

## Research Article



# A new phantom to evaluate the tissue dissolution ability of endodontic irrigants and activating devices

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### Conflict of Interest

No potential conflict of interest relevant to this  
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## ABSTRACT

**Objective:** The aim of this study was to introduce a gelatin/bovine serum albumin (BSA) tissue standard, which provides dissolution properties identical to those of biological tissues. Further, the study evaluated whether the utilization of endodontic activating devices led to enhanced phantom dissolution rates.

**Materials and Methods:** Bovine pulp tissue was obtained to determine a benchmark of tissue dissolution. The surface area and mass of samples were held constant while the ratio of gelatin and BSA were varied, ranging from 7.5% to 10% gelatin and 5% BSA. Each sample was placed in an individual test tube that was filled with an appropriate sodium hypochlorite solution for 1, 3, and 5 minutes, and then removed from the solution, blotted dry, and weighed again. The remaining tissue was calculated as the percent of initial tissue to determine the tissue dissolution rate. A radiopaque agent (sodium diatrizoate) and a fluorescent dye (methylene blue) were added to the phantom to allow easy quantification of phantom dissolution in a canal block model when activated using ultrasonic (EndoUltra) or sonic (EndoActivator) energy.

**Results:** The 9% gelatin + 5% BSA phantom showed statistically equivalent dissolution to bovine pulp tissue at all time intervals. Furthermore, the EndoUltra yielded significantly more phantom dissolution in the canal block than the EndoActivator or syringe irrigation.

**Conclusions:** Our phantom is comparable to biological tissue in terms of tissue dissolution and could be utilized for *in vitro* tests due to its injectability and detectability.

**Keywords:** Tissue dissolution; Phantom; Endodontic irrigants; Gelatin; Bovine serum albumin

## INTRODUCTION

Irrigation of the root canal system during endodontic therapy is an essential step in removing pulp tissue and debris and eradicating microorganisms from the complex root canal apparatus [1-3]. Currently, the irrigant of choice in endodontic therapy is sodium hypochlorite (NaOCl) solution due to its optimal tissue-dissolving capabilities and anti-microbial effectiveness [4,5]. Additionally, NaOCl serves as both a lubricant and a disinfectant, as it is capable of effectively dissolving necrotic pulp tissue and killing microbes in the canal system [6]. As an effective lubricant, NaOCl is able to penetrate the intricate structures of the canal

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and easily percolate through the root canal architecture due to its low viscosity, resulting in low friction between the instrument and root canal walls [7]. Furthermore, NaOCl is an effective disinfectant that is able to effectively rid the root canal system of organic pulp tissue, loose debris, and microbial biofilms [2,8].

Due to these desirable characteristics, numerous studies have measured and assessed the tissue-dissolving capabilities of NaOCl in various conditions [9-13]. While these studies independently hold significant merit, they used a wide range of different tissues, including bovine pulp tissue [14], rat abdominal tissue [15], porcine palatal mucosa [5,16], rabbit liver tissue [17,18], and bovine tendon collagen [19]. The variability in the results of these studies could be due to the varying experimental protocols, materials, and tissues used, making it difficult to draw clinically relevant conclusions [20]. The ideal biological tissue to investigate is dental pulp tissue because its histological characteristics are distinct from those of other anatomical tissues [21]. Dental pulp tissue is composed of loose, fibrous connective tissue with type I and type III collagen with a gelatinous consistency [22]. Bovine pulp tissue is more readily available and more easily preserved than other pulp tissues and is therefore a good benchmark for studies evaluating tissue dissolution [23].

The aim of this study was to introduce a gelatin/bovine serum albumin (BSA) tissue standard that can be used in lieu of pulp tissue to provide consistent results regarding the tissue dissolution properties of endodontic irrigants, including NaOCl. BSA and gelatin were used to mimic the dental pulp tissue in terms of its connective tissue composition and gelatinous consistency. Ultimately, this tissue-mimicking material could be potentially used in future research to standardize results when assessing different properties of endodontic medicaments. In this study, the tissue dissolution rate of this tissue phantom was evaluated in comparison to bovine pulp tissue by exposing various concentrations of the tissue phantom to NaOCl solution at 3-time intervals. In addition to its tissue-dissolution properties, the cleanliness of the developed tissue phantom material was examined with radiopaque fluorescent molecules.

## **MATERIALS AND METHODS**

### **Preparation of bovine pulp tissue**

Bovine pulp tissue was obtained to determine a benchmark of tissue dissolution properties. Bovine pulp tissue was extracted from bovine teeth by splitting the bovine teeth in half and elevating the pulp from the teeth with anatomic forceps. The bovine pulp tissue was stored at  $-15^{\circ}\text{C}$ . Next, the gelatin (G9391-500G, Type B, Sigma-Aldrich, St. Louis, MO, USA)/BSA (A2153-100 G, Sigma-Aldrich) tissue standard was created. This was also stored at  $-15^{\circ}\text{C}$ . Of note, at room temperature, this tissue standard gelatinizes in order to mimic the consistency of biological pulp tissue.

### **Tissue-mimicking procedure**

Rectangular samples of bovine pulp tissue with similar size ( $10 \times 4$  mm) and mass ( $45 \pm 3$  mg) and different compositions of tissue-mimicking material, composed of 5% BSA and 7.5%, 8.5%, 9%, or 10% gelatin ( $n = 10$ ) were prepared. Saline was used as a control group.

Then, 2 mL of 5.25% NaOCl solution (14.5%, Alfa Aesar Chemicals, Haverhill, MA, USA) as the root canal irrigant of choice was used at room temperature ( $25^{\circ}\text{C}$ ). Before immersing the

samples into the NaOCl, each sample was weighed using a precision balance (Mettler Toledo Co., Columbus, OH, USA). Each sample was then placed in an individual test tube filled with 2 mL of 5.25% NaOCl solution. The samples with different compositions were inserted into the test tubes for 1, 3, and 5 minutes each. The samples were then removed from the solution, gently blotted dry, and weighed again. The remaining tissue weight was calculated as the percent of the initial tissue weight in order to determine the tissue dissolution rate when using 5.25% NaOCl for different concentrations of the pulp tissue-mimicking material under different exposure times to the irrigant. The amount of tissue dissolution was compared between groups using the t-test for statistical analysis.

### Cleanliness procedure

Qualitatively, a radiopaque agent (sodium diatrizoate) and a fluorescent dye (methylene blue) were added to the phantom to enable easy quantification of the phantom. Methylene blue is strongly fluorescent, with an emission peak at 686 nm ( $\lambda_{\text{ex}}$ , 665 nm) [24]. However, it was first necessary to test the effect of these chemicals on phantom dissolution to ensure that it showed similar dissolution rates to the bovine pulp. The 9% gelatin (G9391-500 G, Teye B, Sigma-Aldrich) + 5% BSA (A2153-100 G, Sigma-Aldrich) phantom was injected into an endodontic block (GuttaCore 4-Canal Practice Block, Dentsply Sirona, Charlotte, NC, USA) with 1 main canal and 2 lateral canals. During the synthesis of the tissue-mimicking material, methylene blue (0.01%) (code: 229801000, lot No. A0362510, Acros Organics, Pittsburgh, PA, USA), was added to the composition; therefore, after injecting the material into the endodontic block, it was detectable under the fluorescent microscope.

Next, the endodontic block was instrumented using EndoSequence endodontic files up to size 40/04, with 2 mL of NaOCl irrigation after each file change. The canal was then irrigated using 3 different methods with 5 mL of NaOCl solution: 1) conventional syringe irrigation with NaOCl, 2) ultrasonic irrigation with EndoUltra, and 3) sonic irrigation with EndoActivator according to the manufacturer's instructions. Finally, the remaining tissue was observed under a fluorescent microscope. The obtained images were analyzed using the ImageJ program (LOCI, University of Wisconsin, Madison, WI, USA). The outcome variable determined was the amount of remaining tissue, which was calculated as a percentage of the initial tissue to determine the tissue dissolution rate. All measurements were conducted by 1 operator.

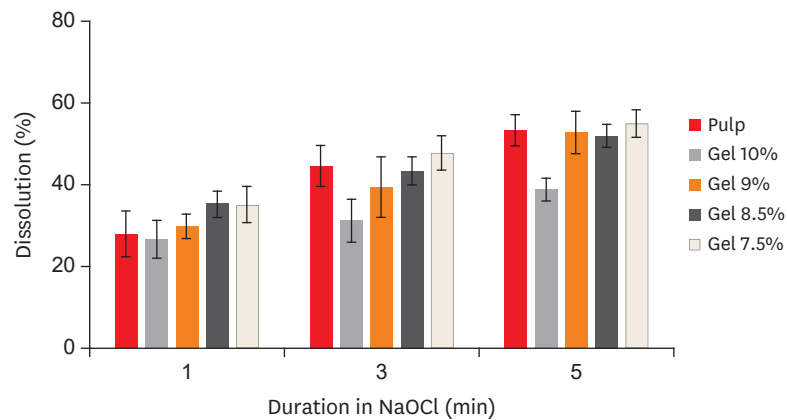
### Statistical analysis

To compare the overall tissue dissolution at room temperature with the corresponding values obtained under various conditions, the sums of remaining area (%) in all main canals per model were averaged for each model ( $n = 10$ ) and compared using the paired *t*-test. All tests were conducted with 95% confidence intervals ( $p < 0.05$ ).

## RESULTS

### Tissue-mimicking material

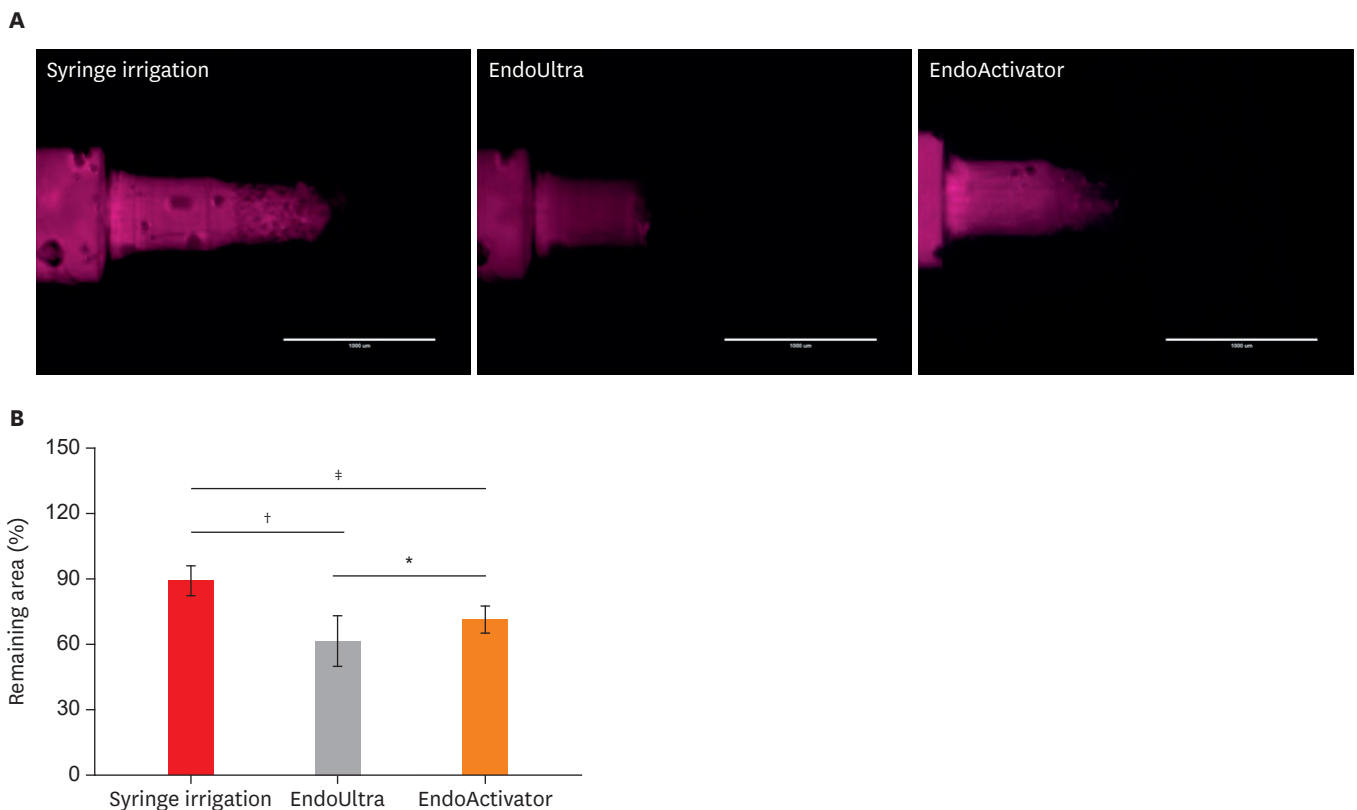
The results demonstrated that the 9% gelatin + 5% BSA phantom showed statistically equivalent dissolution to bovine pulp tissue at all time intervals (**Figure 1**). Furthermore, there was no significant difference in the 9% gelatin + 5% BSA phantom dissolution before and after adding reporter molecules ( $p > 0.05$ ). Therefore, the 9% gelatin + 5% BSA phantom was chosen as the optimum composition for the cleanliness experiments.



**Figure 1.** Dissolution (%) of the different phantoms at all time intervals. NaOCl, sodium hypochlorite.

### Cleanliness

**Figure 2A** shows the results of fluorescence microscopy of endodontic blocks that were instrumented with endodontic files and then irrigated using 3 different methods. The results obtained for remaining tissue (%) showed that the endodontic blocks irrigated with EndoUltra (61.89% ± 11.57%) had a significantly higher dissolution rate than those irrigated using the other methods (syringe irrigation, 89.54% ± 6.71%; EndoActivator, 71.61% ± 6.18%) (**Figure 2B**).



**Figure 2.** (A) Fluorescence microscopy of the endodontic blocks that were instrumented with endodontic files and then irrigated using 3 different methods. (B) Tissue remaining (%) in the endodontic blocks that were irrigated using 3 different methods. \* $p < 0.05$ ; † $p < 0.001$ ; ‡ $p < 0.0001$ .

## DISCUSSION

In the present study, a method of preparing a gelatin/BSA phantom to simulate the dissolution properties of dental pulp tissue was described. Phantoms that simulate various characteristics of tissues are commonly used in biomedical applications to mimic living tissue properties for reliable reference measurements, to provide quantitative information, and to calibrate systems such as ultrasound imaging, spectroscopy, and dosimetry [25-27]. Acoustic and optical properties are among the most studied properties using phantoms in the literature [25,27]. In particular, magnesium silicate-based materials, agarose-based materials, condensed milk-based materials, polyvinyl alcohol-based materials and gelatin-based materials are widely used as tissue substitutes [25].

The implementation of soft tissue substitutes in dental research is a novel topic. In the current endodontics literature, tissue dissolution properties have been studied in a vast variety of vital and necrotic tissue samples from different tissue sources such as human pulp tissue, porcine oral mucosa, and bovine muscle samples [28]. Due to the wide range of variation, the need for standardized models with known and controllable dissolution properties is of paramount importance in order to obtain repeatable and comparable results.

The loose connective tissue of the pulp is roughly composed of 75% water and 25% organic material (mainly collagen) [29]. In the present study, aqueous solution of gelatin/BSA was proposed as a substitute for pulp tissue. Gelatin is derived from collagen by destruction of cross-linkage of polypeptide chains and has a long history of application as a tissue-mimicking material for ultrasound phantoms [30].

Extensive research has evaluated the tissue-dissolving ability of NaOCl. It has been demonstrated that the solvent capability of NaOCl depends on its concentration, volume, pH, temperature, time, agitation, and the type, amount, and surface area of the tissue [12,13,31,32]. As such, the considerable variation in these factors across studies makes it difficult to draw comparative conclusions based on the existing research and to assess the relative significance of each factor [33].

The present study examined the influence of agitation on the capability of NaOCl to dissolve organic materials in a standardized setting and demonstrated that activation improved tissue dissolution. Previous research has ascertained that the tissue-dissolving ability of NaOCl solution decreases if it is diluted [31,34]. It is also worth mentioning that the results obtained from the present study showed that 5% NaOCl was effective. The significance of agitation on the tissue-dissolving ability of NaOCl has been reported in the literature, but relatively few studies have specifically analyzed the effects of agitation [35].

An aqueous solution of NaOCl is a dynamic balance of NaOH and HClO. When NaOCl is in contact with organic material, NaOH reacts with fatty acids to form soap and glycerol, in a reaction known as saponification. It also reacts with amino acids to form salt and water (neutralization). Additionally, HClO reacts with amino acids to form NH<sub>2</sub>Cl (chloramine) and H<sub>2</sub>O. These reactions, which generally occur at the surface, cause the liquefaction of organic tissue [36].

In the meantime, the molecules of NaOCl that take part in these reactions are consumed, bringing about a decrease of nearby activity. Thus, it is critical to supply active hypochlorite to

the region and to expel the remnants of dissolved tissue. In this study, the effects of different agitation techniques on the tissue-dissolving ability of NaOCl were evaluated, and it was found that agitation of the solution enhanced its dissolving ability to a statistically significant extent.

It was noteworthy that after injecting the tissue-mimicking material into the simulated canals, we let the material set at room temperature for 15 minutes, after which the process of instrumentation and irrigation and data acquisition should be performed as soon as possible. The reason for this is that the tissue-mimicking material dries out over time; this also occurs with natural pulp tissue, since water is a major component of both materials. Therefore, handling ought to be performed cautiously and immediately to preserve the similarity with *in vivo* conditions.

The utilization of ultrasound energy in root canal fabrication and irrigation has long been a disputed issue. Although some researchers reported the successful use of ultrasonic tools in root canal cleaning [37-40], others did not find ultrasound to be advantageous compared with root canal irrigation utilizing a syringe [41,42].

The mechanism of passive ultrasonic action has been proposed to involve acoustic streaming (microstreaming) and cavitation. Sonic energy has a comparable mechanism to that of ultrasonic energy, although the pattern of the oscillating file is different [43]. However, as indicated by Estrela *et al.* [36], cavitation is constrained to a distance of under 100  $\mu\text{m}$ . It is important to point out that acoustic streaming results in a stirring action and causes rapid movement of the liquid away from the energy source. This is most likely the mechanism through which ultrasonic energy influences the cleaning of the peripheral sections of the root canal *in vivo*. Within the limitations of the present study, the method of agitation enhanced tissue dissolution.

## CONCLUSIONS

In summary, our developed phantom in this study is comparable to biological tissue in terms of tissue dissolution and could be utilized for *in vitro* tests due to its injectability and detectability. Furthermore, the data obtained from the cleanliness experiments demonstrated that the percentage of remaining material in the endodontic blocks irrigated with EndoUltra was significantly lower than in the blocks irrigated using the other methods.

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