

Glucose reactivity with filling materials as a limitation for using the glucose leakage model

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Abstract

Shemesh H, Souza EM, Wu M-K, Wesselink PR. Glucose reactivity with filling materials as a limitation for using the glucose leakage model. *International Endodontic Journal*, **41**, 869–872, 2008.

Aim To evaluate the reactivity of different endodontic materials and sealers with glucose and to assess the reliability of the glucose leakage model in measuring penetration of glucose through these materials.

Methodology Ten uniform discs (radius 5 mm, thickness 2 mm) were made of each of the following materials: Portland cement, MTA (grey and white), sealer 26, calcium sulphate, calcium hydroxide [Ca(OH)₂], AH26, Epiphany, Resilon, gutta-percha and dentine. After storing the discs for 1 week at 37°C and humid conditions, they were immersed in 0.2 mg mL⁻¹ glucose solution in a test tube. The concentration of

glucose was evaluated using an enzymatic reaction after 1 week. Statistical analysis was performed with the ANOVA and Dunnett tests at a significant level of $P < 0.05$.

Results Portland cement, MTA, Ca(OH)₂ and sealer 26 reduced the concentration in the test tube of glucose significantly after 1 week ($P < 0.05$). Calcium sulphate reduced the concentration of glucose, but the difference in concentrations was not significant ($P = 0.054$).

Conclusions Portland cement, MTA, Ca(OH)₂ and sealer 26 react with a 0.2 mg mL⁻¹ glucose solution. Therefore, these materials should not be evaluated for sealing ability with the glucose leakage model.

Keywords: glucose, leakage model, optical density, reactivity.

Received 29 January 2008; accepted 10 April 2008

Introduction

A new leakage model was suggested recently (Xu *et al.* 2005) using glucose as the tracer. Several studies were published using this model (Shemesh *et al.* 2006, 2007, Kaya *et al.* 2007, van der Sluis *et al.* 2007, Zou *et al.* 2007, Xu *et al.* 2007, Ozok *et al.* 2008) and more are being conducted. This model is based on measurements of glucose concentrations in an apical chamber using a sensitive enzymatic reaction. A coloured substance is produced and optical density (OD) is determined by a spectrophotometer, translated

later to concentration units. The advantages of this model are the relative ease of assembly and operation, the availability of the materials and equipment and the great sensitivity of the test (Shemesh *et al.* 2006).

As leakage studies are constantly being scrutinized for their clinical relevance and reproducibility (Editorial Board of the Journal of Endodontics, 2007), a critical approach to the various leakage models is required. One of the problems of using a tracer for evaluating leakage is that the tracer itself could chemically react with some materials challenging the reliability of such a test in evaluating sealing ability. For example, methylene blue, which is often used for dye-leakage studies, was previously shown to discolour when in contact with certain filling materials (Wu *et al.* 1998), whilst root canal fillings might directly inhibit bacteria in bacterial leakage studies (Pitout *et al.* 2006). The purpose of this study was to determine the reactivity of

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glucose solution with different dental materials to assess whether glucose is an acceptable tracer for all tested materials.

Materials and methods

The following materials were mixed according to the manufacturer's instructions and inserted into round plastic moulds 2 mm deep with a diameter of 5 mm : Portland cement (Gamma, Leusden, the Netherlands), MTA grey (Angelus, Londrina, Brazil), MTA white (Angelus), Sealer 26 (Dentsply, Petrópolis, Brazil), calcium hydroxide powder (Merck, Darmstadt, Germany), calcium sulphate (Sigma-Aldrich, Steinheim, Germany), AH26 (Dentsply-Maillefer, Tulsa, OK, USA) and Epiphany sealer (Pentron Clinical Technologies, Wallingford, CT, USA). The following thermoplastic materials were adapted into similar moulds with a warm instrument: Resilon cones (Pentron), Gutta-

percha cones (Dentsply De Trey, Konstanz, Germany). Ten moulds were filled with each material. An additional 10 dentine discs of similar dimensions were created from extracted molar teeth. All moulds and discs were maintained at 37°C and humid conditions. After 1 week, the set materials were carefully removed from the moulds, forming round discs. Each of the discs was then inserted to a small test tube with 4 mL of glucose 0.2 mg mL⁻¹ solution. Additional 10 test tubes were used as controls and contained only 4 mL of glucose 0.2 mg mL⁻¹ solution. All test tubes were kept at 37°C. A sample of 0.1 mL of the solution was removed after 1 week from each test tube and was analysed with a Glucose kit (Megazyme, Wicklow, Ireland) in a spectrophotometer (Spectra max 384 plus; Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 340 nm. Results were expressed as OD. Statistical analysis was performed with the ANOVA and the Dunnett test at significance level of $P < 0.05$ (SPSS version 15.0, SPSS Inc., Chicago, IL, USA).

Table 1 Average optical density (OD), SD and P -value of the OD measured after 1-week immersion with different materials

Material	Average OD \pm SD	P -value (compared with glucose solution)
Water	0 \pm 0.005	<0.001
Glucose stand.	0.290 \pm 0.010	–
Dentine	0.293 \pm 0.007	0.999
Portland cement	0 \pm 0.007	<0.001
MTA-grey	0 \pm 0.004	<0.001
MTA-white	0 \pm 0.007	<0.001
Sealer 26	0 \pm 0.027	<0.001
Ca(OH) ₂	0 \pm 0.005	<0.001
Calcium sulphate	0.275 \pm 0.007	0.054
AH26	0.282 \pm 0.007	0.645
Epiphany	0.290 \pm 0.017	1.000
Resilon	0.300 \pm 0.015	0.382
Gutta-percha	0.297 \pm 0.008	0.781

The kit used has a detectable threshold of OD 0.05.

Results

The results are presented in Table 1 and Fig. 1.

The glucose kit used has a detectable threshold of OD 0.05, so all readings lower than 0.05 were ignored. The glucose test tubes with no test material showed the expected OD from a 0.2 mg mL⁻¹ glucose solution (OD 0.3 \pm 0.05).

Amongst the materials tested, MTA, Portland cement, sealer 26 and Ca(OH)₂ lowered significantly the OD of the solution after 1 week ($P < 0.05$). Dentine, Resilon, Epiphany, AH26 and gutta-percha did not significantly alter the concentration of glucose. Calcium sulphate reduced the concentration of glucose but the difference in concentrations was not significant ($P = 0.054$).

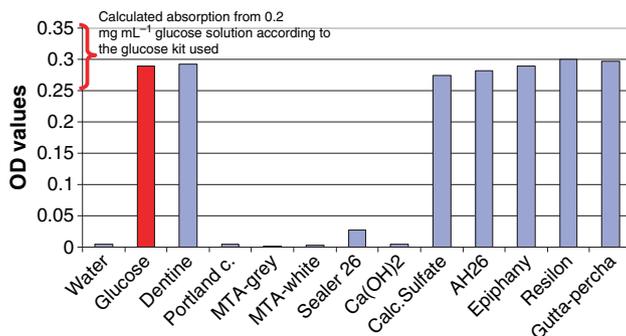


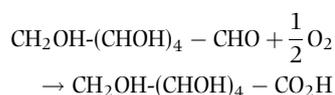
Figure 1 The measured optical density (OD) of 0.2 mg mL⁻¹ glucose solution after 1-week immersion with different materials.

Discussion

After 1 week in contact with glucose solution, a number of the materials tested reduced significantly the OD of the glucose reaction to the level of water (Fig. 1).

As all published glucose leakage tests checked the penetration of glucose for periods ranging from 20 days (Zou et al. 2007) to 56 days (Shemesh et al. 2006) an observation period of 1 week seems relevant when assessing the reactivity of different materials with the tracer.

Glucose (an aldohexose) in an alkaline solution is slowly oxidized by oxygen, forming gluconic acid:



In the presence of sodium hydroxide, for example, gluconic acid is converted to sodium gluconate (Sowden & Schaffer 1952). This means that glucose will not be detected by the glucose kit and could thus mask leakage in the glucose leakage model.

Interestingly, Sealer 26 releases $\text{Ca}(\text{OH})_2$ (Duarte et al. 2000), whilst MTA contains $\text{Ca}(\text{OH})_2$ (Camilleri 2007). It seems that $\text{Ca}(\text{OH})_2$ containing products react directly with glucose. This reaction could also be initiated by traces of $\text{Ca}(\text{OH})_2$ left in the canal when used as an intra-canal medication. Thus, the leakage measured by glucose concentrations could be influenced by this chemical reaction.

Zou et al. (2007) used the glucose leakage model and concluded that calcium sulphate significantly improved the sealing ability of 1 mm perforations repaired with composite resin. Although the statistical tests used in this study resulted in a nonsignificant influence of calcium sulphate on glucose ($P = 0.054$), the P -value is small and it cannot be excluded that calcium sulphate might have an influence. Thus, calcium sulphate could be better evaluated for its sealing properties by another tracer or model.

In the glucose leakage model, a 1 mol L^{-1} glucose solution is used as the tracer and placed in the upper chamber of the model assembly (Xu et al. 2005). This concentration is much higher than the linear range detected by the glucose kits: $4\text{--}80 \mu\text{g}$ of glucose per assay (Glucose–HK assay procedure, Megazyme International Limited, 2004). However, the range of glucose concentrations measured in the apical chamber during a leakage test is dependent on the amount of glucose that penetrated through the canal and can demon-

strate a large range of concentrations (Shemesh et al. 2006). In this experiment, a glucose concentration of 0.2 mg mL^{-1} ($20 \mu\text{g}$ per assay) was chosen as it lies within the linear range of detection of the glucose kit used and could give more accurate information on the variations in glucose concentrations after immersion with different materials.

Conclusion

The observation that certain materials react with glucose suggests that evaluating these materials with the glucose leakage model might lead to erroneous conclusions. An investigation of the influence of tested materials on glucose should always precede glucose leakage tests to validate the conclusions from such studies.

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