 μm fiber diameter. The porosity was 0.87 m³/1 m³ for RCG and 0.88 m³/1 m³ for NRCG. The RCG samples contained 3% water and 1% impurities, whereas NRCG samples had 4% water and 3% impurities. All specimens were 3 mm thick.

Sodium hypochlorite aqueous solutions at 1%–5% concentrations were prepared from 10% sodium hypochlorite solution (Merck KGaA, Darmstadt, Germany). All other
reagents used for iodometric concentration determination were of analytical quality. Sodium hypochlorite solution was agitated and heated by using a Falc Heidolf magnetic agitator (model AG2; Nitech, Bucharest, Romania) that had a built-in thermostat. The pH and chloride concentration were determined by using a Jenway model 3310 pH meter with incorporated ionometer (Fisher Scientific, Loughborough, UK), which was equipped with specific electrodes (FB61546 for pH and PH-905-014P for chloride ion concentration) and a temperature probe.

All the samples were weighed precisely by using a Kern & Sohn analytical balance (model ACS/ACJ; Balingen, Germany).

Twenty-three samples of RCG were divided into 5 groups, 3 containing 5 samples and 2 containing 4 samples. Whereas 5 samples were used for each of the 1% (10 g/L), 2%, and 3% sodium hypochlorite treatments, 4 samples were used for each of the 4% and 5% sodium hypochlorite treatments.

The 20 samples of NRCG were divided into 5 groups, each of which contained 4 samples dissolved into concentrations of 1%, 2%, 3%, 4%, or 5% sodium hypochlorite.

For all of the dissolved samples, the temperature of the sodium hypochlorite solution varied from 21.9°C to 34.1°C. The pH of the solution varied with a range of 12.9–13.9. The solution was heated. The temperature and concentration of the sodium hypochlorite solution and the mass of the samples were recorded before dissolving each sample.

The method used to dissolve each sample was as follows:

1. The collagen matrix samples were cut to dimensions of 5 mm long, 3 mm wide, and 2 mm thick.
2. Samples were weighed and the mass was recorded.
3. 250 mL sodium hypochlorite solution was prepared at the desired dilution, placed in a glass beaker on the Falc Heidolf magnetic heater agitator, and the pH ionometer temperature probe was placed in the solution.
4. The temperature was set, and the solution was agitated to the speed of 1 m/s.
5. The samples were placed on a glass probe, immersed in solution, and maintained in position.
6. The chronometer was started and watched until the sample completely disappeared.
7. The time, pH of the solution, and voltage of the ionic chloride probe were recorded. After the dissolution of each group of samples, the concentration of the solution was tested by iodometric titration. The test results were recorded in tables and represented in Figure 2 and Figure 3.
The time for complete dissolution of RCG and NRCG samples by using various concentrations and temperatures of sodium hypochlorite solution was recorded for each sample. The mass of the sample was divided by the time, which yielded the rate of dissolution that was recorded.

**Results**

The rate of dissolution was calculated for each sample, and the results are shown in Figures 1 and 2. Comparison of these figures shows that the rate of dissolution for RCG was an order of magnitude slower than that for NRCG. The concentration and temperature of the hypochlorite solution both had a positive influence on the dissolution rate. The groups of RCG were compared by using Student test two-tailed two-sample unequal variance. Statistically significant differences \( P < .05 \) were found between group 1% and groups 4% and 5%.

The same test showed significant difference when group 1% of NRCG was compared with the rest of the groups \( P < .05 \). To evaluate the influence of temperature on the rate of collagen dissolution, the results for RCG were arranged in 2 groups corresponding to temperatures below and above 30°C. The Student test revealed statistically significant differences \( P = .01 \). The same method was used for NRCG, but there was no statistical difference \( P = .55 \).

Although the relationship between increasing temperature and the rate of dissolution was exponential, the relationship between increasing hypochlorite concentration and the rate of dissolution was defined by a power function. The following equation describes the dependence of dissolution rate on temperature and concentration.

\[
G_{md} = k \exp \left( \alpha \left( 1 - \frac{t_0}{T} \right) \right) \left( \frac{c_{hp}}{c_0} \right)^m \quad \text{(equation 1)}
\]

The lowest temperature \( t_0 \) was 20°C, and the lowest concentration \( c_0 \) was 1% (10 g/L).

The parameters \( k, \alpha, \) and \( m \) were unknown. They were determined by using a method for the identification of parameters that requires the availability of experimental results. The minimizing of function given by equation (2) gave the unknowns \( k, \alpha, \) and \( m \). Equations (3) and (4) show the dependence of the rate of dissolution on the concentration and temperature of the hypochlorite solution for the RCG and NRCG samples tested.

\[
F(k, \alpha, m) = \sum_{i=1}^{n} \left( G_{md, i} - k \exp \left( \alpha \left( 1 - \frac{t_0}{T_i} \right) \right) \left( \frac{c_{hp, i}}{c_0} \right)^m \right)^2
\quad \text{(equation 2)}
\]

\[
G_{md}^{(RCG)} = 2.58410^{-5} c_{hp}^{1.17} \exp \left( 4.252 \left( 1 - \frac{t_0}{T} \right) \right) \quad \text{(equation 3)}
\]

\[
G_{md}^{(NRCG)} = 1.76610^{-4} c_{hp}^{1.15} \exp \left( 1.092 \left( 1 - \frac{t_0}{T} \right) \right) \quad \text{(equation 4)}
\]

To check the validity of parameter identification, the values obtained by equations 3 and 4 for rate of dissolution are represented alongside the values obtained experimentally (Fig. 3). The plots are aligned diagonally; therefore, validation of the parameter identification was confirmed.

Having verified the relationships between hypochlorite concentration, solution temperature, and rate of collagen dissolution, we were able to enter various values within the practical range of temperatures.
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hypoiodite concentrations and temperatures to test the predictive value of the equation.

To determine the best combination of concentration and temperature for optimal pulp dissolution, equation 3 was used to draw the graphs in Figure 4.

The value $g_{\text{m,diss}} = 10.5 \text{ mg/min}$ was obtained for 5% sodium hypoiodite at 20°C, which was the same as for 1% solution at 36°C. The same rate of dissolution was obtained for 5% sodium hypoiodite at 20°C, 4% solution at 20.8°C, 3% solution at 23.5°C, 2% solution at 26.9°C, and 1% solution at 36°C.

**Discussion**

The maximum temperature 34.1°C was chosen because it is reasonably easy to achieve in a clinical environment. However, because the method included mathematical handling of the data, we can predict the rate of dissolution at higher or lower temperatures. Because of the setting of the experiment it is difficult to maintain or achieve a given temperature. The solution is constantly agitated and heated. When the heating stops, the temperature immediately drops. Therefore, it would have been impossible to dissolve more samples at the same exact temperature. Instead, the temperature was recorded for each sample. However, there were samples dissolved in solutions of similar temperatures but different concentrations, which gives the experiment validity as per statistical results.

The samples were cut roughly the same size to have about the same weight. The aim was to obtain light samples to observe complete dissolution because it is less likely to be inaccurate compared with partial dissolution.

Because sodium hypoiodite is potentially toxic, with its toxicity increasing with increasing concentration (14), it is preferable to use a reduced concentration at a higher temperature, provided it delivers the same result. It is preferable to increase the temperature of the solution rather than the concentration of sodium hypoiodite, and devices to heat sodium hypoiodite have been advocated (15).

A previous study used bovine pulp tissue in sodium hypoiodite solution maintained at a fixed temperature without agitation (16). In that study, the dissolution rate was calculated by dividing the weight of the pulp tissue fragment by the dissolution time, which gave a value $g_{\text{m,diss}} = 0.396 \text{ mg/min}$ for bovine pulp dissolution at 20°C in 0.5% sodium hypoiodite solution. By using equation 3 in the present study, the rate of collagen dissolution was calculated when the temperature was 20°C and the concentration was 0.5%. The result was $g_{\text{m,diss}} = 0.78 \text{ mg/min}$ for RNC. At the same temperature and concentration, the rate $g_{\text{m,diss}}$ was 4.7 mg/min. The value obtained for RCG was comparable to that obtained in the previous study, although it was higher, which could be explained by the agitation of the solution in the present study.

An equivalent value was calculated for the conditions used in a different study (17). The experimental result was 1.29 mg/min at 20°C and 1% concentration, and the result by using equation 3 was 1.54 mg/min. The results obtained by calculation were in proximity to the experimental values, which therefore proves the predictive value of equation 3. Furthermore, the dynamics of bovine pulp tissue seem to be similar to those of reticulated collagen.

**Conclusion**

The temperature and concentration of sodium hypoiodite solution both increase the rate of collagen dissolution. The same rate of dissolution was found for 5% sodium hypoiodite at 20°C, 4% solution at 20.8°C, 3% solution at 23.5°C, 2% solution at 26.9°C, and 1% solution at 36°C.

Further investigations are required to establish the dynamics of pulp dissolution in the root canal because factors besides the temperature and concentration of sodium hypoiodite are involved. The equation that describes the variation in the rate of collagen dissolution with temperature and concentration of sodium hypoiodite can be used in the future to design a mathematical model to simulate irrigation in the root canal and to design experiments to determine the optimal temperature and concentration of sodium hypoiodite solution for root canal irrigation. Collagen is well-suited for experimental simulation of root canal irrigation because it exhibits similar dissolution properties to those of bovine tooth pulp.

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**References**