

A Systematic Review of *In Vitro* Retrograde Obturation Materials

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Abstract

The purpose of this review was two-fold: (a) to determine which retrograde obturation material(s) best prevents dye/ink penetration *in vitro*; and (b) to determine whether *in vitro* results agree with *in vivo* results. A MEDLINE search was conducted to identify *in vitro* studies published between January 1966 and October, week 4, 2003, conducted on human teeth, and published in English, German, or French language, testing the resistance to retrograde penetration of retrograde filling materials. The MEDLINE search identified 278 published articles. Of those, 115 studies examined the resistance to penetration of various retrograde filling materials, *in vitro*. Thirty-four studies met all the inclusion and validity criteria. The results indicate that, beyond 10 days *in vitro*, the most effective retrofilling materials, when measured by dye/ink penetration are: composites > glass ionomer cement > amalgam > orthograde gutta-percha > EBA. The results of these *in vitro* studies are not congruent with *in vivo* study results, suggesting a need to re-evaluate the clinical validity and importance of *in vitro* studies.

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The purpose of this systematic review was: (a) to determine which retrograde obturation (root-end filling) material(s) best prevent(s) retrograde dye/ink penetration *in vitro*, and (b) to compare these results to the results of *in vivo* studies.

In vitro dye penetration studies have been carried out for decades to assess the leakage of endodontic filling materials. The assumption was that this surrogate measure (dye/ink penetration) is a good indicator of sealing ability. The additional assumption was that sealing ability is a good predictor of endodontic success. Since the early 1980s, attempts have been made to correlate *in vitro* and *in vivo* studies with minimal success (1, 2).

A recent systematic review of *in vivo* success of retrograde obturation materials indicates that, in studies using a randomized controlled trial design (level 1 evidence) (EBM: <http://cebm.jr2.ox.ac.uk/docs/levels.html>) (69), glass ionomer cement is more effective than amalgam (3). In contrast, for studies employing a nonrandomized controlled trial design (level 2 evidence) (EBM: <http://cebm.jr2.ox.ac.uk/docs/levels.html>) the data is more widely variable. Some studies indicate that amalgam is more effective than glass ionomer, others that composites, or that EBA is more effective than amalgam (3).

The results from *in vivo* studies of high evidence level can be directly applied to humans (patients). Experimental studies that test new materials and their properties *in vitro* and compare them with other materials are valuable, as well, if their results can also be extrapolated to *in vivo* results. This study was undertaken to begin to determine this relationship.

Materials and Methods

Literature Search

An electronic search of MEDLINE January 1966 to October, week 4, 2003 was conducted (Table 1).

Inclusion Criteria-Validity

Two independent reviewers examined all the identified abstracts to determine whether they met the following inclusion criteria: Study *in vitro*; used human teeth; tested and compared the sealing ability of more than one retrograde obturation materials, placed at the root end following the classic rules of apicoectomy (root-end resection) and retrograde (root-end) obturation; measured the amount of penetration from the apex to the root canal of the teeth; quantitative results provided; and written in English, German, or French language.

Whenever it was not possible to make this determination, the article in full text was examined. Subsequently, all relevant articles were obtained and a determination made by two reviewers if they met the inclusion criteria. It is important to state that only studies measuring the passive retrograde dye leakage were included. The studies that examined the orthograde leakage (dye leakage under pressure, bacterial leakage, electrochemical, and radioisotope leakage), the studies that examined the dye leakage after applying different mechanical methods for the resection of the apex and the preparation of the retrograde cavity and the studies providing qualitative results were excluded. All articles that met the inclusion criteria were assessed for validity. Validity was determined on a seven-point scale (Table 2) and studies not meeting five or more of the seven validity criteria were excluded (Table 3).

TABLE 1. MEDLINE Search Strategy (278 articles)

| # | Search History | Results |
|----|--|---------|
| 1 | Apicoectomy | 1105 |
| 2 | Apicectomy | 95 |
| 3 | Root-end resection | 50 |
| 4 | Root end resection | 70 |
| 5 | Pulpectomy | 893 |
| 6 | Pulpotomy | 953 |
| 7 | Root canal therapy | 11664 |
| 8 | Root canal filling materials | 3529 |
| 9 | Dental pulp test | 704 |
| 10 | Dental pulp disease | 6088 |
| 11 | Periapical abscess | 1272 |
| 12 | Endodontics | 15901 |
| 13 | 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 | 22321 |
| 14 | Random* ¹ | 317435 |
| 15 | Compar* ² | 2012298 |
| 16 | 14 or 15 | 2180989 |
| 17 | (retrograde) or (retro-filling) or (retro filling) or (retrofilling) or (root-end) or (root end) | 35673 |
| 18 | (obturation material) or (obturation) or (filling*) or (filling material*) | 29506 |
| 19 | 13 and 16 and 17 and 18 | 278 |

¹ Random*: randomized, randomly, randomization, etc.

² Compar*: compare, comparatively, comparison, compared, etc.

TABLE 2. Validity Assessment Criteria*

| |
|--|
| 1. Was the assignment of teeth of treatment randomized? |
| 2. Was the randomization list concealed? |
| 3. Was the duration of the experiment sufficiently long and complete? |
| 4. Were all teeth analyzed in the groups to which they were randomized? |
| 5. Were teeth and examiners blinded to the treatment being received? |
| 6. Aside from the experimental treatment, were the groups treated equally? |
| 7. Were the groups similar at the start of the trial? |

* Derived from (69).

Data Analysis

Data from the studies that met inclusion criteria were extracted and plotted as scatter plots and linear regression used to compare penetration.

Ranking the materials was a two-step process. First a linear regression of dye penetration versus time was determined from scatter plots. Then dye penetration was examined for those materials experimental results longer than 10 days.

Results

The MEDLINE search identified 278 articles (Table 1). From those, 115 appeared to meet inclusion criteria. Of these, 25 studies were then excluded because they tested orthograde penetration (from the root canal to the apex and periapical area) and not the retrograde penetration. Eighteen studies were excluded because they examined different mechanical preparations. Eight studies were excluded because of language limitations. Eight studies were excluded because they did not provide quantitative results. Twenty-two studies were excluded because they met less than five of seven validity criteria (Table 3).

Thirty-four studies met all of the inclusion and validity criteria. Details of the included studies are presented in Table 4. The 34 included studies examined 17 different groups of materials, eight different types of indicator agents (Tables 4 and 5), and a range of testing periods (Tables 4 and 5). Thus the number of variables was large. From those 17 groups of materials, seven material groups were distilled to match the material groupings already tested in vivo (3, 4) (Table 5). Dye

TABLE 3. Excluded Studies: not meeting validity criteria

| Reference | 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|-------------------------------|----|----|----|----|----|----|----|
| Abdel Aziz & Fahim, 1986 | N | N | Y | Y | N | Y | Y |
| Barry et al, 1975 | N | N | Y | Y | N | Y | N |
| Chong et al, 1991 | N | N | Y | Y | N | Y | N |
| Dalal & Gohil, 1983 | N | N | Y | Y | N | Y | N |
| Escobar et al, 1986 | N | N | Y | Y | N | Y | N |
| Fournier et al, 1991 | N | N | Y | Y | N | Y | Y |
| Gerhards & Wagner, 1996 | N | N | Y | Y | N | Y | N |
| Higa et al, 1994 | N | N | Y | Y | N | Y | N |
| High & Russell, 1989 | N | N | Y | Y | N | Y | N |
| Kadohiro, 1984 | N | N | Y | Y | N | Y | Y |
| Kaplan et al, 1982 | N | N | Y | Y | N | Y | N |
| Negm et al, 1982 | N | N | Y | Y | N | Y | N |
| Negm, 1988 | N | N | Y | Y | N | Y | N |
| O'Connor et al, 1995 | N | N | Y | Y | N | Y | Y |
| Pretorius & van Heerden, 1995 | N | N | Y | Y | N | Y | N |
| Roy et al, 2001 | N | N | Y | Y | N | Y | N |
| Sultan & Pitt Ford, 1995 | N | N | Y | Y | N | Y | Y |
| Sutimuntanakul et al, 2000 | N | N | Y | Y | N | Y | Y |
| Szeremeta-Browar et al, 1985 | N | N | Y | Y | N | Y | Y |
| Tanzilli et al, 1980 | N | N | Y | Y | Y | Y | N |
| Torabinejad et al, 1995 | N | N | Y | Y | N | Y | N |
| Tuggle et al, 1989 | N | N | Y | Y | N | Y | N |

penetration of these materials is plotted in Figs. 1 (scatter plots) and 2 (linear regression).

Amalgam was examined 39 times by 26 different groups using a variety of experimental procedures and indicators (Tables 4 and 5). It was tested from 0.1 to 180 days. Amalgam appears to lose its sealing ability over time (Figs. 1 and 2), with penetration ranging from 0.3 to 6.9 mm.

Orthograde condensed gutta-percha was used to obturate the root canals conservatively. When the apices were resected no retrograde cavity was prepared nor was any other material added over the already existing gutta-percha. This material was examined 16 times by 12 different groups over a period of 0.1 to 180 days (Table 5). Gutta-percha appears to maintain its sealing ability over time (Figs. 1 and 2). However, the penetration ranges from 0.5 to 10.4 mm (Fig. 1).

TABLE 4. Included studies: Procedures and retrograde obturation materials tested

| Reference ¹ | Indicator ² | Storage Material before/during Experiment ³ | Irrigation solution ⁴ | Orthograde obturation ⁵ | Resection angle ⁶ | Severance procedure/Retrocavity preparation ⁷ | Retrograde obturation materials ⁸ | Time(days)/Best material, as suggested by the authors of the studies ⁹ | Penetration (mm) ¹⁰ | Number of teeth used ¹¹ |
|----------------------------------|------------------------|--|----------------------------------|---|------------------------------|--|---|---|--------------------------------|------------------------------------|
| Cathers, Roahen, 1993 | India Ink | Ringer's solution | None | None | 45° | High speed bur/ Bur | Amalgam/ Amalgam + varnish/ Amalgam + Dentin bonding agent | 90d/ Amalgam + Dentin bonding agent | 1.17 | 94 |
| Smee et al, 1987 | India ink | Saline + NaOCl (5.25%) | NaOCl (2.5%) | Laterally condensed gutta-percha with sealer | 45° | High speed bur with water/Not mentioned | Amalgam + IRM + Composites + Orthograde gutta-percha + Teflon | 2d Teflon, Composites | 0.06, 0.19 | 36 |
| Lloyd et al, 1997 | India Ink | (De-ionized water + thymol) + NaOCl (5.25%) + (water + thymol) | NaOCl (5.25%) | Thermoplasticized gutta-percha with sealer | 1/2:45° + 1/2:90° | Bur with water/ 1/2:bur + 1/2:ultrasonic | Amalgam + Composites, in (10%citric acid + 3%FeCl ₃) | 14d/ Composites | 0.1 | 172 |
| Chong et al, 1995 | India ink | Water | Water | None | 45° | Bur/Bur | Amalgam + IRM + Glass Ionomer Cement | 3d/ Glass Ionomer Cement, IRM | 0.0, 0.0 | 80 |
| Aktener, Pehliln, 1993 | India ink | NaOCl (5.25%) + formalin (10%) | NaOCl (5%) | Laterally condensed gutta-percha with sealer (then stored in water) | 45° | Not mentioned/ Bur | (varnish + Amalgam) + (Cermet Ionomer Cement + varnish) + Cermet Ionomer Cement | 3d/ Cermet Ionomer Cement + varnish | 1 | 54 |
| Olson et al, 1990 | India ink | Formalin (10%) + NaOCl (2.5%) | Saline | Laterally condensed gutta-percha with sealer | 90° | Low-speed bur + saline/Low-speed bur | (varnish + Amalgam) + Amalgam + Glass Ionomer Cement + Thermoplasticized gutta-percha | 3d/ Glass Ionomer Cement, (varnish + Amalgam.) | 1.5, 1.5 | 111 |
| McPherson et al, 1989 | India ink | Saline + NaOCl (2.5%) | Saline | Laterally condensed gutta-percha with sealer | 45° | Low-speed bur + saline/Low-speed bur + saline | (varnish + Amalgam.) + Thermoplasticized gutta-percha | 3d/ Thermoplasticized gutta-percha | 1.67 | 51 |
| Beltes et al, 1988 | India ink | Saline (isotonic) + NaOCl (5%) | NaOCl (5%) | Laterally condensed gutta-percha with sealer | 90° | High-speed bur with water/Low-speed bur | (Amalgam + varnish) + Super EBA + Glass Ionomer Cement + Orthograde gutta-percha | 2d/ Super EBA, (Amalgam + varnish) | 0.24, 0.45 | 40 |
| Staribratova-Reister et al, 2003 | Methylene blue dye | Ethanol (20%) + thymol (0.9%) | NaOCl (2%) | Laterally condensed gutta-percha with sealer | 90° | High-speed bur/ Ultrasonics | Glass Ionomer Cement + Orthograde gutta-percha | 2d/ Orthograde gutta-percha | 1.2 | 102 |
| Agrabawi J, 2000 | Methylene blue dye | Saline | NaOCl (5.25%) | Vertically condensed gutta-percha with sealer | 90° | High-speed bur/ Ultrasonics | Amalgam + Super EBA + MTA | 3d/ MTA | 1.4 | 79 |

TABLE 4. (continued)

| Reference ¹ | Indicator ² | Storage Material before/during Experiment ³ | Irrigation solution ⁴ | Orthograde obturation ⁵ | Resection angle ⁶ | Severance procedure/ Retrocavity preparation ⁷ | Retrograde obturation materials ⁸ | Time(days)/Best material, as suggested by the authors ⁹ | Penetration (mm) ¹⁰ | Number of teeth used ¹¹ |
|-------------------------|------------------------|--|----------------------------------|---|------------------------------|---|--|--|--------------------------------|------------------------------------|
| Subay, Subay, 1999 | Methylene blue dye | Ringer's solution + sodium azide (0.2%) at 0°C | NaOCl (2%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/High-speed bur with water | (Dentin bonding agent + Amalgam) + (Dentin bonding agent + Amalgam + dentin bonding agent) | 30d/ Dentin bonding agent + Amalgam | 0.85 | 96 |
| Torabinejad et al, 1994 | Methylene blue dye | Water (sterile). | NaOCl (5.25%) | Laterally condensed gutta-percha with sealer | 90° | High-speed bur/ High-speed bur with water | Amalgam + Super EBA + IRM + MTA + Orthograde gutta-percha | 3d/ MTA | 0.3 | 90 |
| Starkey et al, 1993 | Methylene blue dye | Ringer's solution + sodium azide (0.2%) | NaOCl (2.5%) | Vertically condensed gutta-percha with sealer | 90° | High-speed bur/ High-speed bur | Amalgam + Composites | 7d/ Composites | 2.22 | 84 |
| Al-Ajam, McGregor, 1993 | Methylene blue dye | Saline (4°C) | NaOCl (2%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur/ Not mentioned | Amalgam + Silver Glass Ionomer Cement | 2d/ Silver Glass Ionomer Cement | 2.1 | 56 |
| Ozata et al, 1993 | Methylene blue dye | Deionised water + water | NaOCl (5%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/Not mentioned | Amalgam + Glass Ionomer Cement + Cermet Ionomer Cement + Glass Ionomer Cement + Cermet Ionomer Cement + (Amalgam + Glass Ionomer Cement) + varnish | 14d/ Glass Ionomer Cement + varnish | 0.55 | 70 |
| Peters, Harrison, 1992 | Methylene blue dye | NaOCl (5.25%) | NaOCl (5.25%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur/ Not mentioned | Amalgam + IRM + Orthograde gutta-percha + [(Amalgam + IRM + Orthograde gutta-percha) + Citric Acid] | 0.1d/ Orthograde gutta-percha, IRM | 0.63, 0.67 | 148 |
| Nixon et al, 1991 | Methylene blue dye | De-ionized water + thymol | NaOCl (5.25%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/Low-speed bur | Amalgam + Silicones | 7d/ Silicones | 1.31 | 100 |
| Negm MM, 1990 | Methylene blue dye | Saline | Water | Laterally condensed gutta-percha with sealer | 90° | High-speed bur with water/Low-speed bur | Amalgam + Orthograde gutta-percha | 180d/ Orthograde gutta-percha | 2.6 | 118 |
| Zakhary SY, 1990 | Methylene blue dye | NaOCl (2.25%) + 100% Humidity for 1mo and 6mo | NaOCl (5.25%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/Low-speed bur | Amalgam + Orthograde gutta-percha + Heat sealed gutta-percha + Glass Ionomer Cement | 2d/ Amalgam, Glass Ionomer Cement | 2.85, 2.95 | 58 |

TABLE 4. (continued)

| Reference ¹ | Indicator ² | Storage Material before/during Experiment ³ | Irrigation solution ⁴ | Orthograde obturation ⁵ | Resection angle ⁶ | Severance procedure/ Retrocavity preparation ⁷ | Retrograde obturation materials ⁸ | Time(days)/Best material, as suggested by the authors ⁹ | Penetration (mm) ¹⁰ | Number of teeth used ¹¹ |
|---------------------------|------------------------|--|---|---|------------------------------|---|--|---|--------------------------------|------------------------------------|
| Shaw et al, 1989 | Methylene blue dye | NaOCl (5.25%) + saline | NaOCl (2.6%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/Bur | Amalgam + (varnish + Amalgam) + Orthograde gutta-percha | 7d/ Varnish + Amalgam | 0.27 | 36 |
| Schwartz, Alexander, 1988 | Methylene blue dye | Formaline (10%) + Water (distilled) | NaOCl (5.25%) | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/Low-speed bur | (Amalgam + varnish) + Silver Glass Ionomer Cement | 7d/ Silver Glass Ionomer Cement | 0.80 | 26 |
| Barry et al, 1976 | Methylene blue dye | Saline | None | None | 45° | High-speed bur/ High-speed bur | Amalgam + Polycarboxylate Cements | 7d/ Amalgam | 1.3 | 400 |
| Boshali et al, 1998 | Basic fuchsin | Chloramines T Solution at 4°C | NaOCl (2.5%) | Vertically condensed gutta-percha with sealer | 90° | High-speed bur with water/Low-speed bur | Super EBA + Composites | 84d/ Composites | 1.8 | 48 |
| Abdal, Retief, 1982 | Fluorescent Dye | Ethanol (70%) | NaOCl (5%) + H ₂ O ₂ (3%) | Laterally condensed gutta-percha with sealer (after apical resection) | 90° | High-speed bur with water/High-speed bur with water | Amalgam + Super EBA + IRM + Glass Ionomer Cement + Composites + Orthograde gutta-percha + Polycarboxylate cement | 7d/ Composites, Orthograde gutta-percha, Glass ionomer cement | 0.41, 0.49, 0.51 | 34 |
| Abdal et al, 1982 | Fluorescent dye | Ethanol (70%) | None | Laterally condensed gutta-percha with sealer (after apical resection) | 90° | High-speed bur with water/High-speed bur with water | Amalgam + (Amalgam + varnish) + Composites + Orthograde gutta-percha + Polycarboxylate Cements | 0d/ Composites, (Amalgam + varnish) | 0.03, 0.37 | 70 |
| Torabinejad et al, 1993 | Fluorescent dye | Water | NaOCl (5.25%) | Laterally condensed gutta-percha with sealer | 90° | High-speed bur with water/High-speed bur with water | Amalgam + Super EBA + MTA | 1d/ MTA | 0.4 | 30 |
| Hosoya et al, 1995 | Fluorescent dye | Saline + NaOCl (5.25%) | NaOCl (5.25%) | Laterally condensed gutta-percha with sealer | 90° | High-speed bur + saline/High-speed bur + saline | Amalgam + Gallium alloy + Glass Ionomer Cement + Orthograde gutta-percha | 84d/ Glass Ionomer Cement | 1.76 | 184 |
| Woo et al, 1990 | Reactive Blue 4 Dye | NaOCl (0.5%) | NaOCl (2.5%) | Laterally condensed gutta-percha with sealer | 45° | Bur/Not mentioned | Amalgam + Thermoplasticized gutta-percha + sealer (Thermoplasticized gutta-percha + sealer) | 0.5d/ Thermoplasticized gutta-percha + sealer | 1.1 | 48 |
| Edmunds, Thirawat, 1989 | Eosin dye | Saline | Saline | Laterally condensed gutta-percha with sealer | 90° | High-speed bur with water/Low-speed bur | Amalgam + Orthograde gutta-percha | 2d/ Orthograde gutta-percha, Amalgam | 5.4, 6.1 | 84 |

TABLE 4. (continued)

| Reference ¹ Indicator ² | Storage Material before/during Experiment ³ | Irrigation solution ⁴ | Orthograde obturation ⁵ | Resection angle ⁶ | Severance procedure/Retrocavity preparation ⁷ | Retrograde obturation materials ⁸ | Time(days)/Best material, as suggested by the authors of the studies ⁹ | Penetration (mm) ¹⁰ | Number of teeth used ¹¹ |
|---|--|--|--|------------------------------|---|--|---|--------------------------------|------------------------------------|
| Thirawat, Edmonds, 1989 | Saline | Saline | Laterally condensed gutta-percha with sealer | 90° | High-speed bur with water/Low-speed bur + 10% H ₂ O ₂ | (Amalgam + varnish) + Super EBA + Glass Ionomer Cement + Composites + Dentin bonding agent + (Composites + Dentin bonding agent) + Orthograde gutta-percha | 2d/ Composites, Dentin bonding agent, (Composites + Dentin bonding agent), Glass Ionomer Cement | 0.58, 1.15, 1.33, 1.4 | 70 |
| Holt, Dumsha, 2000 | Water | NaOCl | Laterally condensed gutta-percha with sealer | 45° | Bur with water/Ultrasonics | (Amalgam + varnish) + Super EBA + Acrylic cement + (Composites + Dentin bonding agent) | 0.1d/ Amalgam + varnish | 0.59 | 90 |
| McDonald, Dumsha, 1990 | None | H ₂ O ₂ + NaOCl (5.25%) + saline | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/Bur | Amalgam + (Amalgam + varnish) + Orthograde gutta-percha + (Composites + Dentin bonding agent) | 0.1d/ Composites + Dentin bonding agent | 0.35 | 158 |
| McDonald, Dumsha, 1987 | Water | NaOCl (5.25%) + saline + H ₂ O ₂ | Laterally condensed gutta-percha with sealer | 45° | High-speed bur with water/Bur | Amalgam + Composites + (Composites + Dentin bonding agent) + Orthograde gutta-percha + Polycarboxylate cements | 0.1d/ Composites + Dentin bonding agent | 0.28 | 122 |
| Fitzpatrick et al, 1997 | Saline Palladium | NaOCl (2.5%) | Laterally condensed gutta-percha with sealer | 90° | High-speed bur with water/Ultrasonics | Super EBA + IRM | 0d/ Super EBA | 1.5 | 31 |

1. The references are presented (#32 studies).
 2. One can see that India ink, methylene blue dye, fluorescent and reactive blue 4 dyes, eosin, basic fuchsin, silver nitrate and gold-palladium have been used to show the degree of the penetration the materials examined allowed. The molecular weight of all the different dyes/inks varied between 169.9 for silver nitrate to 755.9 for fluorescent reactive dyes ([http://www.sigmaaldrich.com](http://chemdat.merck.de/en/catalog/index.html)).
 3. Ten different solutions in various concentrations, combinations with each other and duration of application were used to store the teeth before and/or during the experiments. In some experiments no storage media was used, at all. This data exist at the 3rd column.
 4. Four different irrigation solutions in various concentrations and/or combinations with each other were used to irrigate the root canals during the root canal preparation. Also, in some tests no irrigating solutions were used.
 5. Six different materials, combinations and techniques were used to obturate the root canals. In some tests no root canal filling was used.
 6. The apical 3 to 4 millimetres of the roots were resected at a 45° or 90° angle.
 7. Six different methods were used to resect the root end (presented before the slash) and seven different methods were used to prepare a retrograde cavity (presented after the slash) 2 to 3 millimetres deep. In some experiments the authors did not mention how they proceeded to the apical resection and the retrograde cavity preparation.
 8. Sixteen different groups of materials were examined for their sealing ability. When one material is used in combination with another they are both inserted into a parenthesis.
 9. Before the slash the time the teeth were immersed into the indicating solution (dyes, inks) converted in day's periods—for this systematic review needs—is presented. These time periods varied from 0 to 180 days with 12 different time intervals (0, 0.1, 0.5, 1, 2, 3, 7, 14, 30, 84, 90, 180 days). No pressure was applied to the immersing solutions during the teeth immersion. After the slash, the "best" material, as suggested by the authors of each study, is written in bold characters. When there is/are more than one material/s statistically significantly better than the rest, but still not statistically significantly different among each other they are all written with bold characters. When the "best" material, as suggested by the authors of each study, is not statistically significantly better than the next—"best"—tested material(s), it is written with normal characters.
 10. The penetration the "best" material(s) allowed—as suggested by the authors of each study—converted in millimetres for the needs of this systematic review.
 11. The number of the teeth that were used in each experiment.

TABLE 5. Results on Time, Penetration and Numbers of Studies and Tests

| Material | Time (days) | Penetration (mm) | Number of studies/Times material tested |
|-------------------------|-------------|------------------|---|
| Amalgam | 0.1–180 | 0.3–6.9 | 31/39 |
| Orthograde Gutta-percha | 0.1–180 | 0.5–10.4 | 15/16 |
| Glass Ionomer Cement | 2–84 | 0–4.3 | 12/16 |
| EBA cement | 0–84 | 0.2–12.6 | 9/9 |
| Composites | 0.1–14 | 0.03–2.2 | 10/12 |
| IRM | 0–7 | 0–2.3 | 6/7 |
| MTA | 1–3 | 0.3–0.4 | 3/3 |

At the 1st column, the materials examined are presented, at the 2nd column, the range of the periods of time (in days) the tests lasted, at the 3rd column, the range of the penetration (in millimetres) each material allowed and at the 4th column, before the slash the number of studies that examine each material are presented and after the slash, the number of times each material was tested.

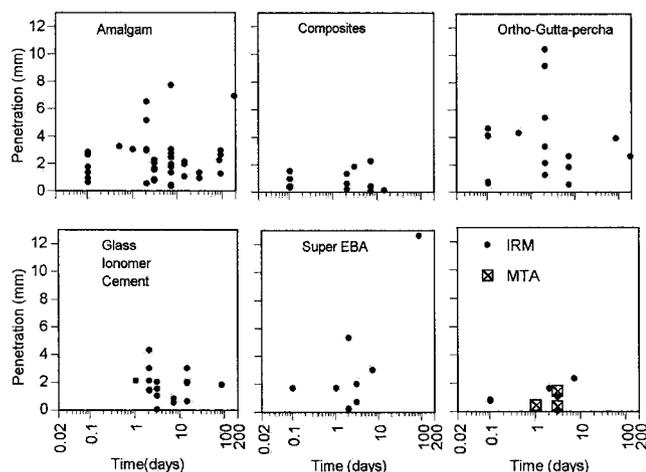


Figure 1. (Paired Scatter) For each material, on the X-axis the time in days (logarithmically) the materials were immersed into the indicator solution is presented. On the Y-axis the penetration in millimeters the dye ran through the mass of the materials as measured from the apex to the crown of the teeth is presented.

Glass ionomer cement was examined 16 times by 12 different groups over a period of 2 to 84 days. Glass ionomer cement appears to increase sealing ability over time (Table 5, Figs. 1 and 2), with penetration ranging from 0 to 4.3 mm.

EBA was examined nine times by eight different groups over a period of 0 to 84 days. EBA appears to lose its sealing ability over time (Table 5, Figs. 1 and 2) with penetration ranging from 0.2 to 12.6 mm.

Composites were examined 12 times by seven different groups from 0.1 to 14 days. Composites appear to maintain their sealing ability over time (Table 5, Figs. 1 and 2), with penetration ranging from 0.03 to 2.2 mm.

IRM was examined seven times by six different groups over a period ranging from 0 to 7 days. IRM appears to have reduced sealing ability over time (Table 5, Figs. 1 and 2) with penetration ranging from 0 to 2.3 mm.

MTA was examined three times by two groups over a period ranging from 1 to 3 days. MTA appears to have reduced sealing ability with penetration ranging from 0.3 to 1.4 mm, however, the number of trials were too few and the time frames were too short for definitive inference (Table 5, Figs. 1 and 2).

Based on the linear regression analysis (Fig. 2), the materials that provide the best sealing after 10 days were, in decreasing order of

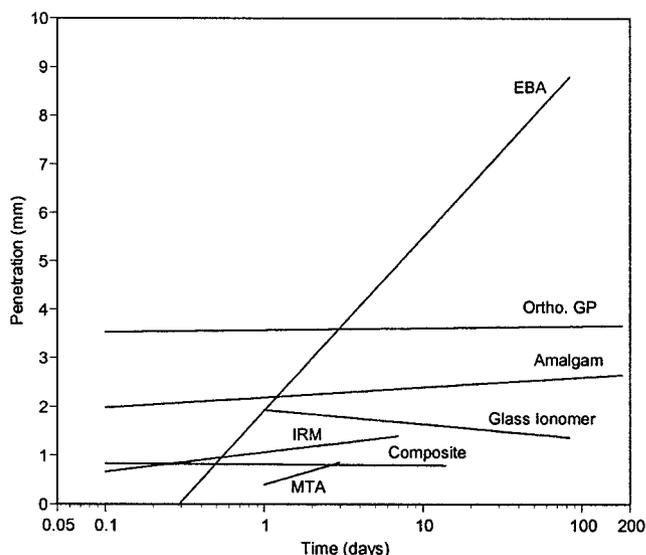


Figure 2. (Linear Regression) For each material, on the X-axis the time in days (logarithmically) the materials were immersed into the indicator solution is presented. On the Y-axis the penetration in millimeters the dye ran through the mass of the materials as measured from the apex to the crown of the teeth is presented.

efficacy: composites ($r^2 = .34$) > glass ionomer cement ($r^2 = .60$) > amalgam ($r^2 = .84$) > orthograde gutta-percha ($r^2 = .69$) > EBA ($r^2 = .86$).

Discussion

This systematic review identified 34 *in vitro* studies that examined the ability of materials used in retrograde obturation to inhibit dye/ink penetration of extracted human teeth following apicoectomy. The results of this study indicated that for time points beyond 10 days the materials that provide the best sealing were, in decreasing order of efficacy: composites, glass ionomer cement, amalgam, orthograde gutta-percha, and EBA. Interestingly, more than one-half of the identified studies lasted less than 10 days.

These results can be contrasted with a recent systematic review of *in vivo* retrograde obturation materials (3). The previous systematic review identified two randomized controlled clinical trials indicating that glass ionomer cement is slightly more effective than amalgam (22, 23). These results are similar to the current results.

However, the *in vivo* nonrandomized controlled clinical trials identified in the previous systematic review, while examining more materials, yielded different data than the randomized controlled trial and different results than the current review. Three different studies indicated that orthograde gutta-percha (27), EBA (25), or composites (26) are more effective than amalgam. Another group of studies indicated that amalgam is more effective than retrograde gutta-percha (24).

According to the above remarks, glass ionomer cement and composites seem to work better than amalgam when examined both by *in vitro* and *in vivo* experiments. A final conclusion cannot be stated for the other retrograde obturation materials for which the *in vitro* studies provide different results than the *in vivo* ones.

This disparity in the results clearly raises issues around the congruency of *in vitro* and *in vivo* studies. The variances might be attributed to experimental procedures. For example, the *in vitro* studies examined here used 8 different indicating agents, 10 different storage solutions, four different irrigation solutions, five different ways, and combinations of materials to orthograde fill the root canals, two different resection

angles, six different ways to resect the apices, seven different ways to prepare the retrograde cavity, and 12 different time periods of indicator immersion. The multiplicity of combinations is well over 1 million. Thus one long-term consideration is to begin narrowing the focus and increasing the standardization of *in vitro* studies to better anticipate *in vivo* results. Clearly, additional *in vitro* studies following the same protocol in every step of the experiment would augment useful information about the sealing ability and other properties of the materials used as retrograde fillings.

Finally, there are two caveats: first, these are *in vitro* studies and the results may not match the results from *in vivo* studies; and second, the maximum time period the materials were tested was 180 days (by only one study), which is very small when compared to the time period of clinical use of these materials. Thus, until *in vitro* methods are developed that can predict *in vivo* effectiveness, one must question their utility.

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